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Title of Dissertation: Homing in the Benthos: Navigation and Orientation in a Mantis Shrimp

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ABSTRACT

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Stomatopods, better known as mantis shrimp, are crustaceans which commonly inhabit holes in benthic marine environments for use as burrows. Many stomatopod species forage at extended distances before returning to their burrows, risking predation. By using large, semi-naturalistic arenas, I investigated the navigational strategies these animals use to find their way home. First, by laterally displacing foraging stomatopods, I demonstrated that the mantis shrimp, *Neogonodactylus oerstedii*, uses path integration, a vector-based strategy, to navigate home, making them the first fully aquatic path-integrating animals yet discovered. Next, by passively rotating stomatopods during foraging, I found that they use celestial and idiothetic (self-motion) orientation cues during path integration. By manipulating the apparent position of the sun and by rotating overhead polarization patterns while animals were foraging, I demonstrated that *N. oerstedii* hierarchically rely on these cues when orienting. During these experiments, I found that path integration in *N. oerstedii* was prone to error proportional to error accumulated over

the course of foraging paths. To combat this error inherent in path integration, stomatopods enacted stereotyped search patterns when path integration did not lead them directly to their burrows. I found that this search behavior forms continuously expanding, non-oriented loops that are centered near the point of search initiation. Also, the radius of this search appeared to be scaled to the animal's accumulated error during path integration, improving the effectiveness of the search. Next, by comparing homeward paths in the presence and absence of a landmark placed near the burrow and by displacing the landmark to an alternate location while animals were foraging, I showed that stomatopods navigate using landmarks in parallel with their path integration system. Finally, I aimed to understand what makes a landmark salient to a mantis shrimp when identifying it. Using dichotomous choice behavioral tests, I, with a team of undergraduates, found that the shape of an object is more important than its color for identification by *N. oerstedii*, suggesting that *N. oerstedii* identifies landmarks more by their shapes than their colors. These experiments uncover for the first time the robust navigational toolkit *N. oerstedii* relies upon to find home.

HOMING IN THE BENTHOS: NAVIGATION AND ORIENTATION IN A
MANTIS SHRIMP

By

RICKESH N. PATEL

Dissertation submitted to the Faculty of the Graduate School of the
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Dedication

To my parents,

who have been a constant source of support throughout my life.

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First, I would like express my heartfelt gratitude to my graduate mentor, Dr. Thomas Cronin, whose guidance and friendship over my graduate career have been invaluable. Tom made my research with mantis shrimp possible and has deeply integrated me in the scientific community, something that he does in an unparalleled way for his students. I would also like to thank my graduate committee members, Dr. John Layne, Dr. Bernard Lohr, Dr. Tamra Mendelson, and Dr. Phyllis Robinson, for the advice they have offered me over the years. A large portion of my work would not be possible without the generous offer from Dr. Jon Cohen at the University of Delaware, his graduate students (especially Victoria Simmons), and the UD College of Earth, Ocean, and Environment to host me, offering space for my large experimental setups, for which I am very grateful.

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Chapter 1: Introduction

Research Motivations and General Research Questions

Stomatopods, better known as mantis shrimp, are predatory crustaceans that mostly reside in shallow tropical marine waters worldwide. These animals are renowned for their ballistic strikes and intricate visual systems. Complex color vision, linear and circular polarization receptivity, and UV vision are achieved by 16 distinct photoreceptor classes in most stomatopod retinas (Cronin et al., 2014). Even though their visual physiology has been well studied, an understanding of the ecological significance of mantis shrimps' elaborate eyes is limited. Due to their physiologically taxing nature, animal eyes have not evolved to efficiently relay all available visual information, but to detect biologically significant information (Zeil and Hemmi, 2006). Therefore, by examining the stomatopod behavioral repertoire and determining how stomatopods may use their elaborate visual systems, a better understanding of the evolution and processing mechanisms of these systems may be gained.

An intuitive place to investigate stomatopod behavior is within the context of what is perhaps a stomatopod's most important resource, its burrow: an inhabitable hole or crevice in marine substrates around which a stomatopod's life is centered. Although some species excavate their own burrows, the availability of naturally occurring burrows is a limiting resource in many natural stomatopod populations (Steger, 1987). From these burrows, stomatopods may ambush their prey, molt, mate, and brood eggs safely concealed from predators (Reaka, 1980). Due to their

usefulness, burrows are highly prized resources, which are guarded and fought over by conspecifics and heterospecifics alike (Caldwell, 1979).

Despite the security that burrows offer, many stomatopod species leave their burrows for tasks such as foraging and finding mates in the open, where they are vulnerable to predation (Caldwell et al., 1989; Dominquez and Reaka, 1988; Basch and Engle, 1989). These trips away from the burrow may extend up to four meters in some *Neogonodactylus* species, a sizeable distance for animals typically around three to five centimeters long (Caldwell et al., 1989; Dominquez and Reaka, 1988). Basch and Engle (1989) observed *Hemisquilla californiensis*, a larger stomatopod species, foraging at distances greater than 60 meters away from their burrows.

Due to the importance of their burrows, stomatopods have likely evolved efficient mechanisms to navigate back to their homes after foraging, raising the following questions: On expeditions, how do stomatopods navigate back to their burrows? In other words, what navigational strategies do they employ? Stomatopods possess well-developed sensory systems and can potentially acquire spatial information through multiple modalities. What cues do stomatopods use for navigation?

Navigation and Orientation

Many animals move to satisfy specific biological needs whether it be to find food, mates, or suitable habitats to rest and/or raise young. Orienting (an animal's determination of the relative direction to a goal in its environment) and navigating (an animal's act of following a route to a goal) in this complex mosaic allows animals to

efficiently find their way when moving from one place to another, whether it be over the span of a meter to global distances, improving their chances of survival.

Navigational strategies in animals can be divided into three main categories: piloting, path integration, and true navigation. When piloting, an animal uses a landmark or an array of landmarks to locate a goal (Fuller, 1983; Figure 1.1A). A landmark can be defined as an earth-bound feature that is unique and easily distinguishable from the background, reliably long-lasting in a fixed position, and somehow relevant to the navigational goal (Zeil et al., 2009). Piloting is the most familiar way humans naturally navigate through their environment. Examples of other piloting animals can be found in diverse taxa, including hymenopteran insects (bees (Cartwright and Collett, 1983), ants (Collett et al., 1992), and wasps (Tinbergen, 1932)), rodents (Redhead et al. 1997), and butterflyfishes (Reese, 1989) to name a few. However, in the absence of unique landmarks or during long distance navigation in which learned landmarks may no longer be observed, other navigational strategies must be employed.

Path integration (also known as dead reckoning) is the process by which an animal continually monitors the directions and distances of its movements as it travels from a reference point in order to determine its position relative to that point. From this information, the most direct path to the reference point (the home vector), is continually updated (Figure 1.1B). An excellent example of path integration can be observed in the remarkable homing behavior of the desert ant, *Cataglyphis fortis*. These Saharan ants are thermophilic scavengers, foraging for dying or dead animals at the hottest time of the day while avoiding predation. While foraging, these ants

constantly note the directions and distances travelled to calculate the most direct path back to their nest (Wittlinger et al., 2007). They primarily do so using celestial polarization patterns for directional measurements (Wehner and Lafranconi, 1981) and a pedometer to keep track of the distances travelled during their outbound routes (Wittlinger et al., 2007). Both these components are used to calculate home vectors in order to make straight paths back to a refuge from the oppressive desert sun (Muller and Wehner, 1988).

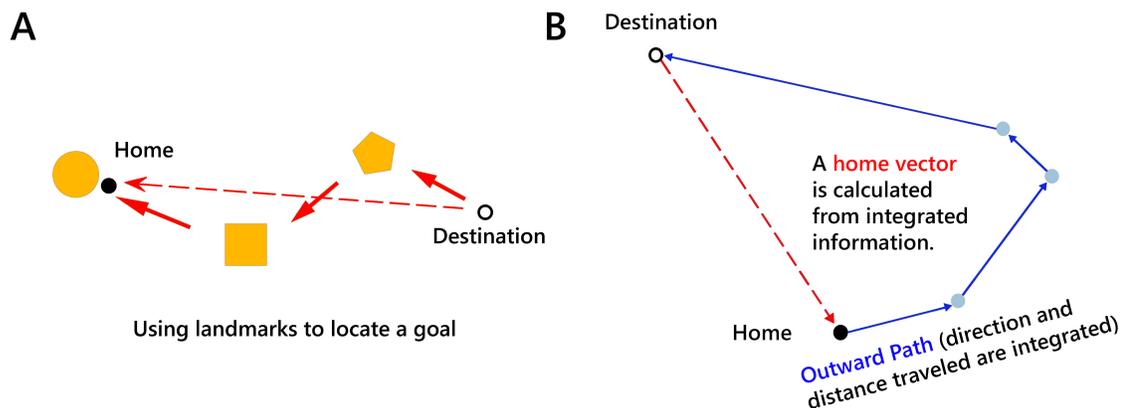


Figure 1.1. Strategies for local navigation. **A.** Piloting refers to the process by which an animal uses a landmark or array of landmarks (in gold) to locate a goal, including following a landmark (beaconing, dashed arrow) or chain of landmarks (route finding, solid arrows) to a goal. **B.** During path integration, an animal continually monitors the direction and distance of its outward path (blue arrows) from a reference point in order to determine its position relative to that reference point. This home vector (red arrow), which is composed of the direction and distance to its starting location, is continually updated during its outward path.

Path integration can be accomplished using multiple mechanisms best described by two parameters: the frame of reference in which the goal is encoded (an egocentric vs. exocentric frame of reference) and the source of spatial information used to compute the goal vector (an idiothetic vs allothetic source of information).

First, considering the vector reference, an animal which uses an egocentric frame of reference keeps the goal direction specified by an angle relative to the animal's head or specific body axis at all times. For example, fiddler crabs radiate from underground burrows, keeping the transverse axis of their bodies aligned with their burrows throughout their foraging excursions (Zeil, 1998). Animal navigators that use an exocentric frame of reference (also called geocentric, allocentric, or earth-bound frame of references) use external stimuli as references for determining the goal vector. Examples of animals which use an exocentric frame of reference include those who locate the goal by noting the position of the sun in the sky or the Earth's geomagnetic field (for a further discussion of these terms see Layne et al., 2003 and Heinze et al., 2018).

The source of spatial information used to compute the home vector can either be idiothetic or allothetic. Idiothetic information is a product of an animal's own motion. Examples include the integration of leg proprioceptors or efferent commands to measure distances travelled (examples include fiddler crabs (Layne et al., 2003; Walls and Layne, 2009) and desert ants (Wittlinger et al., 2007)) or turns estimated from a vestibular system (such as what the golden hamster is thought to use during nocturnal foraging (Etienne et al., 1998)). Optic flow, the pattern of apparent motion of objects in the visual scene when moving, is another example of idiothetic information used to compute distances traveled by some animals such as honeybees (Esch et al., 2001). Conversely, allothetic sensory information is derived from external cues that are independent of an animal's movement. Examples include the use of celestial cues, as is used by honeybees (von Frisch, 1949), or the geomagnetic

field, as is relied on by spiny lobsters (Boles and Lohmann, 2003), to determine the direction of travel.

Animals which successfully home after being displaced to an unfamiliar location in the absence of any cues during their displacement are considered to be capable of true navigation. Animals capable of this feat are able to determine their position relative to a goal from local cues that differ along at least two separate axes rather than information collected during outbound journeys (as occurs during path integration) (Boles and Lohmann, 2003).

The Earth's magnetic field offers an animal the most comprehensive map from a single modality; the animal's position in relation to a goal along two axes can be assessed with the polarity, inclination, and intensity of the Earth's magnetic field alone. It is no surprise that the few well-studied animals that exhibit true navigation, sea turtles (Luschi et al., 2007), spiny lobsters (Boles and Lohmann, 2003), and some birds (Mora et al., 2004; Chernetsov et al., 2017), primarily use magnetoreception to determine their position on Earth.

Many stomatopods are central-place foragers, animals which to return to a home location between foraging bouts. Other central place foragers from a wide taxonomic breadth employ path integration to return home after foraging (Muller and Wehner, 1988; Seguinot et al., 1993; Zeil, 1998). Therefore, it is plausible that stomatopods use path integration to find their burrows after foraging as well. Also, the shallow reef environments in which many stomatopod species reside are structurally complex, offering many potential visual landmarks. Due to the complexity of typical stomatopod visual systems and the relatively high visual acuity

of their compound eyes (*Gonodactylus chiragra* has an acute zone with a resolution of 0.8 cycles/degree (Marshall and Land, 1993)), it is likely that these animals can use landmarks when navigating. Due to the relatively short distances mantis shrimp traverse as adults, it is unlikely they use true navigation when navigating to their burrows after foraging. Even true navigating animals, once they reach close proximity to a goal, are thought to switch to other navigational strategies, such as homing to olfactory cues or following landmarks in the local environment (Endres et al., 2016).

Stomatopod Sensory Systems

Many stomatopods possess intricate visual systems, including complex color vision, linear and circular polarization receptivity, and UV vision. Typical stomatopod eyes are composed of three distinct segments, a dorsal and ventral hemisphere and an equatorial midband which separates them (Manning et al., 1984; Figure 1.2). Generally, the ommatidia of the dorsal and ventral hemispheres possess linear-polarization-sensitive rhabdomeric photoreceptors which maximally absorb blue-green light centered around 500 nm (Cronin and Marshall, 1989; Marshall et al, 1991). Additionally, a UV-sensitive retinular cell is immediately distal to these photoreceptors in each ommatidium. However, it is in the six-rowed midband of most stomatopod eyes that the remarkable complexity of those eyes are found. Due to an array of tiered rhabdoms in conjunction with vesicular colored filters, eight distinct photoreceptor classes responsible for color vision can be found in the dorsal four rows of the midband (Cronin and Marshall, 1989). Distal to these photoreceptors, a combination of two spectrally distinct UV visual pigments associated with four UV-

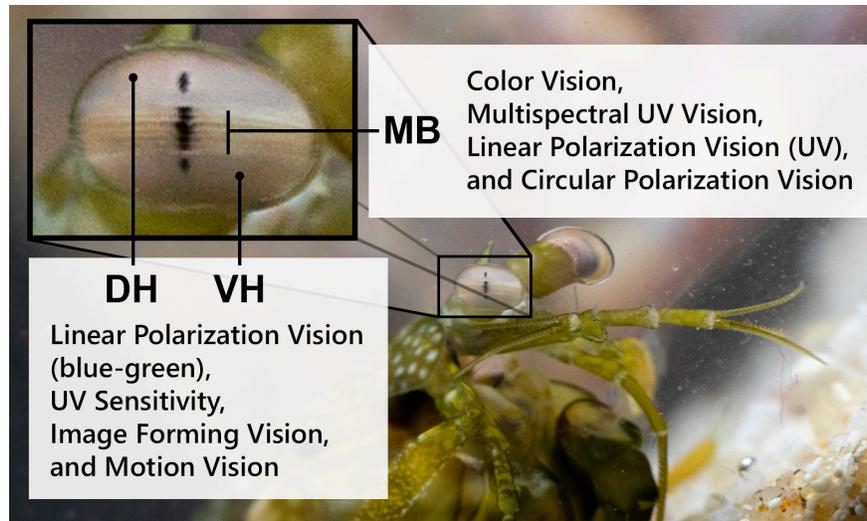


Figure 1.2. Regional specializations of stomatopod eyes. Stomatopod eyes are composed of three segments, a dorsal and a ventral hemisphere (DH and VH) and an equatorial midband (MB) which separates them. The dorsal and ventral hemispheres contain linear-polarization channels in the blue-green spectral range and contain non-polarization-sensitive UV receptors, responsible for image resolving and motion vision. The midband imparts color vision, multispectral UV vision, linear polarization vision in the UV spectral range, and circular polarization vision.

absorbing mycosporine-like amino acid filters likely impart six distinct UV-sensitive photoreceptor classes (Bok et al., 2014). Finally, the ventral two rows of the midband possess specialized distal retinular cells which convey linear-polarization sensitivity in the UV wavelength range. These specialized retinular cells can act as quarter-wave retarders, transforming circular-polarized light into linear-polarized light (and vice-versa). This gives the photoreceptors which lie behind them circular polarization sensitivity, a trait so far unique to stomatopods (Chiou et al., 2008). The sensory capacities described here have been shown to be behaviorally pertinent, as mantis shrimp can distinguish novel visual targets based on color and linear and circular polarization information (Marshall et al., 1996; Marshall et al., 1999; Chiou et al.,

2008; Thoen et al., 2014). This ocular complexity and propensity for learning visual stimuli indicates that visual information is important in influencing stomatopod behavior.

Stomatopods also possess a well-developed chemosensory system, of which the primary sensory structures are the aesthetascs located on the antennules (Derby et al., 2003; Cheroske et al., 2009). Behavioral experiments have shown that the stomatopod, *Gonodactylus festai*, can chemically determine if a cavity is or has been recently occupied by a conspecific individual and can identify specific individuals based on chemical cues alone (Caldwell, 1979). They also have been observed to learn the chemical cues of another species (Caldwell, 1981). Chemical cues may be used for identifying locations; however, the spatial precision of chemosensory systems is low in comparison to visual and auditory sensory systems, and chemical cues rapidly disperse with distance in water. Therefore, visual and auditory cues may prove to be more spatially and temporally reliable for stomatopods during navigation.

Crustaceans typically lack air-filled spaces in their bodies, and are therefore not thought to be receptive to the far-field pressure component of sound. However, mechanosensory setae are present on the antennules, legs, and maxillipeds of stomatopods, which are hypothesized to be receptive to flow and the nearfield particle displacement component of sound (Bok et al., 2013 in Schram et al., 2013).

Supporting the possibility of stomatopod acoustic reception, *Hemisquilla californiensis* males have been observed to produce low-frequency rumbles, which is hypothesized to be used for territory maintenance and/or mate attraction (Patek and Caldwell, 2006; Staaterman et al., 2011). Therefore, typical flow and infrasound

signatures of the local environment may offer stomatopods basic directionality while foraging.

Orientation Cues

An animal capable of path integration must be able to measure the directions and distances it travels. Due to the wide breadth of robust sensory modalities stomatopods possess, many directional cues are available for orientation in a stomatopod's aquatic environment. These may include visual cues, such as a panorama compass, a solar compass, and a celestial polarization compass. Since many stomatopod species occupy shallow waters, celestial cues such as the sun and celestial polarization patterns could be viewed through Snel's window, the overhead view of the world above the water's surface, refracted to angular size of 97 degrees through the air-water interface. Some aquatic animals have been shown to use celestial cues to orient their behaviors, as exemplified by escape behavior orientation in the grass shrimp, *Palaemonetes vulgaris* (Goddard and Forward, 1989; Goddard and Forward, 1991).

Orienting and navigating are complex and difficult feats to accomplish. In many animals, cues from multiple modalities are widely used for orientation behaviors (ex. celestial cues and the landmark panorama are used in soldier crab feeding orientation (Luschi et al., 1997); celestial cues and wind direction are used in straight line navigation in dung beetles (Dacke et al., 2019); optic flow odometry and stride integration are used in desert ant path integration (Pfeffer and Wittlinger, 2016)). With multiple redundant cues, animal navigators are more likely to complete

their tasks successfully. A few potential orientation cues an aquatic navigator may find useful are described below.

The Solar Azimuth

The sun is an ideal compass for orienting. Due to its relative constancy and great distance from the Earth, this cue allows animals to reliably steer straight line courses for long distances and for great lengths of time (if the solar compass is time-compensated such as is found in bees (Lindauer, 1960)), at a scale which Earth-bound landmarks cannot offer. As is expected with such a useful directional cue, many animals from a wide taxonomic breadth have been found to use the solar azimuth as a fundamental aspect of their orientation systems. An aquatic example of such an animal is the grass shrimp, *Palaemonetes vulgaris*, which orients its offshore escape response primarily by the sun (Goddard and Forward, 1989). In vertebrates, it has been shown that various pelagic larval fishes from the Mediterranean Sea and the Great Barrier Reef use a solar compass to return to the shores and reefs where they hatched to settle as adults after being swept away by a prevailing current (Leis and Carson-Ewart 2003; Faillettaz et al., 2015).

Celestial Polarization Patterns

Celestial polarization patterns are created by the scattering of sunlight in the earth's atmosphere. Light scatters in media with the strongest degree of polarization perpendicularly from the light source. Scattering of sunlight in the Earth's atmosphere generates a band of strongly polarized light that can be observed extending across the

sky 90 degrees from the sun's location (Figure 1.3A and C). This band of polarized light can be used as a compass by animals, keeping track of the body's angle in relation to this pattern.

First shown by von Frisch in honeybees (1949), many animals have been found to use celestial polarization patterns as cues for orientation. As Horváth and Varjú (1995) and Cronin and Shashar (2001) showed, these polarization patterns can be viewed underwater through the air-water interface of Snel's window, allowing marine animals to take advantage of this cue. Examples include the grass shrimp, who uses celestial polarization patterns to orient when the sun is covered by clouds (Goddard and Forward, 1991), the crab, *Dotilla wichmanni*, who primarily orients using these patterns during foraging (Luschi et al., 1997), and the rainbow trout, *Oncorhynchus mykiss*, who has been shown to orient to artificial overhead polarization patterns in the laboratory (Hawryshyn and Bolger, 1990).

Generally, light increasingly scatters at shorter wavelengths. Due to this, polarization patterns created by scattering are strongest among shorter wavelengths. Consequently, many photoreceptors responsible for polarization sensitivity in animals which orient using celestial polarization patterns tend to be maximally sensitive to ultraviolet light, such as those in the dorsal rim of the eyes of bees and ants (Frisch, 1949; Labhart, 1980; Nilsson et al., 1987), as well as in the UV-sensitive cones of immature rainbow trout (Hawryshyn and Bolger, 1990). In the marine environment, Cronin and Shashar (2001) found that at a depth of 15 meters, the peak radiance was in the blue spectral region (450-500 nm), but the degree and angle of polarization varied only slightly with wavelength. These observations may change in shallow

marine waters, where some stomatopod species, such as *Neogonodactylus oerstedii*, can be found. There, UV light has travelled through less scattering and attenuating medium. In this situation, the strongest polarization patterns may be in the UV wavelength range, especially at short distances. Furthermore, in freshwater at a depth of 4 meters, Novales-Flamarique and Hawryshyn (1997) show that wavelengths with the highest percent polarization are in the UV and shortwave visible range.

Stomatopods eyes have the potential for multiple polarization vision channels, in the blue-green (around 500nm) and the UV (around 350 nm) wavelength regions (Marshall et al., 1999), spectrally matching the environmental wavelengths of maximum polarization. It is important to note that some polarization-sensitive animals, like some fiddler crabs, which possess excellent polarization vision (How et al., 2014), apparently do not rely on a polarization compass when orienting (Layne et al., 2003).

Celestial polarization patterns change over the course of the day. Dominguez and Reaka (1988) observed that *Neogonodactylus oerstedii* and *Neogonodactylus spinulosus* left their burrows for longer and traveled farther from their burrows during dawn and dusk and that cloudiness of the sky delayed times of emergence at dawn and advanced times of seclusion at dusk. Caldwell et al. (1989) observed heightened crepuscular activity in *Neogonodactylus bredini* as well. Similarly, Basch and Engle (1989) observed *Hemisquilla californiensis* foraging away from their burrows primarily during crepuscular periods. At these times, the celestial polarization pattern would be strongest directly overhead (light scatters greatest at a 90 degree angle from the sun), cutting through Snel's window (Cronin and Shashar, 2001). Crepuscular

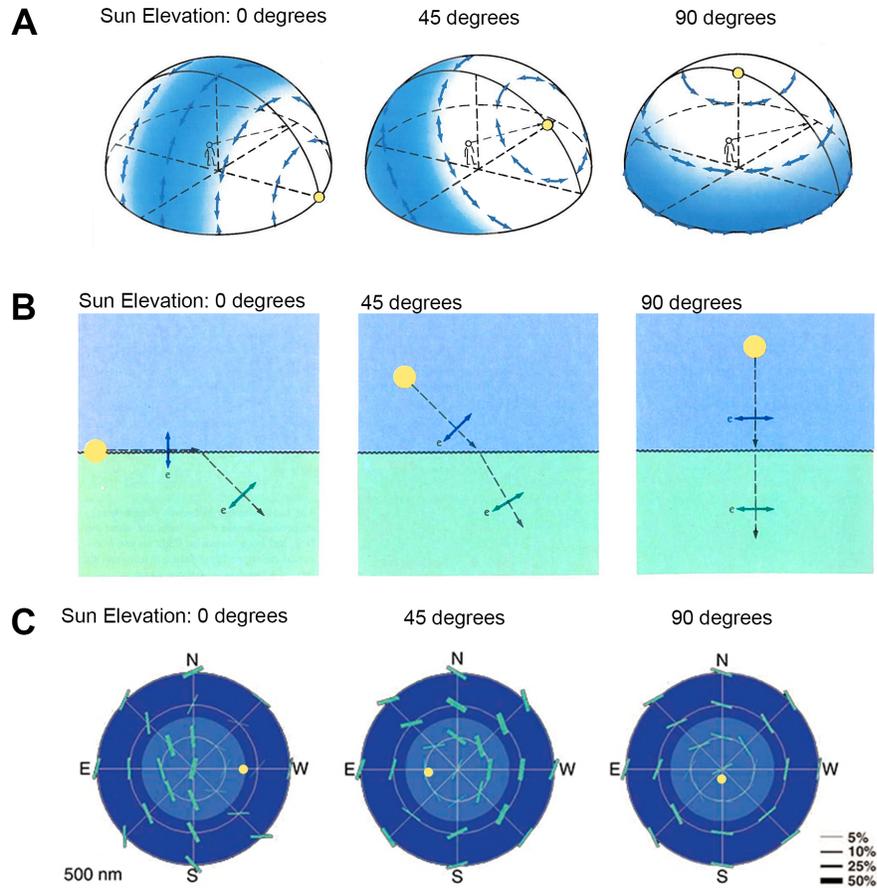


Figure 1.3. Solar-based polarization patterns. **A.** Celestial polarization pattern maxima can be observed in the sky at a 90° angle from the sun's location, due to the scattering of solar rays. **B.** Since underwater light is down-welling, maximally polarized light is scattered horizontally, creating a field of horizontally polarized light in water. The sun's position in the sky affects the angle in which light primarily enters Snel's window, causing this polarization field to be oriented nearly 49° from horizontal when the sun is at the horizon. **C.** Submarine polarization patterns in tropical marine water viewed from an observer looking directly to zenith at a depth of 15 m. The concentric rings represent viewing angles of 30° , 60° , and the horizontal. The yellow spot represents the position of the sun overhead as viewed through Snel's window, marked by the light-blue circle. The e-vector orientation of 500 nm light is indicated by the angle of the green bars relative to the tangent of the circle passing through that point. The thickness of the green bars represents the percent of polarization according to the key at the bottom right. **A.** and **B.** Adapted from Waterman (1989). **C.** Adapted from Cronin and Shashar (2001).

periods have been described as quiet times on the reef when animals adapted for diurnal activity swap places with animals adapted for nocturnal activity (Hobson, 1972). This may explain these observed patterns in stomatopod behavior, when the turning over of potential predators offers a time of relative safety to explore away from the burrow. Whatever the reasons for heightened crepuscular activity may be, strong zenithal celestial polarization patterns are typically present at this time.

Horizontal Submarine Polarization Patterns

Even though celestial cues are very reliable, use of them in an underwater environment can be problematical, especially at depth. Light only penetrates the sea's surface through Snel's window. Therefore, only through this opening to the above world can an underwater animal observe celestial cues. Furthermore, at depth, the image of Snel's window is attenuated by wave action and the absorption and scattering of light. However, as in air, light is scattered with the highest degree of polarization 90 degrees from its source in water. Since underwater light is downwelling, scattered light creates a field of horizontally polarized light in the water column. The sun's position in the sky affects the angle in which light enters Snel's window. Due to this, the underwater polarization field is only truly horizontal when the sun is directly overhead. When the sun is at the horizon, the underwater field of greatest polarization due to this phenomenon is nearly 49 degrees from horizontal (Waterman and Westell, 1956; Waterman, 1989) (Figure 1.3B).

Lerner et al. (2011) found horizontal submarine polarization patterns to be predictable for use as a compass in clear water. It is conceivable that some marine

animals may use these predictable submarine polarization patterns as a compass for orientation even though direct evidence of its use has yet to be shown. However as Cronin and Shashar (2001) reported, at a depth of 15 meters, even though horizontal polarization patterns are observable, the strongest submarine field of polarization at sunset can be observed directly overhead due to celestial polarization patterns observed through Snel's window (Figure 1.3C).

The Panorama Compass

Landmarks need not only be used for piloting; the surrounding landmarks in a navigator's panorama may also be used as a compass. The ocypodid crab, *Dotilla wichmanni*, uses celestial polarization patterns to orient when foraging. However, on overcast days when celestial information is not available, *D. wichmanni* relies on visual landmarks in the panorama as a compass to orient (Luschi et al., 1997). A panorama compass has been observed to be used by ants and bees as well (Collett, 1996; Graham and Cheng, 2009; Wystrach et al., 2011; Narendra and Ramirez-Esquivel, 2016).

Overview of Dissertation

Here, I describe navigation behavior in a mantis shrimp for the first time. In Chapter One, I demonstrate that the mantis shrimp *Neogonodactylus oerstedii* uses path integration to find its way home after foraging, making it the first aquatic path integrating animal described. I also show that *N. oerstedii* possesses a hierarchal reliance on multiple orientation cues, including the solar azimuth, celestial

polarization patterns, and idiothetic cues, during path integration. Chapter Two focuses on the source of error in the mantis shrimp's path integrator and describes how mantis shrimp locate their burrows when their path integrators do not lead them directly home— by enacting a stereotyped search behavior that is adjusted in scope by error in the path integrator. In Chapter Three I show that, when landmarks are available in the local environment, mantis shrimp will use them to locate home, exchanging the stereotyped search for one directed by the landmark. In this chapter, I also demonstrate how the landmark navigation and path integration systems appear to run in parallel by placing the two navigational systems in conflict. Since *N. oerstedii* uses landmarks during navigation, in Chapter Four I present work with a team of undergraduate researchers, where we investigated what visual information mantis shrimp use when recognizing an object by comparing the ability of a mantis shrimp to learn the shape and color of an object. I conclude with a discussion of potential future research directions stemming from my findings and the contribution of my work to the understanding of animal navigation and the neural basis of behavior.

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Chapter 2: Mantis shrimp navigate home using celestial and idiothetic path integration

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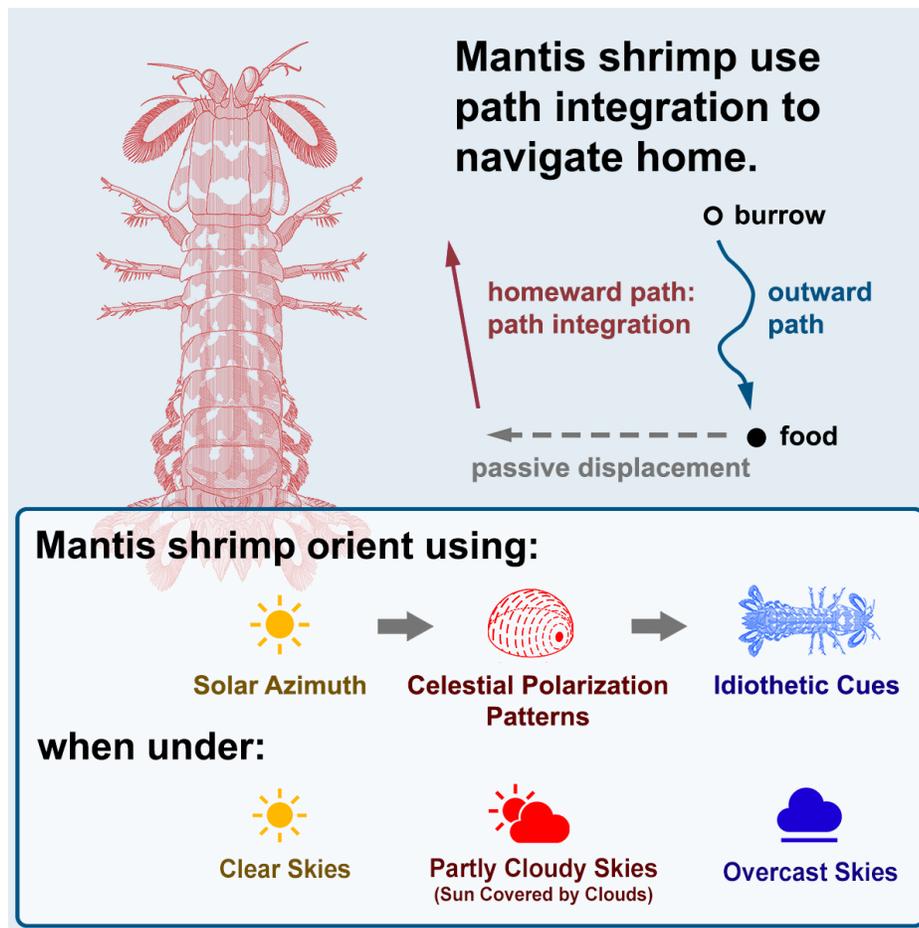
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Graphical Abstract:



Summary

Path integration is a robust mechanism that many animals employ to return to specific locations, typically their homes, during navigation. This efficient navigational strategy has never been demonstrated in a fully aquatic animal, where sensory cues used for orientation may differ dramatically from those available above the water's surface. Here we report that the mantis shrimp, *Neogonodactylus oerstedii*, uses path integration informed by a hierarchical reliance on the sun, overhead polarization patterns, and idiothetic (internal) orientation cues to return home when foraging, making them the first fully aquatic path-integrating animals yet discovered. We show that mantis shrimp rely on navigational strategies closely resembling those used by insect navigators, opening a new avenue for the investigation of the neural basis of navigation behaviors and the evolution of these strategies in arthropods and potentially other animals as well.

Introduction

Many central place foragers, animals that to return to a home location between foraging bouts, efficiently navigate to their homes using path integration. During path integration, an animal monitors its angular and linear movements using compass and odometer cues. From this information, a home vector, the most direct path back to the reference point, is calculated and continually updated, allowing the animal to return to its original location [1-5].

Path integration has been most thoroughly studied in social hymenopterans, which primarily rely on celestial cues for orientation and on idiothetic cues for odometry [2, 6-9]. This navigational strategy is also used by other terrestrial taxa,

including spiders [1], rodents [3], and fiddler crabs [4, 5], but has not been demonstrated in any fully aquatic animal. Sensory cues underwater differ dramatically from those available in terrestrial environments; for example, visual cues which are prominent on land are obscured over relatively short distances underwater.

Stomatopods, better known as mantis shrimp, are predatory crustaceans that mostly inhabit shallow marine waters. Many stomatopod species occupy small holes in their benthic environments where they are safely concealed from their predators. Most species leave these burrows, risking predation, for tasks such as foraging and finding mates [10-12]. These trips away from the burrow may extend to four meters or more in some *Neogonodactylus* species, a substantial distance for animals typically around three to five centimeters long [10-12]. We found that *Neogonodactylus oerstedii* in shallow waters off the Florida Keys make multiple excursions from and back to a home burrow and that these excursions extend up to a few meters from the burrow (Fig. 2.1). Further, the densities of occupied burrows were fairly high in some locations, with burrows of multiple animals as close as 10 centimeters to one another. Due to the aggressive territoriality *Neogonodactylus* (and many other stomatopods) exhibit when defending their burrows and the powerful weaponry they possess [13], accurate navigation back to the correct home burrow is an important task for a mantis shrimp.

These considerations led us to investigate the mechanisms used by *Neogonodactylus oerstedii* to navigate back to its home when foraging.

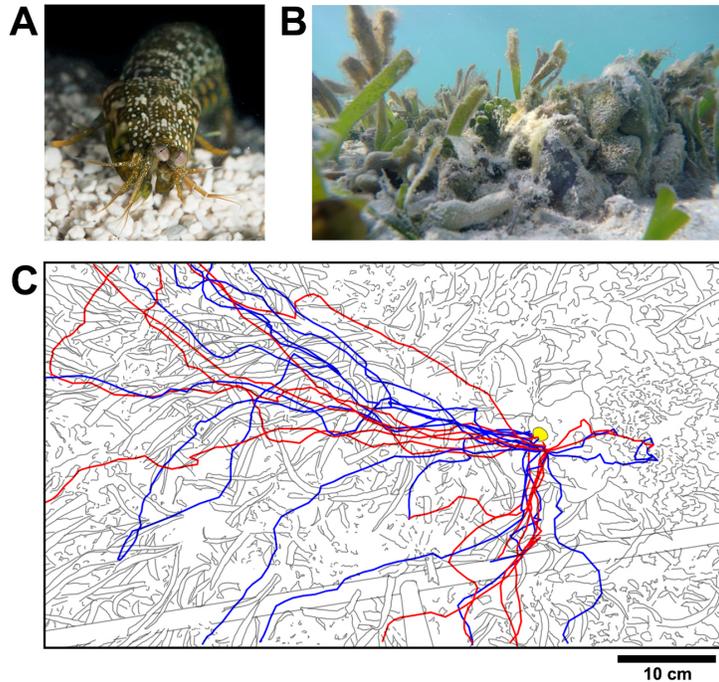


Figure 2.1. *Neogonodactylus oerstedii* makes multiple foraging trips from and to its burrow in nature. **(A)** *Neogonodactylus oerstedii* **(B)** *N. oerstedii* peering out of its burrow in nature. **(C)** Foraging routes of *N. oerstedii* from (blue tracings) and to (red tracings) its burrow (filled in yellow) in nature viewed from above. Data were obtained over three hours during a single evening.

Results and Discussion

Mantis shrimp navigate using path integration

In initial experiments, we placed individual *N. oerstedii* in relatively featureless sandy-bottomed circular arenas filled with sea water in a glass-roofed greenhouse. Vertical burrows were buried in the sand so that they were hidden from view while animals were foraging. Snail shells stuffed with pieces of shrimp were placed at one of two fixed locations approximately 70 cm from the burrow's location.

Foraging paths to and from the location of the food were video recorded from above (Fig. 2.2).

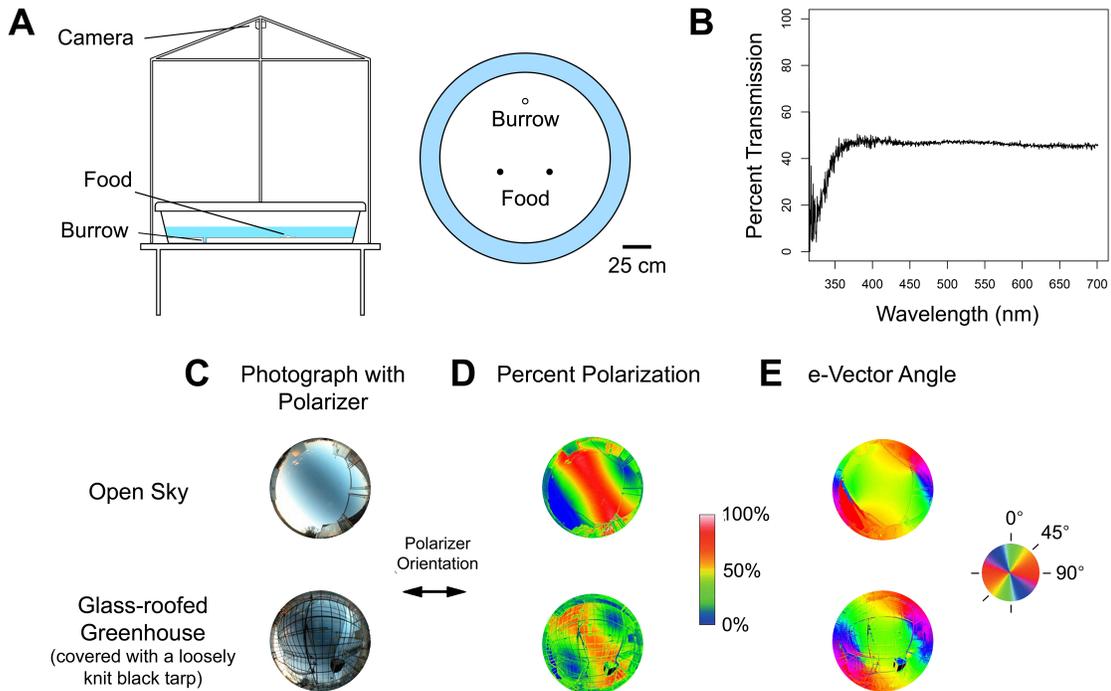


Figure 2.2. Greenhouse arena design. (A) Navigation arenas 150 cm in diameter contained a burrow (empty circle) buried in the base of the arena 30 cm from the arena’s periphery. During trials in the greenhouse when animals were not manipulated, food was placed at one of two positions 50 cm from the periphery of the arena (filled circles). Trials were video recorded from above. (B) Transmission of irradiance spectra through the glass-roof of the greenhouse on November 24, 2015 at 15:30. The spectral transmittance of light through the glass roof of the greenhouse is nearly constant for all wavelengths greater than ~ 360 nm. (C-E) Celestial polarization patterns are transmitted through the glass roof of the greenhouse. (C) Photographs of the sky at sunset on a day with very few clouds (November 24, 2015) using a fisheye lens and linear polarizer set in the east-west direction (as indicated by the arrow in the legend). Photos were taken inside and outside the glass-roofed greenhouse used for the initial set of experiments. (D) Percent polarization. Warmer regions in the images indicate higher percent polarization and cooler regions indicate lower percent polarization (see key). (E) e-Vector angle, indicated by the color corresponding the key to the right of the images. See also Figure 2.3.

During trials, stomatopods made tortuous paths away from their burrows until they located the food in the arena. After animals found the food, they generally executed a well-directed homeward path to the burrow's location. If the burrow was not encountered at the end of the homeward path, a search behavior was initiated (Fig. 2.3B). During these foraging paths, mantis shrimp would both walk and swim to locomote. To differentiate homeward paths from ongoing arena exploration, paths from food locations were considered to be homeward paths when they did not deviate more than 90° from their initial trajectories for at least one-third of the beeline distance (the length of the straightest path) from the food location to the burrow. Search behaviors were determined to be initiated when animals turned more than 90° from their initial homeward path trajectories.

Homeward paths of these animals were significantly oriented towards the burrow ($-7.8^\circ \pm 5.36^\circ$ (mean from the burrow where the burrow is $0^\circ \pm$ S.E.M.), $P < 0.001$; all statistical outcomes are presented in Tables 2.1 and 2.2; Fig. 2.3C, E). This observation suggested that *N. oerstedii* may use path integration to locate its burrow while foraging.

To determine conclusively if *N. oerstedii* homes using path integration, homeward paths of foraging animals were observed after they had been passively translocated in the arena. This was accomplished by placing food on a thin platform on a track. Once an animal found the food, the platform (and animal) were carefully displaced along the track. If *N. oerstedii* path-integrate when foraging, animals should orient parallel to the direction of the burrow had they not been displaced (i.e. towards the expected burrow location). Conversely, if animals oriented towards the burrow's

actual location, either *N. oerstedii* do not path-integrate but instead locate their burrows by some other method (perhaps using an odor emanating from the burrow or structural components of the greenhouse as orientation cues), or the experimental animals were aware of and integrated their passive displacement (Fig. 2.3A).

When animals were displaced, homeward paths were oriented towards the expected location of the burrow rather than towards the actual location of the burrow (Fig. 2.3C, D). Homeward orientations of stationary animals were significantly different from those of displaced animals measured in reference to actual burrow ($p = 0.0016$); however, they were not different when compared to homeward orientations of displaced animals measured in reference to the expected burrow position ($p > 0.1$; Fig. 2.3E). Additionally, home vector lengths from both experiments were close to the beeline distance to the burrow (Not Manipulated: $104.9\% \pm 5.08\%$ of beeline distance, Animal Displaced: $102.7\% \pm 7.3\%$ of beeline distance (Mean \pm Standard Error); Fig 2.3F). These results strongly support the hypothesis that foraging *N. oerstedii* use path integration when homing.

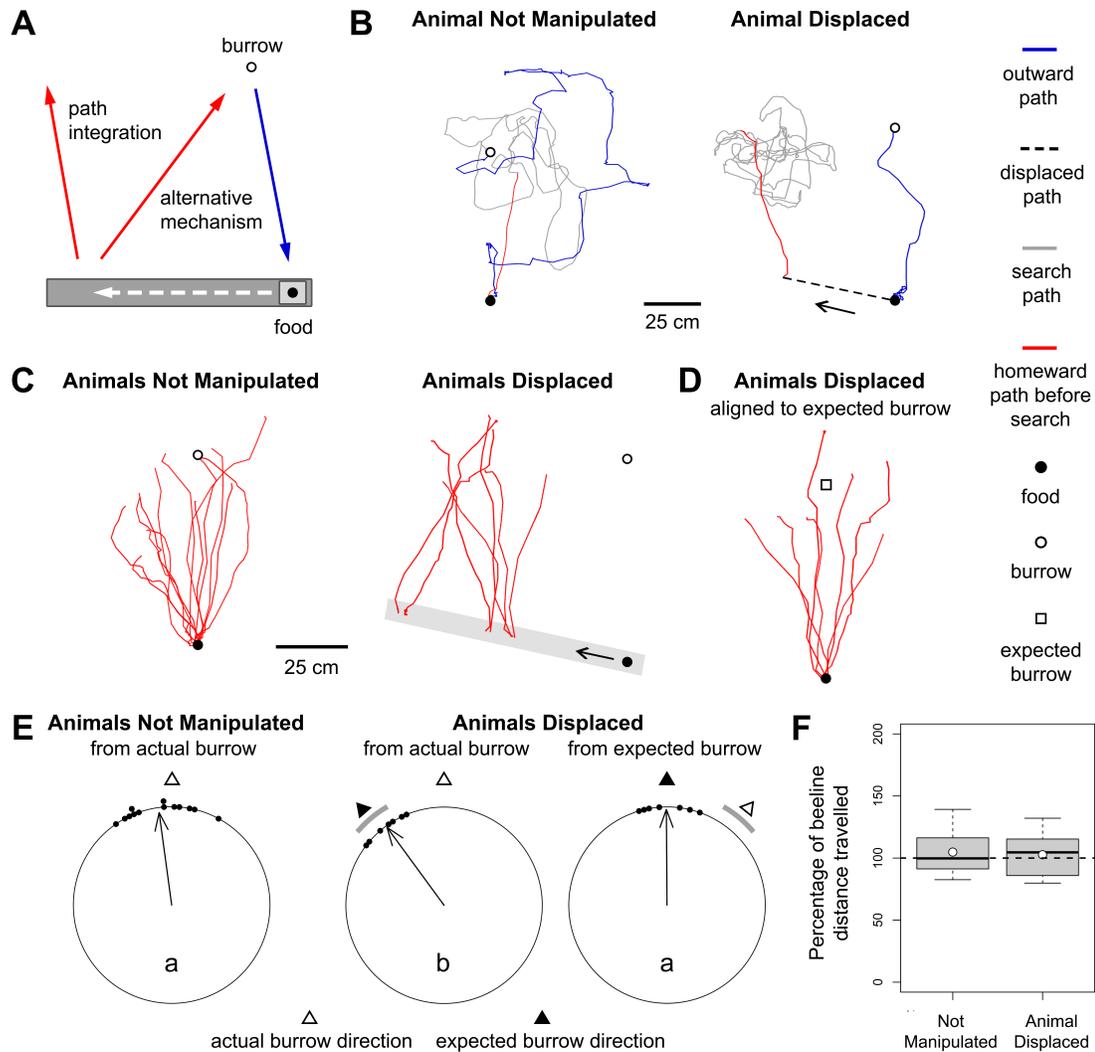


Figure 2.3 *Neogonodactylus oerstedii* uses path integration to navigate back to its burrow while foraging. (A) Experimental design. After passive displacement (dashed arrow), *N. oerstedii* should orient parallel to the direction of the burrow had it not been displaced if it uses path integration while homing. (B) Examples of foraging paths from and to the burrow when an animal was not manipulated and when an animal was passively displaced. (C) Data from all homeward paths. The grey rectangle represents the track on which animals were displaced and the empty circle represents the average burrow location with respect to the track. (D) Homeward paths when animals were displaced aligned to the position of the burrow had the animals not been displaced (the expected burrow). (E) Orientations of homeward paths at one-third the beeline distance from the location of the food to the burrow. In all orientation diagrams, each point on the circumference of the circular plot represents the orientation of the homeward path of one individual with respect to either the actual position or the expected position of the burrow. Grey arcs in the “Animal

Displaced” orientation plots represent the range of the directions of the either the expected or actual burrow from at the location of the food. Arrows in each plot represent mean vectors, where arrow angles represent vector angles and arrow lengths represents the strength of orientation (\bar{R}). Different letters within orientation plots denote a significant difference between groups (Animals Not Manipulated vs. Animals Displaced (actual burrow): $P < 0.001$, Animals Not Manipulated vs. Animals Displaced (expected burrow): $P = 0.38$). (F) The percentage of the beeline distance traveled during homeward paths before initiation of search behaviors. Data are from all trials in both conditions. Bars represent medians, points represent means, boxes indicate lower and upper quartiles, and whiskers show sample minima and maxima. The horizontal dashed line marks the beeline distance from the location of the food to the burrow. See also Figures 2.2 and 2.12 and Tables 2.1 and 2.2.

Mantis shrimp orient using celestial and idiothetic cues during path integration

Path integration requires that an animal possess a compass to determine its headings and an odometer to measure the distances it travels. Due to the abundance of potential cues available for orientation, we examined whether the compass of *N. oerstedii* relies on cues external (allothetic) or internal (idiothetic) to the body. To distinguish between these two potential classes of cues, a rotatable platform was centered in a 1.5-meter diameter circular arena placed outdoors in an open, level field. Food was placed on the center of this platform 60 cm from the burrow. Trials were conducted in three environmental conditions: under clear skies, under partly cloudy skies when the sun was hidden by clouds, and under heavily overcast skies when celestial cues were obscured (Fig. 2.4). Trials were video recorded from cameras on tripods from above.

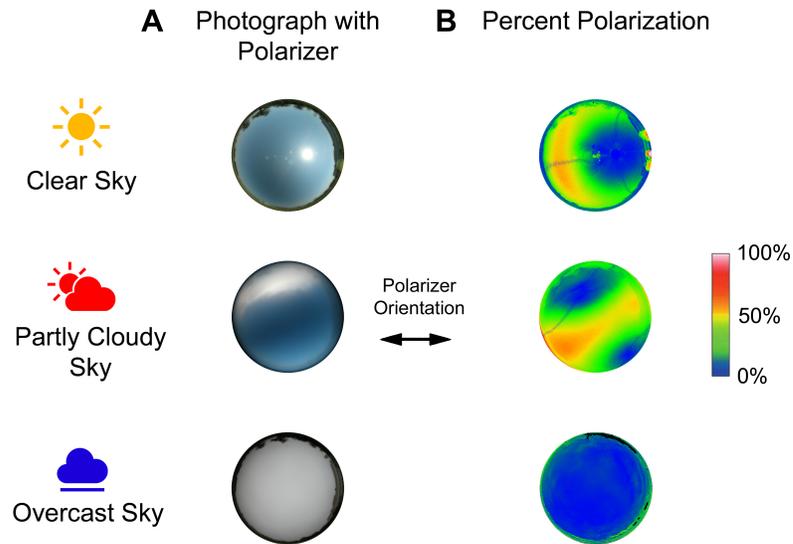


Figure 2.4. Celestial conditions during outdoor rotation experiments. (A) Photographs of the sky on days with clear, partly cloudy (when the sun is covered by clouds), and heavily overcast skies in June 2018 taken using a fisheye lens and linear polarizer set in the east-west direction (as indicated by the arrow in the legend). Photos were taken in a field at the University of Delaware’s College of Earth Ocean and Environment in Lewes, DE. **(B)** Percent polarization. Warmer regions of the images indicate higher percent polarization and cooler regions indicate lower percent polarization (see key). See also Figure 2.5.

Once an animal found the food, the platform was carefully rotated 180°. If *N. oerstedii* used an allothetic compass for orientation, homeward paths should be oriented towards the location of the burrow, despite the animals’ passive rotation. Alternatively, if an idiothetic compass was used, homeward paths after rotation would be oriented approximately in the opposite direction (Fig. 2.5A). During additional trials, the tripods placed over the arenas were rotated either approximately 60° or 180° when animals were not manipulated to control for their presence as a potential orientation cue.

When animals were not manipulated, they oriented significantly towards the burrow under all celestial conditions (clear: $3.27^\circ \pm 9.14^\circ$, $P < 0.001$, partly cloudy: $-7.45^\circ \pm 11.54^\circ$, $P < 0.001$, overcast: $-12.93^\circ \pm 20.27^\circ$, $P = 0.001$). During experiments in which animals were rotated on the central platform, animals continued to orient towards the burrow under clear ($-18.66^\circ \pm 5.54^\circ$, $P < 0.001$) or partly cloudy skies ($-5.88^\circ \pm 4.56^\circ$, $P < 0.001$). In contrast, when animals were rotated under heavily overcast skies, homeward paths were oriented away from the burrow ($-168.02^\circ \pm 17.2^\circ$, $P < 0.001$). Finally, during trials when only the tripod was rotated, homeward paths were oriented towards the burrow ($-1.48^\circ \pm 7.06^\circ$, $P < 0.001$; Fig. 2.5B).

These results indicate that when possible, *N. oerstedii* uses celestial cues for orientation; however, when celestial cues are obscured, *N. oerstedii* relies on an idiothetic compass. In general, idiothetic compasses are particularly prone to error accumulated over the course of foraging excursions [14]. Nevertheless, some animals, including rodents [3] and fruit flies [15] are thought to rely largely on idiothetic orientation during path integration. Under clear skies, *N. oerstedii* may use the solar azimuth for orientation. However, even when the sun was obscured, *N. oerstedii* continued to orient correctly despite being rotated as long as patches of clear sky were visible. Potential orientation cues used under this condition may include polarization patterns [6, 8, 16-19], spectral gradients [20, 21], or luminosity gradients in the sky [22, 23].

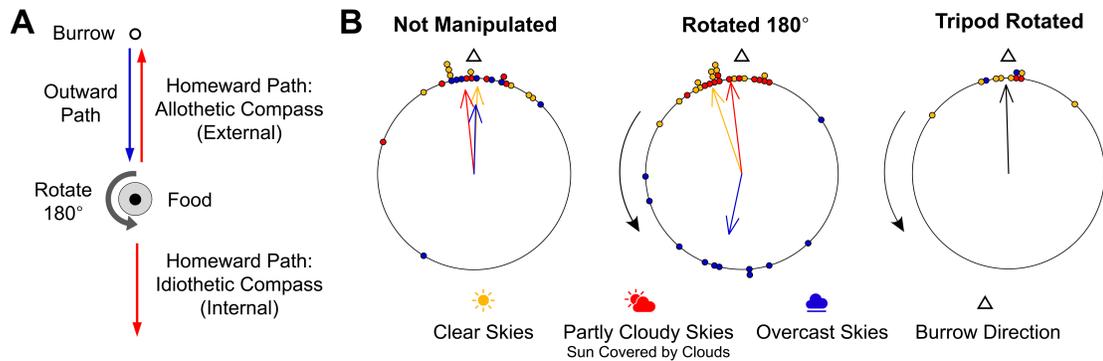


Figure 2.5. *Neogonodactylus oerstedii* uses celestial and idiothetic compasses during path integration. (A) Experimental design. Homeward paths using allothetic compasses were predicted to be oriented towards the burrow while homeward paths using idiothetic compasses were predicted to be oriented away from the burrow after passive 180° rotation. (B) Orientations of homeward paths when skies were either clear, partly cloudy with the sun covered by clouds, or heavily overcast. Animals were either not manipulated, rotated 180° on the platform, or the tripod was rotated during a trial (the direction of rotation is indicated by the curved black arrows). Arrows in each plot represent mean vectors, where arrow angles represent vector angles and arrow lengths represents the strength of orientation (\bar{R}). All groups exhibited significant orientations ($p < 0.01$). See also Figure 2.4 and Table 2.1.

Mantis shrimp use the sun as a compass while orienting

We determined if *N. oerstedii* uses the solar azimuth as a cue for orientation, using the outdoor arenas described above. Trials were conducted when the sun was clearly visible in an open sky at an altitude between 20° and 45° above the horizon. When animals had reached food placed in the center of the arena, the actual location of the sun was blocked by a board and the sun was instead reflected 180° from its original position in the sky by a mirror (Fig. 2.6A). If *N. oerstedii* uses a sun compass for orientation, under this condition homeward paths should be oriented away from the burrow (Fig. 2.6B). In control trials, the sun was concealed by the board but was not mirrored.

During experiments when the sun was concealed and mirrored 180° from its original location, homeward paths were primarily oriented in the opposite direction of the burrow ($-156.3^\circ \pm 8.14^\circ$, $P = 0.023$); however, during control trials when the sun was only concealed, homeward paths were oriented towards the burrow ($-2.11^\circ \pm 4.27^\circ$, $P < 0.001$; Fig. 2.6C). These results indicate that *N. oerstedii* can use the solar azimuth for orientation. Nevertheless, even when the sun was mirrored, three of ten animals ignored the mirrored sun and correctly oriented home. These individuals either primarily orient using a cue other than the solar azimuth (in contrast to the majority of individuals tested) or some other factor may have caused other orientation cues to be ranked over the solar azimuth in these cases.

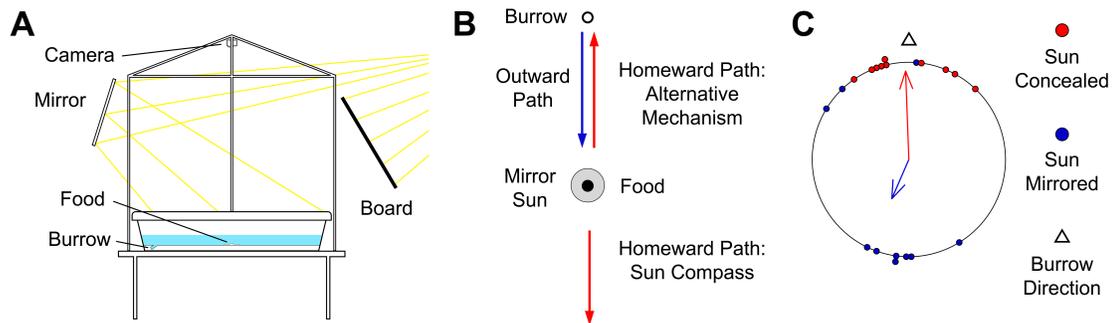


Figure 2.6. *Neogonodactylus oerstedii* uses the solar azimuth as a compass cue for orientation. (A) Sun compass arena design. (B) The sun was mirrored once animals reached the food location. Homeward paths were predicted to be oriented in the direction opposite to the burrow if *N. oerstedii* orients using the solar azimuth. (C) Orientations of the homeward paths when the sun was either concealed with a board or both, concealed with a board and mirrored to the opposite side of the arena. Arrows in each plot represent mean vectors, where arrow angles represent vector angles and arrow lengths represents the strength of orientation (\bar{R}). Both groups exhibited significant orientations ($p < 0.05$). See also Table 2.1.

Mantis shrimp orient using overhead polarization patterns

Since individuals were able to orient correctly after passive rotation under partly cloudy skies when the sun was obscured by clouds or when the sun was concealed by a board, *N. oerstedii* appears to use celestial cues other than the solar azimuth for orientation. Celestial polarization patterns, which are widely used by animals [6, 8, 16-19] and are clearly visible underwater at the depth ranges *N. oerstedii* occupies in nature [24], may have been used under these conditions. To determine if *N. oerstedii* can orient using overhead polarization patterns, indoor arenas were constructed over which an artificial polarization field was created using white LEDs and a composite filter constructed of a polarizer and a diffuser (Fig. 2.7 and Fig. 2.8A). When the polarizer side of the composite sheet faced the arena, light passing through the filter created a linearly polarized light field. When the diffuser side faced downwards, a depolarized light field resulted (Fig. 2.7C-E). Thick black curtains were placed around the arena to create an isolated, nearly homogenous environment.

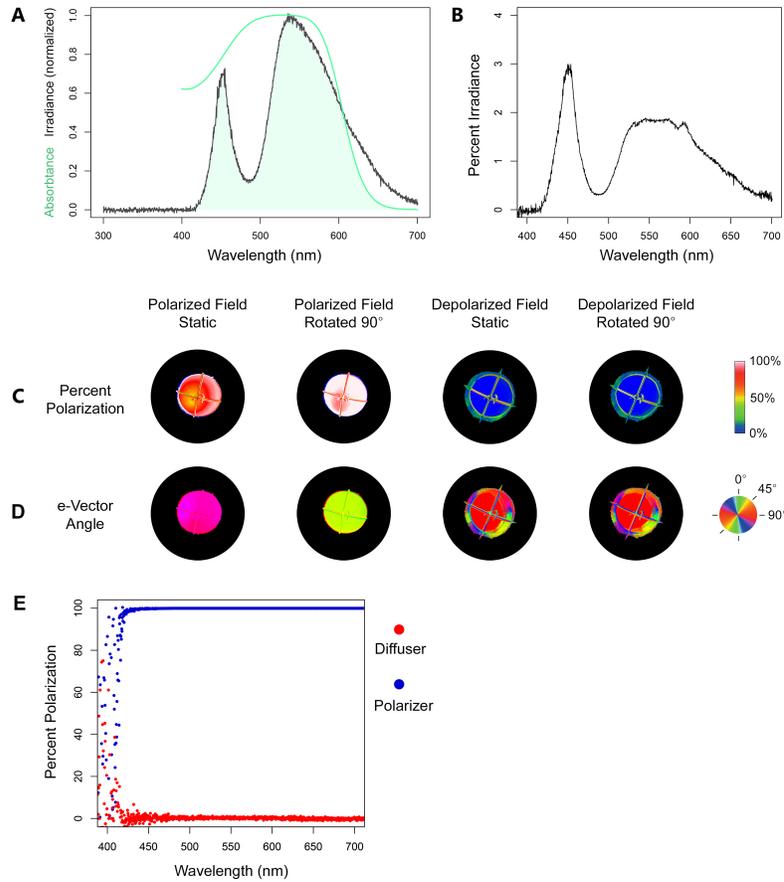


Figure 2.7. Photic conditions in the indoor polarization arenas. (A) Irradiance spectra of light available in the indoor polarization arenas (black line). Absorbance curve of the main rhabdomeric photoreceptors (R1-7) in the peripheral hemispheres of the eyes of *Neogonodactylus oerstedii* (green line; from Cronin and Marshall, 1989 [46]). The shaded area represents light available in the arena absorbed by the main rhabdoms in the peripheral hemispheres of the eye of *N. oerstedii*. (B) Percent irradiance of the indoor polarization arenas compared to an overcast sky. The light environment during outdoor experiments under overcast skies was over 50 times as bright as during those run in the indoor arenas at 540 nm (the brightest wavelength in indoor arenas). (C-E) Polarization information of the overhead feature for all experimental conditions of the indoor polarization experiments. (C) Percent polarization of an indoor artificial polarization field arena near the zenith. Warmer regions of the images indicate higher percent polarization and cooler regions of the images indicate lower percent polarization (see key). (D) e-Vector angles of the same images as in C. e-Vector angle is indicated by the color corresponding to the key on the right of the images. (E) Percentage of polarized light transmitted through the overhead filter used in the indoor polarization experiments when either the polarizer side (blue) or diffuser side (red) faced down over the arena. See also Figures 2.8, 2.9, and 2.10.

To manipulate the polarization field during experiments, once an animal found food centered in the arena, the polarized filter was rotated 90° from its original position. In this condition, if *N. oerstedii* used the overhead polarization field as a compass, homeward paths should be oriented perpendicular to the direction of the burrow (Fig. 2.8B). In control trials, the polarizer remained fixed throughout the experiment. During these experiments, homeward paths should be oriented towards or opposite to the burrow's location (since a polarized light pattern is bidirectionally ambiguous). To control for the rotation of the filter, the filter was positioned to provide a depolarized light field and was rotated 90° when animals were at the location of the food. If experimental animals were only using the polarization field for orientation, homeward path orientations between the static and rotated depolarized light fields should not differ (Fig. 2.7B).

When the polarized field was static, homeward paths were oriented parallel to the direction of the burrow (data were doubled to create a unimodal distribution (see methods), $10.31^\circ \pm 34.03^\circ$, $P < 0.001$); however, when the polarized field was rotated 90°, individuals oriented their homeward paths perpendicular to the direction of the burrow (doubled data, $179.91^\circ \pm 13.74^\circ$, $P < 0.001$). Under a depolarized field, homeward paths were either weakly oriented or exhibited no significant orientation (static: $14.38^\circ \pm 19.59^\circ$, $P = 0.037$, rotated: $-44.35^\circ \pm 25.35^\circ$, $P = 0.39$; Fig. 2.8D and Fig. 2.9). These results indicate that *N. oerstedii* uses overhead polarization patterns in the visible spectrum to orient.

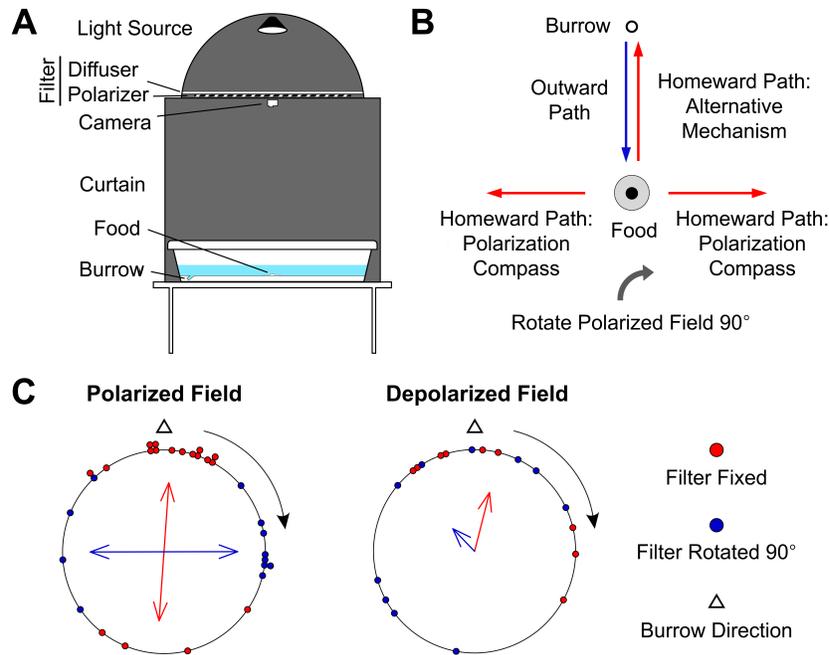


Figure 2.8. *Neogonodactylus oerstedii* uses overhead polarization patterns as a compass cue for orientation. (A) Polarization arena design. (B) The polarized light field was rotated 90° from its original position once animals reached the food location. Homeward paths were predicted to be oriented perpendicular to the direction of the burrow if *N. oerstedii* were using overhead polarization patterns for orientation. (C) Orientations of the homeward paths at one-third the beeline distance from the location of the food to the burrow when the overhead filter was oriented either with the polarizer or diffuser facing down towards the arena. Under each condition, the filter was either fixed in place or rotated 90° in the direction of rotation indicated by the black arrows. Arrows in each plot represent mean vectors, where arrow angles represent vector angles and arrow lengths represents the strength of orientation (\bar{R}). Doubled polarized field data can be reviewed in Figure 2.9. Both groups exhibited significant orientations under polarized fields ($p < 0.01$) and were either weakly or not significantly oriented under depolarized fields (fixed: $p = 0.037$, rotated: $p = 0.39$). See also Figures 2.7 and 2.9 and Table 2.1.

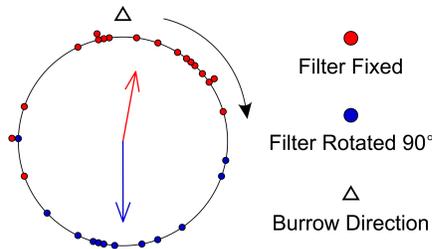


Figure 2.9. Doubled data for the polarized light field experiments. Orientations of homeward paths under a polarized field at one-third the beeline distance from the location of the food to the burrow after the data was doubled to create unimodal distributions (see methods). Red-filled points represent trails when the overhead filter was fixed in place while blue-filled points represent trials when the filter was rotated 90° with the direction of rotation indicated by the black arrow. Each point along the circumference of the circular plot represents the orientation of the homeward path of one individual in respect to the position of the burrow (empty triangle). Arrows in each plot represent mean vectors, where direction of the arrow represents the mean vector angle and arrow length represents the strength of orientation in the mean direction (\bar{R}). See also Figure 2.8 and Table 2.1.

In the outdoor experiments, animals oriented in the opposite direction to their burrows after being rotated 180° under heavily overcast skies, indicating that they relied on an idiothetic compass. Since animals were poorly oriented under indoor depolarized light fields, visual information, such as rotational optic flow fields, may be important for idiothetic orientation in *N. oerstedii*. The photic environment in the indoor arenas differed mainly from the outdoor environment in two ways: UV light was absent in the indoor arenas, and the light available under overcast skies was over 50 times as bright as that available indoors (Fig. 2.7A-B). Visual information may be compromised indoors under these conditions. We also observed that home vector lengths were more varied under indoor conditions when compared to trials under natural lighting ($P = 0.019$, $F = 2.75$, indoor: $n = 27$, outdoor: $n = 23$; Fig. 2.10). This

suggests that visual information such as rotational and translational optic flow fields might play a role in path integration in *N. oerstedii*.

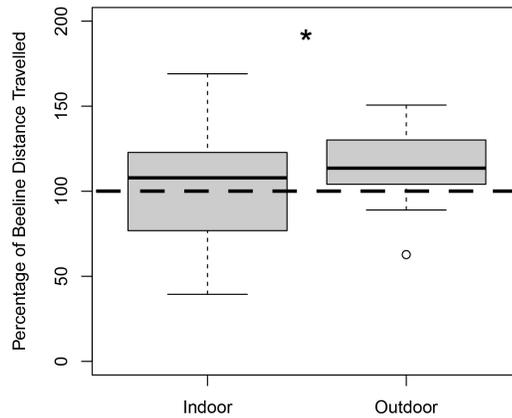


Figure 2.10. Lengths of homeward paths before search behaviors were initiated were more varied under indoor conditions than under outdoor conditions. The y-axis represents the percentage of the beeline distance from the food location to the burrow traveled during homeward paths before search behavior were initiated. The data included are from all trials that occurred outdoors under open skies and indoors in the polarization arenas when animals were not manipulated. The bar represent the median, the box indicates lower and upper quartiles, whiskers show sample minima and maxima, and the dot represents an outlier. The horizontal dashed line marks the beeline distance from the location of the food to the burrow. The variances between the two groups were significantly different ($P = 0.019$, $F = 2.75$, indoor: $n = 27$, outdoor: $n = 23$). See also Figure 2.7.

Hierarchy of compass cues

Our findings reveal a ranked hierarchy of cues used in the compass of *N. oerstedii*. During experiments in which the sun was mirrored, celestial polarization patterns were not affected, yet the majority of animals oriented relative to the displaced sun. This indicates that when the sun is visible in the sky, it is the primary compass cue used by *N. oerstedii*. However, when the sun is obscured, *N. oerstedii* orients using celestial polarization patterns (and potentially other celestial cues not

tested). Finally, when no celestial cue is available, *N. oerstedii* appears to turn to idiothetic cues for orientation (Fig. 2.11). The use of idiothetic or other non-celestial cues for orientation may be particularly advantageous in the aquatic environment, where turbidity, wave action, and the attenuation of celestial cues due to absorption and scattering in the water column may disrupt visual orientation cues. Like many other animal navigators [18, 25-27], *N. oerstedii* uses multiple redundant cues for orientation to navigate successfully.

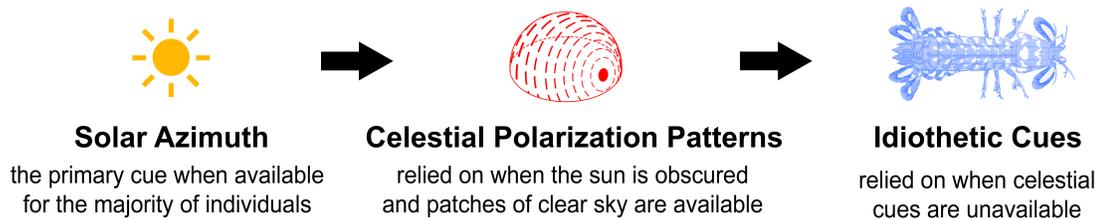


Figure 2.11. Proposed hierarchy of compass cues during path integration in *Neogonodactylus oerstedii*. Other orientation cues not tested during our experiments may be included in this hierarchy as well.

Conclusion

Our results are the first to conclusively demonstrate path integration in a fully aquatic animal. Comparing the sensory cues involved in path integration in *Neogonodactylus* to those used by its terrestrial counterparts can provide insight into how navigational problems are solved in disparate environments with varying properties and challenges. Further, mantis shrimp occupy a wide variety of marine habitats, from clear tropical reefs to silty mud flats. Celestial cues easily viewed

through the air-water interface in calm, shallow water are increasingly obscured with depth, turbidity, and wave action. While *N. oerstedii*, which mostly occupies shallow tropical waters, primarily relies on celestial cues for orientation, compass cue preferences likely differ for deeper-water stomatopod species or those that inhabit rougher, more turbid waters. The Earth's magnetic field, a cue available throughout the water column, is known to be particularly useful for marine navigators [28-30] and, if perceptible to them, may be used by stomatopods for orientation when celestial cues are unreliable as well.

Stomatopod eyes are arranged with a dorsal and ventral hemisphere separated by two to six rows of enlarged ommatidial facets termed the midband. The eyes of *N. oerstedii* contain multiple spectral channels operating in the visible region of the spectrum and an additional one in the ultraviolet [31]. Since our polarization arenas did not include light in the ultraviolet spectrum (Fig. 2.7A), celestial polarization patterns may be viewed throughout most of the retina of *N. oerstedii*. Recent work by Porter et al. (in press) show that reticular cells in the dorsal-most ommatidia of the eye of *N. oerstedii* express opsin transcripts that differ from the rest of the eye. At present, it is unknown whether this regionalization of opsin expression has consequences for polarized-light perception. Nevertheless, in light of our evidence for orientation assisted by the use of overhead polarization patterns, these findings suggest that a dorsal rim area like those many insects possess to orient to celestial polarization patterns [32] might exist in mantis shrimp as well.

In addition to the complex arrangement of stomatopod retinas, stomatopod visual systems incorporate several types of eye movements, including smooth and

saccadic tracking, scanning, large-field optokinetic stabilization, and acquisitional saccades along three axes. Strangely, at least while stationary, stomatopods are capable of performing these eye movements with each eye acting independently [33, 34]. The extreme mobility of stomatopod eyes complicates spatial orientation and raises the question of how a stomatopod's movements can be related to a stimulus' position on the eye. Since stomatopods appear to use visual cues during navigation, they must either account for the motion of their eyes to stabilize their world while moving or refrain from extensive eye movements during locomotion.

This research opens a new avenue into the study of the neural basis of navigation in crustaceans, where insights into the evolution of arthropod brain structures and navigational strategies may be found. The path integration and orientation systems we have found in mantis shrimp closely match those of model insect systems. In insects, a highly conserved region of the brain called the central complex is thought to play a major role in navigation and path integration [35-39]. The neural organization of stomatopod central complexes is remarkably similar to those of insects [40]. Together with the behavioral work we describe here, investigation of the function of neuropils within stomatopod central complexes could help uncover the evolutionary origins of navigation behaviors and the neural architecture of the central nervous system within arthropods; specifically the Pancrustacea, a taxon including both insects and malacostracan crustaceans, which is thought to have diverged from other arthropods over 600 million years ago [41].

Methods

Field Observations

Neogonodactylus oerstedii burrows were located in Florida Bay, offshore of the lower Florida Keys, USA. Burrows were video recorded from above using GoPro HERO 4 Black Edition cameras (GoPro Inc.) mounted to PVC tripods.

Experimental Model and Subject Details

Individual *Neogonodactylus oerstedii* collected in the Florida Keys, USA were shipped to the University of Maryland, Baltimore County (UMBC). Animals were housed individually in 30 parts per thousand (ppt) sea water at room temperature under a 12:12 light:dark cycle for indoor experiments and under local light:dark cycles (Lewes, DE, USA) for outdoor experiments. Animals were fed whiteleg shrimp, *Litopenaeus vannamei*, once per week.

Data were collected from 13 individuals during the initial experiments in the greenhouse and during translocation experiments (5 male and 8 female), 14 individuals during the outdoor rotation experiments (6 male and 8 female), 13 individuals during the sun compass experiments (7 male and 6 female), and 18 individuals during the zenithal polarization compass experiments (11 male and 7 female). All individuals were between 30 and 50 mm long from the rostrum to the tip of the telson.

Experimental Apparatuses

Greenhouse Experiments

Four relatively featureless, circular navigation arenas were constructed from 1.5 m-diameter plastic wading pools that were filled with pool filter sand and artificial seawater (30 ppt, Figure 2.2A). Arenas were placed in a glass-roofed greenhouse on the UMBC campus. The spectral transmittance of light through the greenhouse glass was nearly constant for all wavelengths, excluding the deep-UV-wavelength range (280 to 350 nm; Figure 2.2B). Celestial polarization information was transmitted through the glass roof of the greenhouse (Figure 2.2C-E). Vertical burrows created from 2 cm outer-diameter PVC pipes were buried in the sand 30 cm from the periphery of the arena so that they were hidden from view when experimental animals were foraging. Trials were recorded from above using C1 Security Cameras (Foscam Digital Technologies LLC) mounted to tripods placed above the arenas. During animal displacement experiments, a thin 11 x 82 cm acrylic track with a movable platform was placed 30 cm from the wall of the arena at its closest edge.

Outdoor Experiments

Outdoor navigation arenas were constructed from 1.5 m-diameter plastic wading pools with a white plastic base. 2 cm outer-diameter PVC pipe burrows were placed into holes drilled into the base of the arena 15 cm from the arena's periphery. A rotatable platform was placed in the center of each navigation arena. Arenas were filled with filtered sea water (30 ppt). Trials were recorded from above using GoPro

HERO 4 Black Edition cameras (GoPro Inc.) mounted to tripods placed above each arena. Arenas were placed in a wide empty open field at the University of Delaware's College of Earth, Ocean, and Environment in Lewes, Delaware, USA.

During sun compass experiments, a rotatable 122 cm x 91 cm whiteboard on a vertical stand was used to block the sun while a 41 cm x 41 cm glass mirror was used to reflect the sun to opposite side of the arena.

Indoor Experiments

Arenas used in the outdoor experiments were placed in a dark room. Arenas were surrounded by thick matte black curtains and lit from above using a centered diffused light source (Lepower 50W LED floodlights, 4000Lm, 6500K). Composite filters constructed of a linear polarizer (American Polarizers Inc., 38% transmission visible spectrum) and two sheets of wax-paper sandwiched between two sheets of colorless transparent acrylic were placed under each light source. When the polarizer side of the composite sheet faced downwards towards the arena, light was linearly polarized to an average degree of 99.91% from 420 to 700 nm. For unpolarized fields, the depolarizing waxed paper side faced downwards, reducing the average degree of polarization to 0.04% from 420 to 700 nm. The overhead polarization stimulus had an angular diameter of 27° of when viewed from the center of the arena.

Polarization Imaging

A camera with a polaroid filter and a fisheye lens was used to take photographs of the sky near zenith for all full sky polarization images and zenithal

polarization images of the indoor polarization arenas as per methods of Cronin et al. (2006) [45].

Spectrometry

Irradiance measurements in the greenhouse were taken near midday on a cloudless day using an Ocean Optics USB2000 spectrometer connected to a 3 m long, 400 μm diameter, fiber-optic cable with a cosine-correcting head. The percentage of light transmitted through the greenhouse glass was calculated by comparing the ratio of irradiance measurements taken inside the greenhouse to irradiance measurements taken outside the greenhouse immediately afterwards. Irradiance measurements of light available in the indoor polarization arena and outdoors under heavily overcast skies were measured from the center of the arena using the same spectrometer system.

Transmittance measurements of the polarizing filter were taken using the same spectrometer system without a cosine-correcting head. The percent polarization of light transmitted through the filter was determined by calculating the ratio of the percent transmission of the polarizing filter layered with a second polarizer oriented first perpendicularly and then parallel to the first filter.

Experimental Procedures

Individual *N. oerstedii* were placed in each arena and were allowed to familiarize themselves to the arena for 24 hours. During familiarization, a vertical 2 cm diameter PVC column with alternating 1 cm thick black and white horizontal

stripes was placed adjacent to the burrow, marking it during the animals' initial explorations of the arena.

After familiarization, the column marking the burrow was removed from the arena. Empty *Margarites sp.* snail shells stuffed with pieces of food (whiteleg shrimp) were placed at fixed locations in the arena. During experiments conducted in the greenhouse, food was placed at one of two locations 50 cm from the periphery of the burrow. During experiments in which animals were displaced, food was placed on the movable platform on which animals were translocated. For the outdoor and indoor compass experiments, food was placed on rotatable platforms in the center of the arenas. Each animal was allowed three successful foraging excursions (i.e. food placed in the arena was found) before foraging paths were used for analyses. If an individual did not successfully locate food within one week in the arena, it was replaced with a new individual.

During experiments when animals were not manipulated, food was placed in the arena between two to three hours after sunrise and removed from the arena following sunset. All manipulation experiments were run from sunrise to four hours following sunrise and from four hours preceding sunset to sunset. For manipulation experiments, food was removed from the arena during the middle of the day.

During animal translocation experiments, once animals found food placed on the movable platform, they were carefully displaced along the track to a new location in the arena by the pulling of a thin fishing line tethered to the platform.

During outdoor rotation experiments, trials were conducted under three environmental conditions: clear skies, partly cloudy skies when the sun was hidden

by clouds, and heavily overcast skies. Full-sky polarization images were initially used to determine when trials would be categorized in the heavily overcast condition. It soon became clear that the lack of a visually identifiable solar disk during an overcast day (with clouds completely covering the sky) was sufficient to categorize a trial as occurring under a heavily overcast sky. Therefore, this method was used for the majority of celestial condition designations. During these experiments, animals were either not manipulated, carefully rotated 180°, or the tripods recording trials were rotated either approximately 60° or 180° to control for their presence. During experiments when animals were rotated 180°, animals were carefully rotated by the pulling of thin fishing line tethered to the platform once the animal found food placed on the rotatable platform. Animals were randomly rotated either clockwise or counterclockwise. During data analysis, clockwise trials were flipped so all trials could be analyzed in a counterclockwise fashion. During trials controlling for the presence of the tripod over the arena, the tripod was rotated when the animal was at the food location.

During outdoor solar azimuth compass experiments, trials were run only when the sun was clearly visible at an altitude between 20° and 45° above the horizon. When animals had reached food placed in the center of the arena, the location of the sun was blocked by a board and reflected 180° from its original position in the sky by a mirror. To control for the presence of the board, trials were also conducted when the sun was only blocked by the board but not mirrored.

During indoor experiments in which the polarization field was manipulated, once an experimental animal found food placed in the center of the arena, the

composite polarization filter with the polarizer-side facing downwards over the arena, creating a linearly polarized field, was rotated 90° from its original position. In control trials, the polarizer was touched by the researcher, but remained fixed throughout the experiment. In order to control for the rotation of the filter, the filter was placed wax-paper-side face downward over the arena, creating an unpolarized field, and was rotated 90° when animals were at the location of the food.

Quantification and Statistical Analyses

Foraging paths to and from food locations to the burrow were video recorded. In order to differentiate homeward paths from continued arena exploration, paths from the food locations were considered to be homeward paths when they did not deviate more than 90° from their initial trajectories for at least one-third of the beeline distance (the length of the straightest path) from the food location to the burrow. From these homeward paths, search behaviors were determined to be initiated when an animal turned more than 90° from its initial trajectory.

Paths were traced at a sampling interval of 0.2 seconds using the MTrackJ plugin [43] in ImageJ v1.49 (Broken Symmetry Software), from which the output is given as Cartesian coordinates. From these data, homeward path lengths and beeline distances from the food location to the burrow were determined. From these measures, home vector lengths were calculated as percentages of beeline distances.

Additionally, the orientation of homeward paths when animals were at one-third of the beeline distance from the food source to the burrow (at which point the orientation of the home vector was usually observed) was recorded using ImageJ.

All statistical analyses were run on R (v3.3.1, R Core Development Team 2016) with the “CircStats”, “circular”, “Hmisc”, and “boot” plugins. All statistical analyses for indoor polarization field experiments were performed subsequent to using the doubling angles procedure for bimodal data outlined in Batschelet (1981) [44]. Orientation data were analyzed using the following procedures for circular statistics [44].

All reported mean values for orientation data are circular means and circular standard errors of means.

Rayleigh tests of uniformity were used to determine if homeward paths were oriented within a group for the initial set of experiments and the translocation experiments run in the greenhouse. V-tests of uniformity were used to determine orientation in predicted directions of groups from the compass experiments (for outdoor rotation, sun compass, and zenithal polarization compass experiments).

Watson-Williams tests for homogeneity of means were used to determine if group orientations were significantly different from one another.

No significant difference was observed between homeward orientations of males and females during the initial set of experiments when animals were not manipulated ($p > 0.5$; Figure 2.12)) so data from both sexes were pooled for all experiments.

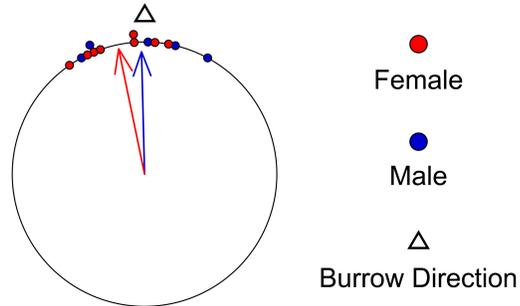


Figure 2.12. Male and female *Neogonodactylus oerstedii* orient towards home equally well while foraging. Homeward orientations of male and female individuals during experiments in the greenhouse when animals were not manipulated. Each point along the circumference of the circular plot represents the orientation of the homeward path of one individual with respect to position of the burrow (empty triangle). Blue-filled circles represent males while red-filled circles represent females. Arrows represent mean vectors, where angles of the arrows represent the mean vector angles and arrow lengths represent the strength of orientation in the mean direction (\bar{R}). Males (n=5) and females (n=8) both exhibited significant orientations ($p < 0.01$ for both groups). No significant difference in orientation was observed between males and females ($p > 0.5$).

An F-test of equality of variances was used to determine if a difference in the variance of homeward vector path lengths could be observed between experiments run in the indoor arenas and experiments run outdoors when animals were not manipulated.

Bonferroni corrections were used for all tests when applicable. All statistical information including sample sizes, test statistics, P-values, means, and standard errors of means are presented in Tables 2.1 and 2.2.

Data Availability

The data from this manuscript are published on Mendeley Data at <http://dx.doi.org/10.17632/wkzygts78t.1>.

Summary of Statistical Outcomes

Table 2.1: Summary of orientation statistics for all experimental groups. Rows with an asterisk (*) were analyzed using Rayleigh Tests of Uniformity. Rows without an asterisk were analyzed using a V-test with a specified mean direction (μ) of 0° (the burrow's direction) with the exception of the "Rotated 180° Overcast", "Sun Blocked and Mirrored", and "Polarized Field Rotated 90° (Doubled)" groups, which had a specified mean direction of 180° . Related to Figures 2.3, 2.5, 2.6, and 2.8.

Experiment	P-value	Bonferroni Correction	N	\bar{R}	Mean + S.E.M.
Animal Not Manipulated*	<0.0001	<0.001	13	0.949	$352.2^\circ \pm 5.36^\circ$
Not Manipulated Males*	0.0058	0.0174	5	0.926	$358.58^\circ \pm 11.1^\circ$
Not Manipulated Females*	<0.0001	<0.001	8	0.968	$348.37^\circ \pm 5.49^\circ$
Animal Displaced (in respect to actual burrow position)*	<0.0001	<0.001	7	0.984	$323.77^\circ \pm 4.2^\circ$
Animal Displaced (in respect to expected burrow position)*	<0.0001	<0.001	7	0.974	$359.58^\circ \pm 5.38^\circ$
Not Manipulated: Clear	<0.0001	NA	8	0.912	$2.27^\circ \pm 9.14^\circ$
Not Manipulated: Partly Cloudy (Sun Covered)	<0.0001	NA	7	0.882	$352.55^\circ \pm 11.54^\circ$
Not Manipulated: Overcast	0.00117	NA	8	0.721	$347.07^\circ \pm 20.27^\circ$
Rotated 180° : Clear	<0.0001	NA	12	0.9	$341.34^\circ \pm 5.54^\circ$
Rotated 180° : Partly Cloudy (Sun Covered)	<0.0001	NA	12	0.959	$354.12^\circ \pm 4.56^\circ$
Rotated 180° : Overcast ($\mu = 180^\circ$)	0.0007	NA	11	0.646	$191.98^\circ \pm 17.2^\circ$
Rotated Tripod All Conditions	<0.0001	NA	11	0.928	$358.52^\circ \pm 7.06^\circ$
Sun Blocked in Isolation	<0.0001	NA	10	0.91	$357.89^\circ \pm 4.27^\circ$
Sun Blocked and Mirrored ($\mu = 180^\circ$)	0.023	NA	10	0.44	$203.7^\circ \pm 8.14^\circ$
Polarized Field Static (Doubled)	<0.001	NA	18	0.677	$10.31^\circ \pm 34.03^\circ$
Polarized Field Rotated 90° (Doubled, $\mu = 180^\circ$)	<0.001	NA	12	0.727	$179.74^\circ \pm 13.74^\circ$
Depolarized Field Static*	0.037	NA	9	0.597	$14.38^\circ \pm 19.59^\circ$
Depolarized Field Rotated 90° *	0.39	NA	10	0.31	$315.65^\circ \pm 25.35^\circ$

Table 2.2: Summary of Watson-Wheeler Tests of Homogeneity of Means. Related to Figure 2.3 and 2.12.

Experiment	P-value	Bonferroni Correction	F
Not Manipulated: Males vs. Females	0.3767	1.0	0.85
Not Manipulated vs. Animal Displaced (in respect to actual burrow position)	0.00016	0.00048	13.78
Not Manipulated vs. Animal Displaced (in respect to expected burrow position)	0.383	1.0	0.8

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Author Contributions: R.N.P. designed and conducted all research, analyzed all data, and prepared the manuscript. T.W.C. provided guidance and research support.

Declaration of Interests: The authors declare no competing interests.

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Chapter 3: Path integration error and adaptable search behaviors in a mantis shrimp

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Summary

Mantis shrimp of the species *Neogonodactylus oerstedii* occupy small burrows in shallow waters throughout the Caribbean. These animals use path integration, a vector-based navigation strategy, to return to their homes while foraging. Here we report that path integration in *N. oerstedii* is prone to error accumulated during outward foraging paths and we describe the search behavior that *N. oerstedii* employs after it fails to locate its home following the route provided by its path integrator. This search behavior forms continuously expanding, non-oriented loops that are centered near the point of search initiation. The radius of this search is scaled to the animal's accumulated error during path integration, improving the effectiveness of the search. The search behaviors exhibited by *N. oerstedii* bear a striking resemblance to search behaviors in other animals, offering potential avenues for the comparative examination of search behaviors and how they are optimized in disparate taxa.

Introduction

Path integration is an efficient navigational strategy that many animals use to return to a specific location. During path integration, an animal monitors its body orientations and distances it travels from a reference point using a biological compass and odometer. From this information, a home vector (the most direct path back to the reference point) is continuously updated, allowing the animal to return to its original location (Seyfarth et al., 1982; Muller and Wehner, 1988; Seguinot et al., 1993). Path integration is especially useful for central place foragers, animals which return to a home location between foraging bouts.

Due to small errors made in angular and odometric measurements during path integration, the home vector is prone to error accumulated over the course of an animal's outward path (the path from the animal's start location to the site of home vector initiation). Therefore, with a longer outward path, an increased error of the home vector is expected (Muller and Wehner, 1988; Cheung et al., 2007; Heinze et al., 2018). To account for this error, some path-integrating animals initiate a stereotyped search behavior if they fail to reach their goal after travelling the distance indicated by their path integrator (Wehner and Srinivasan, 1981; Hoffmann, 1983; Zeil, 1998; Durier and Rivault, 1999).

Many stomatopod crustaceans, more commonly known as mantis shrimp, are central place foragers that inhabit benthic marine environments. These animals occupy burrows in marine substrates, where they reside between foraging bouts (Dominguez and Reaka, 1988; Basch and Engle, 1989; Caldwell et al., 1989). Mantis shrimp of the species *Neogonodactylus oerstedii* employ path integration to

efficiently navigate back to their burrows while foraging. During path integration, *N. oerstedii* exhibit homeward paths that are well oriented and are equal in length to the direct distance from the point where they initiate their return trip to the burrow (Patel and Cronin, 2020). However, the return paths guided by their home vectors often do not lead them directly to their burrows. When this happens, *N. oerstedii* initiate searches to find their homes (Patel and Cronin, 2020). Here we investigate the source of home vector error in *N. oerstedii* and evaluate the means by which *N. oerstedii* copes with this error—the strategies that shape its search pattern.

Results

Path integration in mantis shrimp is prone to accumulated error

In order to investigate the source of home vector error in *N. oerstedii*, individuals were placed in relatively featureless circular arenas with a sandy bottom filled with sea water. Vertical pipe burrows were buried in the sand so that they were hidden from view when experimental animals were away. Snail shells stuffed with small pieces of shrimp were placed at one of two fixed locations in the arena.

Foraging paths to and from the location of the food were observed.

During these trials, animals would make tortuous paths away from the burrow until they located the food placed in the arena (the outward path). After animals located the food, they often executed a fairly direct homeward path (the home vector) before initiating a search behavior if their home vector did not lead them to the hidden burrow (Fig. 1B). We defined the distance from the point of search behavior initiation to the location of the burrow as the path integration error. We found that this

path integration error correlates with outward path lengths during these trials ($p = 0.017$, $r = 0.67$, $n = 12$; Fig. 1C), suggesting that the error of path integration is an outcome of error accumulated over the course of the mantis shrimps' outward paths.

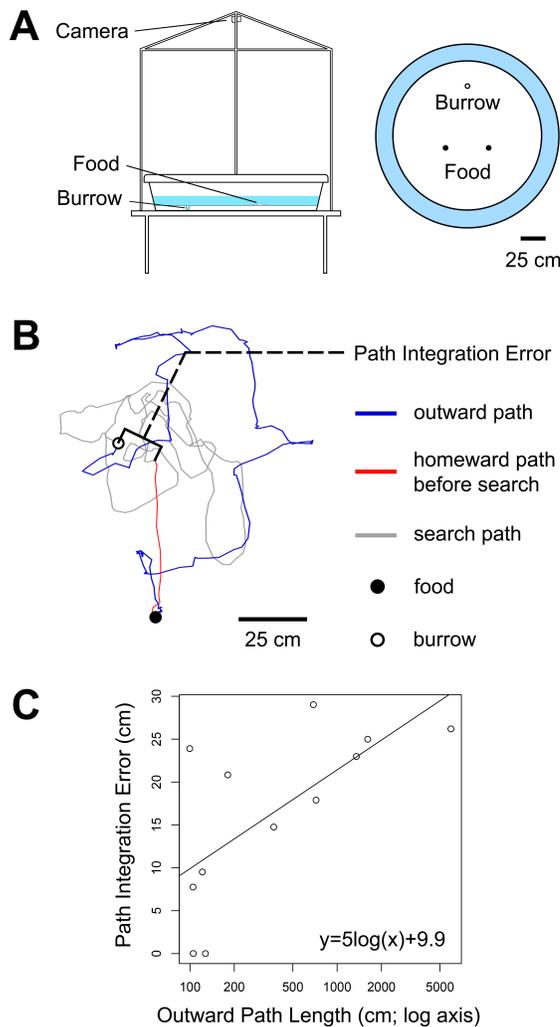


Figure 3.1. Error accumulated during outward foraging paths leads to error in the home vector.

(A) Navigation arenas 150 cm in diameter contained a burrow (empty circle) buried in the base of the arena 30 cm from the arena's periphery. During trials when animals were not manipulated, food was placed at one of two positions 50 cm from the periphery of the arena (filled circles). Trials were video recorded from above. (B) Example of a foraging path of *Neogonodactylus oerstedii*. The distance from the point where search behaviors were initiated to the burrow location is the error of the animal's path integrator. (C) Correlation between outward path lengths (log axis) and the path integration error during trials in which the animals were not manipulated ($P = 0.017$, $R = 0.67$, $n = 12$).

Search behaviors in *N. oerstedii* are stereotyped and flexible depending on error accumulated during path integration.

Mantis shrimp execute stereotyped search behaviors when they have travelled the distances indicated their path integrators without finding their burrows (Fig. 3.2 and 3.3). These search behaviors are composed of loops that start and end near the location where the search is initiated (Fig. 3.2). We defined a loop in the search as a path that increased in distance from the point of search initiation before the animal turned and moved back toward the search initiation point. The loop was determined to be completed when an animal moved closest to the point of search initiation before once again moving away from the search initiation point or when an animal turned more than 90° from its trajectory back towards the search initiation point after returning halfway back to it, whichever occurred first (Fig. 3.2D).

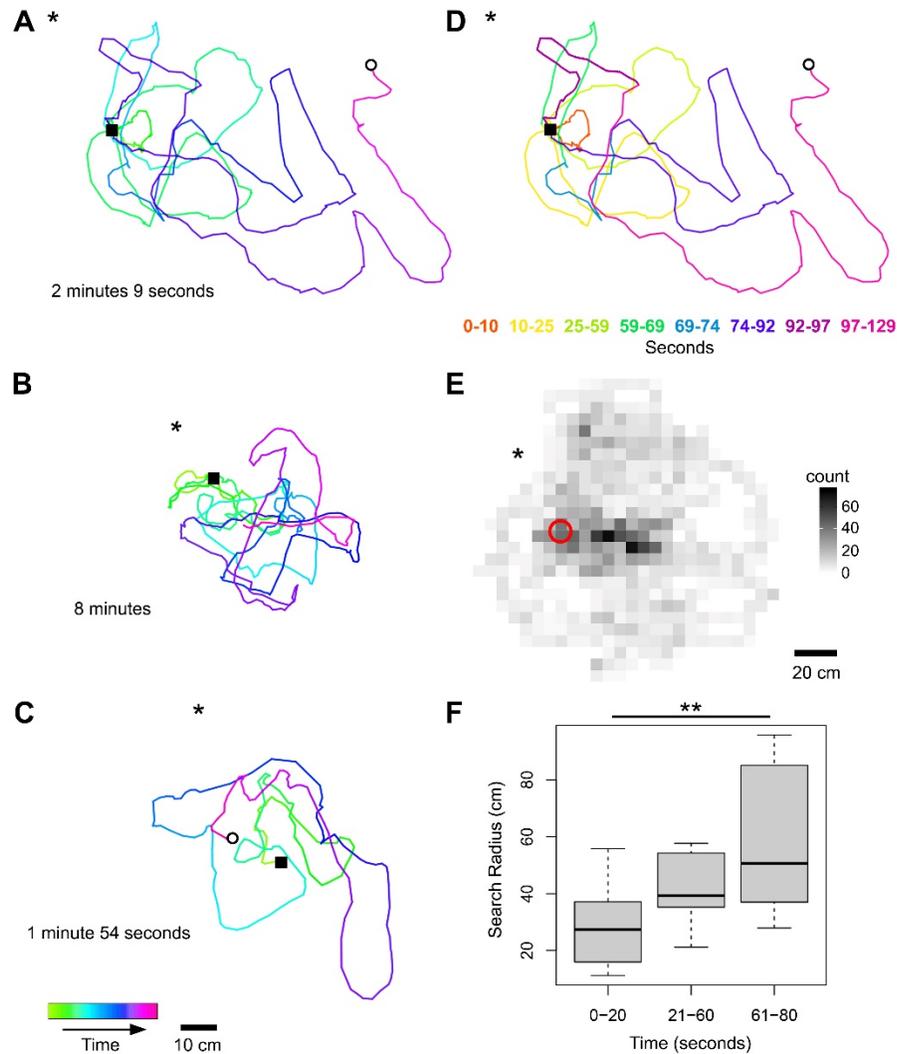


Figure 3.2. Search behaviors consist of a series of consecutive loops of increasing size which start near and return to a central location. Examples of search behaviors during trials when animals were displaced before they initiated a home vector (**A**, **B**) and an animal was not displaced (**C**). Empty circles represent the location of the burrow. Filled squares represent the location of search behavior initiation. Asterisks mark the location of the nearest edge of the arena. Lines are colored with time as indicated in the key in the bottom left corner. (**A**) During this trial, the individual carried a food-filled shell during its homeward path and dropped it once it initiated its search behavior (marked by the filled square). This offered an opportunity to observe the strategy behind the search behavior, where consecutive continually-increasing concentric loops are made from the location of the initiation of the search behavior until the goal has been found. (**B**) This animal did not find its burrow until after eight minutes of searching. The full search can be seen in Fig. 3.3. (**D**) The same search as in (**A**) with search loops color-coded by successive loops according to the key. (**E**) A heat-map of search behaviors compiled from all trials in

which animals were displaced in the arena (n=7). Shades of grey indicate counts of video frames in which animals moving more than one body-length per second were present at that location. Darker areas represent areas in the arenas where animals spent more time searching. The red circle marks the location of search behavior initiation and the asterisk marks the average nearest edge of the arena. Search behaviors are centered near the point of initiation. The observed deviation of the highest trafficked areas from the exact point of search behavior initiation might have been due to the initiation point's proximity to the border of the arena. **(F)** The radii of search behaviors measured between 0-20 seconds, 21-60 seconds, and 61-180 seconds after search initiation. Search behaviors widen over time (ANOVA, $P = 0.0015$, $F = 8.41$, 0-20 seconds: $n = 11$, 21-60 seconds: $n = 10$, 61-180 seconds: $n = 8$). Bars represent medians, boxes indicate lower and upper quartiles, and whiskers show sample minima and maxima. Asterisks indicate a significant difference in search radii between groups ($P = 0.001$).

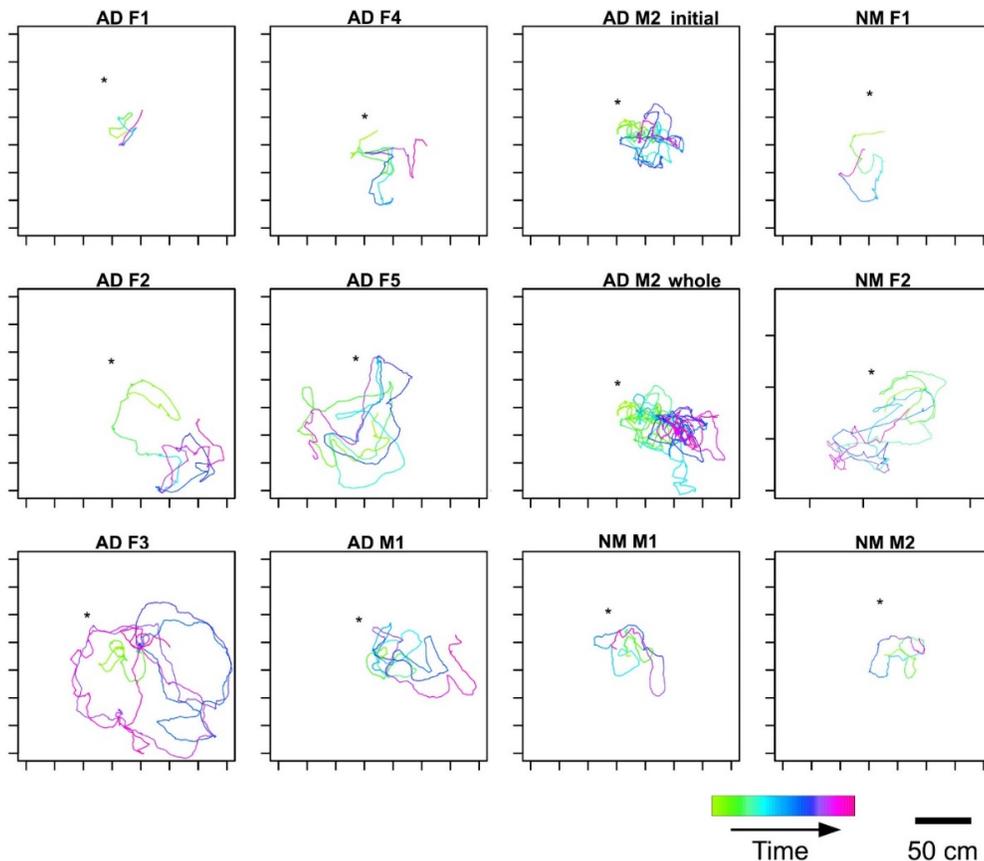


Figure 3.3. Path tracings of all searches. Asterisks mark the location of the nearest edge of the arena. The home vectors preceding the searches are oriented vertically. The search for AD M2 is plotted as both, the first eight minutes of the search (initial) and the whole search (whole).

Loops within single searches were not oriented in a mean direction in most individuals (Fig. 3.4); however, searches in some individuals were biased away from the edge of the arena nearest to the location where the search was initiated (loops were only significantly oriented in two of eleven individuals: $P = 0.03$ and $P = 0.025$; Fig. 3.4 and Table 3.1). These exceptions suggest that *N. oerstedii* can estimate the position of a goal using local structures (here, the walls of the arena) and use these estimates to alter its searches in some cases.

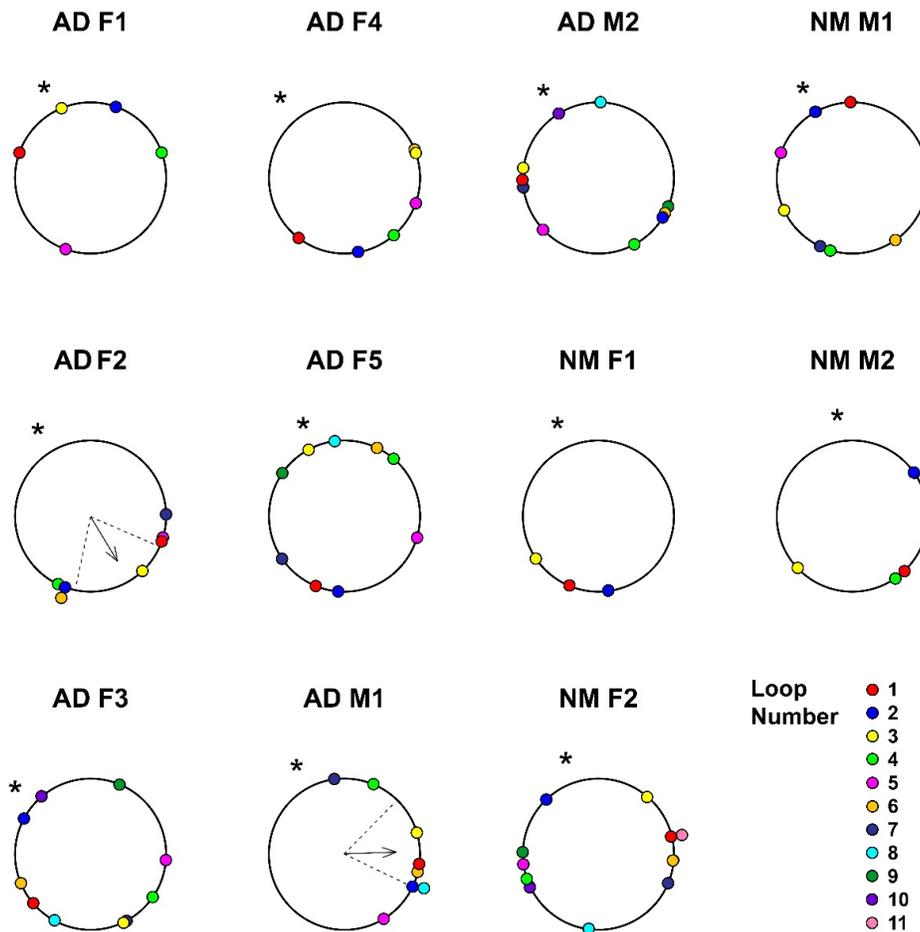


Figure 3.4. Search loop orientations per individual. Asterisks (*) mark the direction of the nearest edge of the arena. The home vectors preceding the searches are oriented to the top of the plot (towards 0 degrees). Arrows in plots represent mean

vectors, where arrow angles represent vector angles and arrow lengths represents the strength of orientation (\bar{R}). Dashed lines represent 95% confidence intervals. Means and 95% confidence intervals were only included in plots with significant orientations (AD F2: $P = 0.03$, $\bar{R} = 0.689$ and AD M1: $P = 0.025$, $\bar{R} = 0.704$). Loops appear to be biased away from nearest edge of the arena in these individuals.

Table 3.1. Orientation statistics of search loops from each individual. Search loop orientations were analyzed using Rayleigh Tests of Uniformity. Rows with an asterisk (*) indicate individuals with significant mean direction of search loop orientations ($p < 0.05$). Related to Figure 3.4.

Individual	\bar{R}	P	Number of loops in search
AD F1	0.322	0.619	5
AD F2*	0.689	0.0297	7
AD F3	0.229	0.605	10
AD F4	0.614	0.101	6
AD F5	0.168	0.786	9
AD M1*	0.691	0.017	8
AD M2	0.156	0.793	10
NM F1	0.897	0.08	3
NM F2	0.036	0.986	11
NM M1	0.378	0.381	7
NM M2	0.540	0.333	4

We also measured the radii of searches (the farthest distance of a search from the point of search initiation) within three time ranges (0-20 seconds, 21-60 seconds, and 61-180 seconds) and found that searches tend to increase in size over time (ANOVA, $P = 0.0015$, $F 8.41$; Fig. 2F). Since search patterns accumulate error along the course of the search, optimal search theory predicts that the search radius should increase as the square root of the search time, $\text{radius}_{\text{max}} = \text{time}^{0.5}$ (Heinze et al., 2018).

Data from desert ant searches (Wehner and Srinivasan, 1981) fitted to power functions match this prediction, where the maximum search radius is proportional to $\text{time}^{0.48}$ (Heinze et al., 2018). We found that mantis shrimp search expansions agree with this prediction, where the exponential factor of time from a fitted power function of the searches we measured that lasted at 60 seconds resulted in a maximum radius proportional to $\text{time}^{0.43}$, indicating that mantis shrimp searches expand in a close to optimal manner (Fig. 4A, B).

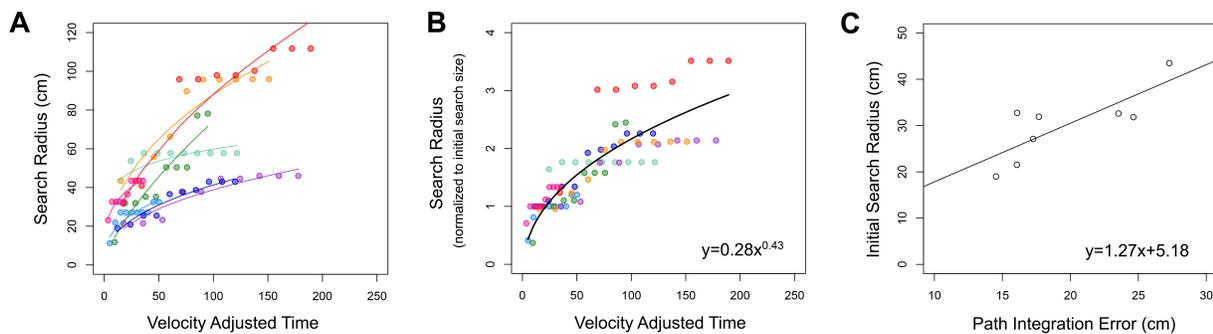


Figure 3.5. Search behaviors expand similarly to those predicted by optimal search theory and are adjusted in size by path integration uncertainty. **A.** The radii of searches that lasted at least one minute plotted every 10 percent of the total search time until the search was completed ($n = 8$). Since individuals were travelling at different speeds during their searches, search time (in seconds) was multiplied by the individual's mean velocity during the search relative to the search with the highest mean velocity. Colors represent individual searches. Each search was fitted with a power function (lines of corresponding colors). **B.** The radii of searches in (A) normalized by the initial search size (measured at 17 seconds into the search, the time at which the radius of the most extended search was first measured). The black line is the power function of best fit for all data, resulting in the search expansion pattern, $\text{radius}_{\text{max}} = \text{time}^{0.43}$. Optimal search theory predicts that searches should expand by $\text{radius}_{\text{max}} = \text{time}^{0.5}$. **C.** Correlation of the initial radii of search behaviors and positional error during path integration. The sizes of search behaviors were larger when error in path integration was greater ($P = 0.018$, $R = 0.794$, $n = 8$).

We found that the radii of searches were variable at similar time-points among searches, with some searches over three times as wide as other searches (Fig. 2F, 4A, S1). We hypothesized that searches were wider when the error in a mantis shrimp's path integrator was higher (i.e. the animal's confidence in its home vector's accuracy was lower). In order to test this hypothesis, we compared the radii of search behaviors lasting at least 60 seconds to the positional error in the home vector (path integration error) during those same trials. We found search radii were correlated with error in the path integrator (Pearson's correlation, $P = 0.018$, $R = 0.79$, $n = 8$; Fig. 4C). This result suggests that the sizes of search behaviors are modulated by the reliability of the path integrator.

Discussion

Path integration in *N. oerstedii* is inherently prone to error, which accumulates over the course of an animal's outward path. Error due to distance estimates are expected to increase linearly with increasing outward path lengths. However, the magnitude of angular errors differ depending on the manner in which angular measurements are taken. If directional information is measured in relation to a stable compass heading or environmental feature, angular errors would be expected to increase in a linear manner, similar to error accumulated from distance measurements; however, if angular information is measured from a previous rotational estimate, angular errors should compound, increasing at a rate greater than a linear relationship over the course of an animal's journey (Cheung, 2014; Heinze et

al., 2018). Some models of error accumulation during path integration suggest that due to this large accumulated rotational error, path integration over extended distances (such as those exhibited by bees and ants) would require the use of a stable compass reference during navigation (Cheung et al., 2007; Cheung and Vickerstaff, 2010; Cheung, 2014; Heinze et al., 2018). This may be true for mantis shrimp as well since the path integration error in our experiments accumulated at a rate less than a linear relationship over the length of outbound foraging paths (Fig. 1C); however, previous work suggests that mantis shrimp do rely on idiothetic orientation during path integration when celestial cues are obscured (Patel and Cronin, 2020). If mantis shrimp are indeed using idiothetic path integration when celestial information is unavailable, they would be relying on cumulative rotational estimates to measure their angular displacements under these conditions. Perhaps the typical limited foraging distances *N. oerstedii* exhibit in nature (usually not greater than a couple of meters (Dominguez and Reaka, 1988; Patel and Cronin, 2020)) allow them to home using idiothetic path integration with reasonable accuracy.

To cope with the error in the home vector, *N. oerstedii* executes stereotyped search behaviors composed of a series of non-oriented loops (unless local features are detected) which increase in size over the course of the search in a manner similar to that predicted by optimal search theory. Even though these searches are stereotyped, their sizes are scaled: they become larger with increased error in the path integrator. This flexible strategy improves the efficacy of the search.

In this study, some mantis shrimp searches were biased away from the edge of the arena nearest to where they initiated the search (Fig. 2 and 3, Fig. S1). This result

suggests that mantis shrimp can estimate the position of a goal from nearby structures, which may act as landmarks. Similar search biases to local features have been observed in other animals. Desert ants alter the geometry of their searches for their nests depending on the apparent image size of the local landmark array on their retinas (Akesson and Wehner, 1997). Trained honeybees also have been demonstrated to use the apparent sizes of landmarks in their environment to focus their searches for a hidden food source (Cartwright and Collet, 1983). Landmark navigation is a reliable way for animals to correct for error accumulated during path integration and is often used by other animals in tandem with path integration to lead them to their targets (Etienne, 1992; Collett, 1996; Wehner, 2003; Heinze et al., 2018). Mantis shrimps, many of which occupy structurally complex environments, may also use landmarks to assist their navigation.

The search behaviors of *N. oerstedii* closely resemble those executed by other animals, such as Cataglyphid desert ants (Wehner and Srinivasan, 1981), cockroaches (Durier and Rivault, 1999), and desert isopods (Hoffmann, 1983). The searches of these animals are similarly composed of ever-expanding loops centered near the animal's estimate of its shelter position and strikingly resemble the searches of mantis shrimp reported in this study. As in mantis shrimp, the sizes of desert ant searches are also flexible (Merkle et al., 2006; Schultheiss and Cheng, 2011). In Cataglyphid ants, the search radii were found to be scaled to the length of the home vector (Merkle et al., 2006), not to the length of the outward foraging path (Merkle and Wehner, 2010) such as we found in *N. oerstedii*, since error accumulated during outward foraging paths contribute to positional error in path integration (Figure 1). However, Heinze et

al. (2018), argue that after extensive search travel, optimal search theory predicts the small differences in search radii of groups with differing outward path lengths measured in Merkle and Wehner (2010) (Merkle and Wehner measured search paths at least 50 m long). Merkle and Wehner (2010) may have noticed greater differences in search sizes between their experimental groups if the radii of shorter searches or of earlier stages in the searches were used for their analyses. Regardless, due to the similarities of searches in insects and malacostracan crustaceans, the neural programs of these search behaviors and the path integration circuits they likely manifest from may either be ancient homologs or remarkable convergences between these disparate groups of animals. Even if the underlying mechanisms of the searches these groups exhibit are homologous, differences in how these searches are manifested and elaborated are likely to be present.

Methods

All data in this study were collected from experiments reported in Chapter 2. Specifically, foraging behaviors from the “not manipulated” and “animal displaced” groups of trials enacted in the greenhouse on the UMBC campus in Chapter 2 were used in the current study.

Animal Care

Individual *Neogonodactylus oerstedii* collected in the Florida Keys, USA were shipped to the University of Maryland, Baltimore County (UMBC). Animals were housed individually in 30 parts per thousand (ppt) sea water at room

temperature under a 12:12 light:dark cycle. Animals were fed whiteleg shrimp, *Litopenaeus vannamei*, once per week. Data were collected from 13 individuals (5 male and 8 female). All individuals were between 30 and 50 mm long from the rostrum to the tip of the telson.

Experimental Apparatuses

Four relatively featureless, circular navigation arenas were constructed from 1.5 m-diameter plastic wading pools that were filled with pool filter sand and artificial seawater (30 ppt, Fig. 3.1A). Arenas were placed in a glass-roofed greenhouse on the UMBC campus. The spectral transmittance of light through the greenhouse glass was nearly constant for all wavelengths, excluding the deep-UV-wavelength range (280 to 350 nm; Fig. 2.2A). Celestial polarization information was transmitted through the glass roof of the greenhouse (Fig. 2.2B-D). Vertical burrows created from 2 cm outer-diameter PVC pipes were buried in the sand 30 cm from the periphery of the arena so that they were hidden from view when experimental animals were foraging. Trials were recorded from above using C1 Security Cameras (Foscam Digital Technologies LLC) mounted to tripods placed above the arenas. During animal displacement experiments, a thin 11 x 82 cm acrylic track with a movable platform was placed 30 cm from the wall of the arena at its closest edge.

Experimental Procedures

Individual *N. oerstedii* were placed in each arena and were allowed to familiarize themselves to the arena for 24 hours. During familiarization, a vertical 2

cm diameter PVC column with alternating 1 cm thick black and white horizontal stripes was placed adjacent to the burrow, marking it during the animals' initial explorations of the arena.

After familiarization, the column marking the burrow was removed from the arena. Empty *Margarites* sp. snail shells stuffed with pieces of food (whiteleg shrimp) were placed at fixed locations in the arena. During experiments when animals were not manipulated, food was placed at one of two locations 50 cm from the periphery of the burrow. During experiments in which animals were displaced, food was placed on the movable platform on which animals were translocated. Each animal was allowed three successful foraging excursions (i.e. food placed in the arena was found) before foraging paths were used for analyses. If an individual did not successfully locate food within one week in the arena, it was replaced with a new individual.

During experiments when animals were not manipulated, food was placed in the arena between two to three hours after sunrise and removed from the arena following sunset. Animal displacement experiments were run from sunrise to four hours following sunrise and from four hours preceding sunset to sunset. During animal displacement experiments, food was removed from the arena during the middle of the day.

During animal displacement experiments, once animals found food placed on the movable platform, they were carefully displaced along the track to a new location in the arena by the pulling of a thin fishing line tethered to the platform.

Data and Statistical Analyses

Foraging paths from the burrow to find food and from food locations back to the burrow were video recorded from above. In order to differentiate homeward paths from continued arena exploration, paths from the food locations were considered to be homeward paths when they did not deviate more than 90° from their initial trajectories for at least one-third of the beeline distance (the length of the straightest path) from the food location to the burrow. From these homeward paths, search behaviors were determined to be initiated when an animal turned more than 90° from its initial trajectory.

Paths were traced at a sampling interval of 0.2 seconds using the MTrackJ plugin (Meijering et al., 2012) in ImageJ v1.49 (Broken Symmetry Software), from which the output is given in Cartesian coordinates. From these data, the lengths of outbound, homebound, and search paths were calculated. The distance of the point of search behavior initiation to the burrow (the path integration error) was also measured using the MTrackJ plugin. Path integration error analyses were only measured from trials when animals were not manipulated ($n = 12$).

Search behaviors lasting over ten seconds with at least one completed loop were analyzed from all trials when animals were either not manipulated ($n = 4$) and or were displaced to a new location in the arena ($n = 7$; $n = 11$ total). We defined a loop in the search as a path that increased in distance from the point of search initiation before the animal turned and moved back toward the search initiation point. The loop was determined to be completed when an animal moved closest to the point of search initiation before once again moving away from the search initiation point or when an

animal turned more than 90° from its trajectory back towards the search initiation point after returning halfway back to it, whichever occurred first.

The radii of search behaviors were measured as the farthest distance of a search from the original point of search initiation (i.e. the end point of the home vector) using ImageJ. The radii of all searches were measured over three time ranges after search initiation: 0-20 seconds, 21-60 seconds, and 61-180 seconds. The radii of individual searches lasting at least 60 seconds were also measured from the beginning to end of the search, every 10 percent of the total search time until the search was completed (up to 10 minutes). Since individuals travelled at different speeds during their searches, search time (in seconds) was multiplied by the individual's mean velocity during the search relative to the search with the highest mean velocity. In order to observe the general expansion pattern of the searches, the radii of searches were normalized by the initial search size (measured at 17 seconds into the search, the time at which the radius of the most extended search was first measured) and were fitted with a power function. Additionally, orientations of search loops when loops were at the farthest distance from the search initiation point were recorded using ImageJ. For these measurements, searches were oriented so the axis of each home vector preceding the searches was at 0 degrees.

All statistical analyses were run on R (v3.3.1, R Core Development Team 2016) with the “CircStats”, “circular”, “plotrix”, “Hmisc”, and “boot” plugins. As reported in Patel and Cronin (2020), no significant difference was observed between homeward orientations of males and females during experiments when animals were

not manipulated ($P > 0.5$; Fig. 2.12)), so data from both sexes were pooled for all experiments.

Rayleigh tests of uniformity were used to determine if all loops within individual searches had a directional bias and if successive search loops between individuals contained some pattern of orientation (Batschelet, 1981). All reported mean values for orientation data are circular means. All circular 95% confidence intervals were calculated by bootstrapping with replacement over 1000 iterations.

An Analysis of Variance Test (ANOVA) was used to determine if the radii of searches differed between the time intervals measured. A Tukey Honest Significant Difference post-hoc analysis was used to determine significant differences between groups.

Pearson's correlation tests were used for all correlative analyses.

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Competing Interests: The authors declare no competing financial interests.

Author Contributions: R.N.P. designed and conducted all research, analyzed all data, and prepared the manuscript. T.W.C. provided guidance and research support.

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Data and Materials Availability: The data from this manuscript are published on Mendeley Data at <http://dx.doi.org/10.17632/whrcjff7jx.1>.

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Chapter 4: Landmark navigation in a mantis shrimp

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Summary

Mantis shrimp are predatory crustaceans that commonly occupy burrows in shallow, tropical waters worldwide. Most of these animals inhabit structurally complex, benthic environments where many potential landmarks are available. Mantis shrimp of the species *Neogonodactylus oerstedii* return to their burrows between foraging excursions using path integration, a vector-based navigational strategy that is prone to accumulated error. Here we show that *N. oerstedii* can navigate using landmarks in parallel with their path integration system, offsetting error generated when navigating using solely path integration. We also report that when the path integration and landmark navigation systems are placed in conflict, *N. oerstedii* will orient using either system or even switch systems enroute. How they make the decision to trust one navigational system over another is unclear. These findings add to the refined navigational toolkit *N. oerstedii* relies upon to efficiently navigate back to its burrow, complementing its robust, yet error prone, path integration system with landmark guidance.

Introduction

Stomatopods, better known as mantis shrimp, are benthic crustaceans renowned for their ballistic strikes and complex visual systems. As adults, most mantis shrimp species reside in shallow tropical marine waters, environments that are often structurally varied and therefore contain many potential visual landmarks [1]. In these environments, mantis shrimp typically occupy small holes or crevices for use as burrows, where they reside concealed for most of the day. During foraging, many stomatopod species leave the safety of their burrows for extended excursions, where they become vulnerable to predation [2-5]. Returning to the burrow efficiently is critical to minimize predation risk and to also reduce the chance that the vacated burrow will be claimed by another animal.

Mantis shrimp of the species *Neogonodactylus oerstedii* employ path integration to efficiently navigate back to their burrows between foraging bouts [5]. During path integration, an animal monitors the distances it travels in various directions from a reference point (usually home) using angular and distance measurements. From this information, a home vector (the most direct path back to the reference point) is continuously calculated, allowing the animal to return to its original location [6-8]. As animals update their home vectors during excursions, small errors in odometric and angular measurements are made. Over the course of an animal's travel, these small errors accumulate in its path integrator. Therefore, with longer outward paths, increased errors of home vectors are expected [7, 9]. Path integration using idiothetic cues (those informed by stimuli anchored internal to the body) are particularly prone to accumulated error. As theory suggests, path

integration in *N. oerstedii* is prone to this accumulated error [10]. To reduce this error, many path-integrators use landmarks to accurately pinpoint their goal [9, 11-14]. We hypothesized that in addition to path integration, *N. oerstedii* uses landmarks when available during navigation. The benthic habitats *N. oerstedii* occupy are structurally complex with an abundance of sponges, coral, rock, and seagrass to serve as potential visual landmarks (Fig. 4.1). Using landmarks during navigation would allow *N. oerstedii* to correct for error accumulated while path-integrating during foraging paths away from the burrow.



Figure 4.1. Examples of *Neogonodactylus oerstedii* burrows in nature. Burrows are indicated by orange arrows. Note the abundance of potential landmarks, including marine vegetation, sponges, coral fragments, and rock rubble, available in the scenes.

Results

Mantis shrimp use landmarks during navigation

We placed *N. oerstedii* individuals in relatively featureless circular arenas filled with sand and sea water in a glass-roofed greenhouse. Vertical burrows were buried in the sand so that they were hidden from view when experimental animals were away. Snail shells stuffed with small pieces of shrimp were placed at one of two fixed locations approximately 70 cm from the location of the burrow in the arena (Fig. 4.2A). Foraging paths to and from the location of the food were video recorded from above.

As described by Patel and Cronin (2020) [5,10], we observed that animals would make tortuous paths away from the burrow until they located the food placed in the arena. After animals located the food, they would usually execute a fairly direct home vector towards the burrow. If the burrow was not found using the home vector, animals would initiate a stereotyped search behavior (Fig. 4.2C).

To determine if *N. oerstedii* use landmarks during homeward navigation when available, a 2-cm diameter, 8-cm tall vertical cylinder with alternating 1-cm thick horizontal black and white stripes was placed adjacent to the burrow to serve as a landmark. Stripe cycles of the landmark would appear to span approximately 0.8 cycles/degree at the location of the food, approximately twice the visual resolving limit of *Gonodactylus chiragra* [15], a closely related mantis shrimp that can be slightly larger than *N. oerstedii*. Trials with the landmark present were compared to the results of previous experiments in which the landmark was absent [5].

Return trips in the presence of the landmark were more direct than trips in the landmark's absence ($P < 0.05$; Fig. 4.2C-D and 3), supporting the hypothesis that *N. oerstedii* uses landmarks during navigation. This was primarily due to the virtual elimination of stereotyped search behaviors at the ends of homeward paths in the presence of the landmark. Instead, short directed searches for the burrow around the landmark were observed. Return trips were initially oriented similarly between the two groups (Groups were oriented: $P < 0.001$ for both groups; Orientations were not significantly different between groups: $P > 0.5$; All statistical outcomes are presented in Tables 4.1-4.3). However, during trials in the presence of the landmark, individuals appeared to correct for their initial homeward error over the course of the homeward path ($P < 0.05$), in contrast to what we observed in the absence of the landmark ($P > 0.5$; Fig. 4.2D-F). These results indicate that in the presence of a landmark, *N. oerstedii* uses both path integration and landmark navigation to navigate back to its burrow.

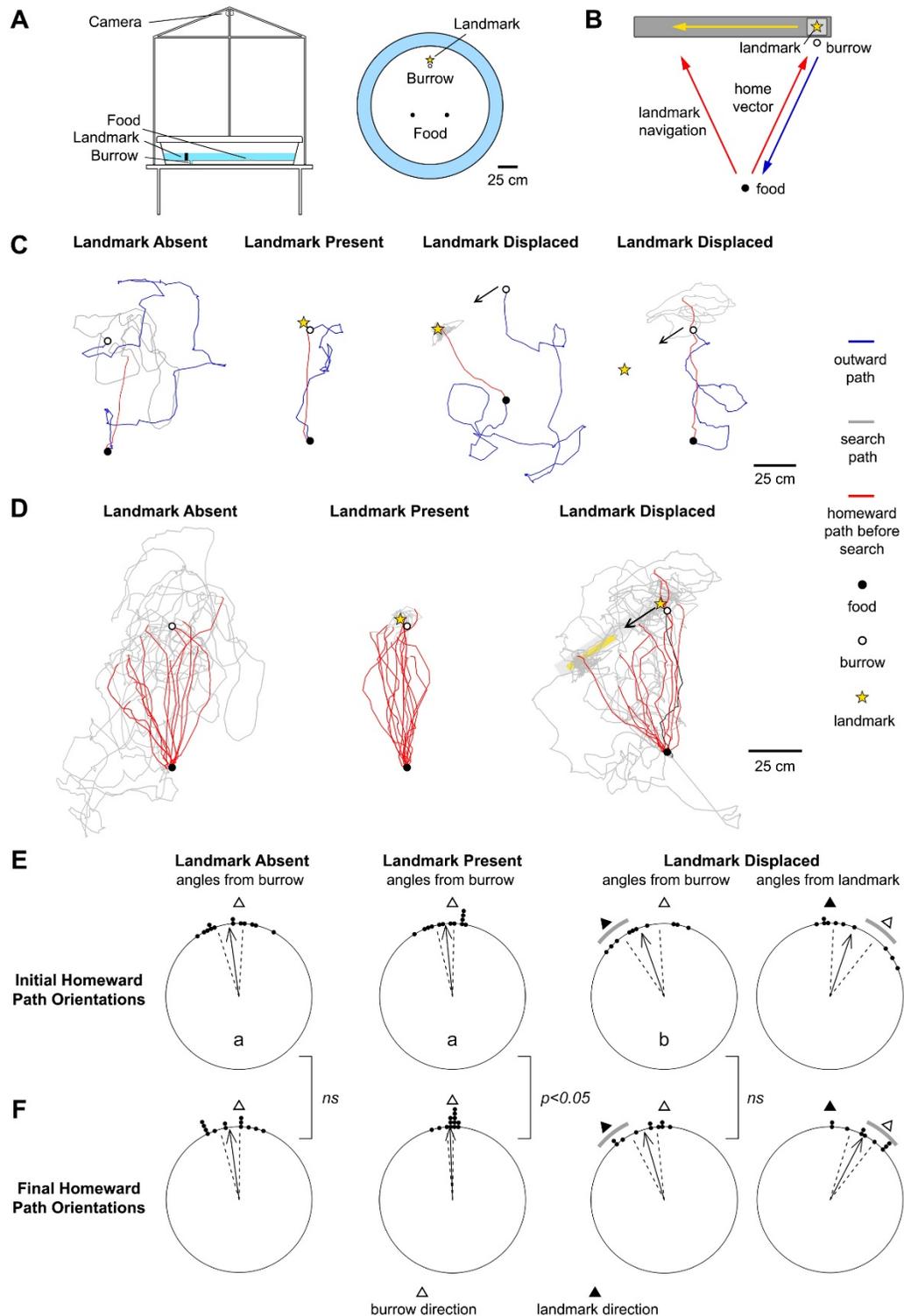


Figure 4.2. *Neogonodactylus oerstedii* uses a landmark to navigate back to its burrow while foraging. (A) Navigation arenas. Each arena was 150 cm diameter. A vertical burrow was set into the base of the arena 30 cm from the edge of the pool so it was invisible at range (empty circle). A landmark was placed adjacent to the

burrow during some experiments (gold-filled star). Food was placed in one of two locations near the center of the pool (filled circles). Behaviors were video recorded from above. **(B)** Landmark displacement experimental design. Homeward paths were observed when a landmark adjacent to the burrow was displaced to a new location in the arena while experimental individuals were away foraging. **(C)** Examples of foraging paths from and to the burrow during the three experimental conditions. Blue lines represent outward paths from the burrow while red lines represent homeward paths before search behaviors were initiated. Grey lines represent homeward paths after search behaviors were initiated. Empty and filled circles represent the location of the burrow and food, respectively. Gold-filled stars represent the location of the landmark. Arrows represent paths of landmark displacements. **(D)** Data from all homeward paths. Lines and filled circles represent the same as in (C). The grey rectangle represents the track along which the landmark was displaced. The gold rectangle marks the range of locations to which the landmark was displaced during landmark displacement trials. The black tracing in the “landmark displaced” group marks the homeward path of an individual on its second run which, after orienting its initial homeward path towards the displaced landmark (in red), it returned to the food location and oriented towards the burrow (in black). **(E)** Orientations of homeward paths at one-third the beeline distance from the location of the food to the burrow (initial orientations). Each point along the circumference of the circular plot represents the orientation of the homeward path of one individual with respect to either the actual position of the burrow (empty triangle) or displaced landmark’s position (filled triangle). Grey arcs in the “Landmark Displaced” orientation plots represent the range of the directions of the either the displaced landmark or the burrow from at the location of the food. Arrows in each plot represent mean vectors, where arrow angles represent vector angles and arrow lengths represents the strength of orientation (\bar{R}). Dashed lines represent 95% confidence intervals. Different letters within orientation plots denote a significant difference between groups ($p < 0.05$). “Landmark Absent” data were obtained from Patel and Cronin (2020a) [5]. **(F)** Homeward path orientations of groups same as in (E) measured immediately before search behaviors were initiated (final orientations).

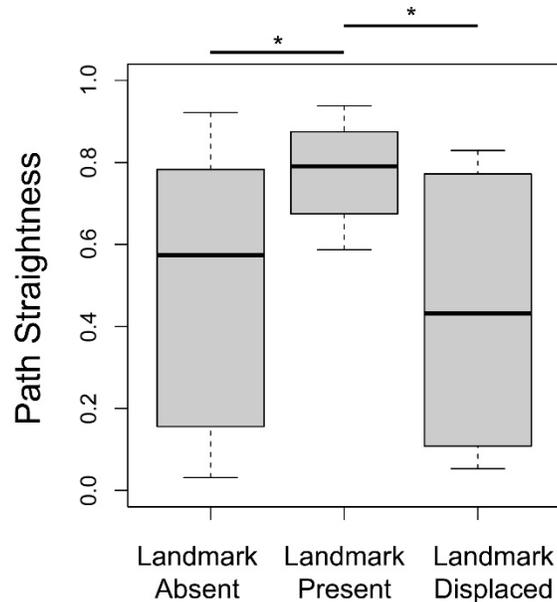


Figure 4.3. Homeward paths were more direct when a fixed landmark was present during navigation than when the landmark was absent or displaced to a new location in the arena during foraging. Straightness of homeward paths from the location of food to the burrow during trials when the landmark was present, absent, and displaced. Larger path straightness values indicate straighter paths with a value of one being a completely straight path from the food location to the burrow (a beeline path). Bars represent medians, boxes indicate lower and upper quartiles, and whiskers show sample minima and maxima. Asterisks indicate significant differences in path straightness between groups ($P \leq 0.05$; Landmark Absent: $n = 13$, Landmark Present: $n = 13$, Landmark Displaced: $n = 10$).

Mantis shrimp exhibit varied homeward paths when landmark navigation and path integration are placed in conflict

In light of the above results, we were interested in the confidence *N. oerstedii* places in its landmark navigation system when it is in conflict with its path integrator. In order to create this situation, homeward paths were observed when a landmark adjacent to the burrow was displaced to a new location in the arena while experimental individuals were away foraging. The landmark remained at roughly the same distance from the food location both before and after displacement. If *N.*

oerstedii navigates using landmarks and trusts a landmark's location over the location designated by its path integrator when homing, animals should orient towards the displaced landmark rather than the burrow's location (Fig. 4.2B).

Homeward paths were less direct ($P < 0.05$; Fig. 4.3) and were differently oriented ($P < 0.05$; Fig. 4.2D-F) when landmarks were displaced compared to when they were left in place, further supporting the hypothesis that *N. oerstedii* navigate using landmarks. Some individuals oriented towards the displaced landmark while others ignored the displaced landmark, orienting towards the burrow (Fig. 4.2C). Several individuals initially oriented towards the displaced landmark, but broke away from their initial trajectories during their homeward paths, orienting towards the burrow instead (Fig. 4.2D). Overall, however, differences observed between initial path orientations and the orientations of homeward paths at the end of the home vector were not statistically significant when the landmark was displaced ($P = 0.36$; Fig. 4.2E-F). One individual initially oriented its homeward path towards the landmark, only to turn around and return to the food location before adopting a revised homeward path oriented towards the burrow (Fig. 4.2D). These observations suggest that the path integrator of *N. oerstedii* is continually updated during foraging, even after homeward paths are initiated.

As just described, when landmarks were displaced some animals adopted paths initially oriented towards the displaced landmark while others ignored the displaced landmark completely, orienting towards the burrow. These results demonstrate that *N. oerstedii* must make decisions when the navigational strategies it relies on are in conflict and raise the question of how these decisions are made.

Due to errors inherent in path integration, *N. oerstedii* exhibit growing home vector errors with increased outward path lengths [10]. When the landmark was displaced, individuals may have evaluated this accumulated error during foraging, choosing to trust the position of the landmark when the accumulated error of the path integrator was high (i.e. confidence in the path integrator was low). However, we found that the orientations of homeward paths during landmark displacement experiments were not significantly correlated with the outward path lengths from the burrow to the food location ($P = 0.16$; Fig. 4.4A); nonetheless, the effect size of this relationship was fairly strong ($r = -0.48$), suggesting this hypothesis should not be completely discounted.

Cataglyphid desert ants are model terrestrial species for studying navigation using path integration and visual landmarks. In experiments with these ants, when their path integrators are placed conflict with their surrounding landmark panorama, displaced desert ants will orient toward either the location indicated by their path integrator or toward a local landmark array depending on their distance from their nest, not on the error accumulated in their path integrators. These ants will orient using their home vectors, ignoring local landmarks, when displaced from at a distance greater than three meters from their nest; however, they will orient using the local landmark array when displaced from near the nest. When displaced from a distance of one meter from their nest, desert ants will orient with a mean vector not clearly directed at either their home vectors derived from path integration or the local landmark panorama, but somewhere in between [16]. Interestingly, orientation results of the desert ants displaced from roughly one meter from the nest are similar to those

of *N. oerstedii* during the landmark displacement experiments described above. Stomatopods in those experiments were around 0.7 m from their burrows when initiating their homeward paths (Fig. 4.3E). These observations suggest that a cue integration mechanism resembling that employed by desert ants may also be present in mantis shrimp.

As an alternative hypothesis to account for the variation observed in homeward paths during experiments when the landmark was displaced, the deviation between the home vector and the landmark's perceived position may have been at a preference threshold for either of the two navigation systems. For example, if the landmark was displaced further away from the burrow, the majority of animals may have trusted their home vector, while if the landmark was not moved as far from the burrow, the animals may have been more likely to trust the landmark's position. However, when homeward path orientations during landmark displacement experiments were compared to the distance of landmark displacement along the track during those trials, no correlation was observed ($P=0.92$, $r = -0.04$; Fig. 4.4B). This suggests that the degree of landmark displacement did not influence the decision to orient toward the home vector or the displaced landmark during these trials.

Finally, we hypothesized that animals that may have observed the landmark's displacement were more likely to disregard its location than those that may not have noticed displacement of the landmark. To investigate this hypothesis, we measured the orientations of all animals' body axes with respect to the landmark while it was displaced, sampled at a rate of 0.2 seconds. We compared the means of these body axis orientations to the orientations of homeward paths and found no correlation ($P =$

0.604, $r = 0.19$; Fig. 4.4C). This suggests that either animals did not notice the landmark's displacement or that observing the landmark's displacement did not influence an animal's decision to determine the burrow's location by using the displaced landmark's position or by using its home vector.

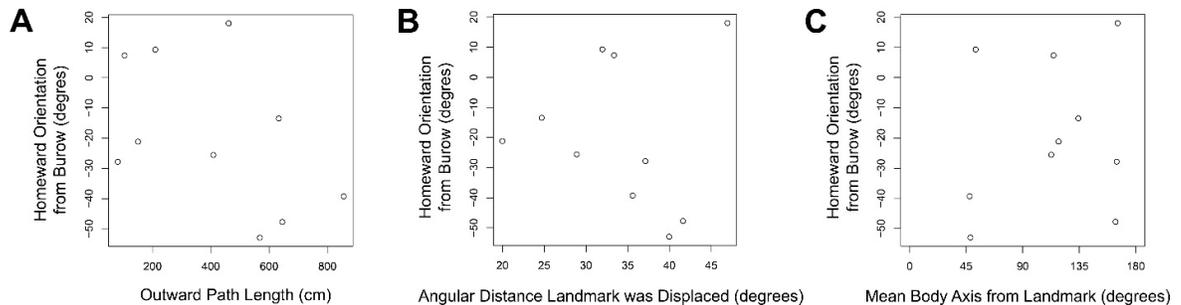


Figure 4.4. It is unclear why *N. oerstedii* chose to trust either the landmark or the home vector while navigating during landmark displacement experiments. (A)

The orientations of homeward paths when the landmark was displaced was not significantly correlated with the length of outward paths from the burrow to the location of food ($P = 0.16$, $n = 10$, $r = -0.48$). **(B)** The orientations of homeward paths when the landmark was displaced was not correlated with the angular distance of landmark displacement along the track when viewed from the location of the food ($P = 0.92$, $n = 10$, $r = -0.04$). **(C)** Homeward path orientations were not correlated with body axis orientations of animals with respect to the landmark during its displacement ($P = 0.604$, $n = 10$, $r = 0.19$). Each point represents the mean body axis orientation of an individual with respect to the landmark measured at a sampling rate of 0.2 seconds during the landmark's displacement.

Discussion

Our results demonstrate that *Neogonodactylus oerstedii* uses landmark navigation together with path integration while navigating back to its burrow while foraging. Landmarks are reliable references which can be used to correct for error

accumulated by path integration; this is especially important during idiothetic path integration, which *N. oerstedii* uses when allothetic cues become unreliable [5].

Landmarks were used in a very basic situation during our experiments— as a beacon to home towards. Many other questions about how landmarks may be used by mantis shrimp arise from this work: Can mantis shrimp estimate the relative position of a goal to multiple landmarks? Do stomatopods use a snapshot mechanism like that employed by some insects to learn landmark arrays [13,17]? Do they possess cognitive maps akin to those thought to exist in mammals [18]? Do mantis shrimp learn to recognize landmarks encountered during foraging routes, exhibiting “trapline foraging”? Further, mantis shrimp are famed for possessing complex color vision, linear polarization vision in two spectral channels, and circular polarization vision [19]. Besides spatial vision alone, do stomatopods use these visual channels to identify landmarks? If so, how?

Mantis shrimp occupy a wide variety of marine habitats and depths, from structurally complex reefs to nearly featureless mud flats. Stomatopod species that occupy landmark-rich environments may weigh the importance of landmarks more heavily during navigation than stomatopods which occupy benthic environments relatively void of landmarks. Further, visual information rapidly attenuates with distance underwater due to extreme scattering of light in water. Therefore, the relative importance of landmark navigation over path integration may differ for mantis shrimp species occupying waters of different depths and turbidities.

Taken together with our previous work on mantis shrimp navigation [5, 10], this work offers an opportunity to study the neural basis of navigation, learning,

memory, and decision making in stomatopods. Mushroom bodies, centers for arthropod learning and memory, are thought to play a prominent role in landmark learning in insects [20-23]. Prominent hemiellipsoid bodies, homologues of insect mushroom bodies, exist in stomatopod eyestalks [24]. As in insects, these neuropils may be crucial for navigation and landmark learning in mantis shrimp. A separate brain region, the central complex, plays a role in landmark orientation in *Drosophila melanogaster*. Here, landmark orientation is neurally based in the ellipsoid body of the central complex [25]. Stomatopods themselves possess a highly developed central complex composed of a collection of neuropils anatomically very similar to those found in insects [26]. Investigation of the function of stomatopod brain regions in light of our work may have implications for the evolutionary origins of navigational strategies and the neural architecture of the brain within the ancient Pancrustacean clade, a taxon which includes all insects and crustaceans [27], as well as in other arthropods.

In summary, *N. oerstedii* possesses a robust navigational toolkit on which it relies to efficiently navigate back to its burrow. First, *N. oerstedii* relies on path integration using multiple redundant compass cues to navigate back to its home [5]. If path integration does not lead *N. oerstedii* directly to its burrow, it relies on a stereotyped search behavior which is scaled to the amount of error it accumulates during its outbound foraging path to locate its nearby lost target [10]. Finally, the stomatopod will use landmarks, if available, to quickly pinpoint its target, offsetting error accumulated during path integration.

Methods

Animal Care

Individual *Neogonodactylus oerstedii* collected in the Florida Keys, USA were shipped to the University of Maryland, Baltimore County (UMBC). Animals were housed individually in 30 parts per thousand (ppt) sea water at room temperature under a 12:12 light:dark cycle. Animals were fed whiteleg shrimp, *Litopenaeus vannamei*, once per week. Data were collected from 13 individuals (5 male and 8 female). All individuals were between 30 and 50 mm long from the rostrum to the tip of the telson.

Experimental Apparatuses

Four relatively featureless, circular navigation arenas were constructed from 1.5 m-diameter plastic wading pools that were filled with pool filter sand and artificial seawater (30 ppt; Fig. 4.2A). Arenas were placed in a glass-roofed greenhouse on the UMBC campus. The spectral transmittance of light through the greenhouse glass was nearly constant for all wavelengths, excluding the deep-UV-wavelength range (280 to 350 nm; Fig. 2.2B). Celestial polarization information was transmitted through the glass roof of the greenhouse (Fig. 2.2D-E). Vertical burrows created from 2 cm outer-diameter PVC pipes were buried in the sand 30 cm from the periphery of the arena so that they were hidden from view when experimental animals were foraging. Vertical 2 cm diameter, 8 cm high PVC columns with alternating 1 cm thick black and white horizontal stripes were placed adjacent to the burrows to function as removable landmarks. Stripe cycle widths of the landmarks were

approximately twice the visual resolving limit of *Gonodactylus chiragra* (0.8 cycles/degree [13]), a closely related mantis shrimp that can be slightly larger than *N. oerstedii*, when viewed from the food location in the arena (a distance of 70 cm). Trials were recorded from above using C1 Security Cameras (Foscam Digital Technologies LLC) mounted to tripods placed above the arenas. During landmark displacement experiments, a thin 11 x 82 cm acrylic track with a movable platform was placed adjacent to the burrow (Fig. 4.2B). A landmark identical to the one used in trials in which the landmark was static, was mounted to the movable platform.

Experimental Procedures

Individual *N. oerstedii* were placed in each arena and were allowed to familiarize themselves to the arena for 24 hours. During familiarization, the striped landmark was placed adjacent to the burrow, marking it during the animals' initial explorations of the arena.

After familiarization, the landmark was either removed for trials in which the landmark was absent or left in place for trials in which the landmark was present. Empty *Margarites sp.* snail shells stuffed with pieces of food (whiteleg shrimp) were placed at one of two locations 50 cm from the periphery of the burrow. Each animal was allowed three successful foraging excursions (i.e. food placed in the arena was found) before foraging paths were used for analyses. If an individual did not successfully locate food within one week in the arena, it was replaced with a new individual.

During landmark displacement experiments, the landmark was carefully displaced along the track to a new location in the arena by the pulling of a thin fishing line tethered to the platform when animals were foraging away from their burrows. The distance from the food location to the landmark remained relatively constant while the landmark was displaced.

Data and Statistical Analyses

Foraging paths to food locations and from them to the burrow were video recorded from above. In order to differentiate homeward paths from continued arena exploration, paths from the food locations were considered to be homeward paths when they did not deviate more than 90° from their initial trajectories for at least one-third of the beeline distance (the length of the straightest path) from the food location to the burrow. From these homeward paths, search behaviors were determined to be initiated when an animal turned more than 90° from its initial trajectory.

Paths were traced at a sampling interval of 0.2 seconds using the MTrackJ plugin [28] in ImageJ v1.49 (Broken Symmetry Software), from which the output is given as Cartesian coordinates. From these data, the inbound and outbound path lengths, beeline distances from food to burrow, and inbound and outbound indices of path straightness were calculated, where

$$Path\ Straightness = \frac{beeline\ distance}{path\ length} = \frac{\sqrt{(x_n - x_1)^2 + (y_n - y_1)^2}}{\sum_{k=1}^n (\sqrt{(x_{k+1} - x_k)^2 + (y_{k+1} - y_k)^2})}$$

n = the last coordinate of the path

Additionally, the orientations of homeward paths when animals were both, at one-third of the beeline distance from the food source to the burrow (at which point the orientation of the home vector was usually observed) and at the end of the home vector (when search behaviors were initiated) were recorded using ImageJ.

We also measured the orientations of the body axes of all animals in respect to the landmark while it was displaced. These body axis orientations were sampled at a rate of 0.2 seconds. From these body axis orientations a mean body axis orientation was calculated for each individual.

Data from the “Landmark Absent” group in this study were taken from the “Not Manipulated” trials of the greenhouse experiments published in Patel and Cronin (2020a) [5].

All statistical analyses were run on R (v3.3.1, R Core Development Team 2016) with the “CircStats”, “circular”, “Hmisc”, and “boot” plugins. Orientation data were analyzed using the following procedures for circular statistics [29]. All reported mean values for orientation data are circular means. All circular 95% confidence intervals were calculated by bootstrapping with replacement over 1000 iterations.

As reported in Patel and Cronin (2020a) [5], no significant difference was observed between homeward orientations of males and females during experiments in the absence of a landmark ($P > 0.5$; Fig. 2.12)), so data from both sexes were pooled for all experiments.

Rayleigh tests of uniformity were used to determine if homeward paths were oriented within a group for all trials. Parametric Watson-Williams tests for

homogeneity of means were used to determine if those group orientations were significantly different from one another. The orientations of groups which did not fit the assumptions of the Watson-Williams test were instead compared using the non-parametric Watson's two sample test of homogeneity. These tests were also used to compare differences between initial homeward path orientations (orientations at one-third the beeline distance from the food to the burrow) and final homeward path orientations (orientations at the initiation of search behaviors) for each group.

Homeward path lengths of trials in which the landmark was present were compared to those in which the landmark was absent using a paired T-test. A paired Wilcoxon signed-rank test was used to compare homeward path lengths of trials in which the landmark was static to those in which the landmark was displaced.

Pearson's correlation tests were used for all correlative analyses.

Holm-Bonferroni multiple testing corrections were used for all tests when applicable.

Summary of Statistical Outcomes

Table 4.1: Statistical outcomes of orientation analyses for all experimental groups. Orientations of homeward paths were measured relative to the burrow at one-third the beeline distance from the location of the food to the burrow (initial orientations) and were measured immediately before search behaviors were initiated (final orientations). Rayleigh tests of uniformity with Holm-Bonferroni multiple testing corrections were used to determine if groups were oriented. Data from this table can be viewed in Figure 4.2E and F.

Experiment	P-value (uncorrected)	Holm-Bonferroni (corrected P-value)	n	\bar{R}	Mean Vector Orientation \pm S.E.M.
Landmark Absent (Initial)	<0.0001	<0.001	13	0.949	354.4° \pm 3.76°
Landmark Present (Initial)	<0.0001	<0.001	13	0.974	352.2° \pm 5.36°
Landmark Displaced (with respect to burrow position; Initial)	<0.0001	<0.001	10	0.920	340.54° \pm 7.76°
Landmark Displaced (with respect to landmark position; Initial)	<0.0001	<0.001	10	0.894	18.79° \pm 8.93°
Landmark Absent (Final)	<0.0001	<0.001	13	0.966	352.32° \pm 4.31°
Landmark Present (Final)	<0.0001	<0.001	13	0.996	358.03° \pm 1.47°
Landmark Displaced (with respect to burrow position; Final)	<0.0001	<0.001	10	0.960	343.73° \pm 5.44°
Landmark Displaced (with respect to landmark position; Final)	<0.0001	<0.001	10	0.956	27.54° \pm 5.74°

Table 4.2: Summary of homogeneity of means circular statistical tests for orientation data. Comparisons of orientation groups in rows without an asterisk were analyzed using a Watson-Wheeler Test of Homogeneity of Means (test statistic is F). Comparisons of groups in rows with an asterisk (*) were analyzed using a non-parametric Watson’s Two-Sample Test of Homogeneity (test statistic is U^2) since they did not adhere to the assumptions of a Watson-Wheeler Test. A P-value of less than 0.05 indicates a significant difference between groups. Data from this table can be viewed in Figure 4.2E and F.

Experiment	P-value	Holm-Bonferroni	Test Statistic
Landmark Absent (Initial) vs Landmark Present (Initial)	0.7355	0.7355	0.1168
Landmark Present (Initial) vs Landmark Displaced (with respect to burrow; Initial)*	<0.02	<0.04	0.2227
Landmark Absent: Initial vs Final	0.9827	1	0.000048
Landmark Present: Initial vs Final*	<0.005	<0.015	0.3373
Landmark Displaced: Initial vs Final	0.7414	0.7414	0.11234

Table 4.3: Summary of homogeneity of means statistical tests for path straightness data. The comparison in the row without an asterisk was analyzed using a paired T-test (test statistic is t). Since the “landmark displaced” group did not adhere to the requirements of a T-test, the row with an asterisk (*) was analyzed using a non-parametric paired Wilcoxon Signed-Rank Test (test statistic is V). The straightness of paths from groups within each comparison were significantly different from one another ($P < 0.05$). The data from this table can be viewed in Figure 4.3.

Experiment	P-value	Holm-Bonferroni	Test Statistic
Landmark Absent vs Landmark Present	0.0216	0.0432	2.64
Landmark Present vs Landmark Displaced*	0.027	0.0432	49

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Author Contributions: R.N.P. designed and conducted all research, analyzed all data, and prepared the manuscript. T.W.C. provided guidance and research support.

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Data and Materials Availability: The data that support the findings of this study are available from the corresponding author upon reasonable request. Correspondence and requests for materials should be addressed to R.N.P. (e-mail: telrickp@gmail.com).

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Chapter 5: Mantis shrimp rank the shape of an object over its color during recognition

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Summary

Mantis shrimp are predatory crustaceans that commonly occupy burrows in shallow, tropical waters worldwide. Most of these animals inhabit structurally complex, benthic environments with an abundance of visual features that are regularly observed, including conspecifics, predators, prey, and landmarks for use in navigation. While these animals are capable of learning and discriminating color and polarization, it is unknown what specific attributes of a visual object are important for its recognition. Here we show that mantis shrimp of the species *Neogonodactylus oerstedii* can learn the shape of a trained target. Furthermore, when the shape and color of a target which they had been trained to identify were placed in conflict, *N. oerstedii* significantly chose the target of the trained shape over the target of the trained color. Thus, we conclude that the shape of a target is more important than its color for its recognition by *N. oerstedii*. Our findings suggest that the shapes of

learned structures, such as landmarks or other animals, are important for *N. oerstedii* during object recognition.

Introduction

Each species of animal living in a given space experiences its own distinct sensory world, known as its “umwelt” (von Uexküll, 1957/1934). The sensory structures responsible for an animal’s perception of its environment are metabolically taxing tissues that are often under strong selection pressures to permit the recognition of biologically relevant stimuli, while ignoring much of the available information an environment has to offer. Despite their complexity, the visual systems of stomatopod crustaceans should follow this generalization.

Better known as mantis shrimp, these animals are renowned for their visual systems which in most species enable spatial and motion vision, color and multispectral UV vision, and linear and circular polarization receptivity (Cronin et al., 2014a). The compound eyes of many stomatopod species have a relatively high visual acuity; for instance, *Gonodactylus chiragra*, an animal typically about 8 cm in length, achieves a resolution of 0.8 cycles/degree (Marshall and Land, 1993). The ability of stomatopods to learn novel visual stimuli has been previously demonstrated with color, linear polarization, and circular polarization cues (Marshall et al., 1996; Marshall et al., 1999; Chiou et al., 2008; Thoen et al., 2014). Taken together, it is clear that visual information is an important part of a stomatopod’s sensory experience and likely critical for its survival.

Mantis shrimp mostly reside in shallow tropical marine waters worldwide. These locations offer some of the most structurally complex and colorful environments on Earth, and therefore contain many visual features. In these environments, mantis shrimp typically occupy small holes or crevices in the marine substrate for use as burrows, where they reside concealed for most of the day. Mantis shrimp consume a variety of prey (deVries et al., 2016), many of which are brightly colored, and they use colored signals to communicate with one another (Hazlett, 1979; Cheroske et al., 2009; Chiou et al., 2010; Franklin et al., 2019). Furthermore, mantis shrimp of the species *Neogonodactylus oerstedii* exhibit impressive navigational abilities when returning to their burrows from foraging excursions. These animals use landmarks, if available, in parallel with path integration to quickly pinpoint the location of their burrows (Patel and Cronin, 2020a,b,c). The benthic habitats *N. oerstedii* occupy are abundant with potential visually informative features including sponges, coral, rock, and aquatic vegetation: structures of distinct shapes and colors.

Since color may be informative in many aspects of a mantis shrimp's life and since these animals use landmarks for navigation when available, this raises the question of what makes an object salient to a mantis shrimp for recognizing it. Considering that mantis shrimp have reasonably acute visual systems and are known to possess color vision, we were interested in determining whether *N. oerstedii* learns to recognize a visual target using its shape and/or its color.

Results

Neogonodactylus oerstedii learns to identify a specific visual target over time

N. oerstedii individuals (n=78) were trained to one of four targets of a specific color and shape combination (either a red rectangle, red triangle, green rectangle, or green triangle) using a paired food reward in a dichotomous choice y-maze (Fig. 5.1). Since stomatopods in previous behavioral experiments successfully learned to discriminate red and green colored targets (Marshall et al., 1996), targets of these colors were chosen for the current study. The target of the alternate color and shape of the trained target was placed in the other arm of the y-maze and was associated with no reward (for example, a rewarded red triangle was paired with an unrewarded green rectangle). Animals on average responded in this situation (i.e. made a choice) approximately half of the time (Fig. 5.2A). From these choices, animals learned to associate food with their respective trained targets over time ($P < 0.05$; Fig. 5.2B). Of the 78 stomatopods that were trained, 20 individuals reached the criteria set to progress to the testing procedure (see the Methods section for the criteria).

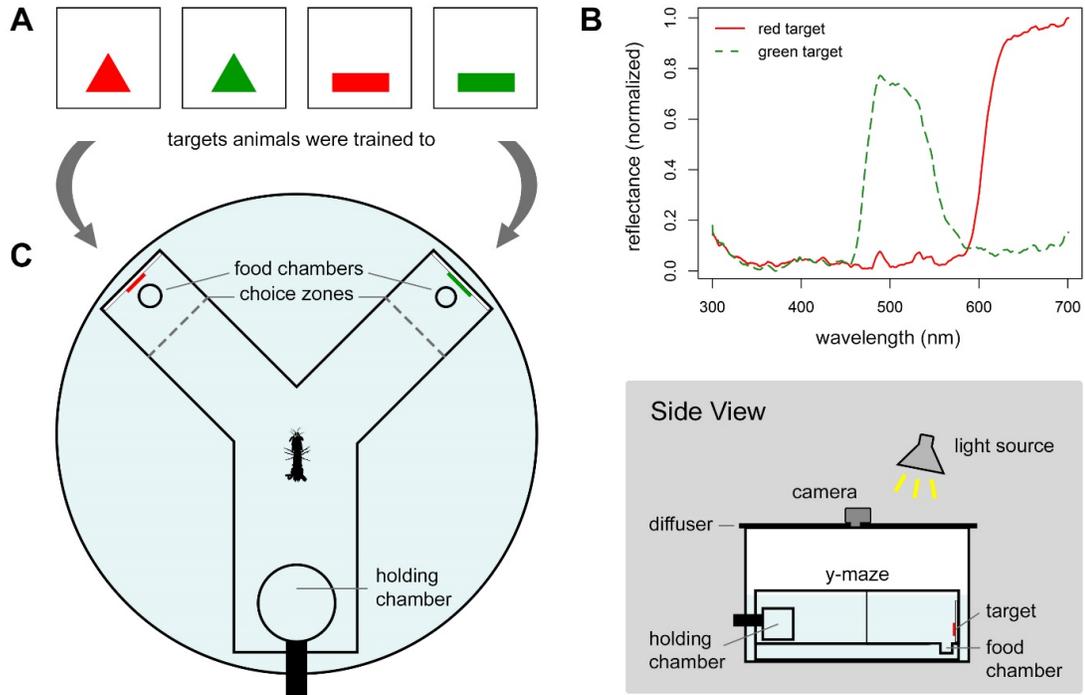


Figure 5.1. Layout of experimental setup. (A) The four targets used during the experiment: a red triangle, a green triangle, a red rectangle, and a green rectangle. (B) Averaged reflectance spectra (300 to 700 nm) of the red targets (solid red line) and green targets (dashed green line). (C) A y-maze was placed in a cylindrical tank with an incandescent light source centered above it. A diffusing filter was placed above the arena. The filter had a centered hole, where the lens of a camera was fit to record each trial. The y-maze contained an entrance arm and two choice arms oriented 90 degrees from one another. A cylindrical holding chamber was centered at the end of the entrance arm. At the end of each choice arm laid a hole set below the floor of the y-maze. A food reward could have been placed in either hole. One of the targets in (A) was placed at the end of each choice arm as indicated.

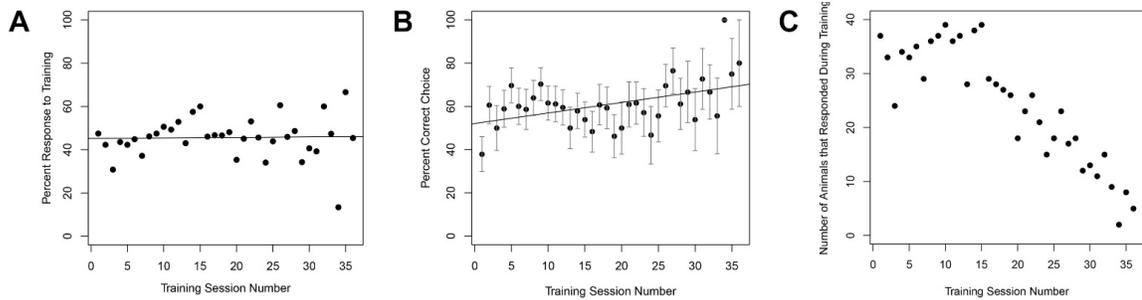


Figure 5.2. Training Results. (A) *Neogonodactylus oerstedii* responded to the training procedure approximately half of the time. (B) *N. oerstedii* associated food with their respective trained targets over time. Each point represents the percentage of animals who correctly chose the target they were training to from all animals who made choices during that training session. Error bars represent standard errors of the means. $P = 0.034$, $r = 0.35$. (C) Sample size per point in (B). The number of animals undergoing training decreased over time because animals either progressed to the testing procedure or died during the course of the study.

Neogonodactylus oerstedii recognized the trained target by its shape, not its color

Once animals reached the performance criteria to enter the testing phase, they were tested in three separate procedures: a shape recognition test, a color recognition test, and a conflicting cue test. During all testing procedures, food was not paired with a target.

During the shape recognition test, both arms contained targets of the color an animal had been trained to, but the target in each arm was of a different shape (e.g. a red triangle paired with a red rectangle). In this experiment, individuals significantly chose the arm with the shape to which they had been trained, indicating that they recognized the shape of their trained target ($P < 0.05$, $Z = 1.976$, $n = 19$; Fig. 5.3 and Table 5.1).

During the color recognition test, both arms contained targets of the shape they were trained to but the color of the target differed per arm (e.g. a red triangle

paired with a green triangle). During this task, stomatopods more often than not chose the arm with the same color target that they were trained to; however, this relationship was not significantly different from a random choice distribution ($P > 0.1$, $Z = 0.934$, $n = 21$; Fig 5.3 and Table 5.1).

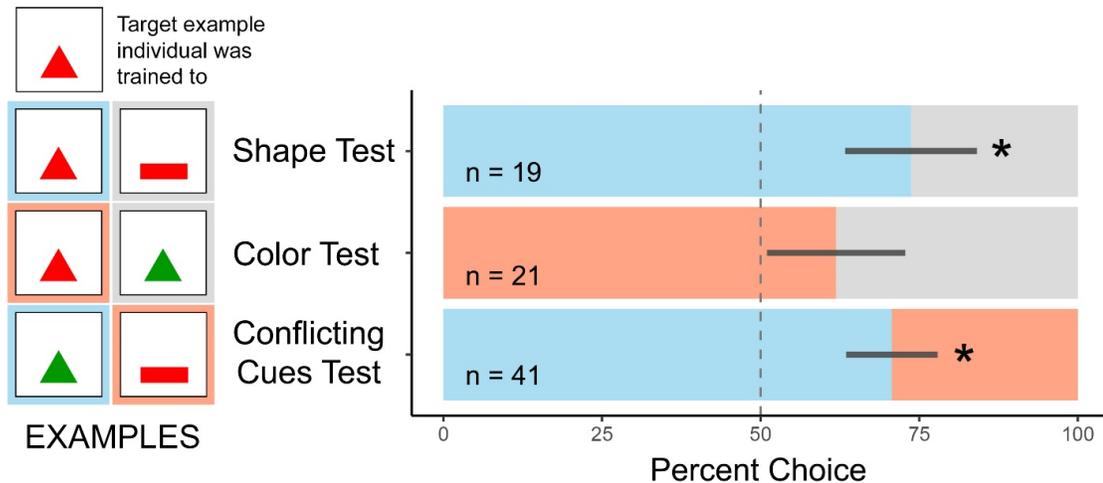


Figure 5.3. *Neogonodactylus oerstedii* recognized an object by its shape, not its color. Blue and red bars represent proportions of choices during testing that were for the target of the correct shape and color, respectively. Grey bars represent proportions of choices during testing that were for the incorrect target. Dark grey lines represent standard errors of the means. Asterisks (*) indicate a significant deviation from a random choice proportion ($P < 0.05$). The vertical dashed line marks a 50% proportion of choices (i.e. a random choice proportion). Examples of targets placed in either arm of each test for an individual that was trained to associate food with a red triangle are found on the left of each bar.

During the conflicting cue test, one arm contained a target with the same shape but opposite color to the target to which they were trained while the other arm had a target with the same color but alternate shape to the trained target (e.g. a green triangle paired with a red rectangle). Consistent with the results from the other tests, stomatopods significantly chose the arm with the trained shape over the arm with the

trained color, implying that the shape of the trained target was more important than the target's color to *N. oerstedii* during recognition ($P < 0.05$, $Z = 1.927$, $n = 41$; Fig. 5.3 and Table 5.1).

Table 5.1: Summary of generalized linear mixed modelling outcomes for all experimental tests.

Experiment	P-value	Z	n (tests)
Shape Recognition Test (Chose correct shape)	0.048	1.976	19
Color Recognition Test (Chose correct color)	0.35	0.934	21
Conflicting Cues Test (Chose correct shape)	0.054	1.927	41
Degree to which repeated measures explain results	P-value	X^2	n (individuals)
Shape Recognition Test*	1	0	12
Color Recognition Test*	0.432	0.619	13
Conflicting Cues Test*	0.227	1.458	15

*Repeated measures do not significantly explain results.

Discussion

Our study is the first to demonstrate that mantis shrimp are able to recognize distinct shapes. We found that mantis shrimp ranked the shape of an object higher than its color when recognizing it (Fig. 5.3). Since mantis shrimp use landmarks during navigation (Patel and Cronin, 2020c), the findings in our study suggest that the shape of a landmark may be more important than its color when being identified by a mantis shrimp during navigation. Similarly, the shapes of prey, predators, and of body structures used in signaling may be critical for recognition and for generating appropriate behavioral responses independent of their roles in navigation.

Identifying an object by its shape might be more effective than recognizing its color when viewed underwater. In water, contrast attenuates with distance and depth due to the absorption and scattering of light. This is especially true for color

information underwater, where the spectral range of incoming daylight or of an object's color is rapidly trimmed to primarily blue light with increasing distance and depth (Cronin et al., 2014b). Because of this, luminosity contrast persists farther than color contrast in water, and the colors of objects vary with the distance and the depth of viewing while their shapes remain unchanged. Therefore, the shapes of objects may be more reliable cues to their identity than their colors when viewed by mantis shrimp in ecologically relevant situations.

Many animals use the edges of objects for recognition, including humans (Shapley and Tolhurst, 1973) and honeybees (Lehrer et al., 1990), so it is reasonable to hypothesize that mantis shrimps do the same. Shape recognition is likely to be critically important to mantis shrimp when they are recognizing landmarks, which they use to locate their home burrow during navigation (Patel and Cronin, 2020c). In other arthropods, landmark navigation involves retinal image matching, where the field of view seen while navigating is matched to a stored retinal "snapshot" of the view of their goal (Cartwright and Collett, 1983; Akesson and Wehner, 2007). During these tasks, the edges of landmarks appear to be important for image matching and distance estimations (Cartwright and Collett, 1983; Harris et al., 2007). Similarly, the overall panorama, especially the upper edge of the horizon, is used for orientation in some arthropods (Collett, 1996; Luschi et al., 1997; Fukushi, 2001; Graham and Cheng, 2009, Wystrach et al., 2011). Therefore, edge detection of objects may be critical during navigation as well as for other aspects of a mantis shrimp's life, such as signal recognition, food identification, and recognition of predatory threats.

Conditioning experiments with other animals have demonstrated that multiple redundant cues can compete during associative learning, allowing one cue to overshadow the learning of another one (Couvillon et al., 1983; Menzel, 1990). In our experiments, we combined shape and color during associative learning. The apparent failure of our experimental animals to choose a target on the basis of color in our experiments suggests that shape was a more relevant cue in the task we gave the mantis shrimp, and therefore may have overshadowed the learning of the color of the target. On the other hand, while color recognition did not reach significance in these experiments, animals selected the color to which they had been trained over 60% of the time in our color recognition test and have significantly learned to recognize and discriminate color in the past (Marshall et al., 1996). These results suggest that further examination of the relevance of color in object recognition is warranted. For example, when shapes are similar, color may become more important in discriminating them.

Most mantis shrimp possess fabulously elaborate color vision systems. While color in our study did not seem to be important for object recognition, mantis shrimp are likely to use color discrimination for other specific tasks. Many mantis shrimp have colorful body surfaces, some of which are used for signaling (Hazlett, 1979; Cheroske et al., 2009; Chiou et al., 2010; Franklin et al., 2019). Due to mantis shrimps' powerful weaponry and aggressive territoriality, signaling intent may be an important way to circumvent a potentially fatal encounter. Many mantis shrimp species possess colorful signals on the inner sides of their raptorial appendages termed meral spots. The colors of these spots often are distinct in coexisting species.

Since multiple stomatopod species are often found occupying the same reef patches, the color of signals such as these meral spots might be useful for species recognition when identifying conspecifics. Color vision might also have other functions for mantis shrimp such as contrast enhancement when hunting and/or avoiding predators at shallow depths (Cronin et al., 2014c). Carl von Hess (1913) and Karl von Frisch (1914), early researchers studying color vision in honeybees, disagreed about the abilities of these animals to discriminate color (even though across the Atlantic, Charles Turner (1910) had already demonstrated that honeybees possessed color vision). The disagreement arose because the researchers chose different behavioral contexts in their studies. We now know that bees use color for nest and flower identification (the contexts in which Turner and von Frisch tested color vision), not for escape runs toward light (von Hess's approach; see Menzel & Backhaus, 1989). Similarly, a mantis shrimp's reliance on color vision surely differs depending on the contextually varied situations it encounters.

Methods

Animal Care

Individual *Neogonodactylus oerstedii* collected in the Florida Keys, USA were shipped to the University of Maryland Baltimore County (UMBC). Animals were housed individually in 30 parts per thousand (ppt) sea water at room temperature under a 12:12 light:dark cycle. Animals were fed whiteleg shrimp, *Litopenaeus vannamei*, once per week when food was not acquired during training sessions. 78 individuals (31 males and 47 females) that survived over four weeks in

captivity were used for the study. Testing data were collected from 22 individuals (9 males and 13 females). All individuals were between 30 and 70 mm long from the rostrum to the tip of the telson.

Experimental Apparatus

A y-maze consisting of an entrance arm and two choice arms oriented 90 degrees from one another was constructed out of white acrylic sheets (Figure 5.1). The end of each arm of the y-maze had a hole in the floor, hidden when viewed from a distance. A food reward was placed in either of these holes. The y-maze was placed in a cylindrical tank with an incandescent light source (Sylvania SPOT-GRO® 65W) centered above it. A diffusing filter was rested on the top of arena below the light source. The filter had a centered hole, where the lens of a small video camera was fit to record each trial. Trials were observed from the screen of this camera. Flat targets made of colored, transparent plastic placed on a solid white background were placed at the end of each choice arm. Four targets were used during the experiment: a red rectangle, a green rectangle, a red triangle, and a green triangle. The rectangle and triangle had an angular size (width x height) of $12^\circ \times 4^\circ$ and $9.3^\circ \times 7.8^\circ$ when viewed from the entrance to the choice arm, respectively. Targets were constructed from transparent plastic colored filters cemented to opaque white acrylic sheets (Figures 24D, 25). A cylindrical holding chamber was centered at the far end of the entrance arm. The holding chamber was designed to be rotated on its side by a researcher, allowing an animal placed inside the chamber access to the rest of the y-maze.

Spectrometry

Reflectance measurements of the colored targets were taken in a dark room using an Ocean Optics USB2000 spectrometer connected to a 3 m long, 400 μm diameter, fiber-optic cable. Reflectances were measured from 300 to 700 nm relative to a "Spectralon" white standard using a PX-2 pulsed xenon light source.

Experimental Procedures

Training

Each *N. oerstedii* individual was randomly assigned to be trained to one of the four target color and shape combinations described above. During training trials, the focal target (ex. red triangle) was placed at the end of a randomly chosen arm with food in the chamber at its end as a reward. The target of opposite shape and color (ex. green rectangle) was placed without food at the end of the other arm. A stomatopod was placed in the holding chamber before a trial and allowed five minutes to adjust to its surroundings. After this time, the holding chamber was turned, allowing the animal to enter the arena, initiating the experiment. Once a stomatopod entered the arena, the first choice arm it traveled down was noted once it entered the choice zone of the arm, two-thirds of the length of the arm. Once the food had been found, the experimental animal was allowed five minutes to eat as a reward before being removed from the arena. If the food was not found within 10 minutes, the animal was removed from the arena. Each animal experienced the training procedure twice per week. After each individual training session, the water in the arena was mixed to prevent olfactory cues from influencing the choice of subsequent training sessions.

At the end of each week, the percentage of correct choices each individual made since the start of training was calculated. Individuals entered the testing phase when they had made a correct choice 80% (or greater) of the time during training trials over the previous four weeks, in combination with a 50% (or greater) response rate during that time. Individuals were required to have been trained for at least one month (eight training trials) to be considered for testing.

Testing

Of the 78 animals that were trained, a total of 20 animals achieved the training criterion and moved on to the testing phase. The procedure of the testing phases was identical to that of the training phase except that no food reward was offered during testing sessions. Trained stomatopods were subjected to three distinct tests: a shape recognition test, a color recognition test, and a conflicting cues test (Fig. 5.3). Individuals experienced these tests in a randomized order. Two training sessions were administered between tests to facilitate reward seeking between tests.

1. The Shape Recognition Test

In order to test if *N. oerstedii* could distinguish the shape of the trained target, the cue of the same shape and color as what the individual was trained to was placed at the end of one arm of the Y-maze (ex. red triangle). The cue of the opposite shape and the same color of what was trained to was placed at the end of the other arm (ex. red rectangle). A correct choice was recorded if

the stomatopod chose the arm displaying the cue with the trained color and shape.

2. The Color Recognition Test

In order to test if *N. oerstedii* could distinguish the color of the trained target, the cue of the same shape and color as what the individual was trained to was placed at the end of one arm of the Y-maze (ex. red triangle). The cue of the same shape and the opposite color of what was trained to was placed at the end of the other arm (ex. green triangle). A correct choice was recorded if the stomatopod chose the arm displaying the cue with the trained color and shape.

3. The Conflicting Cues Test

In order to test if *N. oerstedii* relied more on the shape or color of a target when recognizing it, the cue of the same shape and opposite color as what the individual was trained to was placed at the end of one arm of the Y-maze (ex. green triangle). The cue of the opposite shape and the same color of what the animal was trained to was placed at the end of the other arm (ex. red rectangle). Neither cue was of the identical shape and color combination to the one to which the animal was trained to recognize.

Statistical Analyses

All statistical analyses were run on R (v3.3.1, R Core Development Team 2016) with the “car”, “glmer”, and “lme4” plugins.

A Pearson’s correlation test was used to compare the proportion of correct choices made during training sessions over time.

Generalized linear mixed modelling was used to analyze the data for each of the three tests. Our models used animal choices during testing as the variable of interest, specifying a binomial error distribution (link function "logit"). Since individual stomatopods were tested more than once, the models for each test included individual ID as a random term. Since we used both males and females for our study, sex was also included as a random term for our full models; however, since sex did not significantly increase the explanatory power of our models, it was removed from our final models. Individual ID did not significantly increase the explanatory power of our models, but was left in the final models to account for repeated measures (Table 1).

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Author Contributions: R.N.P. designed the research, analyzed the data, and prepared the manuscript. V.K, L.A., H.D., T.G., S.P, O.P.R., A.S, and B.S collected the data. V.K. oversaw data collection and managed the data. T.W.C. provided guidance and research support.

Competing Interests: The authors declare no competing financial interests.

Data and Materials Availability: The data that support the findings of this study are available from the corresponding author upon reasonable request. Correspondence and requests for materials should be addressed to R.N.P. (e-mail:

telrickp@gmail.com).

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Chapter 6: Future Directions

The research presented in this dissertation leads to several promising new directions of investigation. I describe a few of these future research directions here.

Odometry in Mantis Shrimp

Path integration requires an animal to possess a biological odometer with which to measure the distances it travels from a reference point. Mantis shrimp both walk and swim while foraging. Thus, it is unlikely that these animals use a step integration mechanism animals like Cataglyphid desert ants (Wittlinger et al., 2007) and some fiddler crabs (Walls and Layne, 2009) use as an odometer while navigating. Rather, it is likely that stomatopods use another mechanism, such as biological flow meters to measure water resistance against the body or translational optic flow, as do honeybees (Srinivasan et al., 1997), to monitor distances travelled. Below, I present preliminary work and proposed experiments to test these hypotheses.

Antennal Scale Flow Meters

Stomatopods possess large flat structures with radial hairs modified from a portion of their antenna called antennal scales. These structures lie perpendicular to the body axis with one scale on each side of the body (Figure 21A). Due to the antennal scale shape and their location on the body, I hypothesized that these structures were used as flow meters for estimating either translational or angular displacements.

In preliminary experiments, the antennal scales of two *N. oerstedii* individuals were physically ablated and these animals were placed in the outdoor arenas used in the experiments described in Chapter 2. If *N. oerstedii* use their antennal scales as flowmeters to inform their odometer sense, homeward paths were predicted to be less accurately oriented and homeward path lengths were predicted to be less similar to the beeline distance from the location of the food to the burrow compared to individuals with their antennal scales intact. When the individuals with ablated antennal scales were tested, their homing behavior appeared to be unaffected by the ablations (Figure 6.1). In light of these preliminary observations, *N. oerstedii* do not appear to solely rely on antennal scale input to inform their odometer sense.

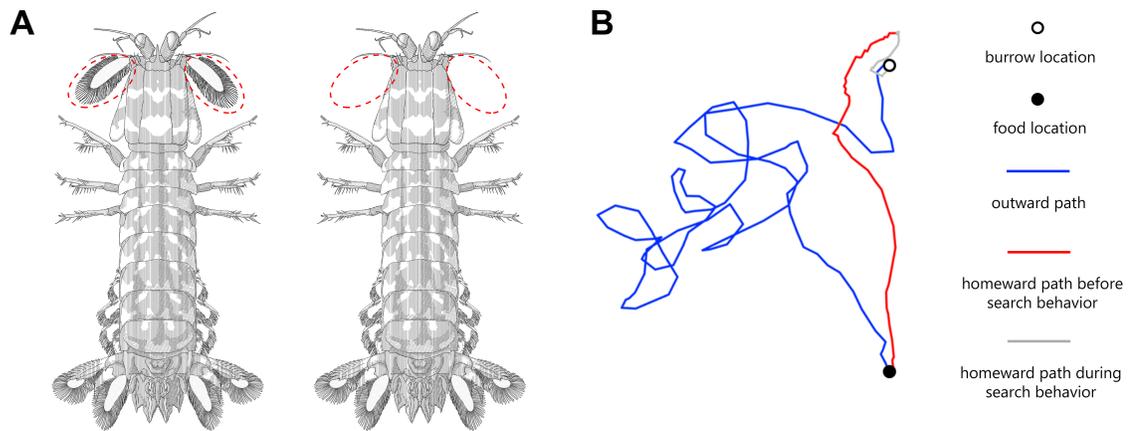


Figure 6.1. *Neogonodactylus oerstedii* does not exclusively rely on its antennal scales for odometry. **A.** *Neogonodactylus oerstedii* with its antennal scales in place (left) and ablated (right). **B.** Tracing of an outward and homeward foraging path of a *N. oerstedii* individual with ablated antennal scales. *N. oerstedii* are able to perform path integration with ablated antennal scales, indicating that antennal scales are not solely relied upon for odometry. Empty and filled circles represent the location of the burrow and food, respectively. The blue, red, and grey tracings represent the outward path, homeward path before a search behavior was initiated, and search path, respectively.

Optic Flow Odometer

Optic flow is the phenomenon by which the world appears to move across the retina when an animal moves in space. This optical effect can be used by animals, such as honeybees (Srinivasan et al., 1997) and desert ants (Pfeffer and Wittlinger, 2016), to estimate their displacement in space.

In the indoor polarization orientation experiments described in Chapter 2, homeward paths lengths under indoor conditions were much less precise compared to homeward paths initiated outdoors ($p = 0.02$, indoor $n=45$, outdoor $n=23$; Figure 2.10). Since the same arenas were used in both the outdoor rotating platform experiments and the indoor polarization experiments, it can be concluded that differences in the light environment between the two sets of experiments (the indoor experimental setups are much dimmer than the outdoor ones, even under heavily overcast skies) are likely responsible for the decreased precision of homeward path lengths. These results indicate that odometry in *N. oerstedii* may be visually informed.

The following experiments offer a few potential ways to test if *N. oerstedii* rely on ventral optic flow as an odometer using indoor arenas similar to those used in the polarization orientation experiments in Chapter 2. These proposed experiments take advantage of the relatively straight paths *N. oerstedii* make from food placed in the center of the arenas. In these experiments, patterns can be projected on the base of a homogenous indoor arena (Figure 6.2A). The first projected pattern consists of concentric black and white rings radiating from the center of the arena (Figure 6.2B). Once animals find food placed in the center of the arena, these concentric rings

should be animated to move either inwards toward the center of the arena, outwards towards the periphery of the arena, or to remain stationary as a control. If *N. oerstedii* rely on ventral optic flow for odometry, it can be predicted that when the concentric ring pattern is animated to move inwards towards the center of the arena, animals will undershoot the distance traveled to the burrow, initiating the search behavior before they would have during control trials. Similarly, it can be predicted that under conditions in which the concentric rings are animated to move outwards, animals will consistently overshoot their burrows.

In a further test, a randomized checked black and white pattern can be projected on the base of the arena (Figure 6.2C). In test trials, during an animal's outward path, this projected image should continuously be in motion in arbitrary directions, swapping the direction of movement, until the animal has found the food location, at which time the image will become stationary. During control trials, the projected image should remain stationary during the entire trial. Under these conditions, if *N. oerstedii* rely on ventral optic flow as an odometer, it can be predicted that both the orientation and length of home vectors during test trials should differ from control trials.

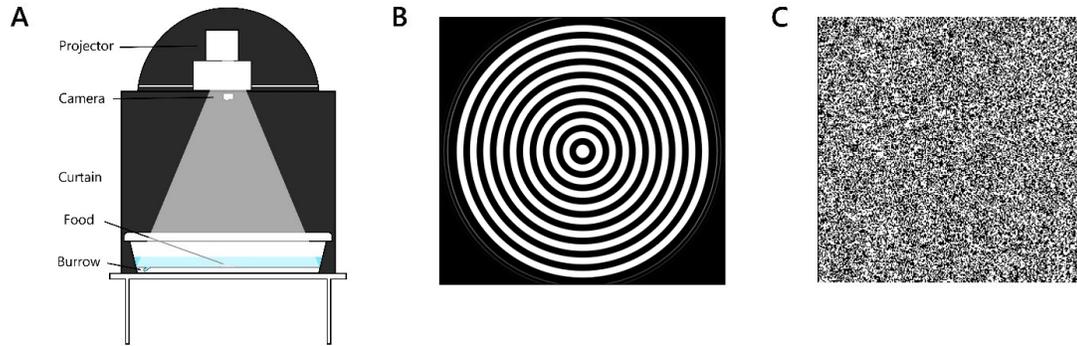


Figure 6.2. Proposed experimental design for testing odometry using optic flow in *Neogonodactylus oerstedii*. A. Experimental design in which flow fields are projected onto the base of an arena. Two projected patterns are proposed, a B. concentric ring pattern and a C. randomly checked pattern.

Navigation Behaviors of Mantis Shrimp Occupying Different Environments

Mantis shrimp occupy a wide variety of marine habitats, from clear tropical reefs to relatively silty mud flats. Celestial cues viewed through the air-water interface, Snell's window, may be prominent in calm, shallow water, but are increasingly obscured with depth, turbidity, and increased wave action. Even though *N. oerstedii*, which mostly occupy shallow tropical waters, appear to primarily rely on celestial cues for orientation, compass cue preferences may differ for deeper water stomatopod species that inhabit rougher, turbid waters. Further, stomatopods which occupy landmark rich environments may weigh the importance of landmarks during navigation more heavily than stomatopods which occupy benthic environments relatively void of landmarks. Examining the navigation behaviors of stomatopod species which occupy different environments may uncover differences in the hierarchies of orientation cues they rely on and their weighting of the importance of different navigational strategies.

Further Investigation of Orientation Cues in Mantis Shrimp

In Chapter 2, I demonstrated that *N. oerstedii* use the sun, celestial polarization patterns, and an idiothetic compass for orientation. In the future, the basis of the idiothetic compass of *N. oerstedii* should be investigated. During the indoor polarization orientation experiments described in Chapter 2, homeward paths under indoor conditions in the absence of an overhead polarization pattern were very weakly or not significantly oriented (Figure 2.5). However, homewards paths were well oriented outdoors under heavily overcast skies (Figure 2.3). In light of these results, I hypothesize that the idiothetic compass of *N. oerstedii* may be informed by rotational optic flow.

Further, *N. oerstedii* may use cues other than those tested for orientation. Additional celestial cues, such as celestial luminosity and spectral gradients may be used in concert with celestial polarization patterns for orientation, something that has been observed in other animals (Rossel and Wehner, 1984; Ugolini et al., 2009; el Jundi et al., 2015; el Jundi et al., 2016).

For stomatopods which occupy deeper and/or more turbid waters where celestial cues are less reliably observed, other allothetic orientation cues may primarily be relied upon for orientation. The Earth's magnetic field, a cue available throughout the water column, is known to be particularly useful for marine navigators (Lohmann and Willows, 1987; Lohmann, 1991; Boles and Lohmann, 2003) and may be used by stomatopods for orientation when celestial cues are unreliable. Further, horizontally-viewed submarine polarization patterns are observable and can be used for orientation (Waterman and Westell, 1956; Waterman, 1989; Lerner et al., 2011);

however, no animal has yet been shown to use this cue when orienting. These orientation cues may also be used by *N. oerstedii* in addition to celestial and idiothetic information.

Investigation of a Dorsal Rim Area in Mantis Shrimp Eyes

Celestial polarization patterns are used by many insects for orientation. The dorsal-most portion of many insect eyes possess multiple adaptations for detecting celestial polarization patterns, including orthogonally arranged rhabdoms with polarization sensitivity and optical units with large acceptance angles (Labhart and Meyer, 1999). Recently, Porter et al. (2020) reported that the dorsal-most portion of the retina of *N. oerstedii* expresses unique opsin transcripts from the rest of the eye. These data, paired with the behavioral evidence of overhead polarization orientation I present in Chapter 2, suggest that, as in insects, a dorsal rim area adapted for viewing celestial polarization patterns might be present in the eyes of mantis shrimp as well.

Stomatopod Eye Movements During Locomotion

In addition to the complex physiology of most stomatopod retinas, stomatopod visual systems incorporate several types of eye movements, bestowing these animals an appearance of curiosity and visual awareness not typically associated with crustaceans. These movements include smooth and saccadic tracking, scanning, large-field optokinetic stabilization, and acquisitional saccades along three axes. Strangely, stomatopods are capable of performing these eye movements with

each eye independent of one another and often switch from one movement to another (Marshall et al., 2014).

The extreme mobility of stomatopod eyes complicates spatial orientation and raises the question of how a stomatopod's movements can be related to a stimulus' position on the eye, since this will change depending on the eye's position (Land et al., 1990). This may present a problem, especially since stomatopods appear to use visual cues during navigation. In hymenopterans, the polarization compass is located in the dorsal rim of eyes fixed in a single position on the head, where polarization-sensitive photoreceptors are spread out in a fanlike distribution over 180 degrees (Wehner, 2003). Therefore, any given celestial polarization pattern evokes a characteristic response pattern in the eyes that depends on the angle between the animal's longitudinal axis and the sun's altitude and azimuth (Waterman, 1989). Movement of the eyes could make such a compass ineffective, throwing an animal's path integration system in disarray. Due to the visually based orientation systems which have been found to be used by stomatopods from the above experiments, the eye movements made during an animal's foraging expeditions should be investigated.

Further Examination of Landmark Navigation in Stomatopods

In the experiments described in Chapter 4, landmarks were only used in the most obvious of contexts: as a beacon to home towards. In the future, it will be interesting to investigate how else stomatopods can relate a goal to a landmark array when navigating. Other organisms, such as desert ants, relate a goal to an array of landmarks by retinal image matching, where an animal matches the visual field while

navigating to a stored memory snapshot of the visual field at a goal in order to locate the spatial position that memory was stored (Akesson and Wehner, 1997; Figure 6.3).

Animals may also encounter landmarks along routes to goals, remembering the sequence of these landmarks in order to navigate effectively between familiar locations (Reese, 1989; Thomson 1995). In nature, stomatopods are likely to possess a spatial memory of familiar landmarks within the local vicinity of their burrows and may navigate between these landmarks to reach known goals. Observations of stomatopod behavior in the field are key to understanding the capacity of a stomatopod’s utilization of landmarks during navigation.

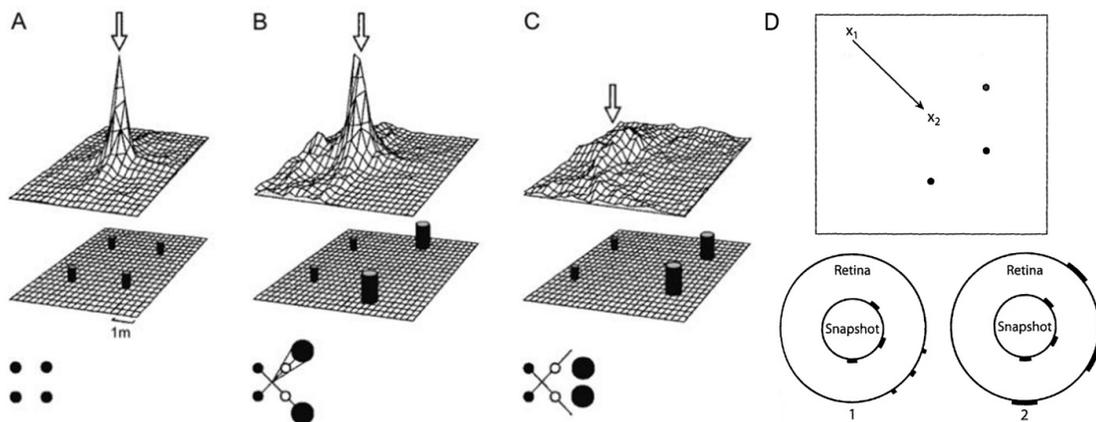


Figure 6.3. Retinal image matching in the desert ant, *Cataglyphis fortis*. Search density distributions (upper row) of ants trained to the center of a 4-cylinder landmark array and tested **A**. within the training array and **B**, **C**. within altered arrays. In **B** the retinal image perceived at the goal is identical with the one in the training situation, but in **C** it is not. Arrows point at the positions at which the view best matches the stored view perceived at the trained goal. **D**. At x_1 , the view of the landmarks does not match the memory “snapshot” to that at the goal (labeled 1). Ants will continue to move towards the landmarks until the view of the landmarks best match the memory “snapshot” at x_2 (labeled 2). **A**, **B**, **C**. From Wehner (2003). **D**. From Cronin et al. (2014).

Stomatopod Landmark Learning Walks

When animals that use landmarks during navigation, such as wasps (Collett and Lehrer, 1993; Ziel, 1993), bees (Leher, 1993), and ants (Fleischmann et al., 2018), leave their nests for the first few times, they make characteristic movement patterns where the local landmark array is learned, termed learning walks or flights. These learning flights in wasps consist of arcing trajectories where the wasp, newly emerged from her nest, continually faces the nest at various distances, presumably familiarizing herself with the landmark array surrounding her nest from multiple points of view (Collett and Lehrer, 1993; Zeil, 1993; Figure 6.4). Similarly, desert ants which have just left the nest will conduct walks of various lengths and will look back at the direction of the nest in a punctuated fashion throughout the walk. Interestingly, the orientation of lookbacks in desert ants are informed by a magnetic compass, a cue that does not influence the path integrator during foraging (Fleischmann et al., 2018).

I have not observed learning walks directly akin to those seen in wasps or ants in foraging stomatopods during my experiments. However, I have noticed that stomatopods during my experiments will often stick their heads out of their burrows in different directions before pulling their heads back into their holes. Eventually, they will leave their burrows for short distances in various directions, before quickly returning to their homes. I have noticed in at least a few instances that these excursions tend to get longer over time and eventually, that the animals will look for food placed in the arena. Future work should characterize these behaviors and use manipulations of landmark arrays placed around the burrow during the period

stomatopods conduct these behaviors to determine if these behaviors are crucial for landmark learning.

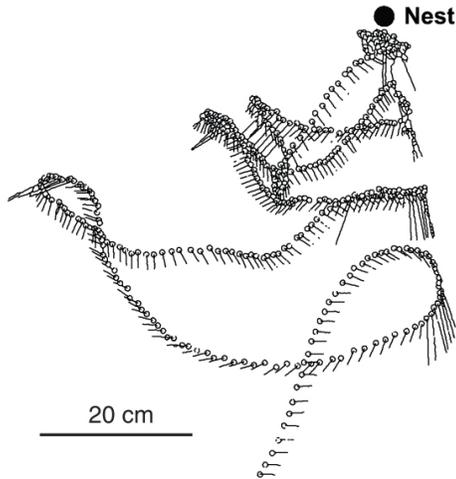


Figure 6.4. Overhead view of the learning flight of the wasp, *Vespula vulgaris*. Body orientations are indicated, where circles mark the head of the animal and tailing lines represent the body relative to the head. Longer tail lines mark when the wasp was looking at its nest. From Collett and Lehrer, 1993.

The Neural Basis of Navigation in Mantis Shrimp

Path-integration behaviors of insects, such as bees and ants, and those of mantis shrimp described here share striking similarities (Figure 6.5A-C). In insects, a highly conserved region of the brain called the central complex has been implicated to have a major role in the neural basis of orientation and path integration (Seeling and Jayaraman, 2015; elJundi et al., 2015; Stone et al., 2017; Heinze, 2017; Giraldo et al., 2018). The central complex of the stomatopod brain has recently been anatomically described and is similar in organization to the central complexes of many distantly related insects (Thoen et al., 2017; Figure 6.5E).

Further, mushroom bodies, centers for arthropod learning and memory, are thought to play a major role in landmark learning in insects (Mizunami et al., 1998;

Heisenberg, 2003; Lutz and Robinson, 2013; Stieb et al., 2010). Prominent hemiellipsoid bodies, homologues of insect mushroom bodies, exist in stomatopod eyestalks (Wolff et al., 2017). As in insects, these neuropils may be crucial for navigation and landmark learning in mantis shrimp.

Insects and malacostracan crustaceans, including stomatopods, are thought to last share a common ancestor over 500 million years ago (Regier et al., 2005; Figure 6.5D). Comparative investigations of the functions of neuropils within stomatopod central complexes and hemiellipsoid bodies could help uncover the evolutionary origins of navigation behaviors and the neural architecture of the central nervous system within arthropods.

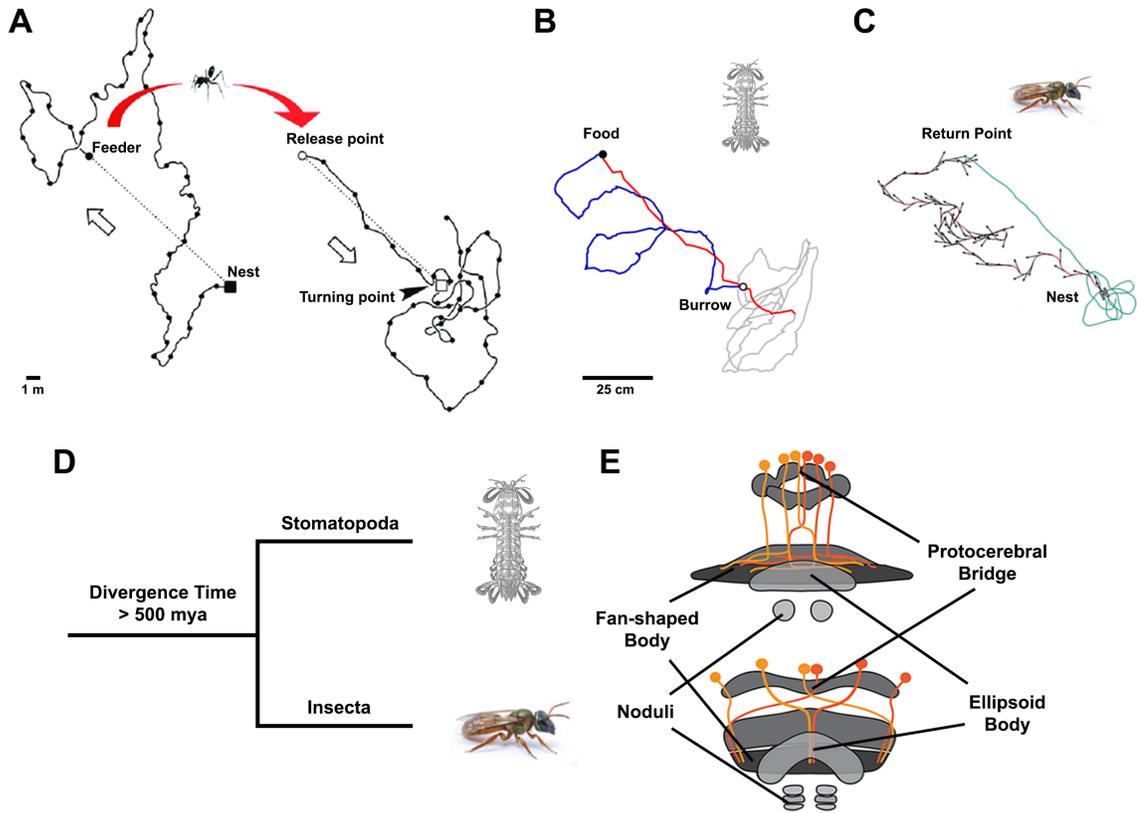


Figure 6.5. Mantis shrimp and insects exhibit similar navigational behaviors and possess similar central brain neuropils. Tracings of path integration behaviors in **A.** the desert ant, *Cataglyphis fortis* and **B.** the mantis shrimp, *Neogonodactylus oerstedii*. **C.** A simulation of path integration generated by a model that was anatomically constrained by the neural circuits present in the central complex of the sweat bee, *Megalopta genalis*. Note the similarities in these three path patterns. **D.** Stomatopods and insects diverged over 500 million years ago (Reiger et al., 2005). **E.** The central complexes of stomatopods and insects share many neuropils, making them uniquely similar among the Pancrustacea. **A.** From Wehner and Wehner (1986). **C.** Repurposed from Stone et al. (2017). **E.** Adapted from Thoen et al. (2017).

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Chapter 7: Conclusion

In conclusion, the research presented here describes the navigational strategies and sensory cues stomatopod crustaceans use for navigation and orientation for the first time.

First, by laterally displacing foraging stomatopods in large, seminatural arenas, I demonstrated that the mantis shrimp, *Neogonodactylus oerstedii*, uses path integration to navigate home, making them the first fully aquatic path-integrating animals yet discovered. Next, by passively rotating stomatopods during foraging, I found that they use celestial and idiothetic (internal) orientation cues during path integration. By manipulating the apparent position of the sun and by rotating overhead polarization patterns while animals were foraging, I demonstrated that *N. oerstedii* hierarchically rely on these cues when orienting.

During these experiments, I found that path integration in *N. oerstedii* was prone to error proportional to error accumulated over the course of outward foraging paths. To combat this error inherent in path integration, stomatopods enacted stereotyped search patterns when path integration did not lead them directly to their burrows. I found that this search behavior forms non-oriented loops that are centered near the point of search initiation and that these loops continuously expand at a rate predicted by optimal search theory. Further, the radius of this search appeared to be scaled to the animal's accumulated error during path integration, improving the effectiveness of the search.

Next, by comparing homeward paths in the presence and absence of a landmark placed near the burrow and by displacing the landmark to an alternate location while animals were foraging, I showed that stomatopods navigate using landmarks in parallel with their path integration system, offsetting error generated when navigating using solely path integration. Finally, working with a team of undergraduate researchers, I show that mantis shrimp rank the shape over the color of an object when learning and recognizing it, suggesting that the relative importance of these visual modalities to a mantis shrimp likely holds true for landmark recognition as well. These experiments uncover the robust navigational toolkit *N. oerstedii* relies upon find to home.

Navigation behaviors are fundamental skills that allow animals to interact with the spatially and temporally dynamic environments in which they live. Due to their importance, the successful acquisition of stimuli useful in navigational behaviors is a significant pressure on the evolution of sensory systems and the neural systems used to process the sensory information they impart (an excellent example of this is the convergence of a dorsal rim area in the eyes of many phylogenetically dispersed insects, which are specifically adapted for detecting celestial polarization information for use as a compass (Labhart and Meyer, 1999)). Therefore, by studying the navigation behavior of stomatopod crustaceans, insights to the ecological relevance of their impressive visual physiology may be gained, potentially uncovering possible selective pressures which may have influenced the evolution of some of the bewildering retinal complexity stomatopods possess. Examining how the sensory cues informing *Neogonodactylus*' path integration system compare to those used by

its well-studied terrestrial counterparts will give some insight on how navigational problems are evolutionarily solved in disparate environments with their own unique properties and challenges to overcome. Further, mantis shrimp occupy a wide variety of marine habitats and depths, from structurally complex reefs to relatively featureless mud flats. Sensory cue preferences for orientation and odometry during path integration and the relative importance of landmark navigation and path integration may differ for stomatopod species that inhabit waters of varied depths, turbidities, and habitat structures. Finally, this work opens an avenue towards the study of the neural basis of navigation in stomatopods, where insights into the evolution of arthropod brain structures and the navigational strategies they manifest may be uncovered.

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