

**The effects of changes in temperature and salinity on the behavior of the
hermit crab *Pagurus longicarpus***

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Abstract

Hermit crabs of the species *Pagurus longicarpus* can be found in many Massachusetts tide pools. Temperatures and salinities in tide pools change far more rapidly than they do subtidally; moreover, climate change is predicted to bring warmer temperatures and increased rainfall to Massachusetts. Hermit crabs have no hard abdominal carapace and must rely on empty gastropod shells for shelter. In this research, I determined the size of shells that hermit crabs preferred in seawater of different temperatures and salinities and studied how changes in temperature and salinity affected their ability to find shelter and food. Hermit crabs in seawater of high temperature (33 °C) and salinity (40 ppt) were unlikely to occupy high quality shells within 24 h, and hermit crabs in low salinity seawater (20 ppt) often did not locate food within 15 min, suggesting that hermit crabs will struggle to find adequate shelter and food in the future.

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Chapter 1

Introduction

The effects of climate change

The Intergovernmental Panel on Climate Change (IPCC, 2014) has detailed how greenhouse gas emissions have increased along with increasing human population size and industrialization since the late 18th century. As carbon dioxide, methane, and nitrous oxide have continued to build up in the atmosphere, temperatures have increased, glaciers have begun to melt, and rainfall patterns have changed, among other things. The IPCC predicts that these changes will intensify in the future. The world's oceans will not escape the effects of climate change (reviewed by Harley et al., 2006). Warming temperatures will affect the survival of marine organisms that are already living close to their maximum thermal tolerance limits, such as some species of coral (reviewed by Hughes et al., 2003), copepods (Kelly et al., 2012), snails (Tomanek & Somero, 1999) and porcelain crabs (Stillman, 2003). Increased water temperatures will also make it possible for species that live in warmer waters to expand their ranges farther away from the equator, allowing them to compete with or parasitize more temperate species (reviewed by Harley et al., 2006). Melting ice and warming temperatures will both contribute to sea level rise (IPCC, 2014), and increasing storm frequency will make it more difficult for marine communities, such as coral communities, to recover from disturbances (reviewed by Harley et al., 2006). Additionally, as the oceans continue to absorb carbon dioxide (roughly a third of emitted carbon dioxide has been absorbed by the oceans so far) (IPCC, 2014), the effects of acidification will include changing the composition of marine communities (Hall-Spencer et al., 2008), weakening gastropod shells (Coleman et

al., 2014; Nienhuis et al., 2010), and interfering with the proper interpretation of chemical signals (de la Haye et al., 2012; Dixson et al., 2010; Munday et al., 2009).

Many of these changes will be felt strongly in the intertidal zone, which is already a stressful place to live. At low tide, which occurs twice daily, the intertidal zone can be a very heterogeneous area. Rocky or sandy beaches are often interspersed with tide pools of various sizes and depths. Sessile marine organisms in the intertidal zone may find themselves completely out of water at low tide, exposed to desiccation (e.g. Diederich et al., 2015; Young, 1978) and ambient air temperature (e.g. Diederich & Pechenik, 2013). Sessile organisms may also be found in intertidal tide pools at low tide. Even mobile organisms can become trapped in tide pools, where conditions change much faster than they do subtidally (Fraenkel, 1960; Koprivnikar & Poulin, 2009; Truchot & Duhamel-Jouve, 1980). Tide pool acidities fluctuate depending on the amount of photosynthesis being performed – pH tends to decrease at night, while pH will increase during the day (Truchot & Duhamel-Jouve, 1980). Hot and sunny days cause temperatures and salinities to rise quickly in tide pools, while rainy days can quickly lower salinities (Fraenkel, 1960; Koprivnikar & Poulin, 2009; Montory et al., 2016). At Nahant, Massachusetts, temperatures reached at least 32 °C in tide pools during the summer of 2016, and salinities reached at least 38 ppt (S.G., pers. obs.). Extreme summer temperatures and salinities will likely become even more extreme as temperatures continue to rise and rainfall patterns continue to change due to climate change (IPCC, 2014).

Intertidal life and hermit crab biology

Depending on the size and location of a tide pool in the northeast United States, the inhabitants for the duration of a low tide might include gastropods, bivalves, crustaceans, sea stars, tunicates, polychaetes, and more (e.g. Diederich & Pechenik, 2013; Gendron, 1977; Heck et al., 1995; Pechenik & Lewis, 2000; Sanders et al., 1962; Valentine et al., 2007). While the fluctuating conditions in the intertidal zone might not make it the ideal environment in which to live, many species are driven there by competition or predation (reviewed by Connell, 1972).

Hermit crabs can be found on many coasts, on the shore or in the water (Hazlett, 1981). Very few species of hermit crab are terrestrial; these breathe air through their gills, which must be kept moist (Cameron, 1981; Hazlett, 1981). Most hermit crabs are marine, and various species can be found from the intertidal zone down to much deeper waters, having been reported at depths of approximately 700 m (e.g. Hazlett, 1981; Pechenik & Lewis, 2000; Provenzano, 1968; Thompson et al., 1985).

Hermit crabs have very soft abdomens (Hazlett, 1981), and thus must find some sort of shelter to avoid predation (Reese, 1969), salinity stress (Pechenik et al., 2001), and desiccation (Brodie, 2005; Taylor, 1981), depending on the habitat in which the hermit crab is found. Fossil records show that hermit crabs likely appeared during the Jurassic Period (200-145 million years ago) (Glaessner, 1969), and occupied empty ammonite shells until empty gastropod shells became abundant during the Cretaceous Period (145-66 million years ago) and provided a thicker, more easily portable shelter for the hermit crabs (Fraaije, 2003). Most

hermit crabs today still seek out gastropod shells as their first choice of shelter (Reese, 1962), entering their first shells as megalopae (Brodie, 1999). The asymmetrical body plans of hermit crabs result in curved abdomens that fit well into these shells. Additionally, their fourth and fifth sets of legs are smaller than their other legs, fitting well against the interior of shells and holding the shells in place (Elmhirst, 1946).

As a result of numerous shell selection studies that have been done on a variety of hermit crab species, we have a fairly good idea of what types, sizes, and quality of shells hermit crabs prefer while experiencing conditions similar to those they would experience in their natural habitats. Hermit crabs are very selective about the shells they'll choose to occupy. Shells that are too small can slow their growth rate (Angel, 2000), shells that are too large require them to carry additional weight (Elwood & Glass, 1981; Osorno et al., 1998, 2005), shells with damage can expose them to predators (McClintock, 1985; Pechenik et al., 2001; Pechenik & Lewis, 2000), and shells with large symbionts attached can take up valuable space or add unneeded weight (Conover, 1976; Li & Pechenik, 2004; Pechenik et al., 2015b). Hermit crabs frequently assess one another's shells to determine if a better shell than their own might be found on another hermit crab (Hazlett, 1966). Such interactions are often called "shell fights" and involve an attacker holding its opponent's shell, probing at the opponent's shell, and repeatedly hitting its own shell against the opponent's (Dowds & Elwood, 1983; Hazlett, 1967). Shell exchanges most often occur after shell fights if both crabs assess that they would benefit from an exchange (Hazlett, 1978).

Study species and questions being addressed

Hermit crabs of the species *Pagurus longicarpus*, the long-wristed hermit crab, can be found intertidally and subtidally along the Atlantic coast of Canada and the United States from Nova Scotia to northeast Florida, and along the Gulf coast from Florida to Texas (Young, 1978). Males reach larger sizes than females (Bach et al., 2006), and individuals from northern populations reach larger sizes than those from more southern populations (Blackstone, 1985); the largest hermit crab we collected from Nahant, Massachusetts in the summer of 2016 had a wet weight of approximately 2 g.

In Massachusetts, long-wristed hermit crabs (except for very small individuals) can often be found occupying periwinkle (*Littorina littorea*) shells (Blackstone & Joslyn, 1984; Scully, 1979; S.G., pers. obs.). Periwinkles were likely introduced to Nova Scotia from northern Europe in the mid-1800s, and then spread down the coast to the northeast United States over the next half-century (Chapman et al., 2007). Long-wristed hermit crabs in New England often prefer these introduced shells over the shells of native gastropod species (Blackstone & Joslyn, 1984).

When periwinkles die, their shells become available to hermit crabs for housing; hermit crabs have been shown to kill gastropods for their shells (Rutherford, 1977), but this likely happens very rarely (Laidre, 2011). Pechenik et al. (2015a) hypothesized that most periwinkle shells become available after cold winter temperatures kill many periwinkles. After an unusually warm Massachusetts winter, long-wristed hermit crabs were found in shells of lower

quality than was typical in other years (Pechenik et al., 2015a). Thus, as temperatures continue to warm, it is likely that these hermit crabs will more often be found in poor quality shells.

As inhabitants of the diverse and heterogeneous intertidal zone, long-wristed hermit crabs employ a number of feeding methods. One method involves scooping up sand and ingesting any edible detritus that is mixed in while discarding any inedible particles (Roberts, 1968), although the sand itself is frequently ingested (Whitman et al., 2001). These hermit crabs can also consume larger pieces of food (i.e. oyster tissue and sea lettuce) by holding the food up to their mouths with their chelipeds (Roberts, 1968). Alternatively, they can hold the food with one cheliped and use the other to rip off smaller pieces to transfer to their mouths (S.G., pers. obs.). Interestingly, in shallow water where foam or larger pieces of food are floating on the surface, long-wristed hermit crabs will roll backwards onto their shell, and then use their chelipeds to grasp the food and bring it closer to their mouths (Scully, 1978; S.G., pers. obs.). The final method of feeding involves producing currents with the maxilla, chelipeds, and mouthparts to bring small food particles towards the mouth, where they are consumed (Whitman et al., 2001).

In coastal Massachusetts, where the studies for my thesis were conducted, temperatures are expected to rise up to 4 or 5 °C relative to 2005 temperatures by the year 2100, and rainfall is expected to increase 10 to 20% (IPCC, 2014). Long-wristed hermit crabs will likely feel the effects of climate change strongly at low

tide in the many tide pools on Massachusetts beaches (Pechenik & Lewis, 2000; S.G., pers. obs.). Thus, I set out to answer three main questions in my research:

1. How do changes in temperature and salinity affect the size of the shells long-wristed hermit crabs choose to occupy?
2. How do changes in temperature and salinity affect the quality of the shells that long-wristed hermit crabs choose to occupy?
3. How do changes in temperature and salinity affect the ability of long-wristed hermit crabs to locate food in their vicinity?

I hope that the results of the studies detailed in the following chapters will help to illustrate how climate change may affect marine intertidal communities. Because of the highly predictable nature of hermit crab shell selection, it is relatively simple to determine whether changes in environmental conditions alter their behavior. If hermit crab shell-selection and food-finding behavior will be affected by shifts in temperature and salinity in tide pools at low tide, it is likely that the behavior of other marine intertidal organisms will be affected as well.

Chapter 2

**The effects of changes in temperature and salinity on the size of shells
selected by the hermit crab *Pagurus longicarpus***

Abstract

Pagurus longicarpus is a species of hermit crab that can be found intertidally and subtidally along the Atlantic coast of North America from Nova Scotia to Florida, and along the Gulf coast of the United States. These hermit crabs rely on empty gastropod shells for shelter. At low tide, they often get stranded in tide pools where changes in temperature and salinity can occur rapidly. We tested how changes in temperature and salinity affected the sizes of the shells occupied by individuals of a range of sizes. Increasing seawater temperature from 22 °C to 32 °C had a significant effect on shell size, as did lowering the temperature to 16 °C. Adjusting the salinity from 30 ppt to 20 ppt or 40 ppt did not have a significant effect, but lowering the salinity to 15 ppt did. This study builds on previous studies of shell size preference of hermit crabs by highlighting the importance of taking the temperature and salinity into account when assigning shells of appropriate size in any future studies. These results also suggest that the effects of climate change may alter the sizes of shells that hermit crabs of the species *Pagurus longicarpus* will seek out in the field.

Introduction

Hermit crabs have no natural protection for their soft abdomens (Hazlett & Provenzano, 1965), which leaves them vulnerable to predators (Hazlett, 1981), desiccation (Herreid, 1969), and salinity stress (Pechenik et al., 2001). In order to survive, they have to find some sort of shelter, preferably a portable one in the form of a gastropod shell (Reese, 1962). When a hermit crab is not occupying a

shell of good quality that is of ideal size for its weight (Angel, 2000; Vance, 1972a), it will investigate almost any shell it comes across, looking for a better option (McClintock, 1985). Occupying a shell that is too small increases the likelihood that a hermit crab will suffer from predation (Vance, 1972b) or desiccation (Taylor, 1981), and occupying a shell that is unnecessarily large means more energy will be expended carrying the additional weight (Osorno et al., 1998; Wilber, 1990).

In any scientific study of hermit crab behavior, it is necessary to provide shells of appropriate size for each individual in order to minimize shell-searching behavior (McClintock, 1985). To this end, there have been a number of studies conducted to determine the shell sizes preferred by hermit crabs of different sizes and species (e.g. Angel, 2000; Briffa & Elwood, 2007; Vance, 1972a). These experiments were performed at temperatures and salinities typically seen in the habitats of the hermit crabs being studied.

One species of hermit crab on which such a study was focused (Angel, 2000) is the long-wristed hermit crab *Pagurus longicarpus*, which can be found along the Atlantic coast of Canada and the United States, as well as the Gulf coast (Young, 1978). These hermit crabs can be found both intertidally and subtidally (McDermott, 1999), and at low tide are often stranded in tide pools (Fraenkel, 1960). Conditions in tide pools can change rapidly depending on the weather on a given day (Fraenkel, 1960). On hot, sunny days the temperature and salinity in a tide pool can increase rapidly (Fraenkel, 1960; Koprivnikar & Poulin, 2009), and on rainy days the salinity can rapidly decrease (Montory et al., 2016). Even more

abrupt changes can occur when a rising tide floods into tide pools, returning the temperature and salinity to more average levels (Berger & Kharazova, 1997; Fraenkel, 1960). As the effects of climate change begin to be felt more strongly (IPCC, 2014), the changes in tide pool physical conditions will likely become more extreme as well.

Previous studies have examined some of the effects of changing environmental conditions on hermit crab physiology and behavior. Changes in temperature have been shown to make the startle response of hermit crabs less predictable (Briffa et al., 2013), and decreases in salinity have been shown to cause hermit crabs to retract into their shells and reduce their rates of oxygen consumption (Davenport et al., 1980). However, we are not aware of any studies that have examined how the sizes of shells chosen by hermit crabs are affected by changes in temperature or salinity. In this study, we set out to determine whether the shell size preference of long-wristed hermit crabs, or their ability to distinguish shells of appropriate size, is affected by levels of temperature or salinity that could be experienced in tide pools currently or in the near future.

Methods

Periwinkle shell collection and preparation

Periwinkle snails (*Littorina littorea*) with shell aperture lengths ranging between 7.6 mm and 19.1 mm (mean length = 14.3 mm, median length = 14.4 mm, S.D. = 2.4 mm, N = 353 shells) were collected haphazardly from the rocky intertidal zone at Beverly, Massachusetts in February 2016. The periwinkles were

put on ice and brought to Tufts University, where they were euthanized by freezing. The dead periwinkles were then placed in room temperature seawater (a mixture of Instant Ocean and 1µm filtered seawater, ~30 ppt salinity) and allowed to decompose. The water was changed periodically over several weeks, until no decomposed tissue was visible in the discarded water. After the tissue decomposition process was complete, the shells were scrubbed with a bristled brush to remove any algal build-up, rinsed several times in tap water, and inspected to ensure they were free of symbionts such as *Crepidula convexa* (Li & Pechenik, 2004) and barnacles (McDermott, 2001).

Hermit crab collection and care

Hermit crabs (*Pagurus longicarpus*) occupying periwinkle shells were collected haphazardly from the rocky intertidal zone at Nahant, Massachusetts in the spring and summer of 2016 and transported to Tufts University, where they were kept in laboratory aquaria containing continuously aerated artificial seawater (Instant Ocean, called “seawater” from now on) at 30 ppt salinity and room temperature (~21 °C). The hermit crabs were fed three times per week in smaller plastic containers (to prevent waste build-up in the larger aquaria) containing seawater on a diet of artificial crab meat (transOCEAN; Alaskan pollock and King Crab) and shrimp pellets (O.S.I.). After feeding they were returned to the aquaria. The hermit crabs were allowed to acclimate to laboratory conditions for one to three weeks before experiments began. No hermit crab was used in more

than one trial. After experimentation, the hermit crabs were returned to the collection site.

Evaluating shell choices made under potentially stressful conditions

Experiments were conducted to determine the shell sizes preferred by hermit crabs of a range of sizes under potentially stressful conditions, following the general procedure of Angel (2000) and Vance (1972). Fifty hermit crabs that had both of their chelipeds were used in each experimental treatment. Each of the hermit crabs was free from visible parasites (McDermott, 2001), although most were occupying damaged shells or shells with symbionts attached such as *Crepidula plana*, barnacles, and coralline red algae, as these were the shells they were collected in (although they may have exchanged shells with one another since the date of collection). Hermit crabs were fed on the day before being used in an experiment, and were then placed in a 20 L aquarium filled with continuously aerated seawater under either control conditions (22 °C, 30 ppt salinity), one of two altered temperatures (32 °C, simulating possible thermal conditions in a tide pool on a hot day, or 16 °C), or one of three altered salinities (40ppt salinity, simulating possible conditions in a tide pool on a sunny, hot day; 20 ppt salinity, simulating possible conditions in a tide pool on a rainy day; or 15 ppt salinity, simulating high levels of precipitation).

The sizes of the shells selected by hermit crabs were examined after 24 h of acclimation to the new temperature and salinity conditions. In addition to the 50 shells occupied by the hermit crabs, another 250 intact, empty shells ranging in

aperture length from 7.6 mm to 19.1 mm (selected haphazardly from those collected at Beverly, MA) were added to the aquarium for a total of 300 shells. The hermit crabs were given 48 h to investigate their options and switch shells, after which time their final shell choice was recorded (Angel, 2000; Vance, 1972a).

The hermit crabs were placed individually in cups with room-temperature, 30 ppt seawater, and one-by-one were removed from their shells by heating in seawater (Pechenik et al., 2015b). Hermit crabs were blotted dry and massed to the nearest mg, and shell aperture length was measured with calipers to the nearest 0.1 mm. Any gravid female hermit crabs or hermit crabs missing one or both of their chelipeds at the end of the experiment were discarded from the analysis. The sizes of the hermit crabs in each treatment are detailed in Table 2.1.

Data analysis

The results were plotted on a log scale in GraphPad Prism version 7.02, and the lines of best fit for each experimental group were compared to every other group by linear regression. Additionally, the number of hermit crabs at the end of each trial that were or were not occupying shells with damage or symbionts (that had not switched into one of the new, intact shells that were provided at the beginning of each trial) were compared using a chi-squared goodness-of-fit test in R version 3.2.2.

In order to determine if the shell sizes preferred by long-wristed hermit crabs (*P. longicarpus*) in the field at Nahant, Massachusetts have remained

constant over the years, a linear regression was used to compare our results to the results obtained by Angel, who collected her hermit crabs at the same study site in 1997 (Angel, 2000). The methods used by Angel were similar to those used in this study, with the notable exception that Angel included gravid females in her results. We approximated the preferred shell sizes for long-wristed hermit crabs recorded by Angel based on a figure from her paper. The equation for the line of best fit obtained by Angel was $y = 0.2623x + 0.4342$, while the equation we obtained based on the placement of points on her figure was $y = 0.2549x + 0.4479$. The laboratory experiment from Angel was conducted at 18 °C, so her results were compared to the decreased temperature treatment (16 °C) and the control treatment (22 °C) in the present study to determine if the shells sizes preferred by these hermit crabs at Nahant, Massachusetts have remained constant.

Results

When plotted on a log scale, the aperture length of each shell occupied by a hermit crab (after 48 h in experimental conditions) increased consistently with the wet weight of the hermit crab that selected each shell (r^2 of at least 0.76 for each treatment) (Figs. 2.1 & 2.2). Linear regressions showed that the slope of the line of best fit for the control treatment (22 °C, 30 ppt) was significantly different from the slopes of the lines of best fit for both the 16 °C treatment ($F = 7.678$, $p < 0.01$) and the 32 °C treatment ($F = 5.849$, $p = 0.02$) (Fig. 2.1), as well as the 15 ppt salinity treatment ($F = 4.485$, $p = 0.04$) (Fig. 2.2a). However, it was not significantly different from either the 20 ppt salinity treatment ($F = 1.638$, $p =$

0.21) or the 40 ppt salinity treatment (1.348, $p = 0.25$) (Fig. 2.2b & c). Every other linear regression between pairs of groups showed no significant difference in the slopes of the lines of best fit for the data.

The number of hermit crabs at the end of each trial that were or were not occupying shells with damage or symbionts (Table 2.2) was not significantly different across temperature trials ($\chi^2 = 0.52$, $df = 2$, $p = 0.77$) but was different across salinity trials ($\chi^2 = 8.33$, $df = 3$, $p = 0.04$).

Linear regressions comparing our data to those from Angel (2000) suggest that the shell size preferences of the long-wristed hermit crab may not have changed over the past 20 years. The slope of our approximated line of best fit for Angel's data was not significantly different from the slope of the line obtained in our study at 16 °C ($F = 0.197$, $p = 0.66$), but was significantly different from the slope of the line obtained in our study at 22 °C ($F = 6.403$, $p = 0.01$) (Fig 2.3).

Discussion

Because the number of hermit crabs that were still occupying shells with damage or symbionts by the end of the experiment did not differ among treatments with different temperatures, the changes in temperature likely did not prevent the hermit crabs from switching shells. Thus, any differences in the shell sizes occupied by the hermit crabs at different temperatures were likely due to altered preferences. We observed significant differences in preferred shell size between hermit crabs under control conditions (22 °C and 30 ppt salinity) and hermit crabs at both the lower temperature of 16 °C and the higher temperature of

32 °C (Fig. 2.1). Generally, at both the lowered and elevated temperatures, small hermit crabs preferred smaller shells than similarly-sized hermit crabs did under control conditions, and large hermit crabs preferred larger shells than similarly sized hermit crabs did under control conditions.

Reducing the salinity from 30 ppt to 20 ppt did not alter shell size preferences, but reducing the salinity to 15 ppt did (Fig. 2.2). As with the hermit crabs at 16 °C and 32 °C, the small hermit crabs preferred smaller shells than hermit crabs of the same size under control conditions, and large hermit crabs preferred larger shells than those under control conditions. Hermit crabs at Nahant will likely not be frequently seeking out different-sized shells as a result of lowered salinity: given an average baseline salinity of 30 ppt or slightly above in tide pools at Nahant during the summer of 2016 (S.G., pers. obs.), it would take enough rain to equal half the original amount of seawater in a tide pool to lower the salinity to 15 ppt.

There was no significant difference in the size of shells chosen by hermit crabs under control conditions versus those held at 40 ppt salinity (Fig. 2.2). We observed seawater in tide pools at Nahant at low tide reaching a maximum salinity of 38 ppt in the summer of 2016. On warmer days as are predicted for the future (IPCC, 2014), with higher levels of evaporation, it is reasonable to expect that the salinity in tide pools may exceed 40 ppt. Future studies thus might consider whether even higher salinity levels might significantly affect the shell size choice of long-wristed hermit crabs.

The number of hermit crabs that were still occupying shells with damage or symbionts by the end of the experiment differed significantly between the treatments with different salinities, suggesting that changes in salinity might have affected the ability of the hermit crabs to recognize or switch into the better shells. The hermit crabs at 40 ppt salinity were found in shells with damage or symbionts more often than the hermit crabs in any other treatment. However, the 40 ppt treatment did not significantly affect the size of the shells occupied by the hermit crabs when compared to the control treatment, suggesting that even if the shift from 30 ppt to 40 ppt did affect some of the hermit crabs' abilities or choices to switch shells, those that did make the switch chose shells of sizes similar to those chosen by their similarly-sized conspecifics under control conditions.

A hermit crab in a shell too small for its mass is more likely to suffer from predation (Vance, 1972b), desiccation (Taylor, 1981), and a decreased growth rate (Angel, 2000) than a hermit crab in a shell of ideal size. Moreover, a hermit crab in a shell that is too large is expending more energy than is necessary to carry a shell that can protect its entire body (Wilber, 1990). We wouldn't expect hermit crabs to knowingly choose to occupy shells that might put them in danger. However, the direction of changes in shell size preference that we observed were consistent in all experimental treatments when compared to the control treatment: small hermit crabs experiencing a change in temperature or salinity preferred smaller shells than the small hermit crabs under control conditions, and large hermit crabs experiencing a change in temperature or salinity preferred larger shells. Excluding the control treatment, no other combinations of treatments

differed significantly from one another. This suggests that the substantial changes in temperature and salinity did not render hermit crabs incapable of distinguishing between different sized shells. The response to shifts to potentially stressful environmental conditions might not be specific to the type or direction of the shift. Instead, it might be a more generalized response to a fluctuating environment. This raises the question of why the hermit crabs might be shifting their shell size preference.

A hermit crab choosing a smaller shell could be making a selection based on shell weight – if higher than usual amounts of energy are being expended to maintain normal functions in response to stressful conditions, a smaller, lighter shell could indicate a desire to conserve as much energy as possible, while still possessing a shelter that provides some protection (Herreid & Full, 1986; Osorno et al., 1998). A hermit crab choosing a larger shell could be seeking out a shelter that it can retract further into than usual, perhaps to better avoid predators when they may not have the energy to fight or flee from them. Additionally, larger shells can hold more water in their interiors, providing a greater buffer against a fluctuating environment (Bertness, 1982). Larger shells also provide more room for females to brood embryos (Hazlett, 1981), and a future study could examine whether females will still brood embryos while experiencing shifts in temperature and salinity.

But none of this explains why smaller crabs experiencing changes in temperature or salinity preferred smaller than average shells while the larger crabs experiencing the same conditions preferred larger than average shells. This could

have been an artefact of the linear regression, as the hermit crabs that were collected haphazardly for the control treatment were on average larger than those collected haphazardly for most of the other treatments. It is also possible that the difference is related to sex, as adult male long-wristed hermit crabs reach larger sizes than adult females (Blackstone & Joslyn, 1984). Future studies could consider the results of males and females separately to determine if their shell size choices differ from one another when water temperature or salinity are changed.

Long-wristed hermit crabs in the intertidal zone could potentially be exposed to temperature shifts multiple times a day (Fraenkel, 1960). At low tide, any individuals that get caught in tide pools will experience increasing temperatures on a hot day. And when tides rise, the incoming water will likely have a lower temperature that quickly cools tide pools. In habitats such as Nahant, Massachusetts where the supply of high-quality empty shells is often limited (Pechenik et al., 2015a), a frequently shifting pattern of shell preference could lead to an increased amount of time spent actively seeking out and fighting for new shells (Hazlett, 1966), time that could otherwise be spent hunting for food or mates. Future studies could examine whether hermit crabs exposed to oscillating temperatures – as might be expected to occur routinely in the intertidal zone – or seasonal changes in temperature will switch shells as often as the temperature shifts.

In addition to changes in temperature and salinity, intertidal organisms experience daily fluctuations in acidity. At night, when tide pool inhabitants perform cellular respiration but no photosynthesis is taking place, pH decreases.

During the day, when sunlight increases the rate of photosynthesis in algae, pH increases. In the coming years, as the ocean continues to absorb carbon dioxide from the atmosphere, the overall pH of seawater will decrease (IPCC, 2014). de la Haye et al. (2011) showed that decreases in pH caused hermit crabs of the species *P. bernhardus* to take longer to switch from a shell that was too small to a shell of optimal size. A future study could determine whether changes in acidity cause hermit crabs to choose shells of different sizes.

The shell sizes preferred by long-wristed hermit crabs at Nahant, Massachusetts have likely not changed over the past 20 years. The laboratory study designed by Angel (2000) was conducted at 18 °C, and while we did not conduct a trial at this exact temperature, the results from her study were not significantly different from our results at 16 °C (Fig. 2.3), and thus lend support to our conclusions. Studies such as these should be replicated with individuals from warmer climates, as long-wristed hermit crabs can be found along the southern Atlantic and Gulf coasts of the United States (Young et al., 2002). It would be interesting to know whether or not individuals acclimated to warmer temperatures show roughly the same shell size preferences at the warmer temperatures that we observed with the Massachusetts hermit crabs. If they do, it would suggest that as the climate continues to warm, the northern long-wristed hermit crabs will probably be found more consistently in smaller shells (smaller individuals) and larger shells (larger individuals). Hermit crabs have been observed to kill snails for their shells (Rutherford, 1977), but this is likely a rare occurrence (Laidre, 2011), so hermit crabs are limited by the number of available empty shell options:

on some beaches, small shells are hard to come by (e.g. Pechenik et al., 2015), while on others large shells might be in short supply (e.g. Vance, 1972b). Thus, the effects of temperature shifts on shell size preferences would likely vary between locations.

Hermit crabs are constantly on the lookout for high quality gastropod shells of appropriate size to use as shelters (Pechenik et al., 2001), and occupying inadequate shells could lead them to spend more of their time and energy on finding shells of better quality (McClintock, 1985). Thus, in any study of hermit crab behavior that is not focused on shell size, it is important to ensure that every individual hermit crab is in a shell of preferred size to avoid any confounding factors that might influence behavior. Our results indicate that the preferred shell size for a hermit crab at one temperature and salinity may not be the same shell size preferred under altered conditions. Thus, whenever designing a study with hermit crabs of any species, it is important to ensure the model being used for preferred shell size was created under similar conditions. In addition, temperature and salinity should be closely monitored during any study to avoid unnecessary and potentially distracting fluctuations.

To summarize, we have shown that it is important to ensure that any study that requires long-wristed hermit crabs (*Pagurus longicarpus*) to be occupying shells of preferred size needs to take temperature and salinity into account, as shell size preferences can clearly change under different environmental conditions.

Tables

Table 2.1. Number and size of hermit crabs (*Pagurus longicarpus*) in each treatment after gravid females and hermit crabs missing one or both of their chelipeds were discarded from the analysis.

Treatment	n	wet weight (mg)		
		mean	median	S.D.
Control (22 °C, 30 ppt)	35	478	352	298
16 °C	34	323	251	248
32 °C	42	490	314	333
15 ppt	44	320	240	285
20 ppt	35	364	277	241
40 ppt	46	293	234	235

Table 2.2 Percent of hermit crabs (*Pagurus longicarpus*) in each treatment that were occupying shells with damage or symbionts after being given 48 h to choose among 250 intact shells of a range of sizes.

Treatment	n	Percent occupying shells with damage or symbionts
Control (22 °C, 30 ppt)	35	20.00
16 °C	34	26.47
32 °C	42	26.19
15 ppt	44	6.82
20 ppt	35	17.14
40 ppt	46	30.43

Figures

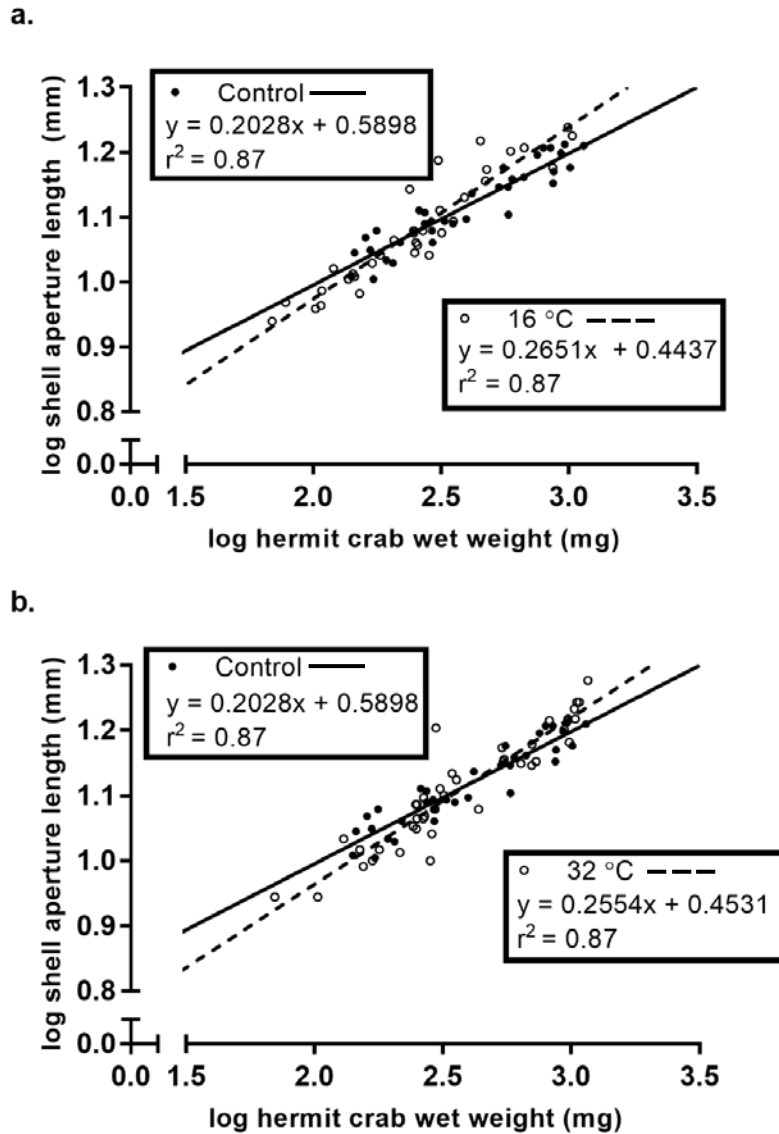


Figure 2.1. Effects of temperature on the relationship between hermit crab (*Pagurus longicarpus*) wet weight and size of shell occupied under control conditions (22 °C, 30 ppt salinity, n = 35, solid circles and line) compared to occupancy at (a) 16 °C and 30 ppt salinity (n = 34) and (b) 32 °C and 30 ppt salinity (n = 42). Hermit crabs were given 250 empty shells to choose from over 48 h.

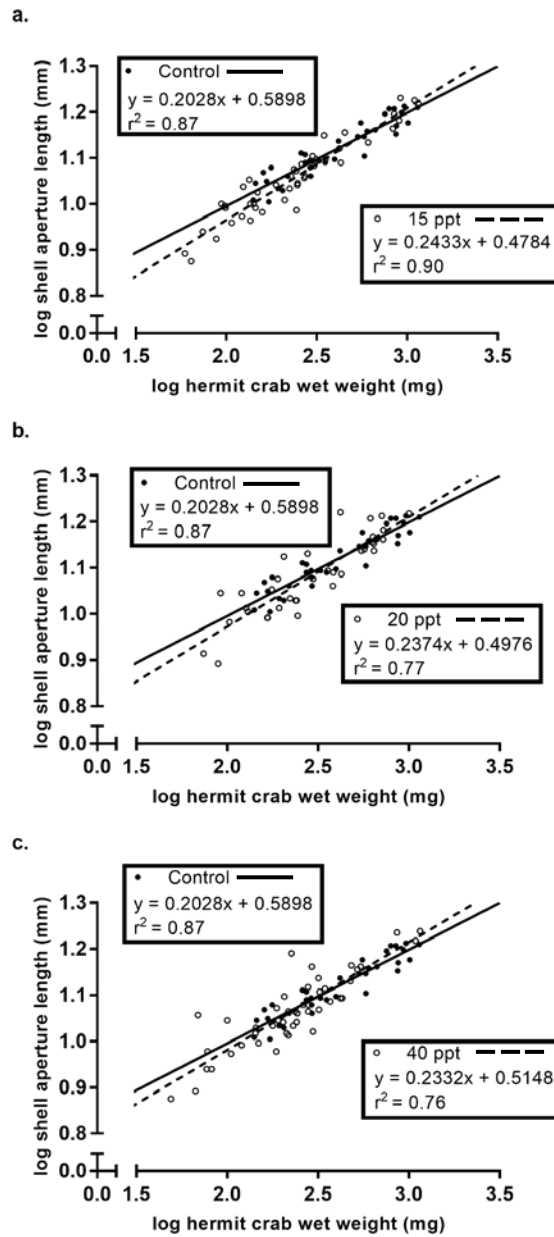


Figure 2.2. Effects of salinity on the relationship between hermit crab (*Pagurus longicarpus*) wet weight and size of shell occupied under control conditions (22 °C, 30 ppt salinity, n = 35, solid circles and line) compared to occupancy at (a) 22 °C and 15 ppt salinity (n = 44), (b) 22 °C and 20 ppt salinity (n = 34), and (c) 22 °C and 40 ppt salinity (n = 46). Hermit crabs were given 250 empty shells to choose from over 48 h.

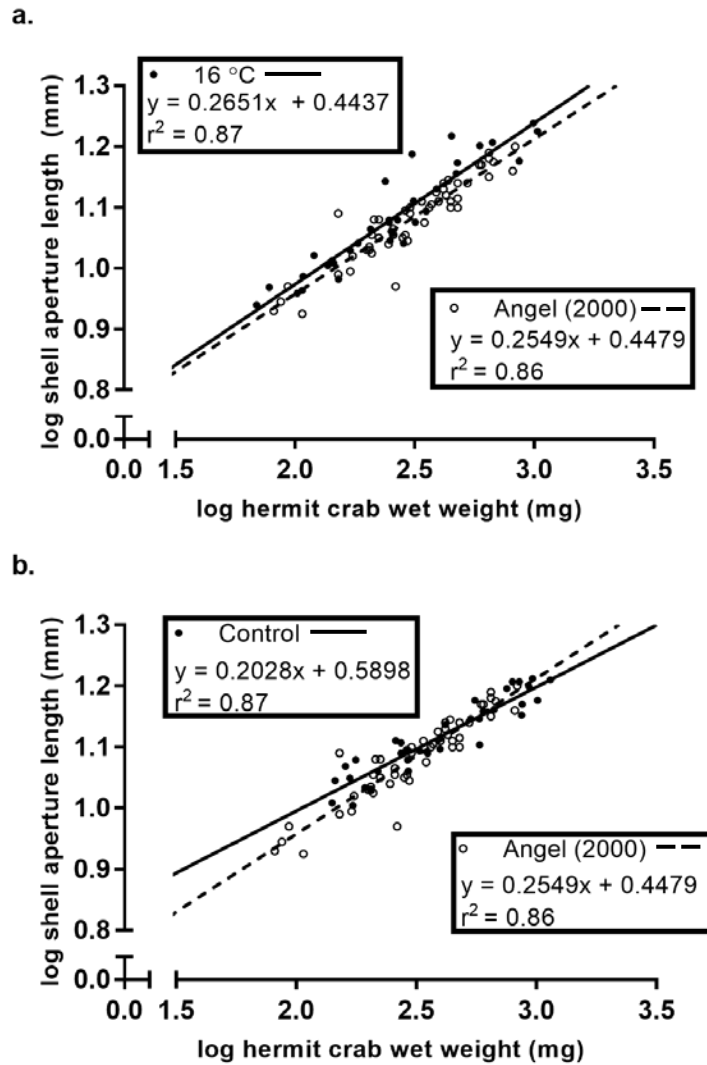


Figure 2.3. Relationship between hermit crab (*Pagurus longicarpus*) wet weight and size of shell occupied under (a) decreased temperature conditions (16 °C, 30 ppt salinity, n = 34, solid circles and line) and (b) control conditions (22 °C, 30 ppt salinity, n = 35, solid circles and line) in this study compared to the 18 °C, 30 ppt salinity conditions (n = 50, hollow circles and dashed line) used by Angel in 1997 (Angel, 2000). In the current study, hermit crabs were given 250 empty shells to choose from over 48 h. Methods used by Angel (2000) were similar, with the notable exception being that gravid females were included in her data.

Chapter 3

The effects of gradual and sudden changes in temperature and salinity on the ability of the hermit crab *Pagurus longicarpus* to choose intact shells of appropriate size to occupy

Abstract

Intertidal zones are highly dynamic environments. At low tide, temperatures and salinities in intertidal tide pools can change greatly depending on the weather conditions on a given day. As we begin to feel the effects of climate change more strongly, conditions in tide pools will likely become even more extreme. Hermit crabs of the species *Pagurus longicarpus* can be found intertidally and subtidally along the Atlantic coast of North America from Nova Scotia to Florida and along the Gulf coast as well. At low tide, they can often be found in tide pools. We tested how the gradual changes in temperature and salinity that might occur in tide pools at low tide, as well as the sudden changes that might occur in tide pools as tides rise, affected the ability of hermit crabs to occupy high quality shells of appropriate size over small shells and shells with drill holes. Increasing both temperature and salinity gradually from 21 °C and 30 ppt to 33 °C and 40 ppt had the greatest effect on the types of shells occupied by the hermit crabs over 24 h, compared to the hermit crabs that remained at the control temperature and salinity of 21 °C and 30 ppt. A gradual increase in both temperature and salinity to 30 °C and 40 ppt also had a noticeable effect, as did increasing just temperature to 33 °C. Sudden changes in temperature from 33 °C to 21 °C, and sudden changes in salinity from 40 ppt to 30 ppt also caused the hermit crabs to take longer to occupy good shells. Our results suggest that as the planet warms and precipitation patterns change, hermit crabs of the species *Pagurus longicarpus* will more often be found occupying shells that are damaged

or not of an ideal size, leaving them more exposed to predation, desiccation, and other stressors.

Introduction

Hermit crabs living in the world's intertidal zones are subject to daily fluctuations in temperature (Fraenkel, 1960) and salinity (Davenport et al., 1980) as tides rise and fall. These fluctuations will likely become more extreme in the near future, as air temperatures are predicted to increase and precipitation patterns are predicted to change over the next 100 years. In Massachusetts, where there are many rocky beaches with tide pools inhabited by hermit crabs at low tide (Pechenik & Lewis, 2000; S.G., pers. obs.), average surface temperature is predicted to increase by up to 4 °C by the year 2100 (relative to temperatures from 2005) and precipitation is predicted to increase by about 10% (IPCC, 2014). There will also be days on which temperature and salinity levels will be more extreme than the predicted average, and tide pool temperatures and salinities will thus fluctuate to more extreme levels. Several studies have shown the limits of hermit crabs' and other marine invertebrates' abilities to survive at elevated temperature (e.g. Fraenkel, 1960; Southward, 1958) and reduced salinity (e.g. Montory et al., 2016; Pechenik et al., 2001) at different stages of development. But how might rapid, non-lethal shifts in temperature and salinity affect the behavior of intertidal inhabitants?

This question is particularly relevant to hermit crabs, as they rely on their mobility and sensory capacity to find food (Briffa et al., 2012), shelter (Reese,

1962), and mates (Hazlett, 1966). In intertidal tide pools where they might be found on hot, sunny days, the temperature will increase far more rapidly than the temperature of deeper water (Fraenkel, 1960). On such days the salinity of the water in tide pools will also be prone to increase, as evaporation reduces the amount of water in the tide pools but not the amount of salt (Koprivnikar & Poulin, 2009). In contrast, on rainy days the salinity of tide pools will decrease much faster than in larger bodies of water (Montory et al., 2016). Just as drastic, and even more sudden, changes will occur when tides rise and the tide pools are flooded with seawater that has a temperature and salinity much less affected by that day's weather conditions (Berger & Kharazova, 1997; Fraenkel, 1960). These abrupt changes, while bringing ambient conditions back to a more average level, will likely be a shock to the hermit crabs that were experiencing the more extreme conditions present in their environment at low tide.

The shelter hermit crabs seek out under these fluctuating conditions is especially important for their survival, as these decapod crustaceans lack the hard carapaces that are found on other crustaceans; in fact, they have no natural protection at all for their soft abdomens (Hazlett, 1981). As a result, they seek out unoccupied gastropod shells as their preferred form of shelter (Reese, 1962). Hermit crabs' curled abdomens fit nicely into the curved interiors of gastropod shells. Their fourth and fifth sets of legs (located on the thorax) are smaller than their other legs, to better fit against the inner walls of shells, holding the shells in place (Elmhirst, 1946). Hermit crabs rarely kill snails for their shells (Laidre, 2011) but will fight one another over that crucial resource (Hazlett, 1966).

Hermit crabs are incredibly selective about the shells they will choose to occupy. They carefully examine shells to determine size (shells that are too small can reduce their growth rate and leave them more vulnerable to predation and desiccation; thus hermit crabs often occupy shells that leave some room for them to grow) (Angel, 2000; Brodie, 2005; Spight, 1985), shape (Arce & Alcaraz, 2012), whether there is any damage that might leave them vulnerable to predators (McClintock, 1985; Pechenik & Lewis, 2000; Pechenik et al., 2001), and whether there are any symbionts attached to the shell that might take up space or add unnecessary weight (Conover, 1976; Li & Pechenik, 2004; Pechenik et al., 2015b).

Shells can become damaged by wear and tear over time, or by the action of predators of hermit crabs or predators of the original gastropods that made the shells (Pechenik & Lewis, 2000). One predator that damages the shells of living gastropods is the moon snail, *Euspira heros* (Pechenik & Lewis, 2000), which uses a combination of an acidic secretion and scraping with their radula to drill perfectly circular holes into the shells of their prey (Carriker & Williams, 1978; Pechenik & Lewis, 2000). While the predation of a gastropod by a moon snail frees up the shell for use by hermit crabs, Pechenik and Lewis (2000) showed that hermit crabs will go to great lengths to avoid occupying such drilled shells. They'll even choose intact shells appropriate for hermit crabs 50% of their size over a shell of appropriate size with a drill hole (Pechenik & Lewis, 2000). This is understandable, because in drilled shells hermit crabs are highly susceptible to predation and eviction by conspecifics (Pechenik et al., 2001).

The long-wristed hermit crab, *Pagurus longicarpus*, can be found in large numbers intertidally in Massachusetts. The adults of this species at our study site are often found occupying shells made by periwinkle snails (*Littorina littorea*) (Pechenik et al., 2015a). The long-wristed hermit crab is an excellent organism to represent the invertebrates of the Massachusetts intertidal zones. As temperatures and precipitation levels continue to change, it will be difficult to predict how organisms will respond in an environment that changes so rapidly. But the shell selection behavior of these hermit crabs is fairly predictable under current, relatively average conditions and has been well documented over the years (e.g. Angel, 2000; Blackstone & Joslyn, 1984; Pechenik & Lewis, 2000), so it should be simple to observe whether or not fairly rapid changes in temperature and/or salinity affect the choices they make and the speed at which they make those choices.

Previous studies have examined some of the effects of changing environmental conditions on hermit crab physiology and behavior. Changes in temperature have been shown to make the hermit crab startle response (the amount of time taken for hermit crabs to emerge from their shells after being disturbed) less predictable (Briffa et al., 2013), and decreases in salinity have been shown to cause hermit crabs to retract into their shells and reduce their oxygen consumption (Davenport et al., 1980). Several studies have also demonstrated that ocean acidification affects the ability of hermit crabs to detect food and identify shells of appropriate size (Briffa et al., 2012; de la Haye et al., 2012, 2011; Kim et al., 2016). But we are not aware of any studies that have

examined how shell selection is affected by changes in temperature or salinity, even though this is clearly an important indicator of how intertidal hermit crabs will be able to resist predation and desiccation in a changing environment.

Here, we examine the shells chosen by the long-wristed hermit crab after gradual changes in temperature and/or salinity (to represent the effects of changing conditions in tide pools at low tide) and the shells chosen following sudden changes in temperature and salinity (to represent the effects of a rising tide suddenly flooding tide pools). If the hermit crabs don't end up occupying intact shells of appropriate size, we can predict that they will become more susceptible to predators and desiccation as the climate continues to change.

Methods

Hermit crab collection and care

Hermit crabs (*Pagurus longicarpus*) occupying periwinkle (*Littorina littorea*) shells were collected haphazardly from the rocky intertidal zone at Nahant, Massachusetts in the summer and early fall of 2016. They were transported to Tufts University, where they were kept in laboratory aquaria containing aerated artificial seawater (Instant Ocean, called “seawater” from now on) at 30 ppt salinity and room temperature (~21 °C). The hermit crabs were fed three times per week in smaller plastic containers (to minimize waste build-up in the larger tanks) containing seawater on a diet of artificial crab meat (transOCEAN; Alaskan pollock and King Crab) and shrimp pellets (O.S.I.). After each feeding they were returned to the aquaria. The hermit crabs were allowed to

acclimate to laboratory conditions for one to three weeks before experiments began. No hermit crab was used in more than one trial.

Evaluating shell choice after gradual changes in temperature and/or salinity

To determine how extreme conditions in tide pools at low tide might affect hermit crabs, experiments were conducted to determine the types of shells hermit crabs would choose to occupy at different temperatures and salinities. In a previous study, we established the shell sizes preferred by locally collected long-wristed hermit crabs at various temperature and salinity levels (Gilliand, unpublished data); the results of that study were used here to ensure that we selected shells of appropriate size for each hermit crab.

Fifteen hermit crabs were used in each treatment. All were free from visible parasites (McDermott, 2001), had both chelipeds, were not gravid, and were not observed to have molted at any point after selection for the experiment. Hermit crabs were fed on the day before being used in an experiment, removed from their shells by cracking with a vise (Pechenik et al., 2015b), gently blotted dry and weighed to the nearest mg, and placed in individual plastic containers (12 cm x 8.3 cm x 3.2 cm) with 180 mL of seawater. Any hermit crabs weighing less than 300 mg or more than 1,600 mg were not used in the study, due to difficulty obtaining shells of appropriate size for them. Each hermit crab was provided with either a shell 75% of ideal size, a shell of ideal size drilled by a moon snail (Carriker & Williams, 1978; Pechenik & Lewis, 2000), or no shell at all. The containers were covered with plastic wrap and aerated by a steady stream of

bubbles delivered through a glass Pasteur pipette poked through the plastic wrap. They were then left to acclimate in the containers overnight.

In order to determine how changes in temperature and salinity affect hermit crabs that start without any shell, and thus are likely desperate for any shelter they can find, we evaluated the types of shells preferred under control conditions (21 °C, 30 ppt salinity), lowered temperature (14 °C), two elevated temperatures (30 °C and 33 °C – simulating possible conditions in a tide pool on a hot summer day), decreased salinity (20 ppt salinity – simulating possible conditions in a tide pool on a rainy day), and increased salinity (40 ppt salinity – simulating possible conditions in a tide pool on a hot, dry, sunny day) on the day after a hermit crab had been removed from its shell.

In order to determine how changes in temperature and salinity affect hermit crabs in poor quality shells, we evaluated the types of shells preferred under the same six conditions listed above, as well as two additional conditions with both elevated temperature and salinity: 30 °C and 40 ppt salinity, and 33 °C and 40 ppt salinity – both simulating possible conditions in tide pools on hot, sunny days.

The hermit crabs were moved to a walk-in incubator set to bring seawater to the appropriate temperature (± 1 °C) and transferred to plastic containers identical to the ones they had been occupying overnight. These were again covered with plastic wrap and aerated through a Pasteur pipette. In the control and adjusted temperature treatments, 180 mL of 30 ppt salinity seawater was poured into each of the containers and allowed 1 h to reach the target temperature

with the hermit crabs in the containers. In the adjusted salinity treatments, we started with 90 mL of 30 ppt salinity seawater in each of the containers, and then gradually added 90 mL of seawater of a calculated salinity over the course of an hour to reach the desired salinity. For the treatments in which both temperature and salinity needed to be adjusted, these two methods were combined.

Additional shells were then added along one of the short edges of each of the containers, so that each hermit crab ultimately had a choice between three types of shells (including the shell they started in): one shell was of ideal size for the hermit crab and free from any visible damage (“good shell”), one was of ideal size but with a hole that had been drilled into it by a moon snail (“drilled shell”) (Carriker & Williams, 1978; Pechenik & Lewis, 2000), and one was 75% of the ideal size (“small shell”). The containers were then re-covered and the shell occupied by each crab was recorded seven times over 24 h. Seawater of an appropriate temperature was added if needed to maintain the water level in each of the containers, and salinity was monitored to ensure it stayed constant.

After each trial the hermit crabs were removed from the incubator, fed, removed from their shells by heating (Pechenik et al., 2015b), provided with new shells, and placed in a communal tank. They were later returned to the collection site.

Evaluating shell choice after sudden changes in temperature or salinity

Experiments were also conducted to examine the effects of sudden changes in temperature and salinity on hermit crab shell selection; such changes

are common when a rising tide floods tide pools, particularly on days on which the air and water temperatures differ considerably from one another, such as during the spring. The temperature changes studied included a shift from 21 °C to 14 °C, a shift from 30 °C to 21 °C, and a shift from 33 °C to 21 °C. These temperature changes represent the tide rising into tide pools that have been heated on a warm day. The salinity changes studied included a shift from 20 ppt to 30 ppt and a shift from 40 ppt to 30 ppt, representing the tide rising into tide pools where evaporation or precipitation has affected the salinity at low tide.

The methods used were similar to those used to study the effects of gradual changes in temperature and salinity, except that the hermit crabs were acclimated to initial temperature and salinity conditions for 12 h with only their starting shell. They were then abruptly placed into new containers with their additional shell options and with a water of a different temperature or salinity. Shell choice was then recorded seven times over 24 h.

Data analysis

Results were plotted in Graphpad Prism version 7.02, and statistical analyses were conducted in R version 3.2.2.

Chi-squared goodness-of-fit tests were used to determine if the number of hermit crabs that died during the experiment differed between treatments.

We chose to focus on the 30 min and 24 h time points for our data analysis, to show what was happening both early on and at the end of each experiment. Multinomial logistic regressions were fit to the data at both the 30

min and 24 h time points for every treatment (one type of starting shell, one temperature, and one salinity). The coefficients of the intercepts of the models were back-transformed from natural logs, giving us the odds of occupying one type of shell versus another. Two-tailed z-tests were used to obtain p-values.

Results

Hermit crab mortality

Hermit crab mortality in a given treatment was most often 0%, but ranged up to 27%. However, there was no significant difference between the treatments in the number of hermit crabs that died after starting in the same type of shell (gradual change in temperature, start without shell: $\chi^2 = 3.86$, $df = 3$, $p = 0.28$; gradual change in salinity, start without shell: $\chi^2 = 1.05$, $df = 2$, $p = 0.59$; gradual change in temperature and/or salinity, start in small shell: $\chi^2 = 13.67$, $df = 7$, $p = 0.06$; gradual change in temperature and/or salinity, start in drilled shell: $\chi^2 = 9.30$, $df = 7$, $p = 0.23$; sudden change in temperature, start in small shell: $\chi^2 = 1.95$, $df = 3$, $p = 0.58$; sudden change in salinity, start in small shell: $\chi^2 = 1.83$, $df = 2$, $p = 0.40$; sudden change in temp or salinity, start in drilled shell: no hermit crabs died).

Behavior of hermit crabs in the control treatments

Regardless of the type of shell (or lack thereof) that they began the experiments with, the hermit crabs that were kept at the control temperature and salinity of 21 °C and 30 ppt were always among the first to occupy intact shells of

appropriate size (“good shells”) (Fig. 3.1a, 3.3a, & 3.5a) and by 24 h were always occupying good shells (Fig. 3.2b, 3.4b, & 3.6b).

Shell choice after gradual changes in temperature and salinity

Hermit crabs that started without shells

With the exception of those that were tested at 33 °C, the hermit crabs that started without a shell chose to occupy intact shells of appropriate size over drilled or small shells at about the same rate across the various treatments with gradual changes in temperature or salinity (Fig. 3.1). Very few hermit crabs were found without a shell after the first 30 min (Fig. 3.1d). Those tested at 33 °C took longer to choose good shells, and were found occupying small shells more frequently than hermit crabs in the other treatments (Fig. 3.1b).

This is highlighted by Fig. 3.2, which shows that within 30 min after the shell options were introduced, the hermit crabs in every treatment were more often occupying good shells than any other shell type, except at 33 °C (Fig. 3.2a). After 24 h, there were significantly more hermit crabs in good shells than any other type of occupied shell in every treatment including the one at 33 °C (Fig. 3.2b, Table 3.1b).

Hermit crabs that started in small shells

The amount of time taken to choose a good shell by hermit crabs that started the experiment in small shells differed greatly between treatments (Fig. 3.3). Those tested at the control temperature and salinity (21 °C and 30 ppt), 30

°C, and 40 ppt were the fastest to occupy good shells, while those at the combined high temperature (33 °C) and salinity (40 ppt) took the longest to occupy good shells, and often never did (Fig. 3.3a). Most of the hermit crabs that were not occupying good shells at any given time point under those adverse conditions remained in their original small shells (Fig. 3.3b), while a few switched to drilled shells (Fig. 3.3c).

None of the hermit crabs in the 33 °C treatment, the 30 °C and 40 ppt treatment, or the 33 °C and 40 ppt treatment had switched out of their small shells by the end of the first 30 min (Fig. 3.4a). After 24 h, hermit crabs were more likely to be in a good shell than in a drilled or small shell in every treatment except the combined high temperature and salinity of 33 °C and 40 ppt; in this treatment, the hermit crabs were significantly more likely to be occupying small shells than good shells.

Hermit crabs that started in drilled shells

As with the hermit crabs that started in small shells, those that started in drilled shells differed greatly in the amount of time taken to choose good shells to occupy under the different environmental conditions tested (Fig. 3.5a). Those at the control temperature and salinity (21 °C and 30 ppt), at 14 °C, and at 30 °C were the fastest to occupy good shells, while those at the combined high temperature of 33 °C and high salinity of 40 ppt again took the longest to switch shells, and often never switched into good shells. Most of those that were not occupying a good shell at any given time were occupying the drilled shells that

they began the experiment in (Fig. 3.5c), while few were found to be occupying the small shells (Fig. 3.5b).

After 30 min, the hermit crabs were not significantly more likely to be in good shells than in drilled shells in any treatment (Fig 3.6a, Table 3.3a). But the hermit crabs were less likely to be in a good shell than in a drilled shell at the high temperature (33 °C), the low salinity (20 ppt), the high salinity (40 ppt) and the combined high temperatures and salinities of 30 °C and 40 ppt, and 33 °C and 40 ppt. But after 24 h, the only treatment in which there were more hermit crabs in drilled shells than in good shells was the one at 33 °C and 40 ppt; however, the difference was not significant (Fig. 3.6b, Table 3.3b). At 24 h in all other treatments, there were more hermit crabs in good shells than in drilled or small shells.

Shell choice after sudden changes in temperature and salinity

Hermit crabs that started in small shells

The hermit crabs that experienced a sudden shift in salinity from 40 ppt to 30 ppt while occupying small shells took the longest time to choose good shells to occupy, if they ever did so (Fig. 3.7a). A number of them switched into drilled shells soon after the salinity changed (Fig. 3.7c). Those that experienced other shifts in temperature or salinity took different amounts of time to choose good shells, but by 3 h at least 50% had done so.

Looking more closely at the results at 30 min (Fig. 3.8a, Table 3.4a), we can see that the hermit crabs that experienced a downward shift in temperature

from 21 °C to 14 °C and those that experienced a salinity increase from 20 ppt to 30 ppt were significantly more likely to be occupying a small shell than a good shell. By 24 h, the hermit crabs were more likely to be in good shells than in drilled or small shells in every treatment, however, the difference between the number in good and drilled shells was not significant when salinity was abruptly decreased from 40 to 30 ppt (Fig. 3.8b, Table 3.4b).

Hermit crabs that started in drilled shells

The hermit crabs that experienced a sudden decrease in temperature from 33 °C to 21 °C while occupying drilled shells took the longest amount of time to occupy good shells, and many never did (Fig. 3.9a). Those at the control temperature and salinity (21 °C and 30 ppt), as well as those that experienced a sudden temperature decrease from 30 °C to 21 °C were the fastest to occupy good shells. Most of those that were not occupying a good shell at a given time were still in the drilled shell in which they began the experiment (Fig. 3.9c).

After 30 min, there were no treatments in which there were more hermit crabs in good shells than in drilled shells (Fig. 3.10a). There were significantly more hermit crabs in drilled shells than in good shells when the hermit crabs had experienced a sudden decrease in temperature from 21 °C to 14 °C or a sudden change in salinity from 20 ppt to 30 ppt or from 40 ppt to 30 ppt (Table 3.5a). In all treatments, by 24 h more hermit crabs were found to be occupying good shells than any other type, but the difference between the number found in good shells and drilled shells was not significant in the treatments where temperature was

suddenly changed from 21 °C to 14 °C or from 33 °C to 21 °C (Fig. 3.10b, Table 3.5b).

Discussion

A large body of previous research has shown that hermit crabs are very predictable in terms of the shells they will choose to occupy (Angel, 2000; Arce & Alcaraz, 2012; Conover, 1976; Li & Pechenik, 2004; McClintock, 1985; Pechenik et al., 2015b, 2001; Pechenik & Lewis, 2000; Vance, 1972a). Their dislike for shells with drill holes and shells that are too small suggests that any time they are found in such an undesirable shell, there are likely either no better shells available or something is preventing them from switching (Angel, 2000; Pechenik et al., 2015a, 2001; Pechenik & Lewis, 2000; Vance, 1972a).

The results of our experiments provide support for these assumptions, as no hermit crabs were found without a shell if they had previously been in a shell, and any hermit crab that switched into an intact shell of ideal size (“good shell”) never left that shell for a drilled or small shell (Figs. 3.1, 3.3, 3.5, 3.7, & 3.9). Additionally, the hermit crabs in our control groups (21 °C and 30 ppt salinity) were always among the fastest to switch into good shells (Figs. 3.1, 3.3, 3.5, 3.7, & 3.9) and by 24 h were always occupying good shells (Figs. 3.2b, 3.4b, 3.6b, 3.8b, & 3.10b), suggesting that these truly are the shells that hermit crabs prefer under relatively average conditions.

The effects of gradual changes in temperature and salinity

Hermit crabs at the high temperature of 33 °C were always among the slowest to occupy good shells, and some never did occupy the good shells (Figs. 3.1, 3.3, & 3.5). The hermit crabs that started without a shell at this temperature were quick to move into a shell – by 1 h they were all occupying some sort of shell (Fig. 3.1). But many of them moved into drilled or small shells, and by three hours only about half of them were occupying good shells; about 30% never did. The hermit crabs that started off in drilled or small shells at this temperature often remained in these initial shells for quite some time (Figs. 3.3 & 3.5). These behaviors suggest that the high temperature of 33 °C doesn't render long-wristed hermit crabs incapable of switching shells, but likely makes them very reluctant or slow to do so.

Why might this be? Hermit crabs in intact shells of ideal size are able to use their legs to seal off the apertures of their shells, a desirable ability that can help protect them from predators as well as harmful environmental conditions (Reese, 1969). It is possible that switching from a drilled or small shell to a good shell posed too much of a risk to the hermit crabs – if they switched to a new shell in a hot tide pool and then found that something was wrong with it, it is possible that another hermit crab could have come along and taken their original shell, leaving them in a more dangerous situation at this high temperature than they were originally in. But why is this high temperature dangerous?

Our data suggest that the high temperature of 33 °C is not a lethal temperature for long-wristed hermit crabs. The hermit crabs at this temperature

were not significantly more or less likely to die than the hermit crabs in any other treatment. And this temperature of 33 °C is typically not high enough even to cause long-wristed hermit crabs from Nahant, Massachusetts to exit their shells, which they typically begin to do around 34 to 35 °C (Fraenkel, 1960; Pechenik et al., 2015; S.G., pers. obs.). A study conducted by Fraenkel (1960) found that long-wristed hermit crabs began to die after exposure to temperatures 35.5 °C or higher, although the exposure in his study was only for 1 h. However, increases in temperature have been shown to have a variety of effects on marine species besides death, and our results show that there is an obvious difference between hermit crab shell selection behavior at 33 °C and the second-highest temperature studied of 30 °C.

It seems unlikely that a change in metabolism or a lack of oxygen caused the behaviors observed in the hermit crabs. Burggren and McMahon (1981) found that the rates of oxygen uptake in two species of intertidal hermit crabs were much less affected by changes in temperature than the rates of oxygen uptake in two species of hermit crabs that only lived subtidally. While high temperature is known to decrease the oxygen affinity of hemoglobins and hemocyanins in blood (Barcroft, 1909; Mangum, 1980), the relationship between temperature and the affinity for oxygen by these oxygen-carrying proteins does not hold true in some poikilotherms that experience fluctuations in temperature. Most relevant to this study, Jokumsen and Weber (1982) showed that hermit crabs of the species *P. bernhardus*, a species closely related to long-wristed hermit crabs, have hemocyanin with an oxygen-affinity that is not affected by temperature.

Assuming long-wristed hermit crabs have hemocyanin with similar characteristics, lack of oxygen was likely not a problem in our experiment, especially considering that we aerated the seawater constantly to mitigate the effects of the lower solubility of oxygen in seawater at high temperatures (Truesdale et al., 1955).

Other negative effects of increased temperature that could be faced by long-wristed hermit crabs as temperatures continue to rise include impairment of heart function, action potentials, mitochondrial activity, membrane fluidity, and protein function (reviewed by Somero, 2002). The impairment of protein function may be mitigated to some extent at certain temperatures by heat shock proteins, which activate under stressful conditions and aid in the folding of other proteins, as well as with their repair or disposal if damaged (Parsell & Lindquist, 1993). We are not aware of any studies that have examined the role that heat shock proteins may or may not play in hermit crab responses to increasing temperatures. But several other marine invertebrates such as snails and mussels have been shown to express heat shock proteins (reviewed by Tomanek & Somero, 1999). If heat shock proteins do serve an important function in hermit crabs, future research could determine the temperatures at which they activate and the temperatures at which they are no longer effective, to better understand how increasing temperature is impacting these populations. It is possible that at temperatures of 33 °C, hermit crab heat shock proteins are beginning to fail, leading to a decrease in activity.

The effects on hermit crab shell selection that we saw at high temperatures were exacerbated when the salinity of the seawater was simultaneously increased. The hermit crabs that started in small shells and experienced gradual changes in temperature and salinity to 30 °C and 40 ppt took longer to occupy good shells than hermit crabs that started in small shells and experienced a gradual change in just temperature to 30 °C or a gradual change in just salinity to 40 ppt (Fig. 3.3). The hermit crabs that started in drilled shells and experienced gradual changes in temperature and salinity to 30 °C and 40 ppt took longer to choose to occupy good shells than the hermit crabs that experienced a gradual change in just temperature to 30 °C and about the same amount of time to choose to occupy good shells as the hermit crabs that experienced a gradual change in just salinity to 40 ppt (Fig. 3.5). The hermit crabs that experienced gradual changes to the highest temperature and salinity of 33 °C and 40 ppt took longer than any other group to occupy good shells – by 6 h they were all still in their original shells, and fewer than half of them had switched shells by 24 h (Figs. 3.3 & 3.5).

The effects of salinity changes have been studied in some other marine invertebrates. Robertson (1953) found that hermit crabs of the species *P. bernhardus* could regulate ion levels in their body fluids in response to changes in ion levels in seawater. With regards to the effects of salinity on metabolism and oxygen uptake, Davenport et al. (1980) found that decreases in salinity from average environmental levels decreased oxygen consumption by the hermit crab species *P. bernhardus*. It remains to be seen whether this holds true for long-wristed hermit crabs and whether oxygen consumption in hermit crabs increases

or decreases at raised salinity levels. At salinities higher than environmental levels, ghost crabs (*Ocypode albicans*) consume more oxygen (Flemister & Flemister, 1951), as do a species of shrimp (*Palaemonetes varians*) (Lofts, 1956).

Interactions of temperature and salinity have been shown to have an effect on the oxygen consumption of crabs in the genus *Hemigrapsus*; however, high salinity increases the tolerance of these crabs to high temperature, a trend that contradicts the results in the current study (Dehnel, 1960). Perhaps the combined efforts of regulating ion levels at our high salinity of 40 ppt and dealing with the possibly harmful effects of high temperature discussed above prevent the long-wristed hermit crabs we studied from having the energy to investigate and switch into new shells. In the treatments in which temperature and salinity were both changed to 33 °C and 40 ppt, we observed no shell-switching at 6 h, but some hermit crabs had switched shells by 24 h (Fig. 3.3 & 3.5). It is possible that these hermit crabs became acclimated to the conditions and could resume some normal activities after more than 6 h, but the mechanism by which this may happen is unknown.

While not as extreme as the results at the highest temperature of 33 °C or the combined high temperatures and salinities (30 °C and 40 ppt, 33 °C and 40 ppt), the hermit crabs that started the experiment in small shells seemed to take longer to switch to good shells at the low temperature of 14 °C, as well as the low salinity of 20 ppt, than at the control temperature and salinity of 21 °C and 30 ppt (Fig. 3.3a). And the hermit crabs that started in drilled shells seemed to take longer to switch into good shells at both the high salinity of 40 ppt and the low

salinity of 20 ppt (Fig. 3.5a). The results at the cold temperature may be related to the behavior of long-wristed hermit crabs as winter approaches – cold temperatures slow their movement, so they move out into deeper water and bury themselves in the mud (Rebach, 1970). The results at 20 ppt and 40 ppt may be due to energy spent regulating ion levels and changing rates of oxygen uptake, as discussed above. These results at low temperatures and both high and low salinities were not seen when the hermit crabs started the experiment without a shell, perhaps because their lack of a shell encouraged them to investigate the available shell options more quickly.

Effects of starting shells

The hermit crabs that began the experiment without a shell were the only ones that were occupying good shells significantly more often than any other type of occupied shell after 24 h, regardless of the gradual change in temperature or salinity that they experienced (Fig. 3.2). We hypothesize that their initial lack of a shell strongly motivated them to investigate the shell options available, as being without a shell in the intertidal or subtidal zone would leave them especially vulnerable to predation (Reese, 1969), salinity stress (Pechenik et al., 2001), and desiccation (Taylor, 1981).

The hermit crabs that began the experiments in drilled or small shells took longer to occupy good shells than those that started without a shell (Fig. 3.1, 3.3, & 3.5). The hermit crabs at the high temperature and salinity combination of 33 °C and 40 ppt were the slowest to occupy good shells after starting in small or

drilled shells, and those at the control temperature and salinity of 21 °C and 30 ppt, as well as the high temperature of 30 °C, were among the fastest to occupy good shells after starting in small or drilled shells.

There were some differences between the hermit crabs that started in small or drilled shells. Previous research has shown that hermit crabs will often select small shells over drilled shells, unless the shells are so small that they are appropriate for hermit crabs 25% of their size (Pechenik & Lewis, 2000). Thus, we expected that the hermit crabs in our experiment might switch out of drilled shells more quickly than they switched out of small shells. This was indeed the case at the high temperature of 33 °C, the low temperature of 14 °C, and the high temperature and salinity combination of 30 °C and 40 ppt (Figs. 3.3 & 3.5). However, at the temperature of 30 °C and the high salinity of 40 ppt, the hermit crabs switched into good shells more quickly when they started in drilled shells. These differences may have just been due to chance, but future studies should investigate whether these patterns repeat and what might be the cause behind them.

The effects of sudden changes in temperature and salinity

When the hermit crabs began the experiment in small shells and were exposed to sudden changes in temperature or salinity, those that underwent the downward shift in salinity from 40 ppt to 30 ppt took the longest to occupy good shells, if they ever did (Fig. 3.7). Interestingly, many of those that didn't switch to good shells from the small shells instead switched to drilled shells. When the

hermit crabs began the experiment in drilled shells, those that experienced a sudden change in temperature from 33 °C to 21 °C took the longest to occupy good shells (Fig. 3.9). We found it surprising that more of the hermit crabs did not struggle to occupy a good shell, given how drastic some of the changes in temperature and salinity were. However, most of these changes (apart from the shift in temperature from 21 °C to 14 °C) shifted the hermit crabs from an extreme environment back to an environment with the relatively average conditions of 21 °C and 30 ppt salinity, perhaps allowing them to resume normal functions. The longer amount of time taken to occupy a good shell by both the hermit crabs that started in small shells and experienced a change in salinity from 40 ppt to 30 ppt and the hermit crabs that started in drilled shells and experienced a change in temperature from 33 °C to 21 °C may have been due to an inability to recover quickly from the stresses of being in the 40 ppt environment or the 33 °C environment for an extended period.

Implications for the future

During the summer of 2016, we measured the temperature and salinity multiple times at low tide at Nahant, Massachusetts. The highest temperature we recorded in a tide pool was 32 °C, and the highest salinity we recorded was 38 ppt. Higher temperatures and salinities may have been reached on days on which we did not take measurements. Our high measurements of 32 °C and 38 ppt salinity fall incredibly close to the high temperature of 33 °C and the high salinity of 40 ppt used in this study, suggesting that the long-wristed hermit crabs in

coastal Massachusetts may already be experiencing the effects of temperatures and salinities that are dangerously high for them. Tide pool conditions should continue to be monitored in future summers.

If hermit crabs are unable to choose intact shells of ideal size to occupy, they will be more likely to suffer from predation (Reese, 1969), desiccation (Taylor, 1981), a decreased growth rate (Angel, 2000), and a loss of energy (Elwood & Glass, 1981; Osorno et al., 1998, 2005). Long-wristed hermit crabs in Massachusetts move between the intertidal zone and the subtidal zone, and it is possible that the populations will leave the intertidal zone if conditions become too dangerous for them. However, many empty gastropod shells can be found in the intertidal zone (S.G., pers. obs.); leaving behind this supply of resources would likely increase competition for shells available subtidally.

In summary, hermit crabs of the species *Pagurus longicarpus* were found to be less likely to occupy good-quality shells when they were subjected to a gradual increase in temperature to 33 °C and gradual increases in both temperature and salinity to 30 °C and 40 ppt, as well as 33 °C and 40 ppt. They were also less likely to be in good shells after sudden changes in temperature from 33 °C to 21 °C, and after sudden changes in salinity from 40 ppt to 30 ppt, depending on the type of shell they were originally occupying. These responses will likely pose a threat to future generations of long-wristed hermit crabs as the effects of climate change begin to be felt more strongly in the dynamic Massachusetts intertidal zones.

Tables

Table 3.1. The odds of hermit crabs (*Pagurus longicarpus*) occupying no shell, a small shell, or a drilled shell instead of a good shell 30 min (a) and 24 h (b) after gradual changes in temperature or salinity. All hermit crabs started with no shell. Significant p-values are bolded.

a.

Shell occupied at 30 min	Temp and Salinity	Odds	p-value
None vs. good	21 °C, 30 ppt	0.30	0.07
	14 °C, 30 ppt	none without shell	
	30 °C, 30 ppt	0.10	0.03
	33 °C, 30 ppt	0.67	0.66
	21 °C, 20 ppt	none without shell	
	21 °C, 40 ppt	0.50	0.33
Small vs. good	21 °C, 30 ppt	none in small	
	14 °C, 30 ppt	0.33	0.10
	30 °C, 30 ppt	0.30	0.07
	33 °C, 30 ppt	1.67	0.48
	21 °C, 20 ppt	0.10	0.03
	21 °C, 40 ppt	0.33	0.18
Drilled vs. good	21 °C, 30 ppt	0.20	0.04
	14 °C, 30 ppt	0.22	0.05
	30 °C, 30 ppt	0.10	0.03
	33 °C, 30 ppt	1.00	>0.99
	21 °C, 20 ppt	0.30	0.07
	21 °C, 40 ppt	0.50	0.33

b.

Shell occupied at 24 h	Temp and Salinity	Odds	p-value
None vs. good	21 °C, 30 ppt	none without shell	
	14 °C, 30 ppt	none without shell	
	30 °C, 30 ppt	none without shell	
	33 °C, 30 ppt	none without shell	
	21 °C, 20 ppt	none without shell	
	21 °C, 40 ppt	none without shell	
Small vs. good	21 °C, 30 ppt	none in small	
	14 °C, 30 ppt	0.08	0.02
	30 °C, 30 ppt	0.08	0.02
	33 °C, 30 ppt	0.22	0.05
	21 °C, 20 ppt	none in small	
	21 °C, 40 ppt	none in small	
Drilled vs. good	21 °C, 30 ppt	none in drilled	
	14 °C, 30 ppt	0.08	0.02
	30 °C, 30 ppt	0.08	0.02
	33 °C, 30 ppt	0.22	0.05
	21 °C, 20 ppt	0.08	0.01
	21 °C, 40 ppt	0.08	0.01

Table 3.2. The odds of hermit crabs (*Pagurus longicarpus*) occupying a small shell or a drilled shell instead of a good shell 30 min (a) and 24 h (b) after gradual changes in temperature and/or salinity. All hermit crabs started in a small shell. Significant p-values are bolded.

a.

Shell occupied at 30 min	Temp and Salinity	Odds	p-value
Small vs. good	21 °C, 30 ppt	0.63	0.41
	14 °C, 30 ppt	2.67	0.15
	30 °C, 30 ppt	0.71	0.57
	33 °C, 30 ppt	all in small	
	21 °C, 20 ppt	0.67	0.53
	21 °C, 40 ppt	0.38	0.15
	30 °C, 40 ppt	all in small	
	33 °C, 40 ppt	all in small	
Drilled vs. good	21 °C, 30 ppt	none in drilled	
	14 °C, 30 ppt	0.67	0.66
	30 °C, 30 ppt	0.43	0.71
	33 °C, 30 ppt	none in drilled	
	21 °C, 20 ppt	0.67	0.53
	21 °C, 40 ppt	0.38	0.15
	30 °C, 40 ppt	none in drilled	
	33 °C, 40 ppt	none in drilled	

b.

Shell occupied at 24 h	Temp and Salinity	Odds	p-value
Small vs. good	21 °C, 30 ppt	none in small	
	14 °C, 30 ppt	0.63	0.41
	30 °C, 30 ppt	none in small	
	33 °C, 30 ppt	0.12	0.05
	21 °C, 20 ppt	none in small	
	21 °C, 40 ppt	none in small	
	30 °C, 40 ppt	0.63	0.41
	33 °C, 40 ppt	4.5	0.05
Drilled vs. good	21 °C, 30 ppt	none in drilled	
	14 °C, 30 ppt	none in drilled	
	30 °C, 30 ppt	0.07	0.01
	33 °C, 30 ppt	0.25	0.08
	21 °C, 20 ppt	0.17	0.02
	21 °C, 40 ppt	0.08	0.01
	30 °C, 40 ppt	0.13	0.05
	33 °C, 40 ppt	none in drilled	

Table 3.3. The odds of hermit crabs (*Pagurus longicarpus*) occupying a small shell or a drilled shell instead of a good shell 30 min (a) and 24 h (b) after gradual changes in temperature and/or salinity. All hermit crabs started in a drilled shell. Significant p-values are bolded.

a.

Shell occupied at 30 min	Temp and Salinity	Odds	p-value
Small vs. good	21 °C, 30 ppt	none in small	
	14 °C, 30 ppt	none in small	
	30 °C, 30 ppt	0.14	0.07
	33 °C, 30 ppt	none in small	
	21 °C, 20 ppt	none in small	
	21 °C, 40 ppt	0.50	0.57
	30 °C, 40 ppt	none in small	
	33 °C, 40 ppt	none in small	
Drilled vs. good	21 °C, 30 ppt	1.00	1.00
	14 °C, 30 ppt	0.67	0.44
	30 °C, 30 ppt	0.57	0.37
	33 °C, 30 ppt	9.00	0.04
	21 °C, 20 ppt	4.00	0.03
	21 °C, 40 ppt	4.00	0.08
	30 °C, 40 ppt	6.00	0.02
	33 °C, 40 ppt	all in drilled	

b.

Shell occupied at 24 h	Temp and Salinity	Odds	p-value
Small vs. good	21 °C, 30 ppt	none in small	
	14 °C, 30 ppt	0.08	0.02
	30 °C, 30 ppt	none in small	
	33 °C, 30 ppt	none in small	
	21 °C, 20 ppt	none in small	
	21 °C, 40 ppt	0.11	0.04
	30 °C, 40 ppt	0.22	0.05
	33 °C, 40 ppt	none in small	
Drilled vs. good	21 °C, 30 ppt	none in drilled	
	14 °C, 30 ppt	0.08	0.02
	30 °C, 30 ppt	0.09	0.02
	33 °C, 30 ppt	0.43	0.22
	21 °C, 20 ppt	0.30	0.07
	21 °C, 40 ppt	0.11	0.04
	30 °C, 40 ppt	0.33	0.10
	33 °C, 40 ppt	1.80	0.29

Table 3.4. The odds of hermit crabs (*Pagurus longicarpus*) occupying a small shell or a drilled shell instead of a good shell 30 min (a) and 24 h (b) after sudden changes in temperature or salinity. All hermit crabs started in a small shell. Significant p-values are bolded.

a.

Shell occupied at 30 min	Temp and Salinity	Odds	p-value
Small vs. good	21 °C, 30 ppt	0.63	0.41
	21 → 14 °C, 30 ppt	5.00	0.04
	30 → 21 °C, 30 ppt	0.33	0.10
	33 → 21 °C, 30 ppt	1.20	0.76
	21 °C, 20 → 30 ppt	5.00	0.04
	21 °C, 40 → 30 ppt	2.00	0.42
Drilled vs. good	21 °C, 30 ppt	none in drilled	
	21 → 14 °C, 30 ppt	none in drilled	
	30 → 21 °C, 30 ppt	0.22	0.05
	33 → 21 °C, 30 ppt	0.20	0.14
	21 °C, 20 → 30 ppt	0.50	0.57
	21 °C, 40 → 30 ppt	3.00	0.18

b.

Shell occupied at 24 h	Temp and Salinity	Odds	p-value
Small vs. good	21 °C, 30 ppt	none in small	
	21 → 14 °C, 30 ppt	0.09	0.02
	30 → 21 °C, 30 ppt	0.08	0.01
	33 → 21 °C, 30 ppt	0.09	0.02
	21 °C, 20 → 30 ppt	0.08	0.02
	21 °C, 40 → 30 ppt	none in small	
Drilled vs. good	21 °C, 30 ppt	none in drilled	
	21 → 14 °C, 30 ppt	none in drilled	
	30 → 21 °C, 30 ppt	none in drilled	
	33 → 21 °C, 30 ppt	none in drilled	
	21 °C, 20 → 30 ppt	none in drilled	
	21 °C, 40 → 30 ppt	0.33	0.10

Table 3.5. The odds of hermit crabs (*Pagurus longicarpus*) occupying a small shell or a drilled shell instead of a good shell 30 min (a) and 24 h (b) after sudden changes in temperature or salinity. All hermit crabs started in a drilled shell. Significant p-values are bolded.

a.

Shell occupied at 30 min	Temp and Salinity	Odds	p-value
Small vs. good	21 °C, 30 ppt	none in small	
	21 → 14 °C, 30 ppt	1.00	1.00
	30 → 21 °C, 30 ppt	0.20	0.14
	33 → 21 °C, 30 ppt	0.25	0.23
	21 °C, 20 → 30 ppt	none in small	
	21 °C, 40 → 30 ppt	none in small	
Drilled vs. good	21 °C, 30 ppt	1.00	1.00
	21 → 14 °C, 30 ppt	12.00	0.02
	30 → 21 °C, 30 ppt	1.20	0.76
	33 → 21 °C, 30 ppt	2.25	0.18
	21 °C, 20 → 30 ppt	14.00	0.01
	21 °C, 40 → 30 ppt	3.67	0.05

b.

Shell occupied at 24 h	Temp and Salinity	Odds	p-value
Small vs. good	21 °C, 30 ppt	none in small	
	21 → 14 °C, 30 ppt	none in small	
	30 → 21 °C, 30 ppt	none in small	
	33 → 21 °C, 30 ppt	0.13	0.05
	21 °C, 20 → 30 ppt	none in small	
	21 °C, 40 → 30 ppt	none in small	
Drilled vs. good	21 °C, 30 ppt	none in drilled	
	21 → 14 °C, 30 ppt	0.40	0.12
	30 → 21 °C, 30 ppt	0.10	0.03
	33 → 21 °C, 30 ppt	0.63	0.50
	21 °C, 20 → 30 ppt	0.07	0.01
	21 °C, 40 → 30 ppt	0.08	0.01

Figures

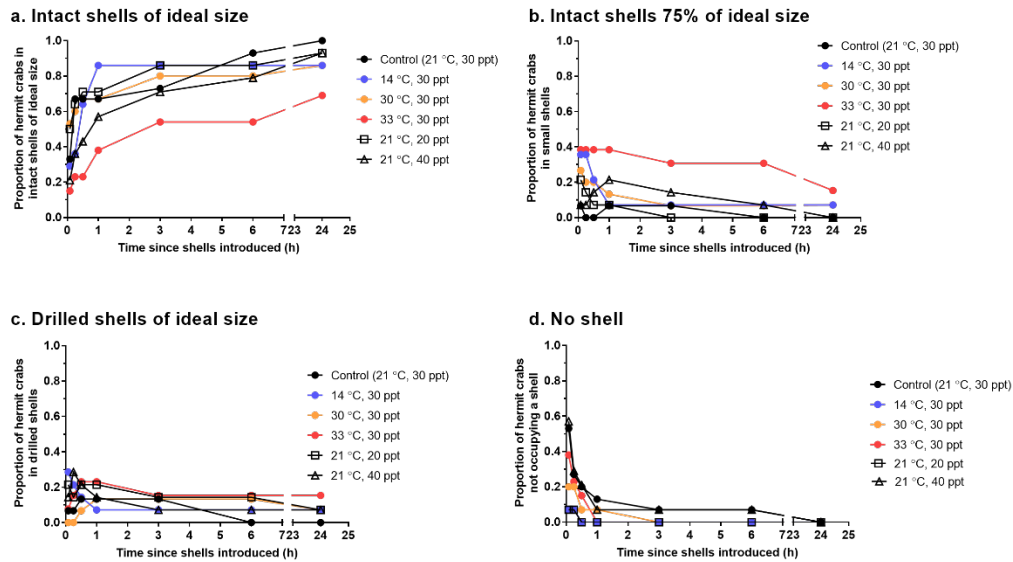
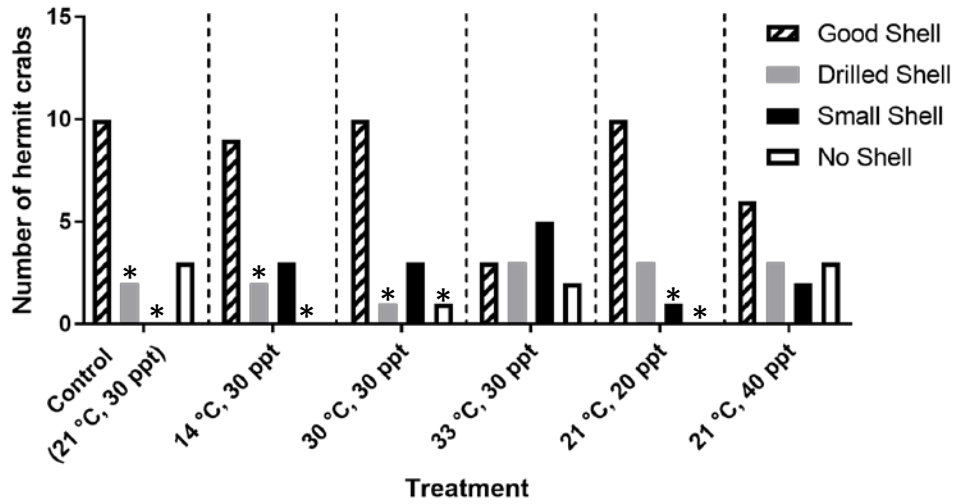


Figure 3.1. Proportion of hermit crabs (*Pagurus longicarpus*) that were occupying intact shells of ideal size (a), intact shells 75% of ideal size (b), drilled shells of ideal size (c) or no shell (d) at various time points over 24 h. The hermit crabs were started off without a shell. After seawater temperature or salinity had been gradually adjusted over the course of an hour, each of the hermit crabs was provided with shell options so that each had access to an intact shell of ideal size, an intact small shell, and a drilled shell of ideal size. $n \sim 15$ hermit crabs per treatment.

a. 30 min



b. 24 h

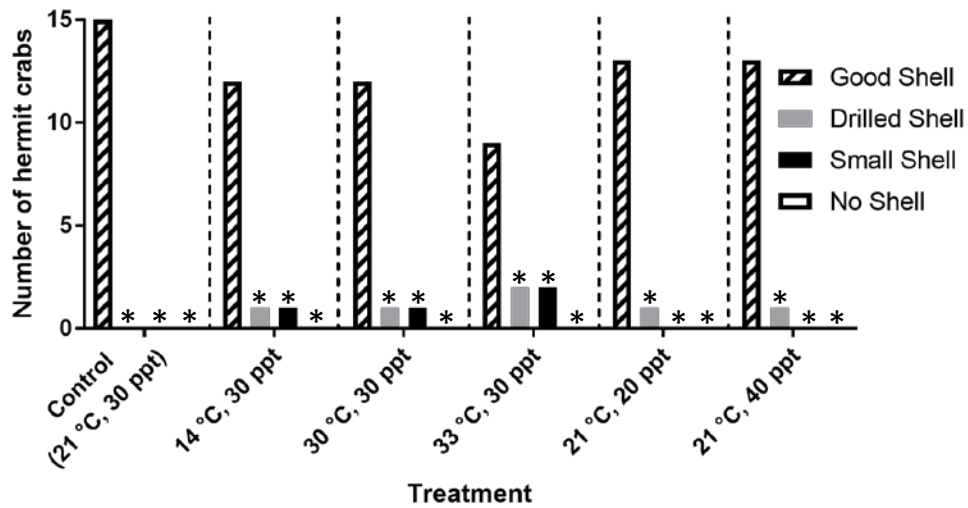


Figure 3.2. The number of hermit crabs (*Pagurus longicarpus*) that started off with no shell that were occupying good shells (intact shells of ideal size), drilled shells of ideal size, shells 75% of ideal size, or no shell 30 min (a) and 24 h (b) after gradual changes in seawater temperature or salinity. See Fig. 3.1 caption for details on methods. Asterisks indicate a significant difference in the odds of being in a drilled shell, a small shell, or no shell instead of a good shell.

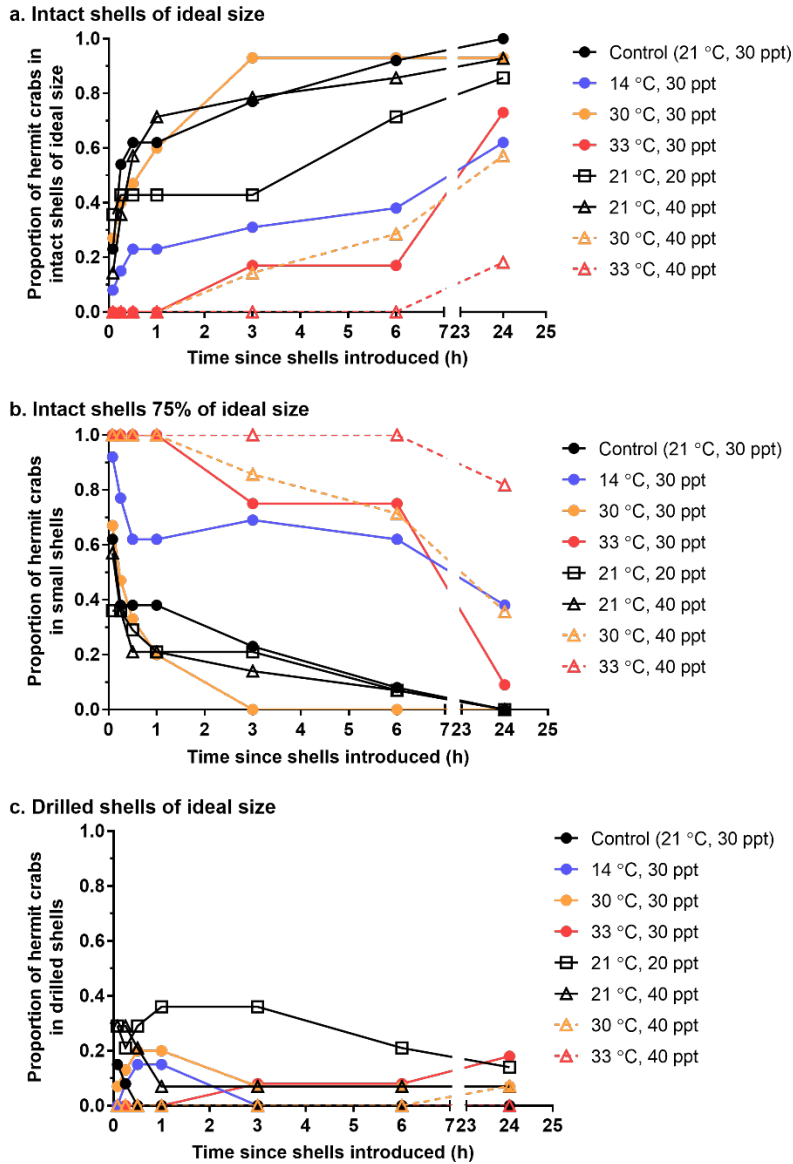


Figure 3.3. Proportion of hermit crabs (*Pagurus longicarpus*) that were occupying intact shells of ideal size (a), intact shells 75% of ideal size (b), or drilled shells of ideal size (c) at various time points over 24 h. The hermit crabs were started off in small shells. After seawater temperature and/or salinity had been gradually adjusted over the course of an hour, each hermit crab was also provided with an intact shell of ideal size and a drilled shell of appropriate size. n ~ 15 hermit crabs per treatment.

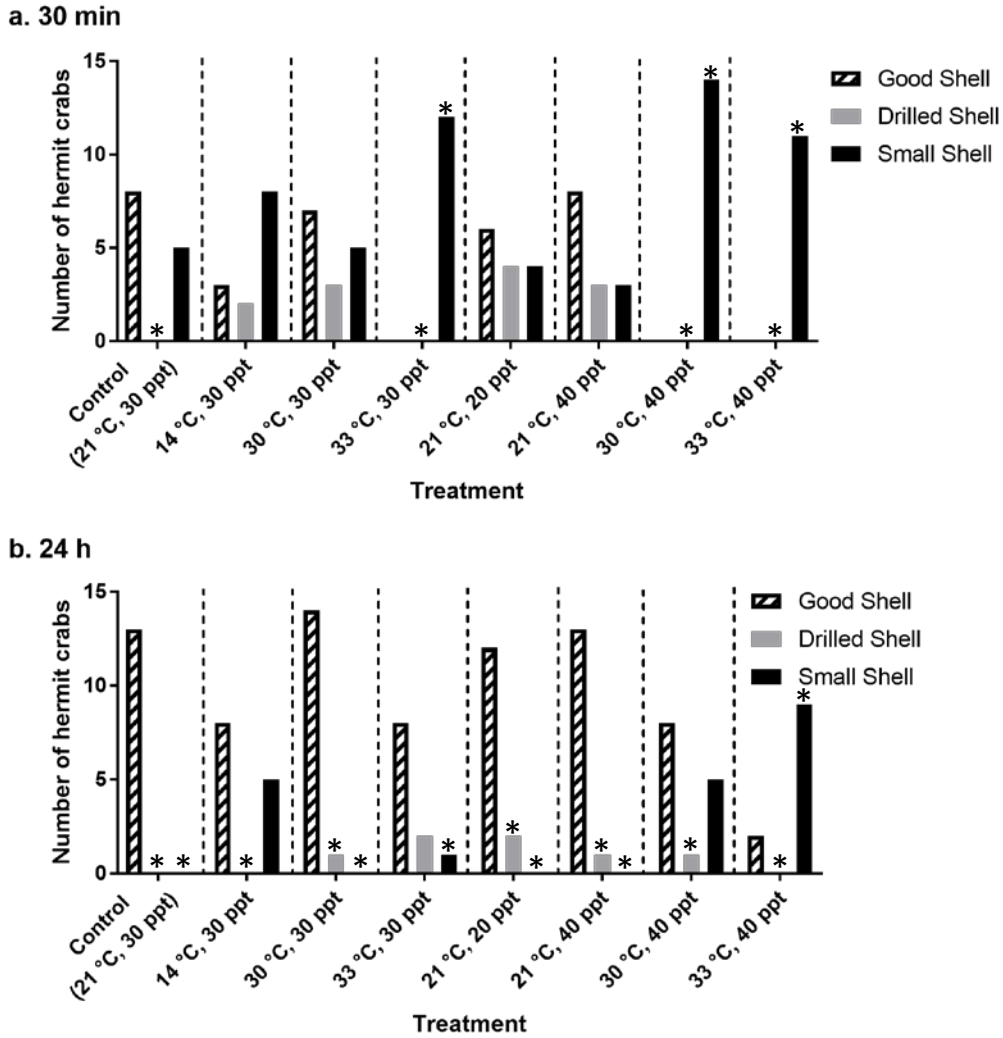


Figure 3.4. The number of hermit crabs (*Pagurus longicarpus*) that started off in a shell 75% of ideal size that were occupying good shells (intact shells of ideal size), drilled shells of ideal size, or shells 75% of ideal size 30 min (a) and 24 h (b) after gradual changes in seawater temperature and/or salinity. See Fig. 3.3 caption for details on methods. Asterisks indicate a significant difference in the odds of being in a drilled or small shell instead of a good shell.

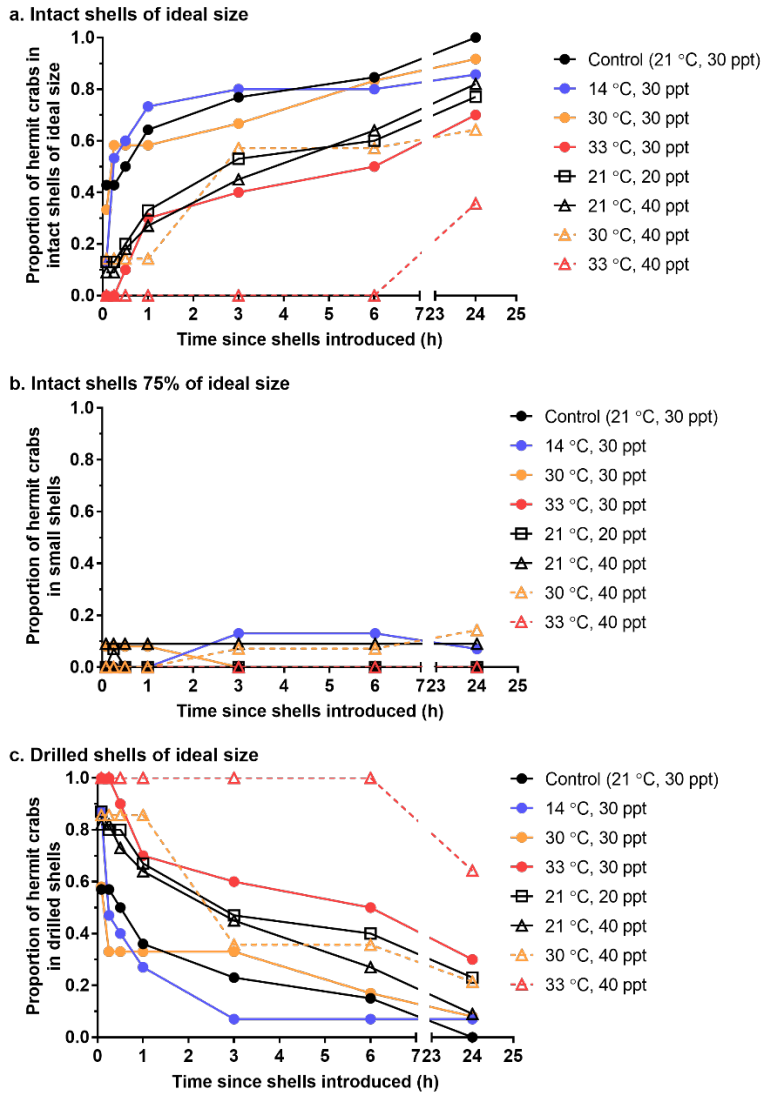
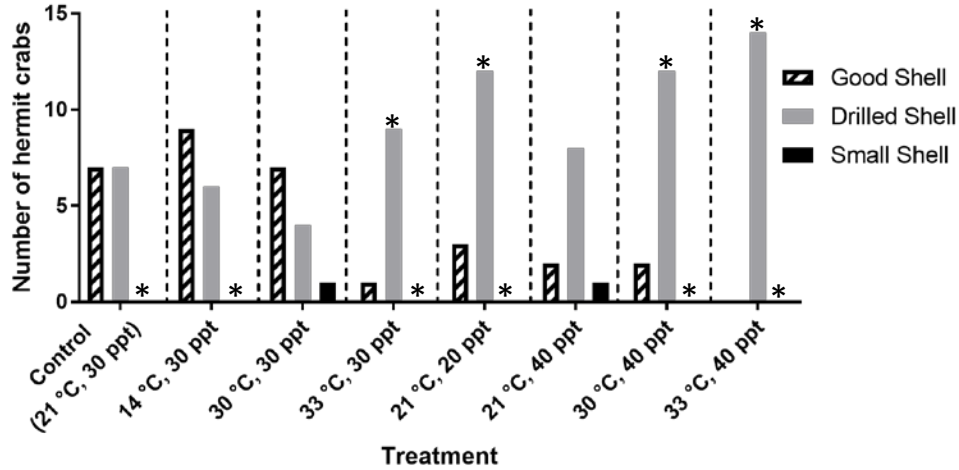


Figure 3.5. Proportion of hermit crabs (*Pagurus longicarpus*) that were occupying intact shells of ideal size (a), intact shells 75% of ideal size (b), or drilled shells of ideal size (c) at various time points over 24 h. The hermit crabs were started off in drilled shells. After seawater temperature and/or salinity had been gradually adjusted over the course of an hour, each hermit crab was also provided with an intact shell of ideal size and an intact shell 75% of ideal size. n ~ 15 hermit crabs per treatment.

a. 30 min



b. 24 h

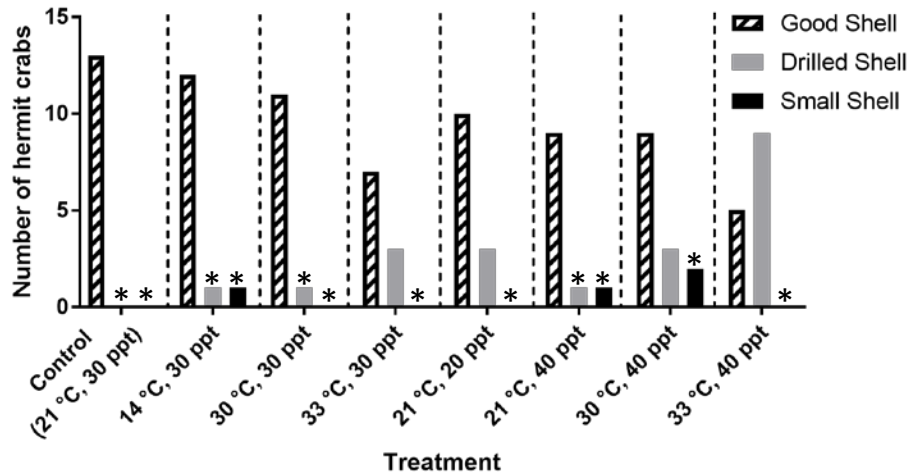


Figure 3.6. The number of hermit crabs (*Pagurus longicarpus*) that started off in a shell of ideal size with a drill hole that were occupying good shells (intact shells of ideal size), drilled shells of ideal size, or shells 75% of ideal size 30 min (a) and 24 h (b) after gradual changes in seawater temperature and/or salinity. See Fig. 3.5 caption for details on methods. Asterisks indicate a significant difference in the odds of being in a drilled or small shell instead of a good shell.

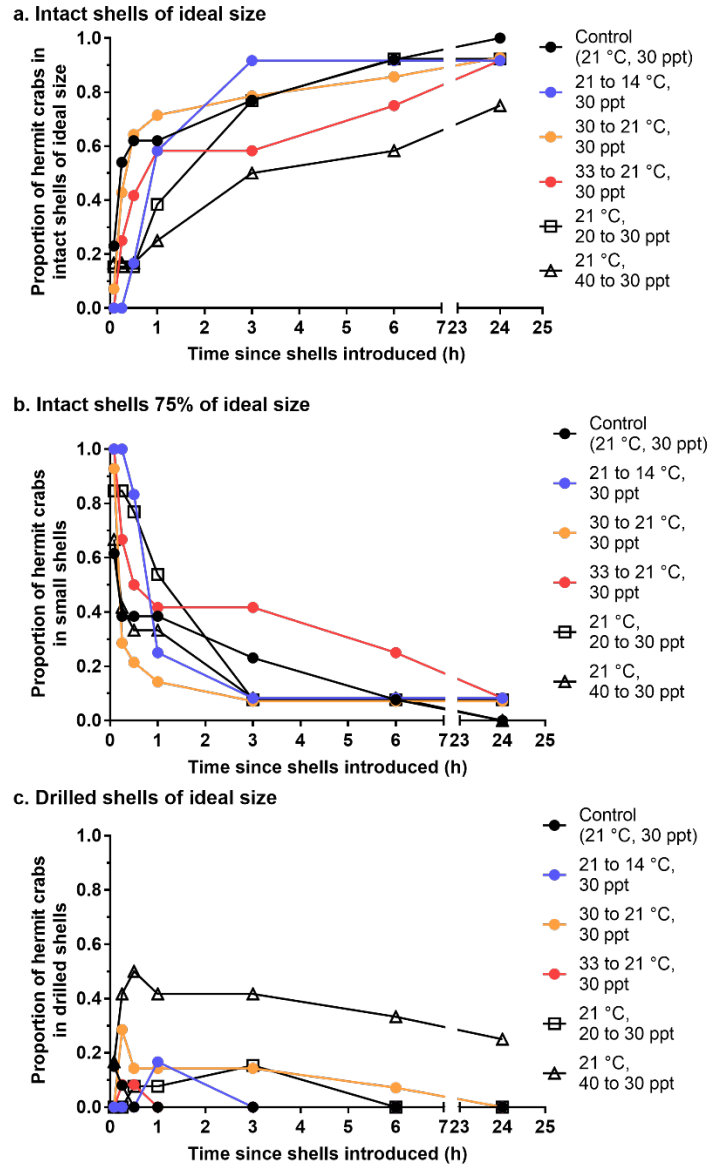
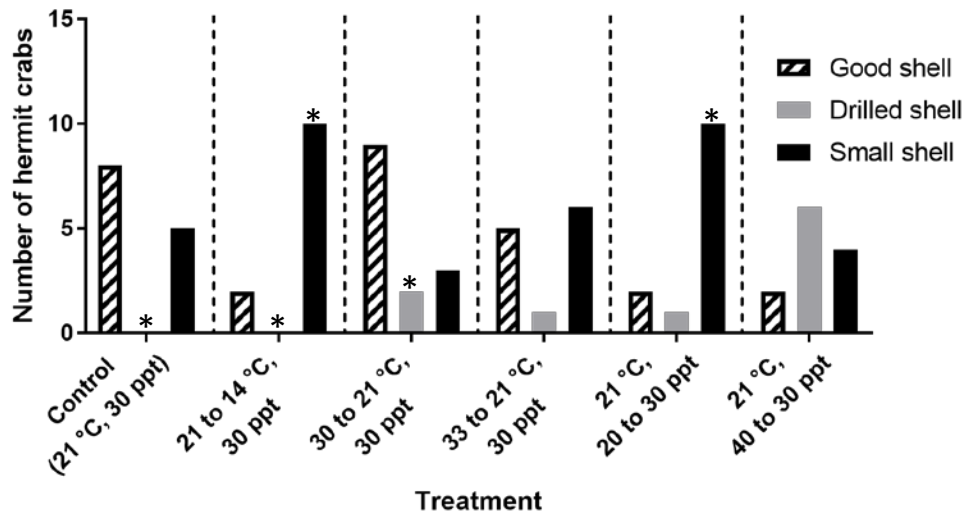


Figure 3.7. Proportion of hermit crabs (*Pagurus longicarpus*) that were occupying intact shells of ideal size (a), intact shells 75% of ideal size (b), or drilled shells of ideal size (c) at various time points over 24 h after sudden changes in seawater temperature or salinity. The hermit crabs were started off in small shells. At the same time that the temperature or salinity was suddenly adjusted, each hermit crab was also provided with an intact shell of ideal size and a drilled shell of ideal size. n ~ 15 hermit crabs per treatment.

a. 30 min



b. 24 h

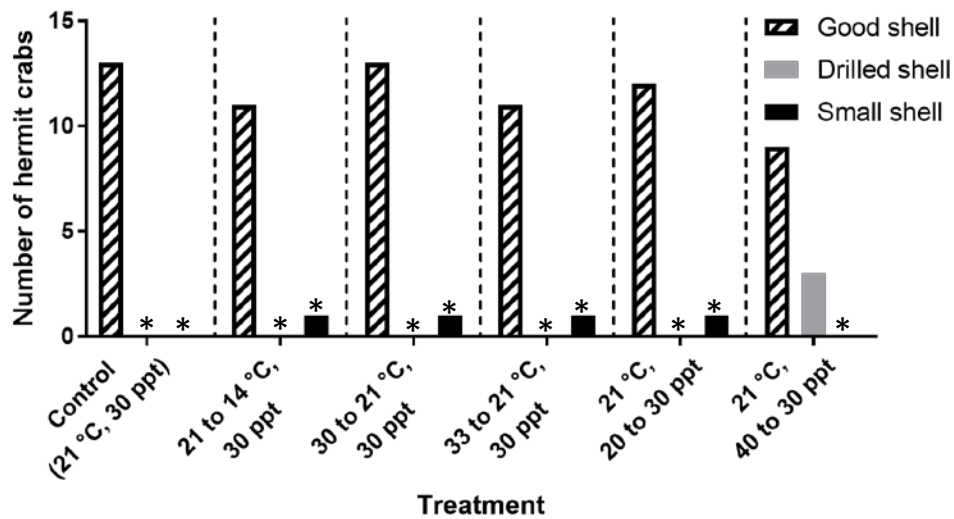


Figure 3.8. The number of hermit crabs (*Pagurus longicarpus*) that started off in an intact shell 75% of ideal size that were occupying good shells (intact shells of ideal size), drilled shells of ideal size, or shells 75% of ideal size 30 min (a) and 24 h (b) after sudden changes in seawater temperature or salinity. See Fig. 3.7 caption for details on methods. Asterisks indicate a significant difference in the odds of being in a drilled or small shell instead of a good shell.

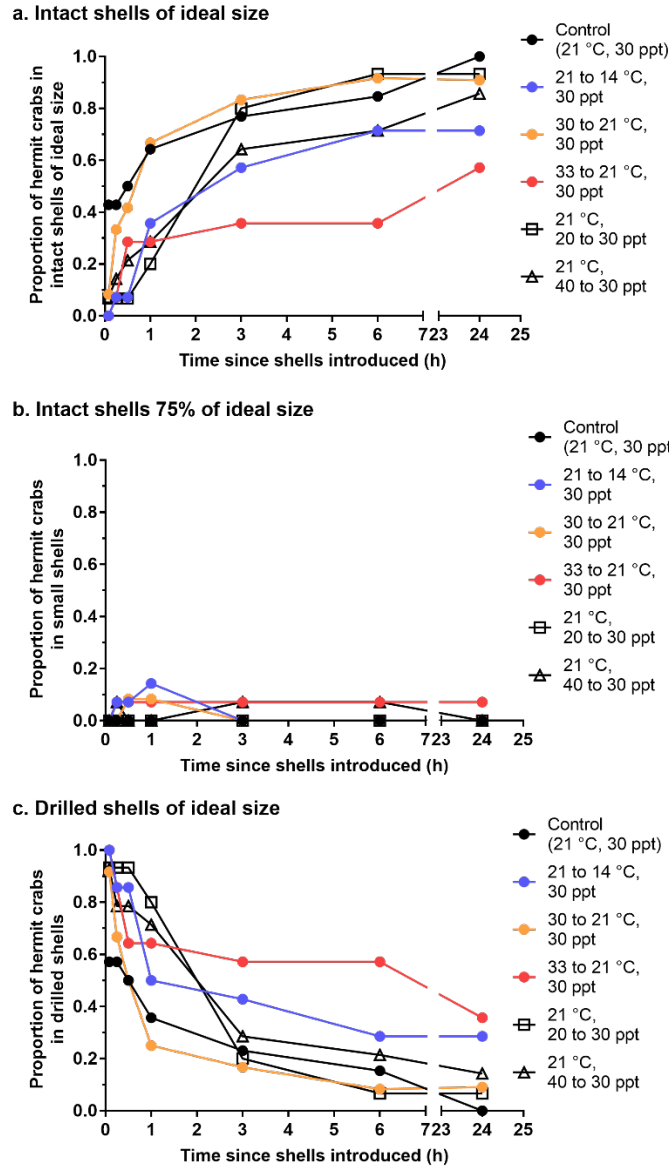
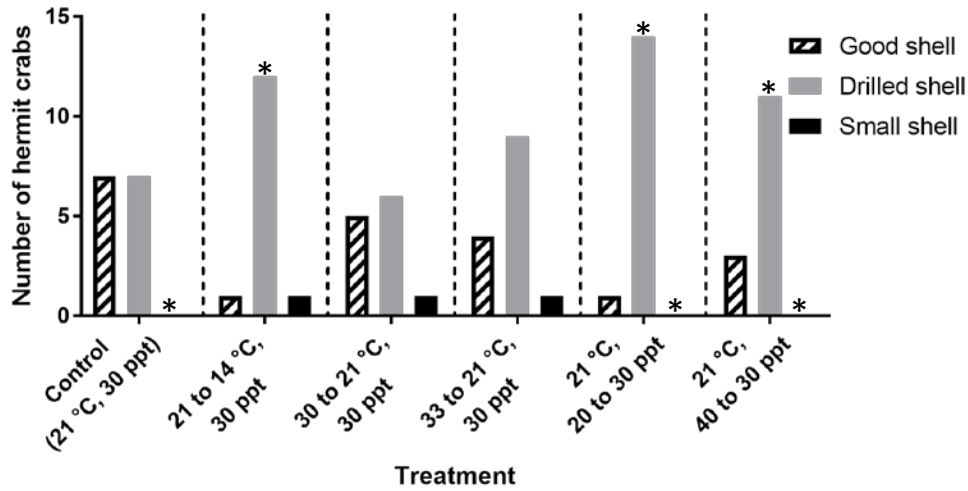


Figure 3.9. Proportion of hermit crabs (*Pagurus longicarpus*) that were occupying intact shells of ideal size (a), intact shells 75% of ideal size (b), or drilled shells of ideal size (c) at various time points over 24 h after sudden changes in seawater temperature or salinity. The hermit crabs were started off in drilled shells. At the same time that the temperature or salinity was suddenly adjusted, each hermit crab was also provided with an intact shell of ideal size and an intact shell 75% of ideal size. n ~ 15 hermit crabs per treatment.

a. 30 min



b. 24 h

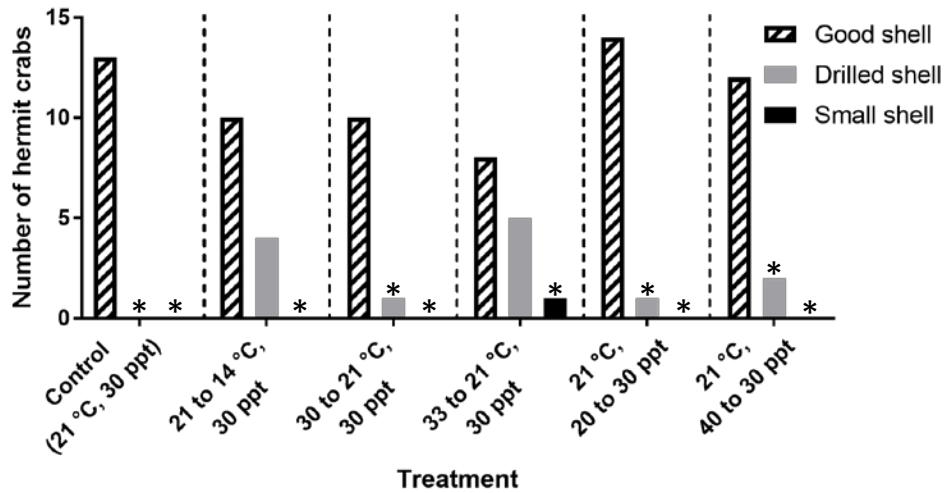


Figure 3.10. The number of hermit crabs (*Pagurus longicarpus*) that started off in a shell of ideal size with a drill hole that were occupying good shells (intact shells of ideal size), drilled shells, or shells 75% of ideal size 30 min (a) and 24 h (b) after sudden changes in seawater temperature or salinity. See Fig. 3.9 caption for details on methods. Asterisks indicate a significant difference in the odds of being in a drilled or small shell instead of a good shell.

Chapter 4

**The effects of changes in temperature and salinity on the ability of the hermit
crab *Pagurus longicarpus* to locate food**

Abstract

Hermit crabs of the species *Pagurus longicarpus* live intertidally and subtidally along the Atlantic coast of Canada and the United States and along the Gulf coast of the United States. They are often found in tide pools at low tide, where conditions can change rapidly depending on the weather on a given day. We studied the ability of these hermit crabs from a Massachusetts population to locate food within 15 min after experiencing a change in temperature or salinity. We found that the low temperature of 14 °C caused the hermit crabs to take at least three times longer to move and contact food after it was placed nearby than hermit crabs at 21 °C and 30 °C. We also found that the low salinity of 20 ppt caused the hermit crabs to take longer to move and contact food than those at salinities of 30 ppt and 40 ppt. These results suggest that the rising temperatures that will result from climate change in the near future will not affect the ability of hermit crabs of the species *Pagurus longicarpus* to find food, but the increasing rainfall predicted for Massachusetts might.

Introduction

Hermit crabs rely on chemoreception to locate resources and to make sense of their surroundings. They can sense the presence of conspecifics, and can even distinguish known from unknown individuals (Gherardi & Tiedemann, 2004). They can also sense predators and food from a distance, which can alert them of the need to leave or move towards a certain area (Brooks, 1991; Hazlett, 1968; Rittschof & Hazlett, 1997). Moreover, chemical signals from decaying

gastropods indicate to hermit crabs that a new shell might soon be available for housing (Rittschof, 1980). In this study, we examined how the chemosensory food-locating abilities of a Massachusetts population of one species of hermit crab, *Pagurus longicarpus*, are affected by changing environmental conditions.

Hermit crabs of the species *P. longicarpus*, also known as the long-wristed hermit crab, can be found intertidally and subtidally along the Atlantic coast of North America from Nova Scotia to Florida, and along the Gulf coast from Florida to Texas (Young, 1978). Analyses of the stomach contents of these hermit crabs have revealed that their diets typically include nematodes, copepods, amphipods, foraminiferans, gastropod larvae, and algae (Caine, 1975; Pechenik et al., 2010; Sanders et al., 1962; Whitman et al., 2001). In the lab, they will consume artificial crab meat and shrimp pellets, as well as mussel and snail tissue (e.g. Blackstone, 1985; Gherardi & Tiedemann, 2004; Gravel et al., 2004; Pechenik & Lewis, 2000; S.G., pers. obs.), using a number of feeding methods (Roberts, 1968; Scully, 1978; Whitman et al., 2001; S.G., pers. obs.). One of these feeding methods, in which the hermit crabs hold large pieces of food up to their mouths with their chelipeds (Roberts, 1968), likely provides hermit crabs with more than half of their food (Caine, 1975) and was the focus of the current study.

Long-wristed hermit crabs can often be found in intertidal tide pools at low tide, where physical conditions can change much more rapidly than in deeper water (Fraenkel, 1960). On hot and sunny days the temperature and salinity in tide pools become elevated quickly (Fraenkel, 1960; Koprivnikar & Poulin,

2009), and on rainy days the salinity decreases quickly (Montory et al., 2016). In Massachusetts, where average air surface temperature is predicted to rise by up to 4 °C between the years 2005 and 2100, and average precipitation is predicted to increase by 10% during that same time frame (IPCC, 2014), we can expect that the fluctuations in the temperature and salinity of tide pools will become more extreme (especially on days when the temperature or amount of precipitation will be above average).

We know that changes in temperature and salinity can affect the ability of long-wristed hermit crabs to occupy high quality shells (Gilliand, unpublished data), but do they also affect their ability to respond to the presence of food? Whitman et al. (2001) measured the rate of suspension feeding by long-wristed hermit crabs and found that hermit crabs in seawater at 15 ppt salinity fed at less than half the rate of those in seawater at 30 ppt salinity, and those at 13 °C fed at slightly more than half the rate of those at 20 °C. Here, we set out to find whether changes in temperature or salinity affect the ability of these hermit crabs to find food in their vicinity. A loss of ability to locate food would suggest that the shifts in temperature or salinity affect the chemosensory capabilities of the hermit crabs, their locomotory abilities, or their desire to consume food.

Methods

Hermit crab collection and care

Long-wristed hermit crabs (*Pagurus longicarpus*) occupying periwinkle (*Littorina littorea*) shells were collected haphazardly from the rocky intertidal

zone at Nahant, Massachusetts in the summer and early fall of 2016 and transported to Tufts University, where they were kept in 20 L laboratory aquaria containing aerated artificial seawater (Instant Ocean, called “seawater” from now on) at 30 ppt salinity and room temperature (~21 °C). The hermit crabs were fed three times per week on a diet of artificial crab meat (transOCEAN; Alaskan pollock and King Crab) and shrimp pellets (O.S.I.). Individuals were moved to smaller plastic containers containing seawater for feeding, to minimize waste accumulation in the larger aquaria. After each feeding they were returned to the aquaria. The hermit crabs were allowed to acclimate to laboratory conditions for one to three weeks before experiments began. No hermit crab was used in more than one trial. After experimentation, the hermit crabs were returned to the collection site.

Evaluating the time taken to locate food under altered temperatures or salinities

We conducted experiments to determine the effects of substantial changes in temperature and salinity in tide pools at low tide on the ability of long-wristed hermit crabs to locate food. Experiments were conducted at temperatures of 14 °C, 30 °C, and 33 °C and salinities of 20 ppt and 40 ppt. A control group of hermit crabs kept at 21 °C and 30 ppt was also tested. Fifteen hermit crabs weighing between 300 mg and 1500 mg were used for each treatment except the 20 ppt, 14 °C, and 33 °C treatments, each of which had one individual molt in the middle of the experiment; molting individuals were excluded from the analysis.

Each hermit crab was placed into an individual rectangular plastic container measuring 12 cm x 8.3 cm x 3.2 cm with 180 ml of seawater, which was then covered with plastic wrap and aerated through a glass Pasteur pipette that was poked through the covering. The water in each container was changed daily, and no food was provided. After 48 h with no food, each hermit crab was removed from its shell by cracking the shell with a vise (Pechenik et al., 2015b), weighed to the nearest mg, and provided with a shell of ideal size for its weight (Angel, 2000; Gilliland, unpublished data).

After 72 h of starvation, the hermit crabs in their individual containers were moved to a walk-in incubator. Those in an altered temperature treatment had the temperature of their 180 ml of seawater gradually adjusted to the new temperature over the course of an hour. Those in an altered salinity treatment were put in 90 ml of 30 ppt seawater, and an additional 90 ml of seawater of a higher or lower salinity (calculated to bring the overall salinity to a desired level) was added gradually over the course of an hour. Those in the control treatment were left to sit for an hour.

After this adjustment period, a single piece of artificial crab meat weighing 100 mg to 125 mg was added to each container, next to the Pasteur pipette that was providing aeration so that the current created by the aeration would carry the food odor towards the hermit crab at the opposite end of the container. The behavior of the hermit crabs was then video recorded for 15 min. After this amount of time, the window of opportunity to locate and consume the food in the intertidal zone in the presence of other, potentially competing,

organisms would likely have passed. The time taken for each hermit crab to move after the piece of food was added and the time taken for the crab to physically contact the food were later evaluated from the video data.

Data analysis

Results were plotted on a natural log scale in Graphpad Prism version 7.02, and statistical analyses were conducted in R version 3.2.2.

A chi-squared goodness-of-fit test was performed on the number of hermit crabs at each temperature and salinity that did or did not move after the food was introduced to their containers, as well as on the number of hermit crabs that did or did not contact the food.

In order to determine whether temperature or salinity had an effect on the time taken for the hermit crabs to move and locate the food, the data were organized in two ways: the first way included any hermit crabs that failed to move and/or make contact with the food during the 15 min experiment as having taken 15 min to do so. The second way removed the hermit crabs that did not move and/or make contact with the food from the analysis.

Data were natural log transformed and Levene's Test was then used to determine if there were significant differences between the amounts of variance in the results of the various treatments. Whenever there was no significant difference in the amount of variance, a one-way ANOVA with a post-hoc Tukey's multiple comparison test was used to compare the time taken for the hermit crabs to move in each treatment after the food had been introduced, and to compare the

time taken to make physical contact with the food. When Levene's test revealed that there was a significant difference between the variances, Welch's ANOVA with a Games Howell post-hoc test was used instead.

Results

In the control treatment and the 40 ppt salinity treatment, all of the hermit crabs moved and made contact with the artificial crab meat within the 15 min allotted after the food was first introduced (Table 4.1). This was not true for hermit crabs tested at 20 ppt salinity or in any of the treatments with altered temperatures. The number of hermit crabs that moved versus the number that did not move was not shown to be significantly different across treatments with different temperatures ($\chi^2 = 4.04$, $df = 3$, $p = 0.26$) but was shown to be significantly different across treatments with different salinities ($\chi^2 = 12.09$, $df = 2$, $p < 0.01$). The number of hermit crabs that contacted the food versus the number that did not contact the food was also not significantly different across temperature treatments ($\chi^2 = 7.29$, $df = 3$, $p = 0.06$) but was significantly different across salinity treatments ($\chi^2 = 12.09$, $df = 2$, $p < 0.01$).

Regardless of whether or not the hermit crabs that never moved were included in the analysis as taking the full time to move, there was a significant effect of temperature on the time taken by the hermit crabs to begin moving after the food had been introduced, with those at 14 °C taking at least three times longer to move than those at the control temperature (21 °C) or 30 °C (including those that never moved: 1- way ANOVA, $F(3,54) = 4.143$, $p = 0.01$; excluding

those that never moved: 1-way ANOVA, $F(3,48) = 5.34$, $p < 0.01$) (Fig. 4.1). The same pattern was observed for the time taken by the hermit crabs to contact the food when those that never contacted the food were included as taking the full time to do so (Welch's ANOVA, $F(3,54) = 12.092$, $p < 0.0001$) (Fig. 4.2a). When those that never contacted the food were excluded from the analysis, the hermit crabs at 14 °C took significantly longer to move than the hermit crabs at any other temperature (1-way ANOVA, $F(3,45) = 14.07$, $p < 1e-5$) (Fig. 4.2b).

For the salinity treatments, when the hermit crabs that never moved were included in the analysis, the hermit crabs at 20 ppt took significantly longer to move (1-way ANOVA, $F(2,41) = 6.57$, $p < 0.01$) and contact the food (Welch's ANOVA, $F(2,41) = 5.68$, $p < 0.01$) than the hermit crabs at the control salinity (30 ppt) and 40 ppt (Fig. 4.3a & 4.4a). However, when those that never moved were excluded from the analysis, salinity had no effect on the time taken for the hermit crabs to move (1-way ANOVA, $F(2,36) = 0.62$, $p = 0.54$) or contact the food (1-way ANOVA, $F(2,36) = 1.43$, $p = 0.25$) (Fig. 4.3b & 4.4b).

Discussion

Discussion of method of data analysis

We conducted the data analysis both with the hermit crabs that never moved or contacted the food (marking them as taking the whole 15 min to do so, similar to Tran (2014)) and without, in order to present a more complete picture of the data. We cannot be sure if or when these individuals would have performed the behaviors in question, so excluding these hermit crabs from the

analysis eliminates this uncertainty. However, including these individuals allows us to account for the fact that the changes in temperature and salinity might be affecting their behavior to the point of preventing them from being able to (or preventing them from choosing to) move or locate the food, as suggested by the significant results of chi-squared analyses for the number of hermit crabs that moved or did not move across treatments with different salinities and the number that did or did not contact the food across treatments with different salinities.

Discussion of results

As we begin to see the effects of climate change more strongly in the coming years, we can expect that conditions in tide pools will become more extreme (IPCC, 2014). Hotter days and changing precipitation patterns will cause both temperature and salinity to fluctuate dramatically with the tidal cycle in the intertidal zone. We know from previous studies (Gilliand, unpublished data) that changes in temperature and salinity can affect the size of the shells that long-wristed hermit crabs choose to live in, as well as their ability to occupy high-quality shells. Additionally, increasing temperatures have been shown to increase and then decrease the movement of long-wristed hermit crabs (Fraenkel, 1960) and to alter the predictability of the startle response (how long they remain withdrawn in their shells after being picked up, inverted, and placed back down) in the closely related species *P. bernhardus* (Briffa et al., 2013). Decreases in salinity can cause hermit crabs of the species *P. bernhardus* to retreat into their

shells (Davenport et al., 1980). Now we have shown that similar changes will likely impact the ability of at least one species of hermit crab to procure food.

The drop in temperature from 21 °C to 14 °C significantly increased the amount of time taken for long-wristed hermit crabs to move (at least a three-fold increase) and contact a piece of artificial crab meat (at least a seven-fold increase) after the food was placed into their containers (Fig. 4.1 & 4.2). An effect at this temperature is not as pertinent to concerns on the effects of climate change, but is perhaps relevant to a hermit crab's behavior as winter approaches. As water temperatures cool in the late fall and early winter, these temperate hermit crabs move into deeper water and bury themselves in the substrate (Rebach, 1970). The colder winter water temperatures slow and even altogether prevent any movement by the hermit crabs, which would leave them exposed to predators and other threats if they did not retreat and cover themselves in such a manner (Rebach, 1974).

Rebach (1974) showed that long-wristed hermit crabs from a population at Narragansett Bay, Rhode Island began moving into deeper water when temperatures declined to 10 °C, which agrees with the water temperature at which Nahant, Massachusetts hermit crabs disappear from the intertidal zone every year (usually in late October) (S.G., pers. obs.). The hermit crabs in Rebach's study ceased all movement at 3 °C, a type of behavior that has also been observed in at least one other species of hermit crab, *Clibanarius vittatus* (Fotheringham, 1975). The low temperature tested in the current study (14 °C) is higher than the temperatures that have been shown to trigger migration to deeper water and

decrease movement in long-wristed hermit crabs (Rebach, 1974). However, the rate at which temperature was decreased in our study was more sudden than would be experienced in the intertidal zone as winter approaches, which may have triggered such changes in behavior. Or, it is possible that the hermit crabs' chemoreceptive abilities or perception of hunger may be affected before the temperature falls low enough to completely prevent movement.

Increasing the salinity from 30 ppt to 40 ppt did not have any noticeable effect on the time taken for the hermit crabs to move or contact the food (Fig. 4.3 & 4.4). This result, combined with the fact that the raised temperatures studied here also did not have an effect, suggests that the warming water temperatures associated with climate change, combined with evaporation on hot, sunny days, will not affect the ability of hermit crabs to locate food. Future studies should examine whether the combined effects of elevated temperature and elevated salinity might have any effect not seen with either variable individually.

The shift from 30 ppt to 20 ppt salinity resulted in the hermit crabs taking 16 times longer to move and 12 times longer to contact food when the individuals that never performed these behaviors were recorded as taking the full 15 min to do so (Fig. 4.3a & 4.4a). However, the analysis of the data excluding the hermit crabs that never moved or contacted the food did not show a significant difference between the treatment groups (Fig. 4.3b & 4.4b), highlighting the fact that the decrease in salinity from 30 ppt to 20 ppt greatly affected these behaviors, to the point of inhibiting them altogether within the experimental window. This could indicate that the increased amount of rainfall that is predicted for Massachusetts

and other areas (IPCC, 2014) will reduce the hermit crabs' ability to find food. However, the problem of low salinity is only a temporary condition in tide pools – as tides rise, water of a more average salinity floods the pools. Future studies should examine how long it takes feeding rates to recover after exposure to low salinity.

The fact that the difference between the results at 30 ppt salinity and 20 ppt salinity changed depending on whether or not individuals that did not move and/or contact the food were included in the analysis highlights the benefits of performing both types of analyses. The authors of future studies with similar designs should also consider analyzing data both with and without individuals that never perform a certain behavior in an allotted time, in order to get a more complete picture of the data.

Whitman et al. (2001) studied the effects of temperature and salinity on the rate at which long-wristed hermit crabs were able to suspension-feed on *Artemia* nauplii. Consistent with the trends observed in the current study, they found that decreased temperature and salinity significantly reduced the rate at which the hermit crabs consumed the nauplii. The impairment of multiple feeding methods suggests that these hermit crabs would likely struggle to consume adequate nutrients while experiencing these environmental conditions.

Other marine organisms have been shown to experience a disruption of chemosensory and olfactory abilities under altered environmental conditions. For example, when exposed to a decrease in pH, hermit crabs of the species *P. bernhardus* moved their antennae less frequently, moved location less, and less

often moved to the source of a food odor (de la Haye et al., 2012). Clownfish (*Amphiprion percula*) larvae in more acidic seawater became attracted to cues that they typically avoid, such as cues from certain shoreline trees, their parents, and even predators (Dixson et al., 2010; Munday et al., 2009). Cheroske et al. (2009) and Franklin et al. (2016) found that briefly immersing stomatopods' antennae in freshwater impacted the rate of antennal flicking and likely prevented them from detecting other stomatopods. It may be that less extreme decreases in salinity might also impact stomatopod chemosensory abilities. Additionally, the presence of pollutants in water has been shown to affect the ability of various predators and prey to detect one another, as well as interfere with conspecific pheromone detection (reviewed in Lürling & Scheffer, 2007).

It is likely that the abilities of long-wristed hermit crabs to sense or respond to other chemical cues are affected by low salinity and temperature as well. Without the ability to sense and accurately interpret predator cues, cues from decaying gastropods, or cues from conspecifics, hermit crabs would likely find themselves unable to avoid dangerous predators, unable to find and occupy shells that adequately protect them from predators and desiccation, and unable to find mates (Gherardi & Tiedemann, 2004; McClintock, 1985; Pechenik et al., 2001; Pechenik & Lewis, 2000; Rittschof, 1980; Rittschof & Hazlett, 1997; Taylor, 1981; Vance, 1972b).

Future research is needed to determine exactly how decreases in temperature and salinity prevent long-wristed hermit crabs from being able to obtain nearby food. The behavior observed here may represent an inability to

detect the food odor, or it may be the result of an inability to move or a lack of motivation to expend energy locating food while experiencing possibly stressful environmental conditions. The impact of environmental changes may cause widespread impairment of chemoreception in marine organisms, but future studies are needed to determine the mechanism behind these responses.

Tables

Table 4.1. Percent of hermit crabs (*Pagurus longicarpus*) in each treatment that, within 15 minutes of a piece of artificial crab meat being introduced into their containers, moved or contacted the food.

Treatment	n	Percent that moved	Percent that contacted food
Control	15	100.00	100.00
14 °C	14	85.71	71.43
30 °C	15	93.33	93.33
33 °C	14	78.57	71.43
20 ppt	14	64.29	64.29
40 ppt	15	100.00	100.00

Figures

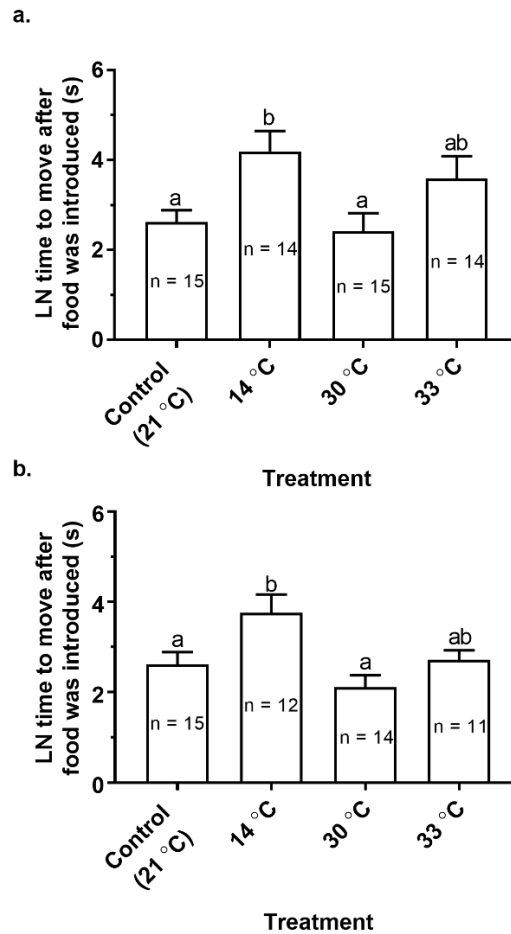


Figure 4.1. Effect of water temperature on average time taken for hermit crabs (*Pagurus longicarpus*) to move after a piece of artificial crab meat was placed at the opposite end of the container that each hermit crab was occupying. Each crab was tested in 30 ppt salinity seawater at either the control temperature (21 °C), 14 °C, 30 °C, or 33 °C. The hermit crabs had been starved for 72 h and were given 15 min to respond to the presence of the food. (a) includes hermit crabs that never moved as having taken the full 15 min to move. (b) excludes hermit crabs that never moved from the analysis. Error bars represent standard error and the letters above each bar represent the results of Tukey's multiple comparisons test.

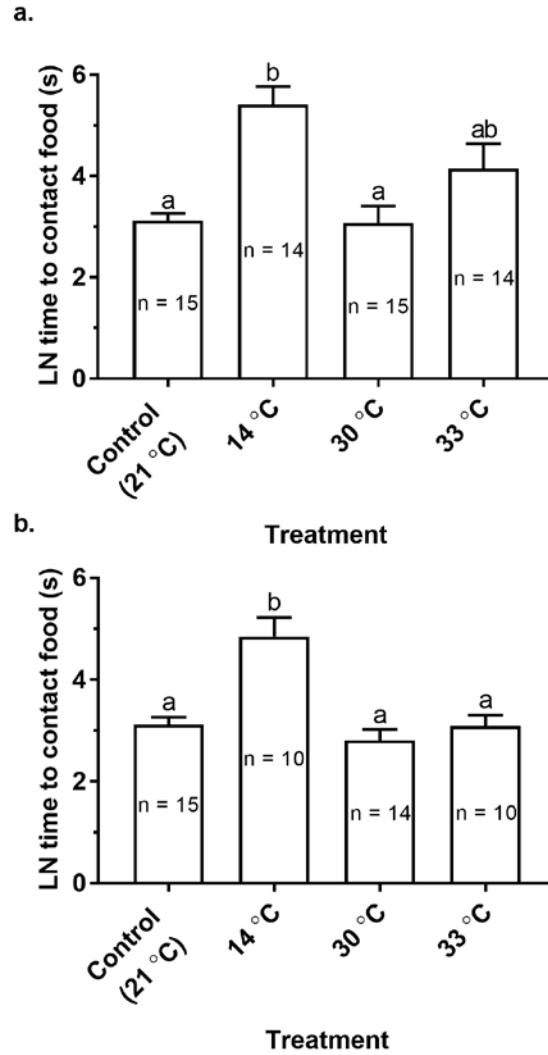


Figure 4.2. Effect of water temperature on average time taken for hermit crabs (*Pagurus longicarpus*) to make physical contact with a piece of artificial crab meat that was placed at the opposite end of the container that each hermit crab was occupying. See Fig. 4.1 caption for details on methods. (a) includes hermit crabs that never contacted the food as having taken the full 15 min to contact it. (b) excludes hermit crabs that never contacted the food from the analysis. Error bars represent standard error. The letters above each bar represent the results of the Games-Howell test in (a) and Tukey's multiple comparisons test in (b).

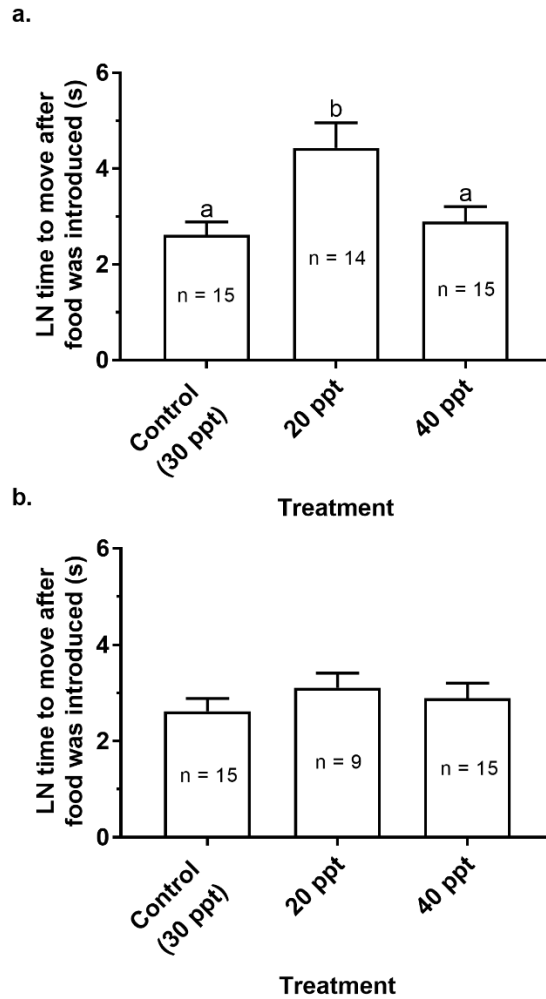


Figure 4.3. Effect of salinity on the average time taken for hermit crabs (*Pagurus longicarpus*) to move after a piece of artificial crab meat was placed at the opposite end of the container that each hermit crab was occupying. Each crab was tested in seawater at 21 °C and either the control salinity (30 ppt), 20 ppt, or 40 ppt. The hermit crabs had been starved for 72 h and were given 15 min to respond to the presence of the food. (a) includes hermit crabs that never moved as having taken the full 15 min to move. (b) excludes hermit crabs that never moved from the analysis. Error bars represent standard error. The letters above each bar in (a) represent the results of Tukey’s multiple comparisons test.

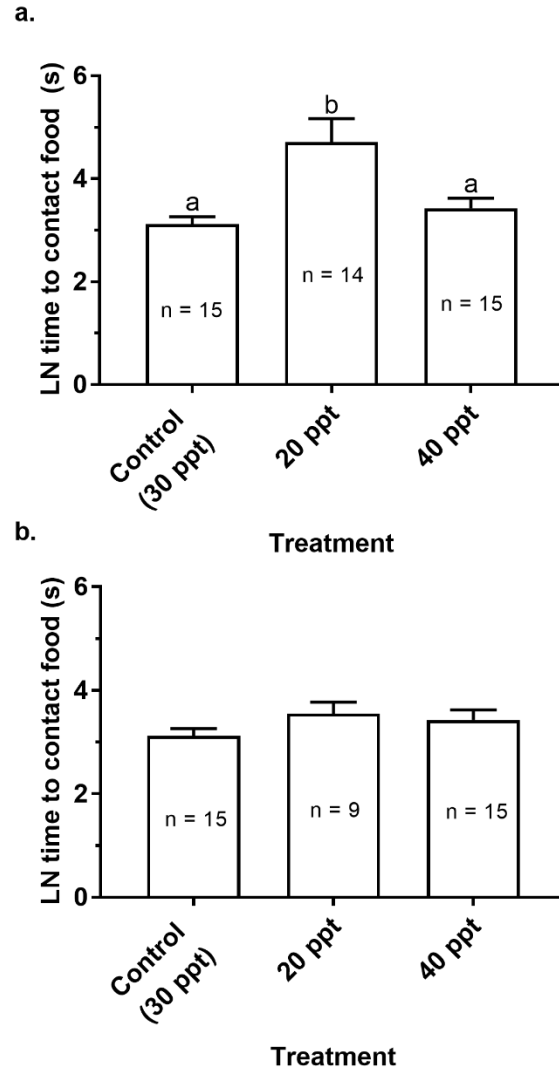


Figure 4.4. Effect of salinity on the average time taken for hermit crabs (*Pagurus longicarpus*) to make physical contact with a piece of artificial crab meat that was placed at the opposite end of the container that each hermit crab was occupying. See Fig. 4.3 caption for details on methods. (a) includes hermit crabs that never contacted the food as having taken the full 15 min to contact it. (b) excludes hermit crabs that never contacted the food from the analysis. Error bars represent standard error. The letters above each bar in (a) represent the results of the Games-Howell test.

Chapter 5
Conclusions

I set out to investigate how long-wristed hermit crabs (*Pagurus longicarpus*) will be affected by future changes in environmental conditions associated with climate change. I designed studies to answer the following three questions:

1. How do changes in temperature and salinity affect the size of the shells long-wristed hermit crabs choose to occupy?
2. How do changes in temperature and salinity affect the quality of the shells that long-wristed hermit crabs choose to occupy?
3. How do changes in temperature and salinity affect the ability of long-wristed hermit crabs to locate food in their vicinity?

I believe that we have made significant progress toward answering these questions with the studies detailed in my previous chapters.

How do changes in temperature and salinity affect the size of the shells long-wristed hermit crabs choose to occupy?

In Chapter 2 of my thesis, I detailed how we found that hermit crabs in seawater at a low temperature of 16 °C, a high temperature of 32 °C, and a low salinity of 15 ppt were occupying shells of significantly different sizes than their same-sized counterparts at the control temperature (21 °C) and salinity (30 ppt). There was not a significant difference between the shell sizes occupied by hermit crabs at salinities of 20 ppt or 40 ppt and those under control conditions. Small hermit crabs in the treatments with raised or lowered temperatures or salinities were typically found in smaller shells than similarly-sized hermit crabs in the

control treatment, and large hermit crabs in the treatments with raised or lowered temperatures or salinities were typically found in larger shells than similarly-sized hermit crabs in the control treatment.

The reason for the different shifts in shell size preference is unknown. Hermit crabs in small shells are more likely to be eaten by predators (Vance, 1972b), be susceptible to desiccation (Taylor, 1981), and suffer from a decrease in growth rate (Angel, 2000). However, they benefit from having less weight to carry (Herreid & Full, 1986; Osorno et al., 1998). Hermit crabs in large shells are using more energy to carry around their shelters (Wilber, 1990), but they may benefit from being able to retract completely into their shells and from having a larger buffer against the outside environment (Bertness, 1982).

Future studies are needed to determine what might be leading different-sized hermit crabs to shift their shell-size preference in different directions. Other studies should investigate whether higher salinities, or even a combination of high temperatures and salinities, might affect the shell sizes preferred by hermit crabs. Additionally, it would be interesting to know whether oscillating temperatures, changes in acidity, or the climate of the area from which the hermit crabs are collected affect these preferences.

How do changes in temperature and salinity affect the quality of the shells that long-wristed hermit crabs choose to occupy?

In chapter 3, I presented the results of the experiments in which we tested the effects of both gradual and sudden changes in temperature and salinity on the

types of shells occupied by hermit crabs. The gradual changes represented what hermit crabs might experience in a tide pool at low tide, and the sudden changes represented what they might experience when a rising tide spills into a tide pool. Our data showed that hermit crabs that began the experiment without a shell were faster to occupy a good shell than the hermit crabs that began the experiment in a small or drilled shell. The hermit crabs were least often occupying good shells of ideal size instead of drilled or small shells after experiencing simultaneous gradual increases in temperature and salinity to 33 °C and 40 ppt from the control temperature and salinity of 21 °C and 30 ppt. Additionally, gradual increases to 33 °C, gradual increases to 30 °C and 40 ppt simultaneously, sudden changes from 33 °C to 21 °C, and sudden changes from 40 ppt to 30 ppt caused the hermit crabs to take longer to occupy good shells than those at the control temperature and salinity.

The high temperature could have impaired heart function, action potentials, mitochondrial activity, membrane fluidity, and protein function in the hermit crabs (reviewed by Somero, 2002). The high salinity could have affected the oxygen consumption of the hermit crabs (Davenport et al., 1980; Flemister & Flemister, 1951; Lofts, 1956) or the amount of energy needed to regulate ion levels (Robertson, 1953). Our results suggest that the temperature and salinity levels that hermit crabs will be exposed to in tide pools in the near future (IPCC, 2014) will impact their ability to occupy high quality shells that will best protect them from predators (Reese, 1969), desiccation (Taylor, 1981), decreased growth

rate (Angel, 2000), and increased energy usage (Elwood & Glass, 1981; Osorno et al., 1998, 2005).

Future research should focus on determining exactly what the mechanisms are behind the hermit crabs' responses to changes in temperature and salinity and what role heat-shock proteins play in hermit crab stress response. Such studies have been performed on other organisms, but much work needs to be done to determine the finer details of the effects of climate change on marine intertidal invertebrates.

How do changes in temperature and salinity affect the ability of long-wristed hermit crabs to locate food in their vicinity?

In chapter 4 of my thesis, I described the results of our experiment in which the time taken for hermit crabs to locate pieces of artificial crab meat was recorded. The hermit crabs were observed for 15 min, and the time they took to move and contact the food was noted. Regardless of whether the hermit crabs that never moved were included in the analysis as taking the full time to do so, the hermit crabs at the low temperature of 14 °C took significantly longer to move than those at 21 °C or 30 °C. When the hermit crabs that never contacted the food were included in the analysis, those at 14 °C took significantly longer to contact the food than those at 21 °C or 30 °C. When the hermit crabs that never contacted the food were not included in the analysis, those at 14 °C took significantly longer to contact the food than those in any other temperature treatment (21 °C, 30 °C, and 33 °C).

When the hermit crabs that never moved were included in the analysis, salinity had a significant effect on the time taken to move, with those at 20 ppt taking significantly longer than those at any other salinity (30 ppt and 40 ppt). When the hermit crabs that never moved were not included, salinity did not have a significant effect. The same patterns were seen in the effects of the salinity treatments on the time taken for the hermit crabs to contact the food.

These results suggest that very hot and sunny days in the near future will not impact the ability of long-wristed hermit crabs to locate sources of food (IPCC, 2014). However, decreased salinity in tide pools, associated with rainy days, may have an effect. Additionally, as winter approaches every year and water temperatures decrease, hermit crabs likely do not move or grasp food as quickly as they do during warmer times of year. This is probably related to the overall decrease in activity caused by cold temperatures that drives them to move to deeper water and burrow into the substrate in the late fall in New England (Rebach, 1970, 1974).

These results raise a number of questions that can be addressed by future research. Do increases in both temperature and salinity have any combined effect on the ability of hermit crabs to locate food? How long does it take feeding rate to recover after changes in environmental conditions? What is the mechanism behind these responses to changes in temperature and salinity?

Final remarks

Long-wristed hermit crabs (*Pagurus longicarpus*) serve as an important food source for Massachusetts intertidal and subtidal predators such as the green crab *Carcinus maenas* (Rotjan et al., 2004) and the rock crab *Cancer irroratus* (Angel, 2000). Additionally, hermit crabs provide a service to symbionts that live on their shells, such as *Crepidula* spp. (Li & Pechenik, 2004; Pechenik et al., 2015b), by preventing the shells from becoming buried in the substrate (Conover, 1975). If the changing temperatures and salinities in tide pools that will result from climate change will prevent hermit crabs from occupying high quality shells, they could initially become a more easily obtainable food source for their predators. But if predation levels on hermit crabs become too high, or if environmental conditions become so extreme that they can no longer survive, their predators and symbionts will suffer too, if those populations haven't already been otherwise harmed by the changing environmental conditions.

The results of our studies suggest that hermit crabs will suffer from an inability to locate food if caught in tide pools on rainy days when salinity will decrease. They will likely suffer from a lack of adequate shelter if not already in an ideal shell if caught in tide pools on hot, sunny days when the temperature and salinity increase. It is possible that long-wristed hermit crabs will move more permanently to the subtidal zone, where they already spend some of their time (McDermott, 1999), to avoid these conditions. However, this would mean leaving behind the abundant supply of shells in the intertidal zone (S.G., pers. obs.). If these Massachusetts hermit crabs will experience problems as a result of

climate change, it is likely that other intertidal invertebrates in the northeast United States will as well. Future research should continue to study the possible effects of climate change on coastal communities, and drastic steps need to be taken to slow climate change and mitigate the harmful effects.

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