Memory of Visual and Topographical Features Suggests Spatial Learning in Nautilus (Nautilus pompilius L.)

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Previous studies demonstrate that soft-bodied (coleoid) cephalopods are adept at learning and remembering features of their environment, but little is known about their primitive relative, nautilus. Nautilus makes nightly migrations from deep to shallow water along coral reef slopes, covering large areas of varied substrate. Memory of its surroundings may be advantageous, but the nautilus brain is the simplest among extant cephalopods, lacking dedicated neural regions that support learning and memory in other cephalopods. The authors hypothesize that the absence of these regions in nautilus may affect memory storage. Here the authors report the first evidence for spatial memory in 2- and 3-dimensional arenas. In a small open-field maze, nautiluses learned the location of a goal within 3 trials, and memory was stable for at least 2 weeks. In 3-dimensional environments, animals habituated within and across trials when their surroundings were unchanged, but activity increased when the environment changed topographically, although not when the change was visual only. These results are comparable to performances of coleoids in similar tasks and are surprising given the far simpler neuroanatomy of nautilus.

Keywords: nautilus, cephalopod, learning, memory, spatial

Cephalopoda (Mollusca) is a diverse and highly successful taxon that today occupies a wide range of marine niches. All modern cephalopods belong to one of two subclasses, the ancient Nautiloidea or the more modern Coleoidea (Teichert, 1988). The coleoids include those cephalopods with an absent or internalized shell (octopuses, cuttlefishes, and squid), while the nautiloids contain only the remnant genera Nautilus and Allonautilus (Ward & Saunders, 1997). Coleoids have a large and centralized nervous system that supports a range of complex and plastic behaviors (reviewed by Hanlon & Messenger, 1996). Their brains contain numerous discrete lobes with dedicated functions, at least one of which (the vertical lobe) is dedicated to learning and consolidation of memory, although other regions may play a role in memory storage (Fiorito & Chichery, 1995; Hochner, Brown, Langella, Shomrat, & Fiorito, 2003; Hochner, Shomrat & Fiorito, 2006; Young, 1960, 1991). There is considerable evidence for spatial learning in coleoids (Alves, Boal, & Dickel, 2007; Alves, Chichery, Boal, & Dickel, 2007; Boal, Dunham, Williams, & Hanlon, 2000; Karson, Boal, & Hanlon, 2003; Mather, 1991; Wells, 1964), as well as numerous examples of associative and nonassociative learning (reviewed by Hanlon & Messenger, 1996, and Mather, 2008).

By contrast, the primitive nautiloids have changed relatively little over the course of their evolutionary history and retain the external, chambered shell that is characteristic of both nautiloid and coleoid ancestors (Teichert, 1988). Their brains are relatively simple, containing fewer lobes and fewer neurons than do coleoid brains (Young, 1965). The ring-shaped brain is less centralized than that of the coleoids to accommodate the large esophagus that passes through its center, and the main lobes are not differentiated clearly from the surrounding tissue, in contrast to the defined, lobular structure of the coleoid central nervous system. Crucially, those regions implicated strongly in learning and memory in coleoids are absent in nautiloids. Although there are some structural similarities between the cerebral nerve cord of nautilus and the inferior frontal lobe in coleoids (Young, 1965), the function of this region in nautiloids is undescribed.

There is some evidence that the neuroanatomy of nautiloids may represent an ancestral condition (Shigeno, Kidokoro, Tsuchiya, Segawa, & Yamamoto, 2001; Shigeno et al., 2007; Young,
1965, 1991), and therefore identifying comparable behavioral expressions of learning in nautilus may provide evidence for an evolutionary antecedent of the “memory” centers present in the modern coleoid brain. Alternatively, it is plausible that the simple nautiloid brain represents a secondary simplification and that neural evolution in cephalopods is bidirectional (e.g., Safi, Seid, & Dechmann, 2005). Similar learning abilities in nautiloids and coleoid cephalopods would therefore suggest a trade-off between reduction in brain volume or complexity and maintenance of behavioral plasticity mediated by different neural substrates in nautiloids.

Learning and memory in nautilus were unknown until recently. Crook and Basil (2008) used an associative learning paradigm to condition Nautilus pompilius to associate a pulse of blue light paired with food odor. Animals demonstrated temporally separated short- and long-term associative memory, similar to results observed in cuttlefishes (Agin, Chichery, Maubert, & Chichery, 2003; Agin, Dickel, Chichery, & Chichery, 1998; Messenger, 1971). The duration of long-term memory (LTM) was surprisingly short in these experiments (under 24 hr), raising the possibility that brief LTM expression is intrinsic to the simple nautilus brain. In the present study we aimed to clarify whether this brief LTM expression was stable across different learning contexts or was instead specific to that associative learning paradigm. Here we focus on learning of visual environmental features, an important component of spatial learning. Such behavior is fundamental to survival in a range of animal species and is potentially an ecologically relevant task for N. pompilius.

Spatial learning is a widely distributed ability among evolutionarily distant taxa and is mediated in vertebrates and among invertebrates by nonhomologous neural structures, suggesting multiple evolutionary origins for this behavior. The absence of coleoid-like neural complexity in nautiloids is thus not necessarily a limitation on spatial learning, although some differences in cue use and strategy might be expected given the different visual systems (Muntz, 1987) and behavioral ecology (Hanlon & Messenger, 1996) of the two subclasses.

Forming representations of space involves stages of exploration, habituation, and memory maintenance (patrolling; Gallistel, 1990; Healy, 1998; O’Keefe & Nadel, 1978; Shettleworth, 1998). If a change to a familiar environment is perceived, then the level of exploratory behavior should increase (dishabituation), presumably because the previously gathered environmental information must be updated (bees: Simonds & Plowright, 2004; cave fish: Teyke, 1989; fish: Welker & Welker, 1958; hamsters: Poucet, Chapuis, Durup, & Thinas-Blanc, 1986). Patterns of staged exploratory behavior have been observed in some coleoids in their natural habitat (e.g., foraging excursions in reef octopuses: Forsythe & Hanlon, 1997; Mather, 1988, 1991). Laboratory studies also suggest spatial learning in octopuses (Boal et al., 2000; Papini & Bitterman, 1991; Walker, Longo, & Bitterman, 1970; Wells, 1964). While cuttlefishes rely primarily on crypsis to avoid detection by predators (Hanlon & Messenger, 1988), laboratory studies indicate that they are also capable of spatial learning (Alves, Boal, & Dickel, 2007, Alves, Chichery, Boal, & Dickel, 2007). There are limited data on squid and on pelagic octopuses.

The possibility that nautilus also uses memory of its surroundings is an intriguing one. In contrast to cephalopod species in which spatial learning has been described, nautilus is a deep-water inhabitant that spends most of its time in darkness (Carlson, McKibben, & DeGruy, 1984). While the brain is simple compared with those of the other cephalopods, nautilus is nonetheless capable of both short- and long-term memory (Crook & Basil, 2008). Demonstrating spatial learning in nautilus would add to our limited understanding of how nautiluses may behave in the wild and, secondarily, characterizing learning behavior across a range of contexts in nautiluses provides an important comparative perspective on the remarkable abilities exhibited by other cephalopod species.

The purpose of this study was to investigate learning and memory of visual environmental cues in wild-caught N. pompilius. We examined learning in a small-scale environment (within 10–30 body lengths), using two techniques: a two-dimensional open-field maze that required animals to locate and remember a fixed goal position in the arena with the aid of visual cues, and an artificial reef construction that tested habituation and dishabituation to a complex, three-dimensional environment that underwent changes in shape and pattern.

Experiment 1: Memory in a Two-Dimensional Environment

This experiment investigated whether animals could learn to locate a goal point within their environment that was marked with a proximate cue. Escape and bright lighting in the arena were used as motivators, because nautiluses typically descend in the presence of bright moonlight in the wild (Ward, Carlson, Weekley, & Brumbaugh, 1984) and will avoid shallow areas in their home tanks (Crook, 2008). Memory relied on a goal–cue association being learned during training.

Method

Animals. This experiment was conducted at Brooklyn College in March and April of 2007. Wild-caught, subadult Nautilus pompilius (N = 10) from the Philippines were obtained through a commercial supplier (Sea Dwelling Creatures, Inc., Los Angeles, CA). Shell diameters ranged from 110 mm to 119 mm. Sexes were undetermined because most animals this size are sexually immature. Animals were housed in two darkened, cylindrical tanks (1.5 m high × 0.8 m diameter) in a 530-L, recirculating, artificial seawater system (Instant Ocean™). The tanks were connected in tandem to a 95-L biofilter that supplied aeration and filtration for the system. Water was maintained at 17°C by a chiller (Aqua Logic 1.3-hp AE4) and was kept sterile by two ultraviolet (UV) filters (Emperor Aquatics 80-W Model 02080) and two protein skimmers (Red Sea™), operating constantly. The 12-hr light/dark cycle (06:00–18:00) alternated between very dim light and complete darkness. Tanks were covered, but two holes in the tank lids (diameter = 3 cm) let dim light into part of the tanks during the light half of the day/night cycle. Animals were maintained in the holding facility for at least 2 weeks before being used in any experimental procedure. We fed each animal a 1.5-cm cube of frozen Tilapia (Oreochromis niloticus) head by head every 4 days. Feeding schedule was altered during experiments to ensure that animals taking part in experimental procedures were not fed immediately prior to the procedure but were always fed several hours after the conclusion of testing. A delay of 3 to 4 hr between
behavioral testing and delivery of food should have prevented any association between the two events forming. A crucial component of this experiment involved animals swimming downward through the water column; thus only animals showing normal buoyancy regulation behavior were used.

**Apparatus.** Trials were conducted in one of the two cylindrical home tanks (Figures 1a and 1b), which had been fitted with a circular open-field maze that was immersed 10–15 cm below the water surface, about the depth of a nautilus-shell diameter. The maze was a circular polyvinyl chloride (PVC) platform made from a modified tank lid (Aquatic Ecosystems, Inc.), resized to fit tightly within the cylindrical home tank. The upper surface of the maze platform was smooth and black, identical in appearance and texture to the inner walls of the home tank. A single hole (diameter = 20 cm) cut into the platform close to one edge served as the only point of exit from above the platform into deeper and darker water (130 cm deep, under the maze) within the home tank. A visual and tactile “beacon” surrounded the goal, acting as both a proximate (tactile) and a distant (visual) navigational aid. The beacon was constructed from a ring-shaped piece of bubble wrap with strips of white tape affixed, forming stripes that radiated from the hole outward. This combination of pattern and texture contrasted strongly with the smooth black surface of the platform. The test arena was lit by fluorescent tubes located directly above the tank. Within the tank, there was a gentle, anticlockwise flow of water (~1 cm/s) from the inlet pipe below the maze platform to the outlet pipe above it.

Trials were videotaped with a digital camera (Hitachi CCD, Model KP-M2U) suspended above the tank, and a Sony DV minirecorder (Sony DV walkman, Model GV-D900). The experimenter, using the minirecorder display, monitored the animal from behind a blind.

**Experimental procedures.** Animals (N = 10) were trained and tested in random order. Five minutes before a trial began, 1 animal was removed from its home tank and placed in an uncovered bucket containing home tank water. This allowed the animal a period of preexposure to ambient light levels in the experimental room before the trial began. The water level above the platform was adjusted to the same depth as the test subject’s shell height such that the base of the animal’s shell would remain in contact with the platform as it swam. The shallow depth of the water above the platform and the bright light in the experiment room acted as aversive stimuli.

Within the tank, the goal location (exit) was marked by the beacon, but otherwise the interior of the tank was featureless, because all surfaces were made from identical black PVC plastic. Flow within the tank provided local hydrodynamic cues. A light gradient within the room was created by an algal-culture stand in one corner lit by broad-spectrum fluorescent lights, a black UV filter casing projected above the tank rim to the left of the escape point, and the wall opposite the algal culture stand painted a darker shade than the other walls (yellow vs. white). The visual sensitivity of nautilus is not well known (Muntz, 1987), and we address use of specific cue types and intra-maze versus extramaze cues in other experiments (Crook, 2008).

Video recording of the experimental arena began 30 s before the animal was positioned in a consistent start location (Figure 1b), which was located opposite (180°) the escape hole. The subject was held in the start position for 5 s, facing into the center of the tank, then released and allowed 10 min to locate and swim through the exit hole and into deeper water. Animals that remained stationary for more than 1 min or attached to the maze surface with their tentacles during training trials were prompted with a tap on the hood with a wooden rod to motivate renewed search behavior. Once subjects descended through the hole, they remained undisturbed in the deeper, dark water for 10 min as a positive reinforcement for completing the task. Animals that failed to complete the task within the 10 min were guided gently by hand into the escape hole as soon as the 10-min trial time expired. This was done either by allowing the animal to attach its tentacles to the experimenter’s fingers, who then towed the animal at its normal swimming speed into the hole, or if the tentacles were retracted, it was pushed from behind at swimming speed until it contacted the hole and exited. Our animals were accustomed to being hand fed, so contact with the experimenter’s hand and being moved manually were experiences with which our animals were familiar. Contact did not appear to have been perceived as threatening, given the normal coupling with feeding and the absence of typical alarm behaviors in response to being moved by hand. Once inside the hole, subjects descended usually without further prompting and were left under the maze for 10 min. After 10 min, animals were

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**Figure 1.** The apparatus set-up for Experiment 1. (a) The maze was immersed in one of the cylindrical home tanks, to a depth similar to the height of the focal animal’s shell. A camera recorded the trial from overhead. The arrow shows the escape path. (b) Maze shown from above. S = start position; E = escape hole; L = landmark. Not to scale.
A sequential Bonferroni correction (Rice, 1989) was used to correct for accumulating alpha error rates arising from multiple post hoc comparisons. All probability values quoted are two-tailed. Hedges’ g effect sizes and their 95% confidence intervals (presented as effect size; upper/lower CI) are quoted for selected significant comparisons.

Results

Escape times. Escape times decreased rapidly across the five training trials. There was an overall difference among trials (Friedman test, $Q = 16.71$, $df = 4$, $p = .002$). In the first trial, 7 of the 10 animals failed to escape within the time allotted and were moved to exit hole at the conclusion of the trial; an escape time of 600 s was recorded for these individuals. In the second trial only 3 animals failed to complete the task within 10 min. The mean escape time decreased significantly from the first trial ($Z = -2.70$, $df = 1$, $p = .007$; Figure 2), but CLs of the effect size overlapped zero ($g = -0.68; 0.22/-1.38$). There was a further significant decrease in the third trial ($Z = -2.55$, $df = 1$, $p = .01; g = -2.04; -0.96/-3.12$), where the mean escape time reached an asymptote at about 1 min, and all animals exited the maze successfully during the trial period. There was no further decrease in Trial 4 ($Z = -1.02$, $df = 1$, $p = .31$) or from Trial 4 to 5 ($Z = -1.37$, $df = 1$, $p = .17$). In Trial 4 and Trial 5, all animals escaped within 10 min. There were no significant differences in comparisons of escape times in the final training trial to any of the memory tests (see Figure 2), indicating retention of memory across the 21-day test period. Although a slight increase in escape times was apparent in the MT at 7 days and 21 days, escape times remained significantly lower than those of naïve animals in Trial 1 (7 days: $Z = -2.39$, $df = 1$, $p = .02$; 21 days: $Z = -2.20$, $df = 1$, $p = .03$).

Figure 2. Mean escape times (±1 standard error) in successive training and testing trials in Experiment 1. Nautiluses ($N = 10$) received five successive, 10-min training trials (intertrial interval, ITI = 15 min), then 6 subjects were selected randomly for memory tests at each of the retention periods (all 10 received a memory test at 7 days posttraining). Escape times decreased rapidly and remained consistently low from the third training trial to at least 21 days posttraining. Points labeled with a different letter are significantly different ($p < .05$, two-tailed).
**Movement patterns.** We computed a simple statistic of movement patterns by comparing the number of times per trial animals changed heading direction by more than 15°, an angle we considered to represent intentional change and not merely oscillations created by the natural swimming motion made during movement across the surface of the maze (see Tourtellot, Collins & Bell, 1991). Complete routes taken by each animal and the mean number of changes of direction per trial are shown in Figure 3. Increasing linearity of escape paths during training indicates that learning was responsible for the decrease in escape times rather than an increase in search activity or swimming distance, producing an increased encounter rate with the exit location. A significant decline in direction changes compared with naive animals occurred in Trial 4 (Z = −2.14, p = .03, g = −1.40; −0.43/−2.38) and persisted in Trial 5 (Z = −1.97, p = .04; g = −1.36; −0.39/−2.33). Paths remained relatively direct across tests at each retention interval, with no significant increases in comparison with the final training trial.

**Experiment 2: Memory of Three-Dimensional Environments**

In Experiment 1 it was apparent that our animals learned to associate the beacon with the reward; however, whether spatial or associative learning was responsible was not clear. Therefore we used a different experimental paradigm to illustrate learning of spatial representations in three dimensions. The purpose of this experiment was to investigate whether

(a) nautiluses would move about a new environment spontaneously;

(b) nautiluses would show reduced activity over time consistent with habituation;

(c) increased activity (dishabituation) would occur when features of the environment were changed; and

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![Figure 3. Route maps show the paths that nautiluses took in each trial of Experiment 1. Positions were plotted every 2 s from the release point (lower right of each large circle) until animals reached the exit (inner circle, top left) or until the 10-min trial expired. The route of one randomly selected animal has been highlighted in black: This individual received five training trials and was tested at the intervals of 18 hr, 96 hr, 7 days, and 21 days posttraining. Paths of all other individuals are shown in gray. Points show the final position of each subject. In training trials (1–5, top panel), paths initially suggested a random exploratory pattern. In successive training trials the paths taken to the exit became progressively straighter. Paths remained relatively direct throughout the retention period (bottom panel), suggesting that memory was retained for at least 21 days after training. The mean number of changes of swimming direction (>15° change in heading angle) are shown below each map. Asterisks indicate significant differences (p < .05, two-tailed) in the number of direction changes. Training trials were compared to behavior of naïve animals in Trial 1; a significant decline appeared in training Trials 4 and 5. Behavior in each memory test was compared to the final training trial, no significant differences were detected.](image-url)
whether changes both to the shape (topographical change) of the environment and to the pattern (visual change) produced an increase of exploratory activity. We used habituation (decreasing activity levels) as a measure of learning in this experiment because the arena did not include aversive shallow areas or a specific goal point.

Method

Animals. This experiment was conducted in July and August of 2007 at the Marine Biological Laboratory (MBL; Woods Hole, MA). Wild-caught animals \((N = 5)\) were obtained from Vanuatu via a commercial supplier (Pacific Island Imports, Artesia, CA). The housing system and experimental arena were cylindrical PVC tanks that were connected to the open seawater system at the MBL. Water temperature increased slowly over the course of the experiment from 19°C to around 21°C. The home tank was covered by light-proof sheeting, which was lifted only during feeding and daily maintenance. Animals were fed by hand with a whole uncooked shrimp every 4 days.

Apparatus. Experimental trials were conducted in a cylindrical PVC tank (diameter 120 cm, depth 120 cm) completely enclosed within a blind of light-blocking black plastic. The tank contained a single inlet pipe about 10 cm below the water surface and an outlet pipe about 2 cm above the floor of the tank. Flow was slow (~10 cm/min) and circular. The arena was lit from directly overhead with a single incandescent blue bulb (40 W), providing consistent dim light throughout the experimental procedures.

Three artificial “reefs” were built into this test arena over the course of the experiment. Each reef was constructed from 10 concrete cinder blocks \(19.4 \times 19.4 \times 39.7 \text{ cm}\) and three short lengths of white PVC plumbing pipe \(10 \text{ cm}, 10 \text{ cm}, 20 \text{ cm}\). Three sides of each block had been painted with inert acrylic paint: five painted white and five painted black. The blocks and pipe lengths were arranged against one side of the cylindrical experimental tank, in three different configurations (Reef 1, Reef 2, and Reef 3; Figure 4) over the course of the experiment. Each reef contained the same number of blocks and pipe pieces, and the number of exposed surfaces and the number of painted sides visible (not abutting another block) were constant across the three different reef configurations. Reef 1 (Figure 4a) was a step-like stack of blocks that contacted approximately a third of the edge of the tank. Reef 2 and Reef 3 (Figures 4b and 4c) were stacked in an identical H shape that contacted a narrow portion of the tank wall but projected into the center of the water column. Reefs 2 and 3 were identical to each other structurally (but were a different spatial configuration than that of Reef 1), but they differed with respect to the arrangement of the black and white blocks. Thus an animal exposed to Reef 1, then Reef 2, would have experienced a structural change and a visual change to its environment (the shape and the pattern of light/dark blocks were different), while an animal exposed to Reef 2 and then to Reef 3 would have experienced a visual (the pattern of light/dark blocks) change only.

All experimental trials were recorded from an oblique overhead angle with a Sony mini-DV handycam (DCR-HC36) equipped with night-shot capability, on a time-lapse recording protocol of 1 frame per 4 s (15 frames per minute). Small visual markers on the inside walls of the tank created a virtual grid for video-analysis purposes, allowing us to estimate movement of the animal in three-dimensional space from this recording position.

Experimental procedures. Before trials for each reef commenced, the trial tank was drained partially, and one of the three reefs was assembled by hand. The tank was refilled with fresh seawater from the open seawater system and at least 5 hr elapsed with continuous flow before an animal was introduced. Animals were exposed first to Reef 1, then to Reef 2, then to Reef 3. For each trial, an animal was collected from the home tank in a darkened bucket and transferred into the trial tank, at the surface of the water and opposite the reef, then allowed to move about the tank freely for 3 hr. At the conclusion of the 3-hr period the animal was removed from the trial tank and returned to the home tank for 6 hr. This process was repeated for five trials, then during the normal 6-hr ITI the tank was drained, and Reef 2 was assembled. Another five trials were given on the same schedule, before the final reef change was conducted (Reef 3), and again animals received five 3-hr trials in the final reef configuration.

Data analysis and statistical procedures. Behavioral data were taken from tapes at the conclusion of the experiment. An assistant who was unfamiliar with the experiment assigned a code number to each tape that was unknown to the videoanalyst until after the analysis was complete.

We recorded the distance that each animal swam to the nearest 10 cm per “active minute” (minutes during which some movement was recorded) in each trial, across the 3 hr of trial time. We also

![Figure 4](image-url)
calculated the percentage of time that the animals spent swimming ("percentage activity") for the whole trial: (minutes during which some movement was observed/180)*100. Within-trial habituation was identified by comparing behaviors between the first and third hours of each trial. Across-trial habituation was identified by comparing behaviors between the first and last trials in each reef configuration. Comparisons between the last trial in each reef configuration and the first trial of the next configuration provided evidence for dishabituation in response to a change of environment. We scored the subject’s proximity to the reef by taking point observations of its position every 60 s (0 = more than 30 cm away; 1 = within 30 cm but not touching the reef; 2 = touching the reef). Proportional data (number of times each score was recorded/180) were compared among trials. The sample size in this experiment was small, and data did not meet assumptions of a normal distribution; thus nonparametric statistics were applied. All probability values are two-tailed.

Results

Within-trial habituation. For Reef 1, there was significant decrease in distance swum between the first and third hours (Wilcoxon signed-ranks test, \( Z = -3.18, df = 1, p = .001; g = -2.40; -0.78/\sim -4.03; \) Figure 5a); neither Hour 1 nor Hour 3 was different from Hour 2. There was also a decline in the percentage of time animals were active between the first and second hours (Wilcoxon signed-ranks test, \( Z = -2.55, df = 1, p = .01; g = -1.64; -0.21/\sim -3.07 \)), but there was no further decline in activity in the third hour (\( Z = -1.95, df = 1, p = .051 \)).

In Reef 2 the pattern was similar. There was a significant decline in distances swum between the first and second hours (\( Z = -2.69, df = 1, p = .007; g = -2.02; -0.50/\sim -3.55 \)). In the third there was a nonsignificant decline from Hour 2 (\( Z = -1.82, df = 1, p = .06 \)). The percentage of time that animals were active also decreased across the 3 hr. There was a significant decline from the first hour to the third hour only (\( Z = -3.41, df = 1, p = .001; g = -1.56; -0.14/\sim -2.97 \)).

In the final reef configuration (Reef 3), the decline was derived from the change in activity between Hour 1 and Hour 2 (\( Z = -3.07, df = 1, p = .002; g = -2.14; -0.59/\sim -3.70 \)). There was no further decline in the third hour (\( Z = -0.29, p = .76 \)). The percentage of time that animals were active declined slightly from the first trial to the
third trial, but this result was not significant after the Bonferroni correction was applied ($Z = -2.11, df = 1, p = .04$).

Across-trial habituation over repeated exposures to identical reefs: Dishabituation in response to reef changes. There were no significant differences across trials in the distances that animals swam. However, the percentages of time that animals were active did show evidence for across-trial changes (Figure 6). Activity declined from Trial 1 to Trial 5 ($Z = -2.55, df = 1, p = .01; g = -1.40; -0.01/-2.78$) and increased when animals experienced the topographic change, from Reef 1 to Reef 2 (Trial 5 to Trial 6; $Z = -2.20, p = .04; g = 1.45; 2.84/0.06$), indicating that some dishabituation to the environment had occurred. By the final trial in Reef 2, activity had declined again (Trial 6 to Trial 10; $Z = -2.32, p = .02; g = -1.46; -0.06/-2.85$), but there was a nonsignificant increase in activity when Reefs 2 and 3 were exchanged (Trials 10 to 11), indicating that a visual change only did not result in increased exploration ($Z = -0.14, df = 1, p = .89$). There was no further change in activity from the first trial to the final trial in Reef 3 ($Z = -0.82, df = 1, p = .41$).

Movement patterns. Time spent both in proximity to (within 30 cm) and in contact with the reef structures typically increased with increasing experience, although variation within subjects was large, and significant differences were few (see Figure 7). There was a significant increase in the proportion of time animals spent

![Figure 6.](image)

Figure 6. There was no evidence for across-trial habituation in (a) the mean distances that animals ($N = 5$) swam per minute of each trial, but there were significant differences in (b) the percentages of time that animals were active. Points show the mean ±1 standard error for each trial. Percentage activity decreased across successive trials in Reef 1, indicating that animals remembered features of the environment across the five-trial period. When the reef was changed from Reef 1 to Reef 2 (a structural change), activity increased, then declined again across successive trials. There was no increase in activity when the environment changed visually only. Asterisks indicate significant differences ($p < .05$, two-tailed) between behavioral measures in each trial.
within 30 cm of the reefs from Trial 1 to Trial 5 ($Z = -2.02, df = 1, p = .04$), and in the time spent in contact with the reef ($Z = -2.02, p = .04$). Although a slight decline in proximity was evident at the change from Reef 1 to Reef 2, neither measure was significant (within 30 cm: $Z = -1.48, p = .13$; in contact: $Z = -1.82, p = .68$). There were no significant changes between the first and last trials in either of the subsequent configurations or at the second change of reef.

Discussion

*N. pompilius* was able to learn about features of its spatial environment rapidly and retained this memory for at least 14 days after training. Despite possessing a considerably simpler brain (Young, 1965) and behavioral repertoire (Basil et al., 2000; Soucier & Basil, 2008) than do coleoid cephalopods, nautiluses displayed comparable associative and nonassociative learning and memory when tested under similar circumstances (e.g., Alves, Chichery, et al., 2007; Boal et al., 2000; Hvorecny et al., 2007; Long, Hanlon, Ter Maat, & Pinsker, 1989; Papini & Bitterman, 1991). We stress that the results presented here do not provide unequivocal evidence for spatial learning in the nautilus, such as the use of path integration vectors or formation of cognitive maps, but instead suggest some mechanisms and types of cues that may be learned and remembered by nautiluses as they navigate about their environment. More study is required to resolve what tactics and cues nautiluses are able to use to navigate and to learn about their environment. In Experiment 1 it was likely that the animals simply learned the association between the proximate visual cue and the exit, although it is possible that extramaze visual cues or other cue types such as hydrodynamic flow were used to navigate.

We investigate use of multiple visual cues and map-based navigation in the nautilus in another study (Crook, 2008).

Despite some uncertainties over the type of learning involved, our study demonstrates several important new findings: very rapid learning of visual cues and retention of memory that was stable for a considerable period.

Acquisition times for nautiluses were similar to those recorded in coleoids: Octopuses placed in a circular open-field maze similar to the set-up in Experiment 1 learned the location of a burrow within the first three trials and retained this memory for at least a week (Boal et al., 2000). In similar spatial memory experiments with cuttlefish, subjects showed a marked drop in escape time after four training trials (Karson et al., 2003), whereas we observed a significant decrease in escape time after a single training trial and further improvement in the next. Habituation was also rapid, in some cases within an hour of exposure. Habituation to stimulus objects, indicated by decreased exploration, has been shown to occur both within and across trials in octopuses. Kuba, Byrne, Meisel and Mather (2006) found that octopuses decreased exploitation of a novel object within 30 min of its presentation and showed reduced exploratory behavior in repeated trials over 5 days. Similarly, Boal et al. (2000) found evidence for habituation to familiar surroundings over the course of 24 hr and memory of those surroundings for a further 24 hr, suggesting that habituation occurs on comparable time schedules in the nautilus and in octopuses.

We found clear retention of memory at 21 days posttraining in Experiment 1, although repeated testing of subjects may have reinforced this memory over the week after the training trials. The 21-day test occurred after the test of all subjects at 7 days post-
training, so memory persisted at least 14 days without any “re-


Unlike other wide-ranging marine species (albatross: Jouventin


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