Homing behavior following shelter displacement in two crayfishes, *Creaserinus fodiens* (Cottle, 1863) and *Faxonius rusticus* (Girard, 1852) (Decapoda: Astacidea: Cambaridae)

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ABSTRACT

Crayfishes are an excellent model for examining complex behaviors such as homing. We examined homing behaviors in two species of crayfish. Individuals of *Creaserinus fodiens* (Cottle, 1863), a primary burrower, and *Faxonius rusticus* (Girard, 1852), a tertiary burrower, were placed in an arena with artificial burrows embedded within a rotating disk. The disks allowed for the displacement of burrows by 90° relative to the original location. Individuals were motivated to perform search behavior with the presence of food. The burrows were displaced once individuals had left the burrows and their return pathways were examined. The paths were digitized and analyzed for kinematic parameters. Crayfishes in the control group located the burrows successfully but individuals in the experimental group (displaced burrow) were unable to locate the burrows successfully. There were significant differences (*P* = 0.001) between the control and treatment groups in heading angles relative to burrow on the return trip, with the heading angles of the control group being significantly lower (*P* = 0.0003). There were species-specific differences in velocity toward the burrow, with the primary burrower being more negatively impacted by the displacement. The results confirm that the effect of burrow displacements severely impacts homing success in both species and a local cue associated with the burrow is not utilized in homing. We demonstrate that there are no significant species-specific differences in homing behavior.

Key Words: burrowing, path integration, spatial learning

INTRODUCTION

An essential behavioral adaptation of many animals is the ability to move between resources. Such adaptations allow leaving a home location, such as a burrow or shelter, for foraging or searching for mates and subsequently returning to their home location (Fagan et al., 2013; Wiener et al., 2011; Geva-Sagiv et al., 2015). The ability to move successfully by navigating between resources can have a profound impact on the survival of any animal (Akesson & Henderstrom, 2007; Fagan et al., 2013). Failure to return to a home location could result in an increased risk of predation (White & Brown, 2013). The inability to return to rich foraging grounds can limit growth and survival by limiting access to the resources that would promote the animal’s growth and fecundity, as healthier individuals would be more likely selected as mates (Piper, 2011). To perform these behaviors, animals exhibit a large range of strategies which are reflected by the scale of the behavior and the sources of information used to navigate. Many of these differences are shaped, in part, by the complexity of sensory cues and the sensory capabilities of the animal in question. The sensory landscape contains the information which will dictate what cues are available for orientation (Jurck & Moore, 2014).

Navigational strategies can be categorized, in part, by the scale of movement and, in part, by the sources of information used to move through the environment. As a subset of navigation, homing...
refers to a goal-oriented behavior in which the animal returns to a previously known location and path integration is a type of homing strategy (Papi, 1992). Within path integration, a home vector is constantly being updated based on internal information about the distance and direction traveled on the outbound journey. The home vector is an estimation of the animal's distance and direction relative to the starting point (Müller & Wehner, 1988; Collett & Collett, 2000; Wehner & Srinivasan, 2005). One of the hallmarks of path integration is the accumulation of errors. Any error that the animal makes in estimating distance or direction is carried forward throughout every subsequent calculation of a new home vector. Thus, the error associated with the home vector continues to increase in size as each recalculation error accumulates. Animals can be experimentally manipulated to accumulate errors by causing them to slip, or by introducing artificial structures that were not present on the outward journey (Walls & Layne, 2009). To determine whether animals are navigating using path integration, one can either displace or rotate the animal in space (relative to environmental cues) to induce errors. The amount of induced displacement or rotation can demonstrate whether the animal overshoots or undershoots the goal by the amount of the induced error.

Research in semi-terrestrial arthropods over the past two decades has focused on examining the mechanisms of homing in species such as fiddler crabs (Layne et al., 2003a, b). While the work has focused on the use of visual cues in certain species of fiddler crabs, there is evidence highlighting the use of path integration in other arthropods. Researchers have most recently proposed that dual mechanisms (path integration and landmark guidance) can be used simultaneously, particularly when one system fails (Cheng et al., 2014; Murakami et al., 2017). Some animals can accumulate considerable home vector errors during homing as a consequence of engaging in mating behaviors or fighting with conspecifics. As a result of these conspecific interactions, animals may rely on other sources of information such as landmarks. The construction of structures such as “chimneys” around a burrow by males has shown to play a role in courtship behavior as well as functioning as beacons associated with the burrow (Ribeiro et al., 2006; Kim et al., 2010; Kim & Christy, 2015). Results from work in fiddler crabs has shown that when rotated on a platform (as part of the experimental setup) the crabs attempt to counteract or compensate for this turning by using visual cues (Layne et al., 2003b). This type of compensation indicates the use of an external cue as a compass reference. Thus, there may be species-specific differences in homing strategies even within closely related species.

Crayfishes have proven to be an excellent model for examining the underlying homing behaviors for several reasons. They have rich behavioral repertoires, ranging from spatial learning to complex social hierarchies, that have been well documented. Crayfishes also possess relatively simple nervous systems that have also been well studied. Despite studies demonstrating their spatial learning abilities, examination of homing behaviors in crayfishes has been largely absent from the literature. Kamran & Moore (2015) demonstrated homing to artificially constructed burrows by two species of crayfishes; one a primary burrower and the other a tertiary burrower. Primary burrowers construct elaborate burrows within which crayfish reside most of their lives and do not have to be connected to water. Secondary burrowers have less complex burrows that are often open or connected to sources of water. Tertiary burrowers are species that while possessing the ability to construct burrows, will spend the majority of their life in open water. In some cases, tertiary burrowing crayfish species have been found to occupy burrows constructed by other burrowing crayfish species seasonally (Grow & Merchant, 1980; Hobbs, 1981). One could hypothesize that primarily burrowing crayfishes may have enhanced homing abilities for returning to the locations of burrows given the energy investment in construction of burrows. Secondary and tertiary burrowers have significantly less energy and time investments into burrow construction and thus may have homing abilities that are less robust than primary burrowers. This would allow us to further examine possible differences in homing behaviors between two species of crayfish of varying burrowing categories.

The purpose of this study was to examine in more detail the possible mechanism of homing in Creaserinus fodiens (Cottle, 1863), a primary burrower, and Faxonius rutilus (Girard, 1852), a tertiary burrower, and to determine if there are differences between primary and tertiary burrowing species. Primary burrowers construct burrows where it resides while tertiary burrowers utilize burrows constructed by other species, if at all. There are two components needed to determine a home vector in path integration - one is an estimation of the distance traveled to or from the goal and the second is the direction component. The experimental design of this study allowed for manipulation of one of these two factors used to calculate the home vector, by displacing the burrow 90° by rotating the disk in which the burrow is embedded. If crayfish are utilizing a mechanism alternate to path integration, one that utilizes a cue directly associated with the burrow, crayfish in the treatment group (where the burrow is displaced) will be able to locate the burrow successfully. These alternative mechanisms could include the use of landmarks (such as burrow hoods) or homing to chemical cues left in the burrow. If crayfish return to the original location of the burrows prior to the displacement, this result supplies more evidence for path integration as a possible mechanism for homing.

**MATERIALS AND METHODS**

**Experimental animals**

Individuals of *Faxonius rutilus* were collected from the Portage River, Wood County, Ohio, USA (41.361398°N, -83.591038°W) during spring and summer 2015. *Creaserinus fodiens* individuals were collected from two local unnamed ponds (41.355585°N, -83.862049°W and 41.395994°N, -83.627857°W), also in Wood County, Ohio during spring and summer 2015. The length of the carapace (2.06 ± 0.09 cm in *F. fodiens*, 2.91 ± 0.54 cm in *F. rutilus*) and length of the chela (1.65 ± 0.09 cm in *C. fodiens*, 2.00 ± 0.05 cm in *F. rutilus*) were recorded. Reproductive and non-reproductive male and females of both species were used in trials. Both species of crayfish were housed in individual flow-through plastic containers to ensure visual and mechanical isolation and were kept on a 12 h:12 h light:dark cycle. All individuals were fed three times weekly with commercial-size rabbit food pellets (Forti-Diet, Central Point and Pet, Walnut Creek, CA, USA). All crayfishes were food deprived for 10 d prior to the start of the experiment to increase motivation for crayfishes to leave the burrows during the duration of a trial. Individuals were used only once during the trials.

**Experimental arena**

Trials were conducted in one of two identical homing arenas, each 80 cm × 80 cm × 35.5 cm. All arenas were placed in an environmental chamber in which temperature was maintained at 23 ± 0.9 °C and kept on a constant 12 h:12 h light:dark cycle. An elevated floor for each of the arenas compromised of egg grating (1.3 cm) and mesh plastic screening (0.13 × 0.13 cm holes) was raised 17 cm above the base of the tank and supported by PVC pipes. The mesh was covered with a thin (1 cm) layer of gravel to provide traction for crayfishes during homing trials. The inside of the arena was painted white to limit reflection of light (which can cause errors during tracking of individuals) and contrasting visual cues. Previous trials have shown that the paint is not toxic to crayfishes and does not alter their behaviors (Camougis & Hichar, 1959; Jarboe & Romaine, 1995a, b). A black cloth was placed around the arena to limit visual cues from the surrounding area.
HOMING BEHAVIOR IN CRAYFISH

Burrow construction
An artificial burrow was embedded in the raised floor of each arena with the burrow opening at the same level and same color as the substrate and flooring. The burrow was embedded within the floor of the arena to limit the presence of any visual cues associated directly with the burrow itself. The burrow was constructed using a single PVC pipe (inside diameter 2.54 cm) and 10.1 cm deep. The inside of the burrows had sand glued to the PVC pipe to provide traction for crayfishes. The substrate floor was constructed with panels that allowed for a rotating disk which contained the burrow to be embedded in the floor. The presence of the rotating disk allowed for the displacement of the burrow. The circular disk was constructed using Plexiglas with a diameter of 21.0 cm. The disk was placed on a pillar attached to the floor of the arena, allowing the disk to be pivoted at the pillar. The design of the rotating disk was such that the rotation would be strictly 90° and no more (in either direction, depending on the arena). The disk allowed for a 90° rotation around a central point in both clockwise and counterclockwise directions which moved the burrow approximately 8 cm. Preliminary work determined that an 8 cm displacement was found to be sufficient displacement for the size of crayfish used, as this distance ranged from 1–3 body lengths of a crayfish. The disk was also covered with mesh and lined with a thin layer of substrate to ensure that the floor was texturally consistent to remove any visual or tactile cue differences between the disk and rest of the tank. (Supplementary material Figs. S1A, B).

Odor stimulus
We used fish gelatin in each trial to provide motivation for crayfishes to leave their burrows during the trial, only after a crayfish had found the burrows (Willman et al., 1994; Wolf et al., 2004). The gelatin was made using 46 g of homogenized canned sardines in oil blended with 600 ml boiling water and 4 packets of Knox’s unflavored gelatin (E.D. Smith Foods, Winona, ONT, Canada). After a crayfish found the burrow, fish gelatin (2 cm × 2 cm × 1 cm: w x l x h) was placed in a weighted mesh bag and returned to the burrow. Crayfishes were allowed a period of approximately 2 h to leave and return to the burrow after the placement of the fish gelatin. The arena was reset for another trial if the crayfish did not return to the burrow in 4 h. These individuals were eliminated from the data analysis of pathways and did not contribute to that final data set. These trials were included in the suc- cess rates that were analyzed statistically. It is possible that given enough time these crayfishes could have found the burrow by random chance or some other mechanism. Thus, we included only those pathways that were a successful completion of the homing task. This decision resulted in the removal of a single C. fodiens and four F. rusticus individuals from the data set. Homing success was defined as the ability of crayfishes to successfully locate the burrow. These time intervals (both to find burrow and return to the burrow following addition of the fish gelatin) were found to be sufficient based on analysis of previous data for both species. After a crayfish left the burrow, the disk holding the burrow was rotated 90°, thus displacing the burrow in space. For each individual, the direction of rotation whether clockwise or counterclockwise was selected at random. The group in which the disk was rotated and the burrow was subsequently displaced was referred to as the treatment group. For the control treatment, the burrows were displaced and then returned to the original location. All trials were video recorded with an overhead camera (SW PRO 530-4PK; Swann, Melbourne, VIC, Australia) using a SWANN DVR (model SWDVK-1430004, Swann). The trials were recorded at 1 frame rate per second.

Data analysis
A single position on the carapace of a crayfish was marked and tracked throughout the trial. This was obtained from digitized trials (1 frame rate per second) using EthoVision XT 8.5 (Noldus Information Technology, Wageningen, The Netherlands). The trials were digitized at a single point per second using the center of the carapace as a reference point (Kamran & Moore, 2015). For a track to be considered a successful homing pathway, a crayfish must have exited a burrow and returned to the burrow within the allotted time. The start of the pathway was defined as the point when the entire body of a crayfish had completely exited the burrow. The pathway was considered complete (for the purpose of further analysis) when a crayfish returned to the burrow or the original location of the burrow prior to displacement. The resolution used has been used previously to describe the spatial parameters of movement pathways in crayfishes (Moore & Grills, 1999; Moore et al., 2015). The locations of the burrow prior and post displacement were also recorded.

Digitized homing pathways were further analyzed for the previously defined homing parameters (Moore & Grills, 1999; Wolf et al., 2004; Adams et al., 2009; Kamran & Moore, 2015). The distance of a crayfish from the burrow (and new burrow location following displacement) at any given point in time was measured in centimeters. The cumulative distance traveled by a crayfish throughout the trial was also measured in centimeters. The average walking speed was defined as the distance traveled per unit time as measured in cm s⁻¹. The walking speed towards or away from the burrow was measured as the vector change in distance from the burrow per unit time (also measured in cm s⁻¹), a negative sign being indicative of movement away from the burrow.

Changes in walking speed and heading angles could be indicative of exploratory versus goal-directed behaviors (Wallace et al., 2006). The path-turning angle was defined as the angle between the path connecting the burrow to the previous position (time t = −1) to present (t = 0) and the path connecting the present position (t = 0) to the next position (t = +1). The path-turning angle was the angle between two sequential movement vectors, i.e., between vector at t = −1 to vector at time t = 0 and between vector at t = 0 and t = +1. This angle was measured in degrees (Supplementary material Fig. S2).

The heading angle to the burrow opening was also measured. This angle was defined as the angle between the path connecting the burrow at the current position (t = 0) of a crayfish and the path connecting the current position (t = 0) to the next (t = +1) position, with an angle of 0° pointing directly at the burrow. These angles were measured to determine the path and orientation of a crayfish relative to the burrow. The net to gross ratio was described as the straight-line distance from the start to end of a path divided by the total path length. This ratio was used to determine the circuitousness of the path; a completely straight path would have a net to gross ratio of 1. The distance and heading...
angles from the initial location of the burrow (prior to rotation) and post-rotation were both recorded.

The homing pathway of crayfishes was divided into the outward and return journeys (Supplementary material Fig S3). The return journey was defined as having begun when a crayfish had ten consecutive points where the distance to the burrow decreased. This definition was used as a benchmark across trials to provide a starting point for the return pathway. Only the return pathways were analyzed since these pathways would be more telling in terms of determining differences in homing behaviors between the species and across treatment groups. Fifteen and fourteen individuals created pathways for F. rusticus and C. fodiens, respectively, for the treatment groups were analyzed. Fifteen pathways for each of the species in the control group were analyzed. Out of the treatment group, the C. fodiens data contained seven males and seven females for the treatments and seven males and eight females for the controls. For the F. rusticus data set, six males and nine females were used in the treatment groups and eight males and seven females were used in the control groups. The clockwise and counterclockwise rotations were evenly split among the treatments and species.

To analyze differences between the two species as well as any differences among treatments for homing success, data were analyzed using a modified Chi-square analysis followed by a Tukey multiple proportions contingency table (Zar, 2007). A non-linear mixed model in R (R Core Team, 2018; Bates et al., 2015) was used for non-binary data such as walking speed. The behavioral variables model was initially constructed with full interactions using four fixed factors each with two conditions (Species, Treatment [rotated or control], Sex [M or F], and Journey [outbound or inbound]) and a single random factor (homing tank). Initial models found no significant difference due to sex of either species of crayfish (F(1,99) = 0.199, P = 0.66) or interactions between sex and any of the other variables (F(1,99) = 1.2, P = 0.26, sex and species; F(1,99) = 0.12, P = 0.73, sex and treatment; F(1,99) = 0.07, P = 0.79). Thus, sex was dropped as a fixed factor in subsequent analysis. When significant differences were found with the interaction terms, differential contrasts were used with a Tukey-HSD post-hoc test to determine where significant differences existed (R Core Team, 2018; Hothorn et al., 2008).

A Watson-Williams Test for homogeneity of the means was conducted for the circular data (mean heading angles relative to burrow). The Rayleigh test of significance of the mean angle was utilized when analyzing heading angles relative to the burrow.

RESULTS

Homing success

Four out of seventeen and one out of fifteen individuals of F. rusticus and C. fodiens, respectively, were able to find the burrow following displacement as opposed to returning to the original location of burrow prior displacement. The remainder of the individuals (N = 13 out of 17 for F. rusticus and N = 14 out of 15 for C. fodiens) returned to the original location of the burrow prior to the displacement. Results were determined from video-recorded movements of individuals with their paths digitized. This finding is consistent with the hypothesis that crayfishes are not relying on local cues directly associated with the burrow, for if they were they would be able to find the burrow following displacement. There were no significant differences between homing success rate between species. Fourteen out of sixteen individuals of both species in the control groups returned to the burrow (Fig. 1). There were no significant differences between the two species in control groups, nor were there any species-specific differences in the treatment group (Tukey Multiple Proportions: Q4,4 = 1.75, P > 0.05). There were significant differences between the treatment and control groups (Tukey Multiple Proportions: Q4,4 = 1.98, P < 0.05; χ2(0.05) = 43.87; P < 0.05).

Figure 1. The homing success rates as defined by the proportion of individuals of Cambarus fodiens and Faxonius rusticus within a group that were able to successfully find a burrow. Homing success for the rotated treatement are in black bars, controls in white bars. N = 15 for all treatments except for F. rusticus rotation group, where N = 17. Bars with different capital letters are statistically different (Tukey-HSD, P < 0.05).

Heading angle

There were significant differences between the heading angles towards the burrow between treatment and control groups, with heading angles of individuals of both species in the group undergoing burrow displacements being significantly higher than those in the control groups undergoing no burrow displacements (F(1,99) = 6.55, P = 0.001). The mean heading angle towards the burrow on the return journey for the treatment groups for F. rusticus (38°) were not significantly different than those of C. fodiens (36.6°). Similarly, this trend was observed when comparing the heading angles of the control groups of both species, with F. rusticus (20.4°) being similar to those of C. fodiens (17.6°). The interaction between species and treatment was also significant (F(1,99) = 4.02, P = 0.047). The heading angles are consistent with the prediction that there is an effect of the displacement of the burrow in the treatment groups as compared to the controls, with control groups having lower heading angles (Rayleigh test of significance, P < 0.05). There were no species-specific differences in heading angles (Fig. 2). There was no significant effect across species, both species in the control groups had lower heading angles which are implicit of goal-oriented behavior whereas in the treatment group, both species had greater heading angles (Rayleigh test of significance, P > 0.05).

Walking speed relative to burrow

We found significant differences in walking speeds relative to burrow between individuals of both species in the control group when compared to the burrow displaced group (Fig 3) (F(1,99) = 11.1, P = 0.001). The mean walking speed relative to burrow for the rotated treatment group for F. rusticus was 0.79 ± 0.20 cm s⁻¹ and 0.43 ± 0.11 cm s⁻¹ for C. fodiens. The return-journey walking speeds relative to burrow for the control group were 0.55 ± 0.12 cm s⁻¹ for F. rusticus and 0.79 ± 0.09 cm s⁻¹ for C. fodiens. These findings demonstrate that while the displacement of burrow appeared to have a significant effect, the displacement seems to affect the two species differently. A pairwise comparison revealed that differences...
in walking speed relative to burrow were different between the two species in the treatment group (Tukey-HSD, \(P < 0.05\)) as well as highlighting significant differences between the treatment and control groups for *C. fodiens* (\(P < 0.01\)).

**Walking speed**

There was a significant difference in overall walking speeds across species or across treatment groups on the return pathways (\(F_{1,99} = 8.26, P = 0.0049\); Fig. 4). There was also a significant interaction between treatment and species. The mean walking speed for treatment groups for both *F. rusticus* (1.25 ± 0.32 cm s\(^{-1}\)) and *C. fodiens* (0.82 ± 0.16 cm s\(^{-1}\)) was significantly different (Tukey-HSD, \(P < 0.05\)). Walking speeds of *F. rusticus* (0.96 ± 0.18 cm s\(^{-1}\)) and *C. fodiens* (1.11 ± 0.11 cm s\(^{-1}\)) were similar in the control groups.

**Path-turning angle**

There was no significant difference in path-turning angles in the two species or across treatments (\(F_{1,99} = 13.56, P = 0.0003\)). The mean path-turning angle of *F. rusticus* (35.88 ± 9.23°) was lower than that of *C. fodiens* (51.09 ± 8.56°) in the treatment groups. There was no significant difference between the mean path-turning angles between the two species, *F. rusticus* (45.71 ± 3.14°) and *C. fodiens* (42.06 ± 6.40°) in the control groups. The path-turning angle of *C. fodiens* was higher than that of *F. rusticus* in the treatment group, but this difference was not significant (Tukey-HSD, \(P = 0.13\)). There were no differences between the two species in the control group.

**Net to gross ratio**

There were no significant differences between the net to gross ratio of the return pathway across treatments or species (\(F_{1,99} = 0.38, P = 0.54\)). The interaction between species and treatment, however, was significant (\(F_{1,99} = 17.2, P < 0.0001, P < 0.001\), respectively). The mean net to gross ratio for *F. rusticus* (0.72 ± 0.19) in the treatment groups was higher than in *C. fodiens* (0.59 ± 0.05). The mean net to gross ratio for *C. fodiens* (0.75 ± 0.03) was nevertheless higher than in *F. rusticus* (0.60 ± 0.03) in the control groups.

**DISCUSSION**

The results demonstrate that *F. rusticus* and *C. fodiens* are likely to be using path integration as a homing mechanism. The homing
success of both species was significantly impacted by the displacement in space of their burrow. *Cambarus fodiens* and *F. rusticus* returned to the original location of the burrow prior to burrow displacement in rotated treatment group (Fig 1). Furthermore, the heading angles relative to the burrow of the return pathways for the control groups for both species were significantly lower than that of the treatment groups ($P = 0.001$). This implies that burrow displacements had an effect in altering the goal-oriented behavior of the crayfishes in the treatment group and that there are no species-specific differences in these changes.

There appeared to be little to no differences in homing success and behavior between the two species. We nevertheless found that homing success to displaced burrows in the treatment groups was significantly reduced (Fig 1). The results of homing success in the control groups were comparable for both species, with no significant differences in mean heading angles relative to the burrow. The mean heading angles of the control groups were also significantly lower for both species when compared to those of the treatment group (Fig 2). The homing-success rate in the treatment groups for both species was significantly lower than those in the control group.

Three general mechanisms can be used for homing to burrow locations. Individuals could use a cue associated directly with the burrow (i.e., “landmark”) or could create a larger map using landscape level cues. Individuals could be using path integration. If crayfishes were using a landmark-style homing mechanism, then the rotation of the disk and subsequent displacement of the burrow should show no effect in homing. Objects could have been used as visual landmarks, such as aerators. Yet, there were no landmarks that rotated with the burrow; thus it may be possible that crayfishes were using these subtle landmarks to return to the original burrow location. It could have been possible that crayfishes were using this mechanism to home to burrows given that individuals in both control and experimental treatments returned to the original location of the burrow.

If crayfishes were utilizing path integration, we would anticipate that individuals would only be able to locate the burrow successfully in the non-rotated (control) treatments. The rotation of the disk with the burrow creates an error in the home vector and we would expect that crayfishes would return to the original location of the burrow prior to displacement. This is exactly the behavior exhibited by crayfishes in the experimental treatment. The increased heading angles (relative to the burrow location) indicates that the movement of the burrow created a homing error that would have been expected if the crayfishes were using path integration. This also further strengthens evidence from prior studies that visual cues directly associated with a self-constructed chimney were not necessary for homing to the burrow (Kamran & Moore, 2015). The results, therefore, are consistent with a path integration homing mechanism.

While crayfishes may not be utilizing a local cue directly associated with the burrow, it is possible that crayfishes could be constructing a spatial map. Crayfishes could have acquired information using tactile localization while exploring the arena and thigmotaxis could have been utilized to construct this spatial map. Crayfishes are known to use of tactile localization in locating objects within their environment during exploratory behaviors as well as detecting possible topographic changes within the environment (Zeil & Sandeman, 1985; Basil & Sandeman, 2000). Blindfolded individuals were able to recognize specific spatial configurations within an arena (Basil & Sandeman, 2000). The input of sensory information in regards to changing spatial configurations was attributed to tactile rather than visual or olfactory stimuli as the changes were created using partitions of material identical to that of the arena. Detection of changes both in texture and structure of spatial configurations of the arena were found in the crayfish *Cherax destructor* (Clark, 1936) (McMahon et al., 2005). The brachyuran crab *Thalamita crenata* (Ruppell, 1830) relies on visual landmarks when homing to dens, and these landmarks may be utilized to form a spatial map (Vannini & Cannicci, 1995; Cannicci et al., 2000). A comparative study of motor learning in blind cave and sighted surface-dwelling crayfishes found that while sighted crayfishes rely on chemosensory and visual information, the absence of visual information motor tasks were still completed (Bierbower et al., 2013).

![Figure 4.](image)

**Figure 4.** The mean (± SEM) walking speed in *Cambarus fodiens* (left) and *Faxonius rusticus* (right). $N = 15$ for all treatments except for *F. rusticus* rotation group, where $N = 17$. Bars with different capital letters are statistically different (Tukey-HSD, $P < 0.05$).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Control</th>
<th>Statistical analysis</th>
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<tbody>
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<td></td>
<td><em>F. rusticus</em></td>
<td><em>C. fodiens</em></td>
<td><em>F. rusticus</em></td>
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<td>Walking speed</td>
<td>1.25 ± 0.32 cm s$^{-1}$</td>
<td>0.82 ± 0.16 cm s$^{-1}$</td>
<td>0.96 ± 0.18 cm s$^{-1}$</td>
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<td>Walking speed relative to burrow</td>
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<td>36.6 ± 2.8°</td>
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<td>Net to gross</td>
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<td>0.59 ± 0.05</td>
<td>0.60 ± 0.03</td>
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<td>Path turning angle</td>
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<td>51.0 ± 8.56°</td>
<td>45.71 ± 3.14°</td>
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<td>Homing success</td>
<td>23.5</td>
<td>6.67</td>
<td>100.0</td>
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</table>

Table 1. Mean (± SEM) values of the orientation parameters measured in the treatment and control groups of individuals of *Faxonius rusticus* and *Creaserinus fodiens* compared. The asterisks indicate which variables were significant.
While a visual cue directly associated with the burrow was not utilized by the two species we studied, it is possible that visual cues within the arena, in combination with tactile information resulting from following the wall of the arena could have also been utilized to form a spatial map in our study. The placement of the burrow was such that it prevented the chelae and antennae of crayfishes to be in contact with the wall of the arena and having contact with the burrow at the same time. The arena was designed in such a way as to minimize information that could be derived from visual cues from each of the walls so that texture and coloration were identical and the placement of the aerators on the walls was identical. Furthermore, the absence of a salient visual cue such as a chimney associated with the burrow failed to provide a robust visual cue associated with the burrow.

There is much evidence from investigations on invertebrates that sheds light on the several mechanisms and cues that are utilized for homing. These range from the reliance on celestial cues (Warrant & Dacke, 2010, 2011; Baird et al., 2012; Dacke et al., 2013), visual landmarks (Collett, 2010; Narendra et al., 2013), or internally computed homes vectors (Layne et al., 2003a, b; Walls & Layne, 2009; Cheng et al., 2014). The diversity in the sensory cues and homing strategies utilized by invertebrates when homing is often a consequence of the everchanging sensory landscape of the animals. Reliance on certain cues for homing would be dictated by not only the environmental conditions at the time of homing but also by the behavioral ecology of the species in question.

Cambarus Fodiens is a primary burrower that constructs elaborate burrows within which individuals reside most of their lives, whereas, F. rusticus is a tertiary burrower possessing the ability to construct shallow burrows but individuals often occupy shelters. Faxonius rusticus has two spatial strategies, peaks of higher locomotor activity alternated with longer periods of slow or null speed, that can influence the use of burrows and/or shelters and thus having homing behavior. Some species of digger wasps appear to show a relationship between the investment to build a nest and the ability to localize the nest (Hoedjes et al., 2011). The costs of burrow construction and maintenance in terms of invested energy has been examined in invertebrates such as the inland scorpion (White, 2001) and the burrowing cockroach (Xu et al., 2014). Individuals of the fiddler crab Minuca rapax (Smith, 1870) that did not invest time in constructing “semidomes” around burrows spent more time conducting other activities such as occupying burrows constructed by others, foraging and displaying courtship behaviors, highlighting that construction is a costly activity (Carvalho et al., 2018). It is thus possible that the value of a burrow may vary greatly between the two species of crayfishes studied, particularly as one invests a great deal of energy in burrow construction. The need to evolve mechanisms that lead to successful homing may be greater in primary burrowing species than tertiary burrowing species.

Our results demonstrate that despite differences in the burrowing ecology of the two species, homing success following displacement was similar. The homing pathways were also similar between the two species, which indicates that the primary mechanism of homing may be the same. The results show that the two species of crayfishes do not utilize a local cue associated with the burrow (olfactory and/or visual) and that path integration may be the primary mechanism for homing. While there still exists the potential that there could be alternate strategies for homing, our study was not designed to measure such strategies. Further investigations should completely limit visual input by blindfolding or ablation to determine the role of visual cues. By limiting the input from the antennae, we may also learn more about the hierarchy of sensory cues utilized in spatial learning or when homing to burrows. Studies in two species of desert ants, for example, have demonstrated that based on their respective environments (cluttered versus featureless) may dictate the propensity for a particular species to switch homing strategies (Buehlmann et al., 2011). While the primary mechanism for homing lends evidence to path integration, additional studies must be designed to examine the effects of displacement of the crayfishes on homing success as well as studies examining the effects of conflicting cues to examine alternate strategies that could be in place.

SUPPLEMENTARY MATERIAL

Supplementary material is available at Journal of Crustacean Biology online.

S1A Figure. Overhead diagram of the homing arena showing rotating disk and food source.

S1B Figure. Side view of the homing arena.

S2 Figure. Diagram defining the parameters to analyze homing pathways.

S3 Figure. Sample return pathways for both species of crayfish under both treatments.

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