SPATIAL FAMILIARITY IN THE BLIND CAVE CRAYFISH, *ORCONECTES AUSTRALIS PACKARDI*

BY

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ABSTRACT

This study examined behaviour of cave-adapted blind crayfish to novel territories of various sizes. Cave crayfish, *Orconectes australis packardi*, were kept within a dark room in individual aquaria for 7 days. They were then placed into a small tank (33 × 28 × 23 cm, 10-15 cm water depth), and a large tank (54 × 37 × 30 cm, 10-15 cm water depth). Time, distance of movements, and length of pauses were recorded. Upon initial placement in a new setting, crayfish will walk around the perimeter where their antenna can contact the side of the tank. The animals will subsequently move around the tank away from the perimeter, or they will begin to dig a burrow. Familiarity within the environment for an individual crayfish was defined as when the animal initiates digging or remains in one place for over 5 minutes. The study demonstrates that time required to become familiar to a new setting depends on the size of the setting. The authors suggest that a balance between sensory input and inner processing, termed "familiarity", can be reached.

RÉSUMÉ

Cette étude examine le comportement de l'écrevisse cavernicole aveugle lorsqu'elle est placée dans des territoires nouveaux de tailles variées. Des écrevisses cavernicoles *Orconectes australis packardi*, ont été maintenues dans une pièce sombre dans des aquaria individuels pendant 7 jours. Les écrevisses ont été ensuite placées dans un petit bac (33 × 28 × 23 cm, 10-15 cm d'eau), et dans un grand bac (54 × 37 × 30 cm, 10-15 cm d'eau). Le moment, la distance de mouvements, et la longueur des pauses ont été notés. Dès son arrivée dans un nouveau cadre, les écrevisses marchent tout autour du récipient, leur antenne étant en contact avec la paroi du bac. Les animaux vont ensuite se déplacer autour du bac, à distance de la paroi, ou bien ils vont commencer à creuser un terrier. La familiarisation avec le nouvel environnement pour une écrevisse a été définie quand l'animal commence à creuser ou reste à un endroit pendant plus de 5 minutes. L'étude démontre que le temps requis pour être familiarisée avec un nouveau lieu dépend de la taille du lieu. Les auteurs suggèrent qu'un équilibre entre la réception sensorielle et le processus interne, nommé "familiarisation", peut être atteint.

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INTRODUCTION

Spatial learning is a process that animals use to gain familiarity with the surrounding sensory cues. This phenomenon has been well-demonstrated by Tinbergen's (1932) studies with digger wasps. When a wasp leaves its burrow it hovers over the surrounding area to become familiar with spatial cues (e.g., rocks, pine cones, and shrubs). This provides orientation and learning of spatial cues so that upon returning to the vicinity the wasp can find its burrow. This type of familiarization with the local environment is important not only for insects, but also for reptiles, birds, and mammals, in order to be able to retreat for protection in the threat of predation. Even cave organisms do have predators and patterns of behaviors which are adaptive to survival (Uiblein et al., 1992).

In invertebrate species, regions of the nervous system that are responsible for spatial learning are not as well-known. But it is known that in crayfish and lobsters tactile, chemosensory, and visual information all project to the central brain. To reduce the complexity of investigating spatial learning in crustaceans, we chose a crustacean that lacks visual sensory structures and, therefore, develops spatial familiarity using its olfactory and tactile senses. The animal we used in our studies was the blind cave crayfish, *Orconectes australis packardi* Rhoades, 1944. We assumed that blind crayfish learn their environment by both tactile and chemosensory cues. Crayfish primarily move their first pair of antennae (i.e., the anntenules) to sample the chemical environment around them, although there are also other chemosensory hairs elsewhere on the body. In stagnant pools within a cave, crayfish can initiate water movement by producing gill currents or by moving through the water in order to better perceive chemosensory cues. In a small pool, the chemical cues may be uniform unless there is a point source of a signal, so chemical signals might not be so informative for the animal to locate its position within the environment. The tactile knowledge of the overall terrain may well provide a blind cave crayfish with information of location, sufficient to allow the animal to be familiar with the local territory. In a novel setting the blind cave crayfish could use a multitude of sensory modalities to gain familiarity. In many animals there is a complexity to learning spatial orientation, which arises by assessing the relative contributions of the different senses (Whishaw, 1998). In order to spatially orientate to the environment, by tactile sense, the animal would need to move over the terrain in which it is to become familiar. This type of sampling is referred to as path integration.

The cave crayfish used in this study inhabit a completely dark cave environment containing streams or pools of water. The cave crayfish, *O. a. packardi*, has elongated lateral antenna as compared to epigean crayfish. It is likely that this morphological characteristic, which enhances the tactile sense, is a cave-adaptive trait (Hüppop, 1985). Studies of the amphipod crustacean *Gammarus minus* Say, 1818, have shown that such traits as elongated antennae and reduced eye size are genetically determined and that natural selection may be responsible for their evolution (Culver et al., 1995).

The purpose of this study is an initial assessment of how cave crayfish become familiar with novel settings. The study was designed to test the hypothesis that blind cave crayfish will preform stereotypical behaviors in order to obtain spatial familiarity in a new setting and that they become familiar with smaller settings faster than with larger ones, independent of fatigue or walking.

Preliminary results from segments of this project have been presented in abstract form (Li & Cooper, 1999).

METHODS

Observations in the laboratory

Nine adult male blind cave crayfish, *Orconectes australis packardi*, of varying size (3.0 to 6.0 cm from the tip of the rostrum to the end of the telson) were collected from Sloan's Valley Cave, near Somerset, Kentucky, U.S.A. Species identification was made by examination of the stylets and carapace traits (Hobbs et al., 1977). On capture, each crayfish was marked with letters or numbers on its dorsal carapace. Only intermolt males were used. Females were not used because of behavioral variations associated with being gravid. Each animal was anatomically complete with a full set of intact antennae, walking legs, and chelae. Animals with noticeably smaller, regenerating limbs were not used. Animals were brought to the laboratory in Lexington, Kentucky and kept in a dark room within individual aquaria (33 × 28 × 23 cm; water depth 10-15 cm) for 7 days. The water in the holding and observation tanks was tap water filtered through carbon to eliminate chloramines and stored in a large holding tank for aeration. Water in the observation tanks was replaced immediately before each interval of behavioral observation.

The individually isolated animals were all treated in the same way during the holding time in the laboratory before experimentation. An index of morphological characteristics is presented in table I. To transfer animals to experimental tanks, they were caught in a 100 ml beaker by placing the open mouth of the beaker behind the animal. The tank was mildly tapped in front of the animal resulting in it walking backwards into the beaker. The beaker was gently picked up at an angle so as to prevent the animal from swimming out. The beaker was placed in the middle of the observation tank. When the animal left the beaker, behavioral observations commenced.

TABLE I
Morphological index of blind crayfish, *Orconectes australis packardi* Rhoades, used

Crayfish #	An. L (cm)	Ab. L (cm)	W (g)
1	3.2	2.1	0.88
2	2.9	1.4	0.51
3	4.1	1.9	0.80
4	4.0	2.2	1.13
5	4.0	2.3	1.19
6	4.7	2.5	1.38
7	4.1	2.0	0.86
8	3.5	2.0	0.66
9	3.3	2.0	0.75

An. L, antennal length; Ab. L, abdomen length; W, body weight; #, the individual crayfish.

Observations in caves

Observations were made with normal white light from flashlights or from flashlights fitted with a filter to provide a dim red light (Edmond Scientific, IR only-model # D43,951). Video recordings in white light were made with a JVC compact camcorder (VHS 18X hyper zoom quick response AF, model GR-AX220U videomovie). Only shallow pools ($<$60 cm) could be monitored by video because of difficulties in focusing through the water. Direct observations were possible in deeper pools when the turbidity was low. The time of day and amount of recent flooding within the cave were recorded. Initial observations were made in Shelta Cave (AL) among the three species of crayfish present in that system (Cooper & Cooper, 1998). Further work was carried out in the Sloan's Valley Cave System in Kentucky, because of better access, and of the greater abundance of crayfish. Assuming that removal of crayfish from this system would not result in alterations to cave ecology, further studies under laboratory conditions became viable. Permits from the Department of Fish and Wildlife Resources (Kentucky) allowed removal of cave crayfish for laboratory and in-cave observation and handling.

In order to examine, within the cave setting, the reactions of individual crayfish transferred to a neighboring pool of water without other crayfish present, individuals were prodded to move backwards into an approximately 2-liter water-filled container. The container was transported to the edge of a neighboring pool, placed in the water, and left on its side so that the crayfish could walk out. Transfer and observations were performed in dim red light.

The particular pool in which three crayfish were tested in succession was 30 cm at it deepest point in the center with very gradual inclines on all sides. The bottom was a thin layer of sediment (0.5 cm thick) on top of bedrock. The edges had various-sized rocks and peninsulas that the crayfish readily walked around. The pool used was about 20 meters from the capture pool at about the same level, but separated by moist mud at a level of only 30-50 cm embankments from the pool's edge.

Spatial familiarity

The initial laboratory study examined how cave crayfish respond to being placed into each of the following novel settings after 1 week in a holding tank: a small ($33 \times 28 \times 23$ cm; 10-15 cm water depth), a large ($54 \times 37 \times 30$ cm, 15 cm water depth), or a large round (102 cm diameter, 15 cm water depth) tank. Each tank contained at least 2.5 cm of sand with a rock on one side on top of the sand and the water temperature was maintained at 19°C. Crayfish movements were traced onto paper that replicated, in miniature, the dimensions of the tank. Observations used an infra-red sensing CCD camera (Toshiba, model IK-537A) fitted with a zoom lens (Pentax TV, zoom 8-48 mm) and a video cassette recorder (Panasonic, time lapse SVHS, model AG6T20). Recordings were analysed by viewing video data on a TV screen (17″ across). Time and distance of movements and duration of pauses were recorded. Habituation behavior criteria used to determine "settling" included digging burrows, or remaining still for more than 5 minutes.

Data analysis and graphing

Crayfish movements were continuously recorded until the habituation criterion was attained, followed by periodic observations within 5-minute intervals. In some cases, settling behaviors took more than 4 hours. All data were analysed by a single observer (H.L.). Indices monitored were: (1) time at which the familiarity criterion is reached; (2) duration of each pre-criterion pause; (3) time at which the animal begins moving around Region 2 and return to Region 1. The width of Region 1 was defined by the antennae length of the crayfish around the tank perimeter. Region 2 was the inner region of the tank excluding Region 1. Location of the animal was monitored by the position of its thoracic tagma. Duration of each travel phase, distances covered in Region 1 and Region 2 during each travel phase, and time spent in Region 1 and Region 2 were recorded. Distances traveled and velocity of movements were normalized for body weight.

RESULTS

In brief, the results demonstrate that when a crayfish was placed into a novel setting, it followed the perimeter until it gained familiarity. The larger the setting, the more time was required for the crayfish to settle down. Having reached a state termed "familiarity" with the perimeter, they began to travel into the center of the tank away from the perimeter. Such stereotypical actions were observed within the natural cave environment as well as within observation tanks within a defined laboratory setting.

In field trials within the cave, crayfish tended to follow the perimeter of unfamiliar pools when placed into them. Also noted was that when crayfish are disturbed or placed in a new setting, their locomotive activity increases. To eliminate the uncontrolled variables within the cave setting, experiments were conducted within the laboratory, with only changing the size of the environment. Independent of the size of the tank, when crayfish are placed in the new environment, they moved quickly until they encountered the tank wall, then they moved along the wall with one antenna contacting it. After some time spent gaining familiarity with the environmental boundary, they will start to explore the tank's center. To illustrate these behaviors, three representative 2-minute periods of crayfish movements are presented in fig. 1: (A) initial exposure to the novel setting; (B) twenty minutes later; and (C) the 2 minutes prior to cessation of movement when the animal starts to dig a burrow. Time spent and distance traveled both increased as the size of the setting increases. The data obtained for each of the 9 animals are shown in table II. The total time consumed in the large setting as compared to the small is significantly different for each animal as well as for the group (paired T-test, $P < 0.001$). Likewise, the total distance covered increased with territory size. To eliminate fatigue as a factor in cessation of movement in the large tank, a larger tank was introduced (round with a 102 cm diameter, 15 cm water depth). When placed in this

TABLE II
Time consumed and distance covered before settling

	Time consumed (sec.)			Distance covered (cm)		
Crayfish #	Small	Medium	Large	Small	Medium	Large
1	4717	5149	15798	5239	7991	84158
2	1449	5651	13878	1486	10418	68573
3	1773	3018	10613	1543	4661	38476
4	4310	6680	10947	9074	24576	68899
5	3510	5142	12172	3234	7544	35282
6	1387	4726	9998	1597	7123	42464
7	5536	7958	11877	5500	13546	27951
8	1587	4552	8352	2068	8493	35167
9	2262	3623	10366	2227	4652	40187
Mean	2948	5167	11555	3552	9889	49017
± SEM*	532	498	738	861	2051	6529

* Standard Error of Mean.

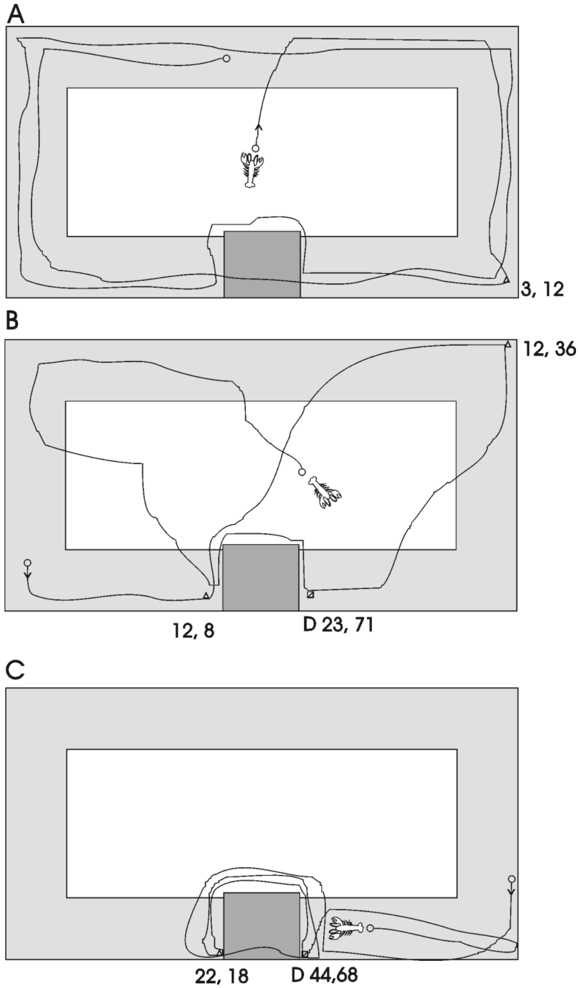

Fig. 1. Three representative 2-minute periods of crayfish movements. A, initial exposure of the animal to the tank; B, 20 min. later; C, 2 min. prior to cessation of movements when burrow-digging has commenced. The tank's area is defined as consisting of Regions 1 and 2. Region 1 made up of the outer perimeter of the tank inward to the length of the antenna perpendicular to the body of the animal (light gray region). Region 2 is the remaining area (white region). The rock is represented by the dark gray square. The line is the path of the crayfish. The open circles together with the arrows indicate where the crayfish began and the direction of movement, and the open triangle represents pauses of no less than 3 seconds. Numbers next to these symbols indicate pause length and time at which they occurred. For example, "3,12" indicates that the animal stopped at this point 12 seconds after the beginning of the 2-minute period and paused for 3 seconds before continuing. Open squares indicate where the crayfish dug a burrow, also noted as "D".

novel setting, the animals covered a substantially greater distance (paired T-test, $P < 0.002$), without settling down until a very long period of time had passed (table II). In this largest setting, settling time and distance covered surpassed the values obtained in the large rectangular tank (Dunnett's test, $P < 0.05$), indicative that fatigue was not a factor for the settling behaviors obtained in the two rectangular tanks used for the rest of this study.

Movement pattern in differently sized settings

The movement pattern of crayfish was quantified by a series of walks and pauses. This movement pattern, which includes walking velocity, walking durations, and pauses, may carry the information of what sensory cues and thus sensory systems are used in spatial learning. To better examine differences in behavior for different-sized settings, parameters were recorded and calculated as described in the following sections.

Pauses

An index over time to the degree of familiarity of a crayfish to a new setting was recorded by the number of pauses and duration of each pause (fig. 2A). As an animal spent more time exploring the environment, the pauses became longer and more frequent until the animal settled. Fig. 2B demonstrates this pattern for the small and large tanks, respectively, which are significantly different (paired T-test, $P < 0.05$). In addition, as shown in fig. 2C, the median of the time span for the combined walking intervals indicates that crayfish had longer intervals between pauses when exploring a larger tank as compared to the smaller one (paired T-test, $P < 0.001$).

Walking speed

Overall velocity in the larger tank was greater since a greater distance was traveled per time. Hence crayfish in larger tanks took longer to settle down, with fewer pauses per minute. In addition, the velocity of movement between pauses was higher in larger tanks. The median velocity, excluding pause time, indicates crayfish walk faster in larger tanks (paired T-test, $P < 0.001$) (fig. 2D).

Pauses, intervals, and velocity for an individual

The variance of response times is somewhat obscured when presented as an averaged response, because of the individual variation, whereas individual plots better represent the trends. Such variations are demonstrated for the three crayfish represented in fig. 3. These three crayfish illustrate the two extremes of the behaviors (crayfish 1 and 3) and the average response (crayfish 2) within the data set (see fig. 3). The sums of pauses, intervals, and distances over time are presented as cumulative plots and thus make visible the trends for particular crayfish in the novel settings. Fig. 3A shows that the number of pauses increases substantially for

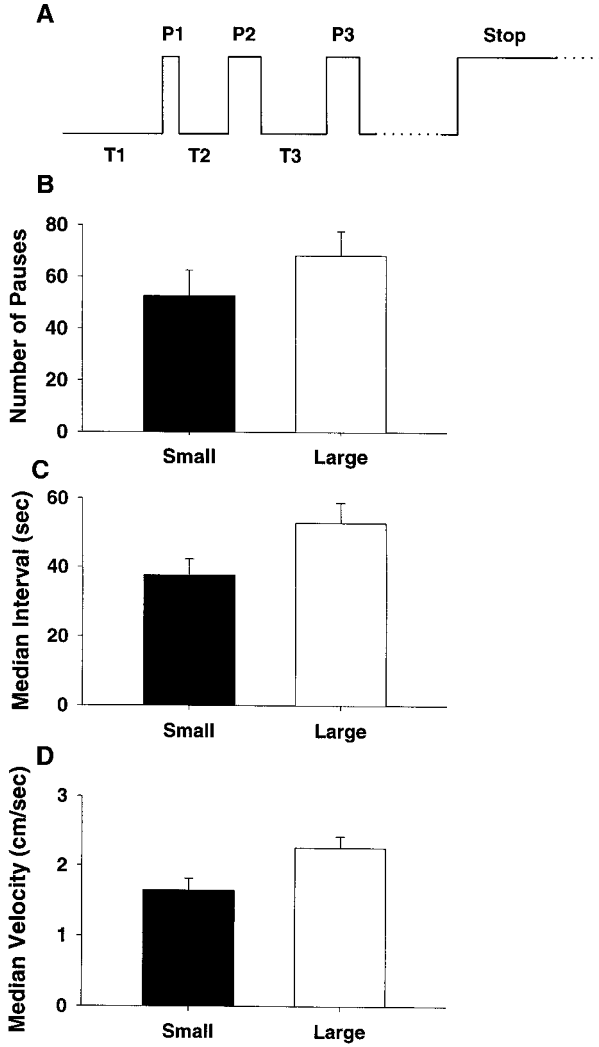

Fig. 2. Elements of the travel and pause process. A, crayfish travel, pause, and habituation behaviors are summarized as a series of travel (T) and pause (P) phases; B, there were more pauses in the large tank as compared to the small tank until the crayfish settled (paired T-test, $P < 0.05$); C, the mean medium of all travel intervals indicates that the crayfish moved longer when placed in a larger tank (paired T-test, $P < 0.001$); D, the mean medium travel velocity, excluding pause time, indicates that the crayfish travel faster in a larger tank (paired T-test, $P < 0.001$).

crayfish 2 and 3 and slightly for crayfish 1 within a short period of time in the small tank (closed circles) as compared to the larger tank (open circles). However, each individual showed this same trend (9 out of 9), although the rate varied among individuals. Thus, the distributions of the data sets overlap for the different tank settings but consistent trends are present. Additionally, intervals between pauses

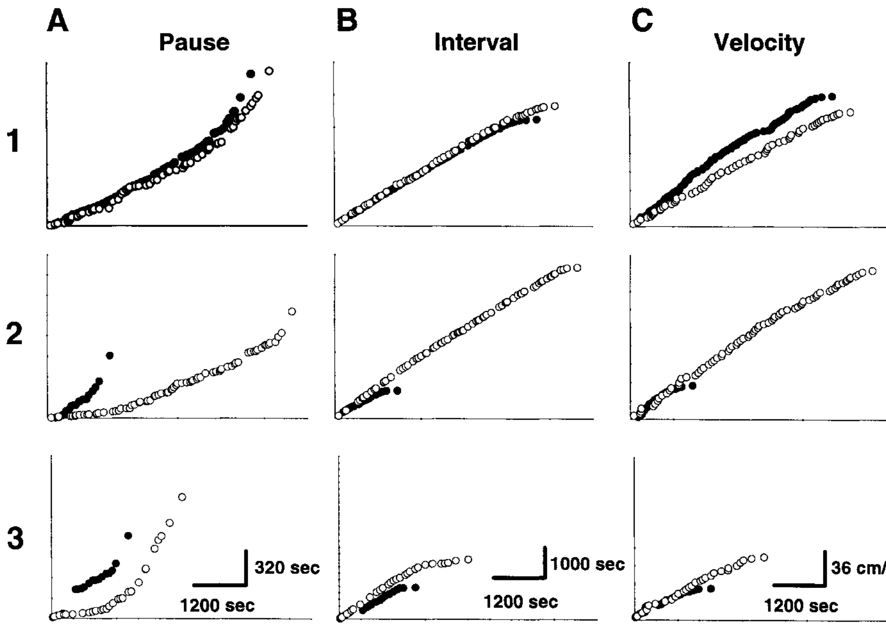

Fig. 3. Crayfish behavioral trends, exemplified. Crayfish #1-3 illustrate trends for individual animals. A, in plotting a cumulative sum of pauses over time spent, pauses increased with time spent in the tank. The open circles represent the large tank and the closed circles represent the small tank. The time to settling took longer in the larger tank. B, the cumulative sum of the time during each travel interval is almost linear until the animal reaches a time at which it begins to settle. This is consistent among the different-sized settings. C, the cumulative sum of velocity during each travel phase is almost linear until the animal begins to settle.

are cumulatively longer in the large tanks (fig. 3B), indicating that an animal takes longer to settle in the large setting. This is particularly so for crayfish 2 and 3, as shown, and only slightly for crayfish 1. As the animals approach a settling behavior the curves tend to plateau. This is also observed in the cumulative sum of velocity during each walking phase, which is almost linear until the commencement of the settling behavior begins (fig. 3C), which is indicated by the plateau.

An examination of the timing and location of where the animals settled indicates that they tended to dig a burrow or physically back into the crevice, with their abdomens between the rock and the tank's edge. Out of a total of 27 accounts of settling, 24 of these took place by the rock. The 3 others took place in Region 1 next to the edge of the tank away from the rock (table III).

The mean values for intervals, pauses, and velocity for each individual are presented in table IV. It is striking that the distance covered over time is much greater during the introduction time to a new setting within the larger tanks as compared to the smaller one (fig. 4A). The trend for all the crayfish is that they travel a greater distance in the larger tanks over time than in smaller tanks. In either

TABLE III
Location and region where the crayfish settle

Tanks	Small			Medium			Large		
Crayfish#	Location		Region	Location		Region	Location		Region
1	Rock	●	1	Rock	●	1	Rock	❍	1
2	Rock	●	1	Corner	❍	1	Rock	●	1
3	Rock	●	1	Rock	●	1	Rock	●	1
4	Rock	●	1	Rock	❍	1	Rock	❍	1
5	Corner	❍	1	Rock	●	1	Rock	●	1
6	Rock	●	1	Rock	●	1	Rock	●	1
7	Rock	❍	1	Edge	❍	1	Rock	●	1
8	Rock	●	1	Rock	●	1	Rock	●	1
9	Rock	●	1	Rock	●	1	Rock	●	1

●, Digging a burrow; ❍, keeping still.

TABLE IV
Mean intervals, pauses, and walking velocity

	Mean intervals			Mean pauses			Mean velocity		
N	S	M	L	S	M	L	S	M	L
1	34.8	59.7	123.1	15.9	24.8	13.1	1.6	2.2	5.9
2	35.1	63.2	111.3	25.3	14.2	9.4	1.7	3.2	5.4
3	48.1	67.5	81.7	40.6	44.2	2.6	1.6	2.6	5.4
4	45.3	78.3	136.7	13.7	12.0	19.7	2.7	4.2	7.2
5	37.7	50.0	82.3	17.2	15.1	21.7	1.3	1.9	3.7
6	38.0	65.5	85.0	17.5	29.0	23.7	1.7	2.2	5.4
7	42.8	49.3	151.5	9.4	12.9	18.1	1.2	2.2	2.6
8	26.0	46.3	66.5	15.8	15.2	17.9	2.1	2.5	5.3
9	36.2	53.8	86.1	15.3	23.3	17.8	1.4	1.8	5.1
Mean	38.2	59.3	102.7	19.0	21.2	17.1	1.7	2.5	5.1
±SEM	2.2	3.5	9.7	3.0	3.5	2.6	0.2	0.3	0.4

N, Crayfish number; S, small tank; M, medium tank; L, large tank.

tank, it is only after a few hours that the crayfish begin to slow down in their almost continuous walking mode. The plateau towards the final phase in exploration, as seen in fig. 3B, and the fact that the number of pauses greatly increases with a reduction in the rate of walking, suggests that the animals are becoming familiar with the environment just prior to exhibiting a settling behavior.

Regional behavior

Most of the time spent walking, as well as the percent of total distance covered (fig. 4A), took place in Region 1 of the tanks. An examination of the mean

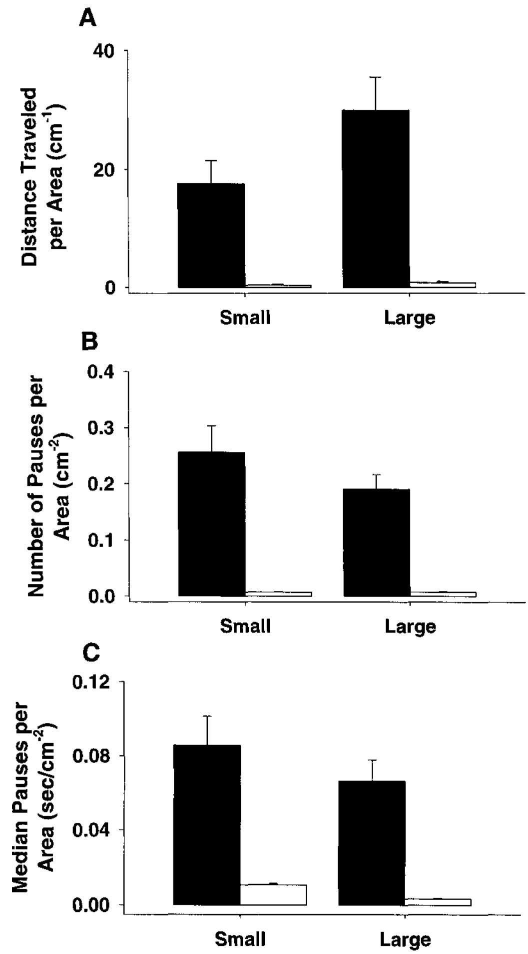

Fig. 4. Regionality of crayfish travel. A, to illustrate the regionality of crayfish travel, the distances covered per unit area in Region 1 and 2 were compared. Crayfish covered a greater distance per unit area in Region 1 than in Region 2 in both sized tanks (paired T-test, $P < 0.002$ and $P < 0.001$, respectively). Comparing the number (B) and medium mean (C) of pauses in Region 1 and Region 2 in tanks of both sizes, the crayfish paused more times in Region 1 for both tank sizes (paired T-test, $P < 0.001$).

distances of all 9 animals revealed that total distance traveled in Region 1 was much larger than that covered in Region 2 for the two different tanks (paired T-test, $P < 0.002$ and $P < 0.001$, respectively). By comparing the number (fig. 4B) and the median (fig. 4C) of the pauses that occurred in Region 1 and Region 2 in both tanks, it can be stated that the crayfish paused more times in Region 1 in both tanks (paired T-test, $P < 0.001$).

Individual variation among animals shows up in both velocity and total time until settling. To ascertain if a particular animal exhibited the same relative activity

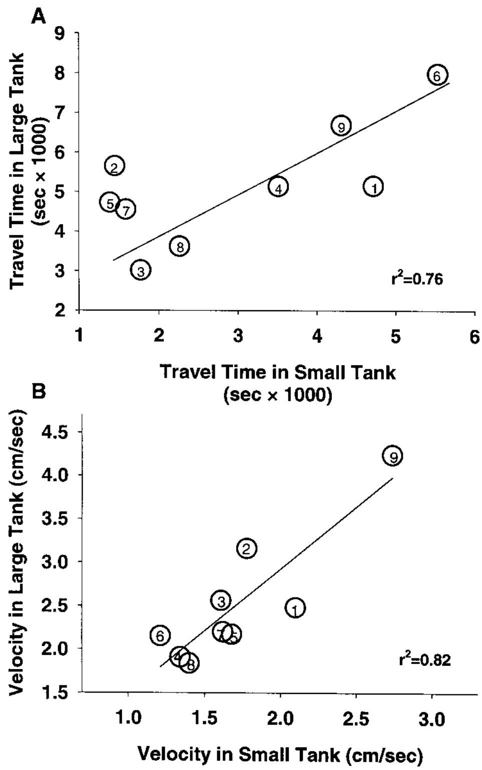

Fig. 5. Velocity and travel in small and large tanks. Individual variation in the degree of activity was consistent across the different experimental observation tanks. A, generally, the total amount of travel time until a particular animal settled was relatively consistent in larger tanks in comparison to the sample of animals examined; B, this relationship was also apparent in the velocity of movements in the different-sized tanks. (Each numbered circle represents one crayfish.)

profile in comparison to the tank size, the values of time spent moving and velocity are compared for the small and the large tanks (fig. 5). The relative amount of travel time for a particular animal until settling occurred was relatively consistent, independent of tank size. This suggests that the intrinsic differences among the crayfish were profiled for both tank environments. This might be useful for measures of the intrinsic state of the animal (fig. 5A). This relationship also held for velocity in tanks of both sizes (fig. 5B).

DISCUSSION

The present study is an attempt to determine if blind cave crayfish show habituation to territorial exploration, inferring familiarity to the surroundings. A composite of behaviors, such as a decrease in the rate of walking movement around the perimeter of the enclosure, an increase in the number of pauses, and digging a burrow or hiding in a crevice were used to aid in distinguishing that the animal became familiar with a novel environment. The behavioral patterns exhibited in the different-sized tanks suggests that these animals become familiar with the smaller tanks faster than with larger tanks. They settled sooner in small tanks. Using a large round tank illustrated that animals did not stop movement due to fatigue in the smaller observation tanks. Regardless of tank size, fatigue was not a factor. Thus, the decreased movement over time indicated the animal chose to cease exploratory movements and begin a construction of a burrow or to settle in one place for an extended amount of time. Since the crayfish showed a characteristic type of intrinsic activity profile in both observation tanks, there is likely an internal state of being that is different for a given individual. Such differences in internal states, as measured by heart rate, have been reported in this species of blind cave crayfish (Li et al., 2000) as well as in epigean crayfish (Listerman et al., 2000). These sightless animals can very quickly sense the size of the novel tank setting. The briefer number of pauses and greater velocity of movement between pauses observed for the larger tanks indicate the animal's awareness that the area available was larger. These behaviors can be depicted before a single circuit is completed around the enclosure, which implies that the animal is aware of the extent of the environment by means that are not understood at present.

The central neural processing involved in gaining familiarity, and which set of sensory cues are primarily needed for familiarity, are not known. In general, it would seem reasonable to conclude that when a memory is matched to incoming sensory signals, an internal state, termed familiarity, would be reached. This familiarity may be within cellular processing or consist of a particular firing pattern among cells. The details of such neural networking are not yet known. Without visual cues, the blind cave crayfish rely on tactile and chemosensory cues for locomotion and spatial orientation. The results of this study show that the crayfish settle faster in a smaller tank, which suggests that they may recognize their environment by various sensory cues during our observation period. Frequent encounters with the same object, such as the rock or the corner of the tank, may cause an integration of their mechanosensory system indicated by the habituation behavior of settling. It is highly unlikely that there is a peripheral adaptation of the primary sensory tactile hairs or cuticular detectors from repetitive use, but instead that incoming sensory signals are repetitive, awaiting a match with previously stored sensory data. A match results in integration within higher neural centers. This is also the likely scenario for chemosensory signals in odor recognition (Atema, 1995; Breithaupt et al., 1999). Determining the exact process of familiarity is beyond the scope of this project. It can be suggested that familiarity is tied to the frequency at which repetitive signals are processed in the integrating centers, since memory itself is limited.

Cave crayfish experience frequent alterations in their natural environment from low pools subject to drought, to slowly rising water, to heavy torrents of water through their pools. Although it is counter-intuitive that they remain in one locality as a result of such changes, field observations indicate otherwise. Following a heavy rain, at one study site, we were able to re-collect 3 of the original 6 marked crayfish. Six crayfish had been marked with red fingernail polish in a relatively small pool (2 m across and 20 m long with a maximum depth of 30 cm). Heavy rain resulted in water completely filling the cave passage (3 m high, 10 to 15 m wide). Over a period of about a week the water subsided substantially, allowing access to the same pools. Although one pool in particular was now about twice as wide and deep, the three marked crayfish were found at its edges. This suggests that crayfish have the capacity to adjust to the changing settings and explore the changing boundaries of their environment.

Environmental pressures such as the availability of oxygen content in the water, may influence crayfish to remain in particular areas of a cave pool. The deeper regions of a stagnant pool might be low in oxygen, while the edges, close to the air-surface interface, provide for better gaseous exchange. Studies of environmental influences on the overall behaviors of crayfish are needed. However, there are several detailed studies on the behaviors and metabolic adaptations of other cave crustaceans to severe hypoxia (Hervant & Mathieu, 1995; Hervant et al., 1997, 1999), which indicate that cave organisms can withstand hypoxia better than their surface cousins (Hervant et al., 1999). There is little literature concerning cave crayfish behavior, although cave crayfish locomotor activity, monitored over a 3-year period by Park et al. (1941), shows that they are arhythmic. This suggests that blind cave crayfish do not have diurnal activity patterns, however, there are other examples of subterranean insects and crustaceans (Lamprecht & Weber, 1985) as well as of mammals, that exhibit some aspects of a circadian rhythm (Ben-Shlomo et al., 1995). Since we conducted the cave observations during the day (about noon) and all the tank observations in the late evening, at least the time of day was kept constant throughout all our laboratory experiments.

We have shown that blind cave crayfish exhibit habituation behaviors that suggest an internal state that we are terming "familiarity". Future studies can be directed toward the testing of spatial learning and spatial maps. The data presented herein can be used to develop indices for the testing of learning and memory in these animals. It may be likely that the blind cave crayfish have developed a better ability than epigean crayfish to spatially learn or become familiar to a novel environment, or to be better in developing a spatial map of a landscape, but this remains to be tested. The loss of a visual system in the cave crayfish does save metabolic needs which may possibly be used to compensate for other needs in their subterranean environment. It does appear that this species of cave crayfish possesses an enhanced neural processing for olfaction as compared to an epigean species (cf. Cooper et al., 2001).

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