An Invertebrate Exercise for Studying Spatial Learning and Memory: Land Maze Navigation in the Terrestrial Isopod, *Armadillidium vulgare*

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The study of spatial learning has naturally been dominated somewhat by work with rodents, although there is a fascinating literature regarding the highly adaptive navigational skills of invertebrates such as ants, cockroaches, and even crickets. The adaptation of methods commonly used with rats (e.g., water and land mazes) has recently highlighted the ability of invertebrates to utilize environmental cues to navigate for purposes of foraging and nest finding. Moreover, such studies reinforce the use of invertebrates as a scientifically interesting and practical choice for the study of spatial learning and memory. In particular, invertebrates can often be collected locally and maintained with minimal specialized care requirements, thus offering a highly economical and manageable resource for undergraduate instruction and research. Here, we describe the use of a widely available invertebrate, the terrestrial crustacean of the order isopoda (*Armadillidium vulgare*) more commonly referred to as a pill bug, wood louse, or roly poly. Using a specially constructed landmaze, student researchers were able to study navigational behavior and assess spatial learning and memory in the context of a project integrating methodological and theoretical perspectives on spatial processing in this species.

Keywords: *invertebrates, isopods, learning and memory, navigation*

The processes underlying spatial navigation are deeply rooted in the evolutionary history of the animal kingdom. Inasmuch as it is highly adaptive to navigate for purposes of securing food and shelter, selecting and obtaining mates, and avoiding predation, this naturally selected survival mechanism has been preserved across a great many species from invertebrates to humans (e.g., Goodrich-Hunsaker et al., 2010; Kesner & Goodrich-Hunsaker, 2010; Manns & Eichenbaum, 2006). Most notably, the study of spatial navigation, particularly in rats and to a lesser extent in humans, has a relatively extensive history which has helped clarify the process by which spatial information is acquired, and has led to the identification of the various anatomical structures involved in the formation of spatial maps (e.g., the hippocampus, O'Keefe & Nadel, 1976; Olton, 1979).

In the laboratory, the study of spatial learning has been dominated somewhat by work with rats, due in part to the popularity of methodological approaches such as the Morris Water Maze (see Kesner & Goodrich-Hunsaker, 2010). Nevertheless, there is a fascinating and intriguing literature regarding the navigational abilities of many other species, including birds, ants, bees, cockroaches, and even crickets (see Alcock, 2010; Mizunami, Weibrecht, & Strausfeld, 1998; 1998; Narendra, Si, Sulikowski, & Cheng, 2007; Wessnitzer et al., 2008).

Of the various species suitable for laboratory studies of spatial learning, the research regarding the navigation strategies among invertebrates is of particular interest for a number of reasons. First, it is impressive to see the systematic approach to foraging, landmark identification, and subsequent landmark-guided navigation among 'less complex' organisms (Collett & Collett, 2000; Collett, Graham, & Durier, 2003; Fry & Wehner, 2002; Narendra et al., 2007). For example, the well documented ability of ants to stabilize the routes they follow from nest to foraging site nicely illustrates the capacity of such species to explore, process environmental cues, map the environment, and form memories (see Collett et al., 2003; also Narendra et al., 2007). Similar work has been done with numerous other invertebrates, fostering an appreciation for the innate navigational facility of such creatures (e.g., crickets, crustaceans; see Hughes, 1992; Moriyama & Takeda, 2007; Ugolini & Pezzani, 1995) and helping to identify the
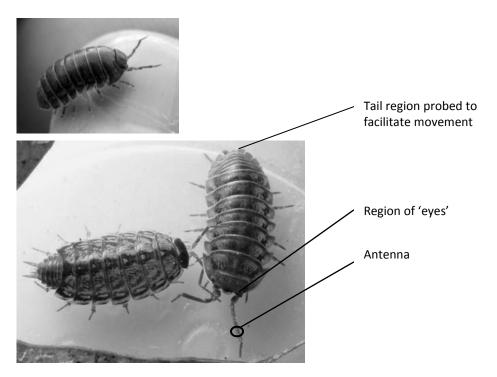
Figure 1. Isopods (*Armadillidium vulgare*) of the type used in this study. Isopods are easily maintained in captivity, and can survive for reasonably long periods of time with adequate care. Materials like dirt, mulch, vegetation, and other natural environmental materials can make for excellent 'bedding' and housing environment. Helpful information regarding the rearing of isopods can be obtained at chamownersweb.net/insects/obtaining.htm.

neural mechanisms involved in spatial processing (e.g., cockroach mushroom bodies, see Mizunami et al., 1998).   Ultimately, this work continues to illustrate the rather impressive navigational skills of invertebrates in much the same tradition as the Nobel laureate von Frisch's (ca. 1973; Dewsbury, 2003) seminal ethological study of the bee waggle dance (see also Menzel & Giurfa, 2006).

Second, and of more practical significance, invertebrates offer an economical and highly manageable option for studying a variety of behavioral processes related to spatial learning and memory. Much has been written about the use of invertebrates in classroom demonstration projects (see Abramson, 1986), providing interesting examples of simple yet effective ways of investigating behavioral principles in simple organisms (e.g., classical conditioning). However, not all invertebrates are alike, and some *behave* in ways more amenable to the study of spatial navigation in the lab.   In addition to the highly mobile ant and cockroach (i.e., locomotor efficiency being a desired characteristic in such studies), terrestrial crustaceans—also known as isopods, pillbugs, or 'roly polys"—have proven to be quite

useful (see Figure 1; refer also to Hughes, 1992; Moriyama & Takeda, 2007). Indeed, isopods readily explore their environment, persistently forage for food and shelter, and nest when locating suitable materials. Large numbers can be maintained in a small space, and they survive well in captivity.

Third, and of particular methodological relevance here, isopods engage in active exploratory behaviors which provide a range of potential dependent measures similar to those commonly employed in rat maze studies (e.g., latencies; time spent in quadrants; path analysis). In previous years, navigational behaviors among other invertebrates (i.e., crickets and cockroaches) have been studied quite successfully by adapting the methods typically used in rat maze research (e.g., creating smaller land maze versions of the water maze; see Mizunami et al., 1998; Wessnitzer et al., 2008). Importantly, such studies reinforce the use of invertebrates as a scientifically relevant and practical choice for the study of spatial learning, and draw attention to the phylogenetic conservation of navigational behaviors across widely different species. Moreover, with appropriate species selection and reasonable familiarity with species-specific behaviors,
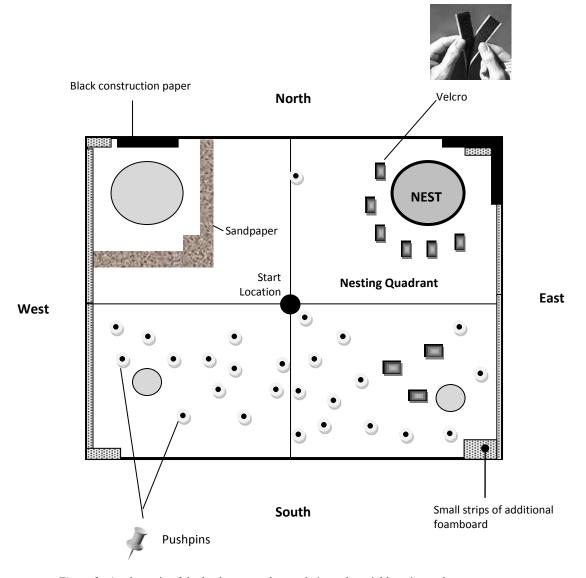
Figure 2. A schematic of the landmaze used to study isopod spatial learning and memory.

researchers can design studies having conceptual continuity with similar research involving higher mammals, yet adapt these to more fiscally conservative and potentially resource limited contexts.

In the current study, our laboratory has taken advantage of the common pillbug or 'roly poly' (*Armadillidium vulgare*) as a highly viable research species. Utilizing a modified landmaze environment, constructed to provide various intramaze and extramaze cues, the results here demonstrate the ability of this species to reliably locate a nesting quadrant and improve nest-finding abilities across successive days. Probe tests of spatial memory (e.g., nest removal; 180° rotation of maze), similar to probe measures used with rats in a water maze, permit the

evaluation of allocentric navigation in this species, and provide an opportunity to discuss various methodological and theoretical perspectives on spatial learning and memory across species. In addition, this study illustrates a fairly novel, scientifically interesting, and highly economical approach to the comparative study of learning and memory.

Methods and Results

Subjects

Isopods are plentiful, and typically found in abundance in wooded regions, often aggregating on the underside of fallen trees, branches, or tree bark.

In addition, they can readily be obtained from similar areas on most college campuses. Indeed, isopods can be collected from diverse locations, yielding an accessible and nearly limitless supply of research subjects.

Collection. In our own work here, isopods have been collected from a regional metropolitan park (Goodyear Heights Metropolitan Park, Akron, Ohio), 'harvested' from among the natural vegetation and debris found alongside footpaths. In our experience, isopods have been collected reasonably early in the spring (i.e., April) and late into November (i.e., typically prior to freezing and snow). Collection generally entails placing isopods in a small container lined with a thin layer of dirt, leaves, and branch fragments. For purposes of this lab, about 30-50 isopods are usually obtained for planned experiments.

Maintenance. Maintaining isopods in the laboratory requires relatively minimal material investment, and a very modest allocation of time. Initially, isopods brought into the lab are group housed in clear plastic containers (e.g., 32.5 cm L x 17 cm W x 20 cm H) lined with dirt, leaves, and small branches from the local environment. Regularly humidifying the contents of the housing container, typically with a water mister to lightly saturate the soil and debris, will insure isopod health and longevity. Isopods readily feed on decaying vegetation, and tomato and cucumber slices have served as excellent food sources.

Isopods have easily been maintained in this lab for a few weeks at a time (i.e., 3-5 week experimental periods), although it is expected that they would survive for extended periods of time when group housed at room temperature (approximately $77°$ *F*) with sufficient humidity and adequate food. Also, isopods have reproduced in captivity, which has promoted our consideration of future developmental studies with this species.

Materials

A land maze has been constructed for use with isopods in this lab. The maze (39 cm L x 39 cm W x 6.5 cm H; see Figure 2) is constructed out of white foam board (1 cm thick, Elmer's®). The walls of the land maze (6.5 cm high) can be affixed to the maze base using tape or glue to provide a tight seal. The northeast quadrant of the maze contained a nesting circle (7.5 cm diameter) cut into the foam board, which was filled with nesting materials obtained when collecting isopods (i.e., wood chips; bark; dirt; acorn husks). Nesting material was piled approximately 1 cm above the surface of the foamboard. To provide surface texture and intramaze cues, pieces of Velcro adhesive strips (i.e., the coarse, rough surface piece; 2.5-3.0 cm) were positioned along approximately one-half of the nesting region rim (see Figure 2, Northeast quadrant). The northeast corner was darkended with pieces of black construction paper. The adjacent northwest quadrant contained a false nesting circle (7.5 cm diameter) filled with a thin layer of dirt (less than .5 cm). This circle was partially bordered by a 2.5 cm wide strip of fine grained sandpaper, with part of the northern wall in this quadrant darkened by a strip of black construction paper.

The southern half of this landmaze contained approximately 29 clear pushpins inserted into the foamboard, thus creating a rather distinct topography, with the southwest and southeast quadrants also containing a 3 cm diameter region cut into the foamboard and filled with dirt or small rocks. The southeast quadrant also contained two pieces of soft-side Velcro strips and one piece of coarse sided Velcro (each approximately 2.5-3.0 cm).

Along the inside border of the east and west walls of the landmaze were placed small strips of additional foamboard, thus adding some texture and dimension to the outer limits of the maze. A videocamera was positioned on a tripod just outside the northeast wall of the maze; to the right of the camera was a set of black filing cabinets. The northern part of the maze was bordered by a laboratory wall, with the researcher remaining seated along the southern portion of the maze.

Spatial Navigation Procedures

Isopods were typically run through landmaze experiments in smaller groups of 5-7 each. Typically, isopods were removed from the larger housing unit and placed in smaller plastic containers (approximately 7 cm diameter, 6 cm high)—each of which held a small amount of dirt and smaller wood chips. During an experiment, this became the housing unit for experimental subjects, and isopods were grouped in these smaller containers for the duration of a study.

Maze Training. The typical maze learning experiment conducted here involved 5 consecutive days of open field navigation, each day consisting of a 30 minute period of maze exploration. The maze was not systematically cleaned throughout the maze training. Prior to each maze session (i.e., learning trial), isopods were transferred from their small
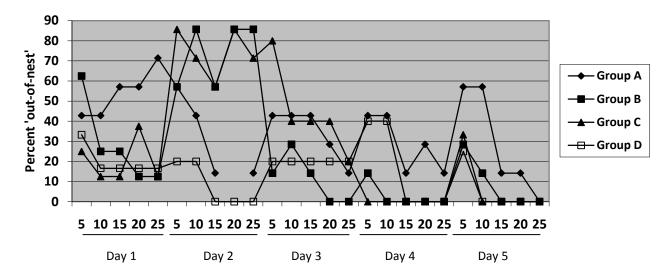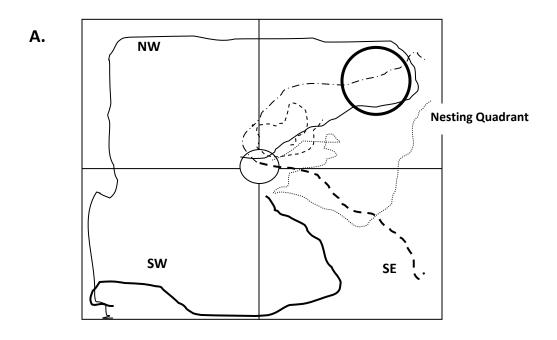
Figure 3. Percentage 'out-of-nest' recordings at 5-minute intervals for 4 groups of isopods (groups A-D) throughout 5 days of maze learning. Note the relative improvement in out-of-nest behavior on days 4 and 5. Typically, isopods find the nest earlier and remain there for the duration of the learning session on days 4 and 5.

housing container into a comparably sized plastic container used to deliver them to the start location in the center of the maze (see Figure 2). There was no effort taken here to control the orientation of isopods at the time of release. Upon release, isopods most commonly move toward the maze perimeter, frequently making contact with perimeter walls and using their antennae to navigate along the maze walls (quite similar to thigmotaxic behavior observed in rats). In our experience, isopods vary in the time it takes to individually make contact with the nesting material (i.e., for each learning session moistened with water and containing tomato fragments), although they commonly remain nested upon reaching the nest. We have adopted the procedure of providing a gentle probe to the tail region of an isopod that remains motionless in the maze for more than 10-20 seconds (i.e., using a small gauge metal probe, or a straightened paper-clip; see Figure 1). This procedure ensures movement and exploration, and typically facilitates nest-finding. The number of such probes per trial was not limited, and some isopods received repeated probes throughout a trial to maintain active exploration. For those not reaching the nest within 25 minutes, gentle direction was provided toward the nest. It should be noted that isopods occasionally attempted to scale the interior wall of the maze, which reinforces the need for experimenter vigilance coupled with the use of the probe to prevent escape from the maze and foster exploration limited to the maze floor.

Isopods were permitted to freely leave and re-enter the nesting material during each 30-minute learning trial. The number of isopods outside of the nest at each 1-minute interval was recorded, and converted to an 'out-of-nest' percentage (for each group). Data for individual isopods was not collected in this study, although future studies could use different non-toxic paint colors to identify individual subjects within a group. Nevertheless, for this study, group performance—expressed as overall percentage of 'out-of-nest' behavior—was used as the unit of analysis (see Figure 3). In this study, isopods typically exhibited more out-of-nest behavior in the first two days of maze learning, but gradually demonstrated more rapid nesting behavior across days three and four. Most notably, on days four and five, isopods characteristically found the nest *earlier* and remained there *longer*—in most cases not leaving the nest (i.e., 0% out-of-nest performance) for the duration of these maze sessions. The reduction in out-of-nest behavior was considered here as one index of improvement across maze sessions.

Probe Measures. As a probe measure of memory, on day six the nesting material was removed from the nesting quadrant, and the isopods were placed as usual in the central starting location of the maze (i.e., initial heading direction/orientation was not controlled). During the first minute of this trial, isopod behavior was videotaped and the recordings were later analyzed for movement patterns. Tracings of isopod movements were made on transparencies laid over the television monitor during the replay of probe trials. As illustrated in Figure 4A, the movement patterns for one group of
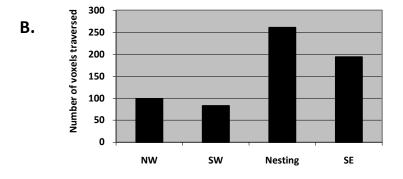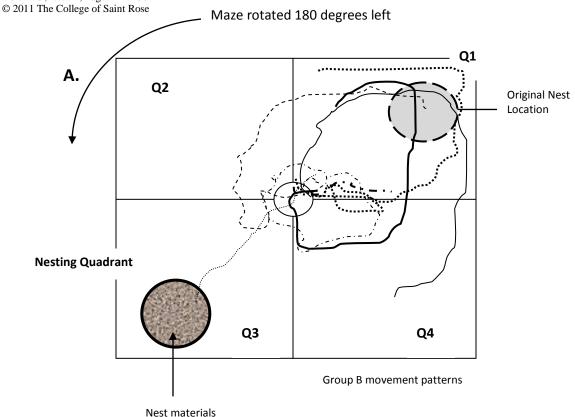
Group A movement patterns

Figure 4. Isopod movement patterns during nest removal probe trial. 4A illustrates the movement trajectories for one group of isopods, reflecting a trend toward nesting quadrant preference. 4B indicates the total number of voxels (i.e., 1 cm x 1 cm grid laid over the isopod movement tracings) traversed for each quadrant.

isopods during this 1-minute probe segment reflected a relative preference for activity in the nesting quadrant, with four of these isopods making movements toward the nesting region (two of these making direct paths through the nesting region). Figure 4B illustrates the number of voxels (i.e., 1 cm x 1 cm grid laid over the isopod movement tracings) traversed by isopods per quadrant across all groups combined. Quantitatively, the greatest amount of activity was recorded in the nesting quadrant (261 voxels), followed by the adjacent northwest quadrant (194 voxels). Clearly, on this probe measure, the remaining quadrants received much less activity (i.e.,

99 and 83), with the southwest quadrant showing the least amount of isopod activity.

The above probe measure was intended to control for the possibility that nest cues (i.e., visual or olfactory) might be principally responsible for directing initial movement patterns in these isopods. Preliminary consideration of this movement data would suggest otherwise. Further consideration of an extra-maze cue configuration used by these isopods to direct behavior (i.e., light gradients; distinct room cues) was evaluated with a second probe measure (i.e., conducted after a return to a standard maze training day) involving a maze rotation, resulting in the nesting quadrant (i.e., containing the nesting
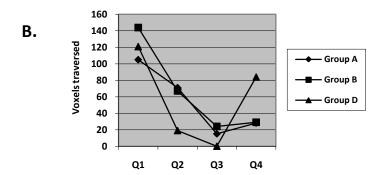
Figure 5. Isopod movement patterns during maze rotation probe trial. 5A illustrates tracings of select isopods during this 1-minute videorecording. 5B indicates the total number of voxels traversed in each quadrant (Q1-Q4) by groups of isopods (Group A, B, and D) during the 1-minute probe trial.

material) now positioned in the southwest quadrant (i.e., now labeled Q3, see Figure 5). This probe measure provides a more stringent test of extra-maze cue use by this species, with movement patterns of the kind reported here (see Figure 5A) reflecting a preference for the quadrant of the original nesting location, which argues for the use of external cues (i.e., light gradient in the room; large dark cabinets; videocamera/tripod) supporting initial navigation. In addition, the measure of voxels covered per quadrant (Figure 5B) reinforces the position that extra-maze cues might be used as part of the navigational system

for these isopods (certainly a contention requiring further experimental work).

Summary

The ability to work with isopods in the laboratory setting is an economical and efficient way to engage students in behavioral research. Isopods are readily obtained, easily maintained, and provide numerous opportunities to investigate various learning and memory issues. In this lab, we have chosen to study spatial navigation, and multiple

undergraduate projects have been initiated using this species. In the original work based on our first attempt at isopod research, Stottlemeyer (2008) nicely framed an argument that has guided a line of thinking and research planning for subsequent investigations:

> "…when the start time began [on the probe trial], many pill bugs went straight towards the old nesting quadrant even though the nest was actually in [a new location]. These isopods spent most of their time searching for the nest in the quadrant where it was previously located [refer to Figure 5 here]. However, isopods did eventually locate the nest and take refuge. The time it took to find the new nest location was not as long as it took during any one of the original trials. Consequently, [it is reasonable to suspect] isopods must be primarily relying on large, extra-[maze] cues to guide them towards a general direction and secondarily rely on intra-[maze] cues to guide them to a goal site. This can easily be understood as larger cues, such as a tree, would help guide a small pill bug in a certain direction, while a certain rock or smaller cue would help pinpoint a specific location…. Narendra et al. (2007) also recognize that unstable landmarks such as rocks and clumps of dirt can easily be replicated throughout the natural environment and they can also easily be overturned or moved; however, when paired with a large visual cue such as a tree [or a videocamera tripod], it can guide…an isopod…to a specific location with greater ease when learned." (p. 18)

Most recently, isopod preferences for light-dark gradients have been explored successfully, in addition to a consideration of intramaze cue *size* as a relevant stimulus dimension for landmark learning (Buzzelli, 2009). Isopod mobility certainly makes them a good invertebrate research candidate, particularly given their natural foraging instincts and nesting behavior. Moreover, recent consideration has been given to studies involving a) various tests of short-term and long-term memory, b) vulnerability to hypothermia-induced amnesia, c) visual, tactile, and olfactory processing in cue identification, and d) effects of antenna removal on shifting a bias toward visual information processing. In addition, it could be of interest to consider the influence of humidity levels on performance, particularly if mild water deprivation procedures were implemented and fluid reinforcement was the goal of maze learning. Given the sensitivity of this species to environmental humidity, this variable (i.e., relative humidity) could be examined for its impact on foraging behavior and learning.

Overall, potential directions for research are nearly as plentiful as isopods themselves. With very modest resources, and an interest in comparative behavioral research, studies like the one described here can be modified, replicated, or adapted to the facilities, finances, and interests of a given researcher.

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