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Magnetic field discrimination, learning, and memory in the yellow stingray (*Urobatis jamaicensis*)

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Abstract Elasmobranch fishes (sharks, skates, and rays) have been hypothesized to use the geomagnetic field as a cue for orienting and navigating across a wide range of spatial scales. Magnetoreception has been demonstrated in many invertebrate and vertebrate taxa, including elasmobranchs, but this sensory modality and the cognitive abilities of cartilaginous fishes are poorly studied. Wild caught yellow stingrays, *Urobatis jamaicensis* ($N = 8$), underwent conditioning to associate a magnetic stimulus with a food reward in order to elicit foraging behaviors. Behavioral conditioning consisted of burying magnets and non-magnetic controls at random locations within a test arena and feeding stingrays as they passed over the hidden magnets. The location of the magnets and controls was changed for each trial, and all confounding sensory cues were eliminated. The stingrays learned to discriminate the magnetic stimuli within a mean of 12.6 ± 0.7 SE training sessions of four trials per session. Memory probes were conducted at intervals between 90 and 180 days post-learning criterion, and six of eight stingrays completed the probes with a $\geq 75\%$ success rate and minimum latency to complete the task. These results show the fastest rate of learning and longest memory window for any batoid (skate or ray) to date. This study demonstrates that yellow stingrays, and possibly other elasmobranchs, can use a magnetic stimulus as a geographic marker for the location of resources and is an important step toward understanding whether these fishes use geomagnetic cues during spatial navigation tasks in the natural environment.

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Introduction

Elasmobranch fishes (sharks, skates, and rays) move across a wide range of spatial and temporal scales, from the annual salmon shark, *Lamna ditropis*, migration between Alaska and Baja California (Weng et al. 2005), to the diel movements of Port Jackson sharks, *Heterodontus portus-jacksoni*, over a few km^2 (O'Gower 1995). The white shark, *Carcharodon carcharias*, displays regular and highly directed movements over long distances to very specific locations in the middle of the Pacific ocean (Nasby-Lucas et al. 2009), yet there are few studies on how elasmobranchs are able to navigate between habitats. The environmental stimuli available to marine species as potential cues for orientation and navigation are comparable to the cues available to terrestrial migrants. However, the physical differences between water and air modify the propagation of stimuli within each medium and influences how stimuli function as navigational cues (reviewed in Lohmann et al. 2008). For example, water is denser than air, which increases the speed of sound and the absorption of light and results in a stratified water column with higher ambient pressures compared to air. Furthermore, the ions in seawater make it a good electrical conductor, whereas air is an insulator, and the movement of water relative to the substrate increases the effects of drift compared to air. Therefore, oceanic species often rely to a lesser extent on visual cues, but experience enhanced auditory and electrosensory perception. They can also detect rapid changes in ambient pressure with depth and chemical cues that are principally confined to horizontal layers of isopycnic water.

The Earth's magnetic field is an omnipresent environmental stimulus that is consistent across habitat transitions, but changes predictably in strength and orientation across large spatial and temporal scales. These qualities make the geomagnetic field (GMF) a reliable source of directional and positional information to magnetically sensitive animals.

The proposed mechanisms for detecting magnetic fields vary depending on the animal in question. Magnetoreception has been documented in bacteria (Blakemore and Frankel 1981), gastropods (Lohmann and Willows 1991), arthropods (Walker and Bitterman 1989), amphibians (Phillips 1986), elasmobranchs (Adrianov et al. 1974; Kalmijn 1978), teleosts (Quinn 1980), reptiles (Lohmann 1991), avians (Wiltschko 1968), and mammals (Mather and Baker 1981). Most organisms are thought to use small particles of biogenic magnetite to detect geomagnetic cues (reviewed in Lohmann and Johnsen 2000; Johnsen and Lohmann 2005, 2008), such as those found in the candidate magnetoreceptor cells of the rainbow trout, *Oncorhynchus mykiss* (Eder et al. 2012). But, the mechanism of magnetoreception in elasmobranchs is not clear. The evidence for magnetite in stingrays (Walker et al. 2003) is indirect and, to our knowledge, not supported by any studies that have isolated a magnetoreceptive cell in this subclass of fishes. Furthermore, the methods used by Walker et al. (2003) do not completely rule out the alternative mechanism, electromagnetic induction (Johnsen and Lohmann 2005, 2008). Elasmobranchs have an extremely sensitive electrosensory system that detects weak bioelectric fields created by their prey due to the exchange of ions with seawater (Wilkens and Hofmann 2005). It has been hypothesized that as an elasmobranch swims in seawater through the Earth's magnetic field that an electric current is induced around the fish (Kalmijn 1974). The magnitude and direction of the induced electric current would be a function of the strength and orientation of the geomagnetic field, which changes predictably with latitude and results in unique geomagnetic signatures for a given geographic location. If elasmobranchs can detect these induced electrical cues, they could potentially use them to derive a cognitive compass and map sense, or a sense of direction and location. However, no studies have shown direct evidence for electromagnetic induction in a shark, ray, or skate (Johnsen and Lohmann 2005, 2008), and this proposed mechanism remains supposition.

The sensory capabilities of elasmobranchs can be studied using a variety of physiological and behavioral assays. Magnetic field sensitivity was first demonstrated in elasmobranchs using neural recordings on the common stingray, *Dasyatis pastinaca*, and the thornback ray, *Raja clavata*, which showed that the central neurons (Adrianov et al. 1974) and electroreceptors (Akoev et al. 1976; Brown and Ilyinsky 1978) of the electrosensory system respond to rapid changes in magnetic field intensity. Early experiments used behavioral conditioning to understand the visual capabilities of lemon sharks, *Negaprion brevirostris* (Clark 1959; Gruber and Schneiderman 1975), bull sharks, *Carcharhinus leucas* (Clark 1963), blacktip reef sharks, *Carcharhinus melanopterus*, grey sharks, *C. menissorah*, (Tester and Kato 1966), and nurse sharks, *Ginglymostoma cirratum*, (Aronson et al. 1967; Graeber et al. 1973), and the hearing capabilities of lemon sharks (Clark 1959; Nelson 1967), and bull sharks (Clark 1963). However, conditioning animals to respond to magnetic stimuli is problematic because the GMF is always present, penetrates most objects, including biological tissue, and does not rapidly change in space or time. Furthermore, testing whether an animal can use geomagnetic cues to orient and navigate is a spatial task that requires the subject to move within a significant amount of laboratory space. Despite these challenges, there have been behavioral experiments indicating magnetic field sensitivity in the round stingray, *Urobatis halleri*, (Kalmijn 1978), the short-tailed stingray, *Dasyatis brevicaudata* (Walker et al. 2003), the sandbar shark, *Carcharhinus plumbeus*, and the scalloped hammerhead shark, *Sphyrna lewini* (Meyer et al. 2005).

The drawback with these behavioral studies is that the researchers used electromagnets to generate conditioning magnetic stimuli, and these devices create electrical artifacts as they are turned on and off. Therefore, it is possible that the sharks and rays were actually responding to the electrical artifact instead of the magnetic field (Johnsen and Lohmann 2005, 2008). Field studies on elasmobranch magnetoreception are lacking, but active tracking of scalloped hammerhead sharks showed highly directional movement between an island and seamount, despite considerable lateral drift due to currents and the absence of obvious visual cues (Klimley 1993). Klimley hypothesized that the sharks could have used the geomagnetic signature of the underlying bottom topography as a navigational aid, but was unable to test this idea due to the observational nature of the study.

Behavioral conditioning of elasmobranchs typically focuses on describing the detection ranges and thresholds for a sensory modality, while the learning and memory capabilities are given as a means to demonstrate sensitivity to specific stimuli. Learning and memory are the foundation of how an animal uses its experience to modify its behavior. Behavioral conditioning requires training a subject to learn and remember an association between a neutral conditioning stimulus (CS+) and an unconditioned stimulus (US+) in order to elicit a conditioned behavioral response (CR+). The recognition and discrimination of sensory stimuli is essential to correctly identify potential mates, predators, prey, and suitable habitats, and

remembering associations makes behavior more energetically efficient. Therefore, in order to understand the salient environmental cues that animals use to orient and navigate we must also study the cognitive processes that underlie spatial behavior. Recent studies on the higher cognitive functions in sharks and stingrays have focused on their ability to orient and retain spatial memories, solve problems, use tools, learn within social contexts, perceive symmetry and illusions, recognize and avoid objects, and retain memories (reviewed in Schluessel 2015). However, the role that instinct, learning, and memory play in the orientation and navigation of fishes, especially sharks and stingrays, is largely unknown (reviewed in Guttridge et al. 2009; Schluessel 2015).

The time it takes to learn a task and the memory retention window for memories depends on the task and can vary between species (Brown 2015). Life-threatening associations, such as hook or net avoidance, can be learned by fishes within one to five trials (Beukemaj 1970; Brown 2001) and can be remembered for up to a year, whereas more subtle learning can take much longer and be forgotten sooner. A short memory retention window is beneficial for species that live in simple and unstable environments where resources are patchy or unreliable (Odling-Smee and Braithwaite 2003). In such cases, remembering the location of predators, prey, or mates that frequently change locations would waste valuable cognitive resources. Conversely, long-term memory retention is useful for species found in complex and stable habitats, or those that alternate between places where profitable resources are more predictable (Odling-Smee et al. 2008). For these animals, it might be less costly to retain metabolically expensive memories (Dukas 1999) than to continually search for valuable resources.

The purpose of this study was to understand the cognitive abilities of elasmobranchs, to determine whether they could use magnetic cues to solve a spatial task, and to see whether they can detect magnetic stimuli without confounding electrical artifacts. This required a species that can detect magnetic fields, could learn to associate two stimuli under a behavioral conditioning paradigm, and could remember this association for a length of time concurrent with the migratory abilities of other elasmobranch species. Due to the aforementioned challenges of magnetic conditioning and spatial tasks, we could not use a highly migratory species; therefore, we assumed that a species living in a complex and stable habitat might be a suitable proxy. The yellow stingray, *Urobatis jamaicensis*, was small enough to perform spatial tasks within a laboratory and thrived in captivity. This species was found year round on Southeastern Florida reefs, hardbottom, and seagrass (Sulikowski 1996; Fahy 2004), and spent most of the daylight hours camouflaged from predators and prey under

a layer of sand. Active acoustic tracking of yellow stingrays over 2- to 28-h periods has shown elevated nocturnal activity with high site fidelity and highly directed forays between habitats (Fahy 2004). Similar diel behavior patterns have been seen in the congeneric round stingray, *U. halleri*, along with movements of greater than 30 km over three months (Vaudo and Lowe 2006). Therefore, it is conceivable that the yellow stingray has spatiotemporal movement patterns similar to its congener and could use geomagnetic cues to derive a sense of direction or location as it migrates between habitats. We hypothesized that the yellow stingray could discriminate between a non-magnetic control and the magnetic stimulus from a permanent magnet; it could learn to associate a magnetic stimulus with a food reward; and it could remember this association for up to 180 days.

Methods

Husbandry

Yellow stingrays ($N = 8$) were captured via hand nets from a wild population in Riviera Beach, FL, under a special activity license from the Florida Fish and Wildlife Conservation Commission. Stingrays were placed in oxygenated seawater and transported 56 km south to the Florida Atlantic University Marine Research Laboratory at the Gumbo Limbo Nature Center in Boca Raton, FL. Stingrays were slowly acclimated to husbandry tanks (1.22×2.44 m) with flow-through seawater on a 12:12-h light:dark cycle and fed a mixture of squid and shrimp (3–5% body weight) every other day. Most animals would eat within seven days, and those that did not eat after 14 days were released back at the point of capture. Subjects were allowed to acclimate to the laboratory conditions for a minimum of 30 days before experimental trials began. Stingrays were separated into three cohorts of mixed size and sex and given an alphanumeric designation indicating the cohort (1–3) and individual within the cohort (a, b, or c).

Behavioral considerations

The natural behavior of yellow stingrays in the wild was similar to that in captivity and dictated certain methods in order to ensure cooperation during behavioral conditioning. Stingrays normally spend the daylight hours camouflaged under a layer of sand (Fahy 2004) and break crypsis when threatened by another organism or when they sense the presence of food. A wild stingray that detected a potential threat would swim away in a rapid and highly directed manner without deviation, then resettle on the substrate and

bury itself in the sand (K. Newton pers. obs.). However, a captive stingray that was threatened could only escape by swimming off the substrate in rapid circles along the periphery, or swimming up the side of a tank. In such cases, the agitated stingray would take several minutes to resettle onto the sand and would not participate in further training. In contrast, a stingray foraging in the wild would swim methodically along the substrate in a directed path using lateral head movements to search for prey (K. Newton pers. obs.) such as benthic fishes and invertebrates (Babel 1967; Quinn 1996). Likewise, a stingray in captivity that sensed the presence of food would search the bottom of the tank in a similar controlled manner until it located the food without any apparent effects due to space limitations. These distinctions were important because a captive stingray could become agitated at the slightest provocation and would display behaviors that were incompatible with conditioning. Therefore, we sought to coax stingrays in and out of the test arena using food odors because it facilitated cooperation of the subjects. One particular stingray would not respond to coaxing with food odorant and initially required a very slow and steady looming stimulus to shuttle into the arena.

Training

Each cohort was fed freely inside the experimental arena (Fig. 1) and acclimatized to the testing procedures (see below) in order to minimize behaviors that hindered the training procedure. When individual stingrays would enter the arena and swim steadily along the sand without expressing behaviors incompatible with conditioning, they were deemed ready for training. All subjects were successfully acclimatized, and none were removed from the study. Cohorts of stingrays were fasted 24–48 h prior to each training session to ensure proper motivation for magnetic stimulus training. We used relatively few trials per session and conducted sessions once every 24–48 h to maintain a high level of motivation in the stingrays. Subjects were trained individually, and the remaining members of the cohort were isolated behind an opaque barrier upstream from the experimental arena. Individual stingrays were placed into one of four randomly predetermined staging areas outside the arena, and each subject underwent one trial from each of the four staging areas for a total of four consecutive trials per training session (Fig. 1). One neodymium magnet (20 mm dia × 2 mm thick) coated in nickel (10^5 μT = 2222 × local geomagnetic field strength) and three non-magnetic controls of nickel (20 mm dia × 2 mm thick) were buried (north pole oriented up for the magnet) at pseudo-randomly determined locations within an arena (100 cm dia × 40 cm high) constructed of 20 mm dia PVC pipe, covered in 6-mm plastic mesh and

black plastic sheeting (Fig. 1). Individual barriers of PVC pipe and plastic sheeting were used to create staging and holding areas within the overall tank (Fig. 1). These barriers could be raised separately to allow the stingrays easy access between compartments and the arena. The magnet and controls were coated in epoxy to eliminate the electric fields generated by galvanic interactions between the metal and seawater, thereby ensuring that only magnetic stimuli emanated from the magnet.

Each trial consisted of pairing the magnetic conditioning stimulus (CS+) with the food reward (US+) in order to elicit foraging behavior (CR+) directed at the magnet. Trials went as follows: Once the stingray was resting calmly on the sand inside the staging area, 25 mL of food odorant (5% squid:seawater homogenate) was injected into the water to encourage the animal to break crypsis and explore the staging area, and then, the nearest edge of the arena was lifted a few cm so that the stingray could enter and swim freely along the bottom. As the subject swam over the location of the hidden magnet, it was immediately reinforced with a morsel of squid ($\sim 0.5\%$ BW) on the end of a sharpened plastic rod. This delivery method guaranteed accurate placement of the food near the mouth and temporal overlap of the CS+ and US+. Automated food delivery systems were impractical due to random placement of the CS+ throughout the tanks, and food dropped from above was impossible to accurately administer to a subject with a ventral mouth in a timely manner. Afterward, the stingray was allowed to swim out of the arena and into a new staging area and thus concluded one trial. Trials lasted for 120 s with a mean intertrial interval (ITI) of 300 ± 120 s. During the ITI, the magnet and controls were moved to new locations within the arena; the sand was cleared of food debris and raked to an undisturbed appearance, thereby eliminating confounding chemical and visual cues.

Memory probes

Stingrays were divided into three new groups to ensure that members from the original cohorts were tested at different intervals and reduce any group effects. The time intervals and group sizes accommodated the constraints imposed by the use of wild animals, limited laboratory space, fasting regimes, and the potential decrease in subject performance as interval duration increased. Memory probes were conducted at 90 ($N = 3$), 175 ($N = 3$), and 180 ($N = 2$) days post-learning criterion in order to maximize the chance that subjects would recall the task after a six-month window. During this time, the stingrays did not experience any magnetic stimuli or pairing of the CS+ with the US+ reinforcement. Prior to testing, the stingrays were fasted and allowed to re-acclimatize to the testing arena as
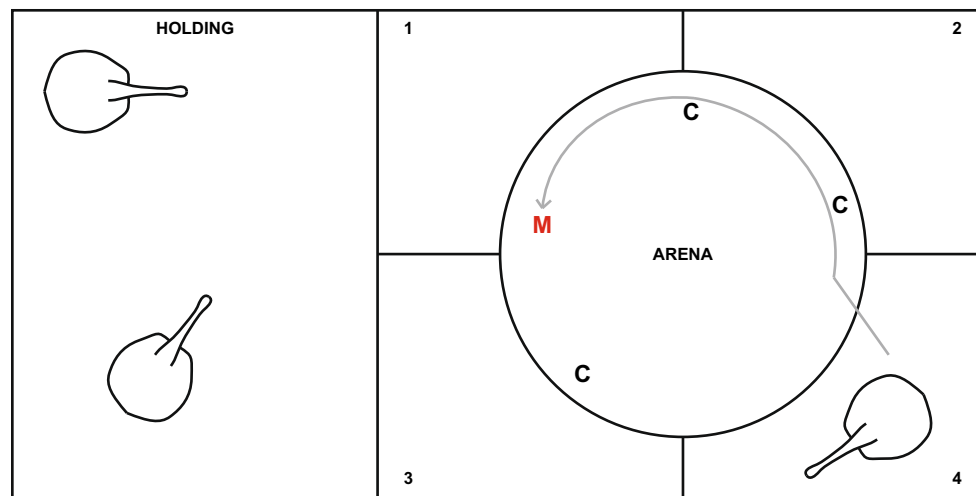
Fig. 1 Overhead view of the holding area, staging areas (*1–4*), and test arena (1 m dia) contained within a 1.22 × 2.44 m tank. *Letters* indicate the pseudorandom locations of three non-magnetic controls (*C*) and one magnet (*M*) buried in the sand for that particular trial. The *gray line* indicates an example of the path that a stingray would take once the edge of the arena was lifted enough to allow entry into the arena, and then, it would swim around the periphery to receive the food reward at *M*

previously described. Memory probes were conducted according to the aforementioned CS+ training procedure with one exception: the US+ was only given during the fourth trial. The first three trials of the memory probe session were unreinforced (US−), which allowed subjects that failed to express the CR+ during the initial trials of the session to demonstrate the CR+ in subsequent trials without any influence of prompts or "reminders."

Behavioral data collection and analysis

All training sessions were recorded on an overhead high-definition video camera for subsequent frame-by-frame analysis. We sought magnetic stimulus detection behaviors that were robust and consistent. Behaviors deemed equivocal were classified according to the most conservative interpretation. The primary observer reliability in identifying all behaviors (92%) was verified for a subset of data (one session for one cohort) by a second observer that was blind to the location of the magnets. Magnetic stimulus detection was determined by observing the following foraging behaviors (CR+) directed only at the magnets: an abrupt stop over a magnet and investigation of the immediate vicinity ($\sim$10 cm dia); an abrupt deviation ($\geq$30°) toward a magnet from an established swimming path followed by investigation; biting the sand over the magnet; digging up the magnet and; tossing the magnet about the arena. The ventral mouth of a stingray prevented the observation of jaw movement by an overhead observer. Therefore, bites were indicated by the ejection of sand from the spiracles, the presence of foraging pits left behind in the smooth sand, or the recurrent elevation of the anterior margin of the disc.

The latency, and the success or failure to find the magnet, was recorded for each trial, and the mean latency ($\pm$SE) and percent correct choice were calculated for each stingray during a session. If the stingray did not demonstrate the CR+ during a trial, or if it displayed the CR+ at any location other than at the magnet, then the trial was scored as a failure and the latency to detect the CS+ as 120 s. Training stopped when each stingray successfully reached the learning criterion of displaying the CR+ at the magnet in at least three out of four trials per session ($\geq$75% correct choice) for three consecutive sessions, and the mean latency for the stingray to complete the task during three sessions had reached a minimum value that was significantly different than the mean latency of the initial three sessions. The learning criterion for each stingray was established for the success of finding the magnet using a Chi-square test (χ^2 (3) $\leq$ 0.05) with a Yates correction. A Wilcoxon sign-rank test was used to compare differences between the mean latency of the initial compared to the final three sessions for individuals, and for the difference in latency between the last session of the learning criterion and the memory probe within the group. All tests were run using JMP (v9. SAS Institute, Cary, NC), and significance levels were set at $p \leq 0.05$.

Results

Overall results

All stingrays reached the learning criterion (Fig. 2) within a mean of 12.6 sessions ($\pm$0.7 SE, range = 10–15), for a mean total of 50.5 trials ($\pm$2.6 SE, range = 40–60). The
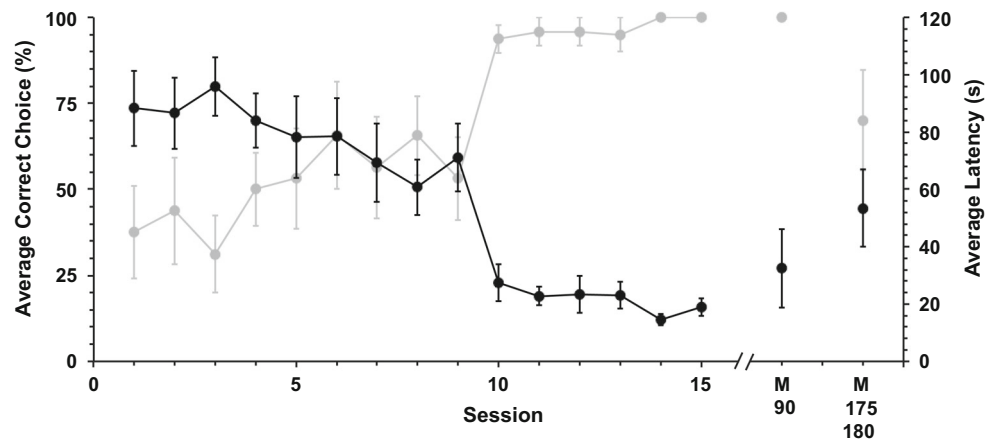
Fig. 2 Learning curve and memory probes for all ($N = 8$) yellow stingrays trained to detect a hidden magnet for a food reward. All data points are the mean for all available stingrays for that session. When an individual reaches the learning criterion, it is removed from the mean of subsequent sessions (i.e., for sessions 1–8, $N = 8$ stingrays; sessions 11–12, $N = 6$; session 13, $N = 5$; session 14, $N = 3$; session 15, $N = 1$). The mean ($\pm$SE) latency (*black line* and *ordinate axis*) to complete the task decreased sharply on the 10th session and the percent correct choice (*gray line* and *ordinate axis*) increased gradually between the 2nd and 11th sessions until the group reached the learning criterion (minimum latency, $\geq$75% correct choice for three sessions) on the 13th session overall. Memory probes were conducted at intervals of 90 days (session M90, $N = 3$) and 175–180 days (session M175/180, $N = 5$). Each session consisted of four 120-s trials

overall mean latency between the initial three and final three sessions for each stingray had significantly decreased ($S = 148.0$, $p < 0.0001$) from 90.2 s ($\pm$11.6 SE) to 22.0 s ($\pm$4.6 SE). Likewise, the overall mean success rate between the initial three and final three sessions for each stingray had increased significantly ($S = 123.0$, $p < 0.0001$) from 37.5% ($\pm$13.0 SE) to 96.9% ($\pm$3.0 SE). Six of eight stingrays (Fig. 3a, d–h) successfully completed memory probes at $\geq$75% proficiency, but the mean latency had increased significantly within these stingrays ($S = 75.5$, $p = 0.0137$). Memory probes indicated that the memory window for the yellow stingray to retain the conditioned association of a magnetic anomaly with a food reward was between 90 and 180 days without reinforcement.

All stingrays showed strong thigmotaxis, frequently swimming along the sand and exploring the edges of the enclosure. Occasionally, a stingray would get startled, swim vertically up the sides of the arena, resettle on the sand after several minutes, and not cooperate in any further training. Thus, we termed these occurrences as "incompatible behaviors" because they impeded effective conditioning or expression of the CR+. As training progressed, the responses of all individuals to the CS+ became more robust and consistent. These responses included: complete stops and investigation of the vicinity ($\sim$10 cm dia); large orientations ($\sim$45–90°) and investigation; biting the sand; digging up magnets; and tossing magnets about the arena. The learning curve for all animals is shown in Fig. 2, whereas the curves for individual stingrays are shown in Fig. 3a–h. Each graph depicts the mean latency ($\pm$SE) and the percent correct choice for each training session of four trials.

Individual results

Stingray 1a (Fig. 3a) was a juvenile male. During sessions 1–10, the large fluctuations in mean latency and percent correct choice were due to incompatible behaviors, such as vertical swimming up the walls. Consequently, the stingray did not reliably demonstrate learning or discrimination between the magnet and controls until session 11, and then, it reached the learning criterion on session 13. The memory probe was conducted successfully after a 175-day interval, and the mean latency had decreased by 9 s.

Stingray 1b (Fig. 3b) was a very small juvenile male. Once conditioning began, this stingray would easily find buried magnets and occasionally find controls, which were scored as failures. Indiscriminate biting at the magnet and controls, coupled with incompatible behaviors, yielded large fluctuations in latency and percent correct choice in sessions 1–9. Evidence of learning to discriminate between the magnet and control is shown by the steady decline in mean latency and the increase in percent correct choice during sessions 7–11. The stingray reached the learning criterion on session 13. After a 175-day interval, the memory was probed and the animal swam up the arena walls and would not perform the task in half the trials. As a result, the mean latency increased by 42 s and percent correct choice fell to 50%.

Stingray 1c (Fig. 3c) was a mature female. She would occasionally remain in the staging area with very little
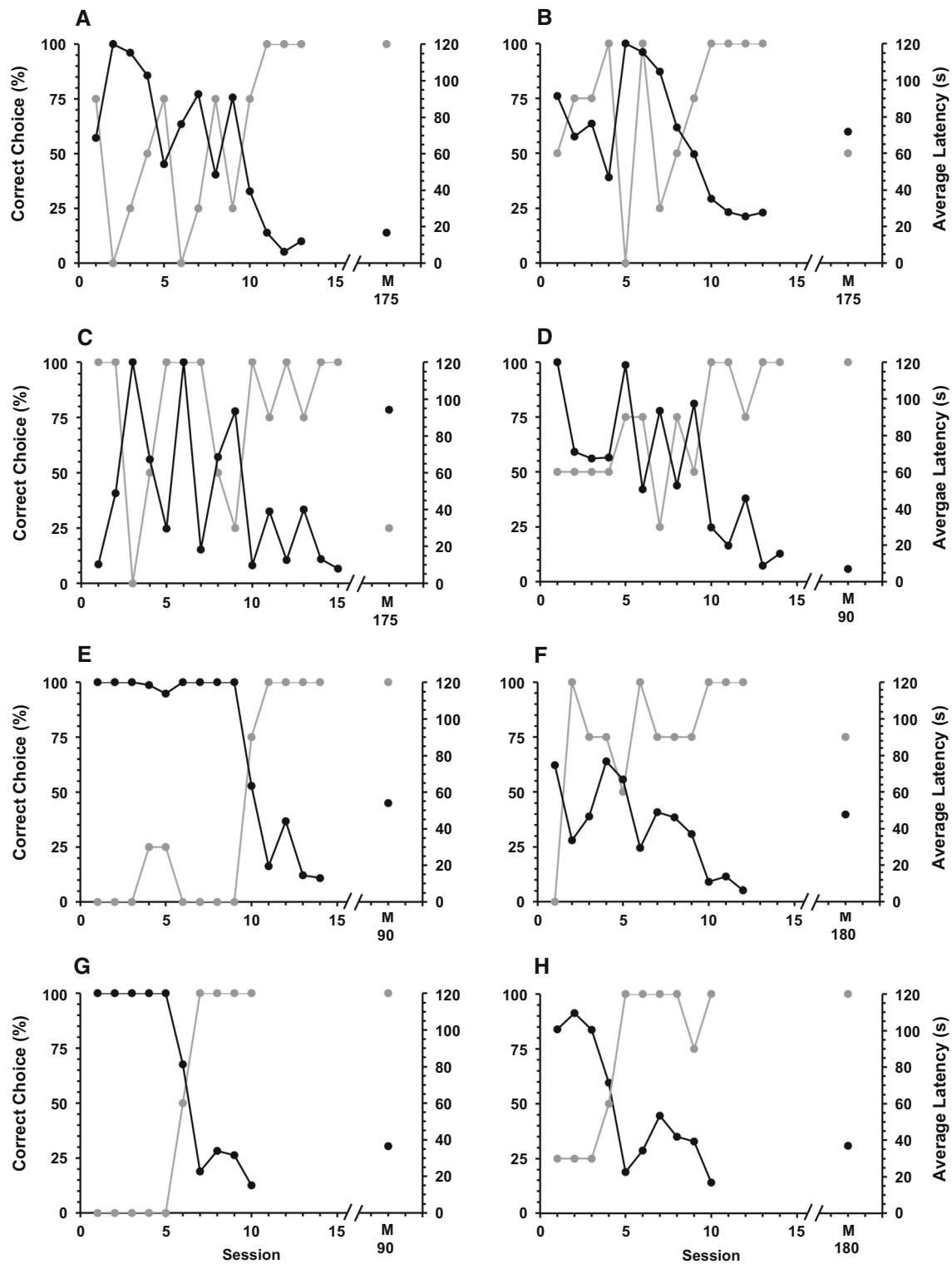
Fig. 3 **a–h** Learning curves for yellow stingrays ($N = 8$) trained to detect a hidden magnet for a food reward. Each training session consisted of four 120-s trails. The mean ($\pm$SE) latency (*black line* and *ordinate axis*) to complete the task decreased and the percent correct choice (*gray line* and *ordinate axis*) increased until each stingray reached the learning criterion (minimum latency, $\geq$75% correct choice for three consecutive sessions). The following **a–h** indicates when each stingray reached the learning criterion: **a**, **b** 13th session, **c** 15th session, **d**, **e** 14th session, **f** 12th session, **g**, **h** 10th session. The break in the x-axis indicates the post-learning criterion interval followed by the memory probe (*M*) and the duration of the interval (90, 175, or 180 days)

motivation to perform and would display intermittent bouts of incompatible behavior. These behaviors led to large cyclical fluctuations in latency and percent correct choice every three to four sessions. However, the fluctuations diminished over time. By session 10, the stingray could reliably demonstrate stimulus discrimination, and it reached the learning criterion by session 15. The memory probe was conducted unsuccessfully after a 175-day interval: the mean latency increased by 86 s and the percent correct choice decreased to 25% relative to that of the criterion. Poor performance on the memory probe was due to behaviors incompatible with CR+ expression.

Stingray 2a (Fig. 3d) was a mature male. Once training began, he would show progress in learning the CS+ and US+ association within a session, but would not demonstrate learning between sessions. This led to high variation in latency and fluctuating percent correct choice for the first nine training sessions. By session 10, the stingray was able to reliably discriminate between the magnet and controls and had achieved the learning criterion by session 14. After a 90-day interval, the memory probe was given, and the stingray performed the task with 100% success rate and a mean latency that decreased by 8 s.

Stingray 2b (Fig. 3e) was a mature female that would not initially perform the task and exhibited no appreciable learning for the first nine sessions. This animal required a slow moving looming stimulus to coax it into the arena, and then, it would swim about very slowly. During session 10, just prior to removing her from the study, she began to swim faster and orient moderately toward the magnet. She performed with rapidly increasing consistency and motivation until she learned the task by session 14. She performed a memory probe with 100% success after a 90-day interval, but the mean latency during the probe had increased by 29 s compared to the criterion due to low motivation and slow swimming speeds.

Stingray 2c (Fig. 3f) was a mature male. During the first nine sessions, this stingray had large fluctuations in mean latency and percent correct choice, due to equal interest in magnet and controls for one trial per session. The stingray reliably exhibited stimulus discrimination by session 10 and reached the learning criterion by session 12. After a 180-day interval, a memory probe was conducted at a 75% success rate and the latency increased by 31 s relative to the criterion due to incompatible behaviors and excessive interest in the controls during the first trial.

Stingray 3a (Fig. 3g) was a very small juvenile female that initially had difficulty adjusting to the procedures and did not respond to training for five sessions. However, during session 6, the stingray displayed a rapid decrease in latency and 100% magnet detection rate by session 7. She learned the task by the 10th session and performed the 90-day memory probe with a 100% success rate and an increase in latency of 21 s compared to the learning criterion.

Stingray 3b (Fig. 3h) was a juvenile female. This individual occasionally displayed the CR+ during the first three sessions and could reliably discriminate the CS+ from the controls with a low latency by the fifth session. Over the next five sessions, the percent correct choice was 75–100%, but during sessions 6–9 the latency to complete the task showed considerable variation, which increased the mean. She demonstrated the fastest learning curve of all stingrays tested and reached criterion by session 10. Her memory was probed after a 180-day interval, and the mean latency had increased from the criterion by 20 s and the percent correct choice remained at 100%.

Discussion

This is the first study of magnetoreception in the yellow stingray, *Urobatis jamaicensis*. Our results support the hypotheses that the yellow stingray can discriminate between non-magnetic controls and a magnetic stimulus from a permanent magnet, can learn to associate a magnetic stimulus with an appetitive stimulus, and can remember this association for up to six months. This experiment demonstrates that stingrays, and possibly other elasmobranchs, can use a magnetic cue as a geographic marker for the location of resources and is another step toward understanding whether these fish use geomagnetic cues during spatial navigation tasks in the natural environment.

All eight stingrays found the hidden magnets faster, more consistently, and more accurately with each training session, as evidenced by the overall decrease in the mean latency to perform the task and the increase in the overall percent correct choice between sessions. Five out of eight stingrays showed interest in the hidden magnets during the initial training sessions (Fig. 3a–d, f), which indicates that naïve stingrays have the ability to detect magnetic fields. However, the variation in the percent correct choice between sessions, and the variation in latency within and between sessions, shows that these stingrays had not learned to associate the CS+ with the US+ and, therefore, could not reliably demonstrate the ability to discriminate between the magnet and controls. We are confident that the only difference between the magnets and non-magnetic controls was the presence of a magnetic field because they were of similar size, shape, mass, composition, and external epoxy coating. It is possible that the epoxy insulation created a minor distortion in the ambient electromagnetic field within the tanks and that this distortion allowed the stingrays to use their electroreceptors to locate the controls,

but this would not explain the consistent and strong association of the CR+ with the CS+.

Our sample size was insufficient to test for differences in performance within the group due to sex or ontogeny. However, individual performance was consistent throughout the experiment. Stingrays that exhibited behaviors incompatible with conditioning during the initial training sessions behaved similarly during the memory probes, and this factor likely impacted their latency or success at finding the magnet (Fig. 3b, c, e, f, h). Furthermore, individuals that were motivated to perform remained motivated, and unmotivated individuals could learn to perform but would revert back to their unmotivated tendency during memory probes.

Magnetoreception in elasmobranchs

To our knowledge, this experiment is only the fourth behavioral conditioning study that demonstrates a magnetic sense in an elasmobranch, and the first to use a permanent magnet as the conditioning stimulus. The neodymium magnets used in our study were a constant source of magnetic stimuli with no electrical artifacts due to galvanic interactions with seawater or the onset of an electromagnet. It is unknown whether our stingrays used magnetite to detect the magnetic field, or their electroreceptors to detect an electrical current induced as they swam through the magnetic field in seawater. However, studies are underway to address this question. Previous work on the round stingray, a sister species to the yellow stingray, showed discrimination of magnetic field polarity within 78–248 stimulus pairings through a combination of appetitive and aversive conditioning (Kalmijn 1978). This was the first behavioral conditioning experiment to test for an elasmobranch magnetic sense, and it yielded robust results despite a small sample size. Short-tailed stingrays were trained to discriminate changes in magnetic field intensity within 30 trials (Walker et al. 2003). The scalloped hammerhead and sandbar sharks learned to associate the onset of a vertically oriented magnetic stimulus that pervaded an experimental tank with feeding events at a station within a tank (Meyer et al. 2005). In this case, the sharks showed a clear increase in goal tracking behavior even when the magnetic stimulus was given and food was withheld. However, this study did not present learning curves or the number of conditioning trials to reach criterion, so a direct comparison with our data is not possible. Additionally, the stingrays in Kalmijn (1978) and the sharks in Meyer et al. (2005) were trained as a group. The freshwater stingray, *Potamotrygon falkneri* (Thonhauser et al. 2013), and the lemon shark (Guttridge et al. 2013) have shown the ability to learn feeding strategies through direct observation of conspecifics. Kalmijn (1978) mentioned that one stingray was removed from his study because it could not compete with the others. Therefore, it is possible that social learning may have facilitated, or competition may have hindered, acquisition in individuals trained as a group to respond to magnetic stimuli.

Learning and memory in elasmobranchs

Direct comparisons between our study and the existing literature are not possible due to wide variations in methods and purpose, but the following will place our results within the broader context of our understanding of elasmobranch cognition. The average training period of 50.5 trials for the yellow stingray to reach criterion is the fastest for any batoid (rays and skates) and is among the fastest for sharks. For example, lemon sharks learned to press a target for food within 30 trials (Clark 1959), and nurse sharks, *Ginglymostoma cirratum*, rang a bell for food within 200–225 trials (Aronson et al. 1967), discriminated black and white objects within 96–203 trials, and horizontal and vertical stripes within 48–213 trials (Graeber et al. 1973). Blacktip reef sharks could differentiate rectangle orientation within 120–609 trials (Tester and Kato 1966); Port Jackson sharks associated an LED and air bubbles with food within 30 trials (Guttridge and Brown 2014). Gray bamboo sharks, *Chiloscyllium griseum*, distinguished visual symmetry within 110–270 trials (Schluessel et al. 2014) and learned a variety of allocentric and egocentric spatial navigation tasks within 37–225 trials (Schluessel and Bleckmann 2012; Fuss et al. 2014a, b). Freshwater stingrays, *P. motoro*, learned an allocentric and egocentric navigation task within 130–190 and 50–110 trials, respectively (Schluessel and Bleckmann 2005). Small spotted catsharks, *Scyliorhinus canicula*, learned to discriminate between AC and DC electric fields within 10 trials (Kimber et al. 2011), and yellow stingrays learned to discriminate electric field polarity within 360–440 trials (Siciliano et al. 2013).

Our result of a six-month memory window in the yellow stingray is the longest for any batoid to date and falls within the range of long-term memory windows for sharks. Memory recall, like learning, has been understudied in elasmobranchs. Clark (1959) found a 10-week window in bull sharks conditioned to associate a visual target with food. Schluessel and Bleckmann (2012) demonstrated a 41-day window in gray bamboo sharks for a spatial navigation study. Kimber et al. (2014) showed a 12-h to three-week window in small spotted catsharks that were trained to use an artificial electric field to locate food. Guttridge and Brown (2014) described a 24-h to 40-day window for Port Jackson sharks, and Fuss and Schluessel (2015) showed a 350-day memory window for a visual discrimination task in gray bamboo sharks.

The cognitive abilities shown in our study could be due to a well-developed telencephalon in the yellow stingray (Walker and Sherman 2001), the region of the forebrain involved in avoidance conditioning, learning, and memory in the gray bamboo shark (Schwarze et al. 2013). The yellow stingray belongs to a group of batoids (Order: Myliobatiformes, Family: Urolophidae) with brains that are 3–10 times larger than sister batoids (Walker and Sherman 2001) and a brain to body mass ratio comparable to that of birds and small mammals (Lisney et al. 2008; Yopak 2012). Learning in our stingrays might have been facilitated because the small neodymium magnets created an intense, localized magnetic stimulus that could have made it easy to discriminate from controls. Preliminary data suggest that more subtle stimuli, such as the relatively weak changes in the strength and inclination angle of the geomagnetic field, are more difficult for the yellow stingray to associate with a US, thereby making learning and memory recall more challenging (Newton and Kajiura, unpublished data). Certain yellow stingrays might have performed memory probes more successfully (Fig. 3b, c, e, f) if they had spent more time re-acclimatizing to the experimental setup prior to the probe trials, thereby reducing their expression of incompatible behaviors.

The reaction of the stingrays to the CS+ during the initial trials and the necessity to prompt the subjects with food odorant raises the possibility that the nature of the learning displayed in this study is more indicative of sensitization and not associative learning. First, the reaction of naïve stingrays to the magnets is contrary to the associative learning requirement that the CS be a neutral stimulus (Molet and Miller 2014). Second, the repeated use of food odorant could have sensitized the stingrays to release foraging behavior. If so, then the resulting behavior would progress in frequency and intensity, last for a long period of time, and be expressed with less and less odorant (Richtand et al. 2001; Webster and Laland 2013). We did observe an increase in the overall intensity of the responses for each individual and the behaviors persisted for a long time. However, we did not use progressively less odorant to see whether stingrays could be coaxed out of crypsis. Therefore, it is possible that sensitization occurred in this study. Animals do not produce magnetic fields, and a magnet should not trigger a predatory response in a stingray. A strong magnet hidden within the ambient GMF could have piqued the interest of the stingrays because it, like prey, produced a novel sensory signal that was localized and stood out against the background. Foraging in stingrays involves searching for hidden prey items by looking for visual, chemical, mechanical, or bioelectric cues in the midst of a featureless field of sand. Foraging requires flexibility to respond appropriately to internal and external stimuli as they occur, and a pattern of behavior that

increases in intensity with progressively less prompting stimuli would likely be maladaptive. Additional experiments with the appropriate design and controls could distinguish between the associative and non-associative components of learning using magnetic cues.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Data availability The datasets created during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Human and animal rights All applicable international, national, and institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the Florida Atlantic University Institutional Animal Care and Use Committee.

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