

MAGNETIC NAVIGATION, MAGNETORECEPTION, AND MIGRATION IN FISHES

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A Dissertation submitted to the faculty at the University of North Carolina at Chapel Hill in
partial fulfillment of the requirements for the degree of Doctor of Philosophy in the
Department of Biology.

Chapel Hill
2022

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ABSTRACT

Lewis Naisbett-Jones: Magnetic Navigation, Magnetoreception, and Migration in Fishes
(Under the direction of Kenneth J. Lohmann)

As the largest and most diverse vertebrate group on the planet, fishes have evolved an impressive array of sensory abilities to overcome the challenges associated with navigating the aquatic realm. Among these, the ability to detect Earth's magnetic field, or magnetoreception, is phylogenetically widespread and used by fish to guide movements over a wide range of spatial scales ranging from local movements to transoceanic migrations. During the last half century, considerable evidence has accumulated that fishes use Earth's magnetic field as a compass for maintaining direction (e.g. toward north or south) as well as a kind of "map" or positional sense that encodes information about their location. Yet, despite significant advances in the field, much about the magnetic navigation in fishes remains enigmatic. How fish detect magnetic fields remains unknown and our understanding of the evolutionary origins of vertebrate magnetoreception would benefit greatly from studies that include a wider array of fish taxa. The research presented in the following six chapters provides new evidence that fishes use Earth's magnetic field in navigation, insights into the possible underlying mechanisms and functional characteristics of the magnetic sense in fishes, as well as advances in methodology for tracking fish movements.

To my family and friends near and far, thank you for your unremitted support – I love and cherish you all.

ACKNOWLEDGEMENTS

This dissertation would not have been possible without the unwavering support of quite a few people; I cannot overstate my gratitude to all of them. I begin by thanking my supervisor, Ken Lohmann, whose careful guidance and support has been key to my successes over the last six years. Ken, you have helped me become the writer I am today; for that, and for the wisdom you have instilled in me, I am forever grateful. I greatly appreciate the entire Lohmann lab for their support. In alphabetical order, I am specifically thankful of Vanessa Bézy, Roger Brothers, Dave Ernst, Kayla Goforth, John Haught, Luke Havens, Dana Lim, Cathy Lohmann, Alayna Mackiewicz, Greg Sollom, Lein Soltan, and Dave Steinberg. Their support, assistance, and camaraderie were a key contributor to my enjoyment at UNC and in the lab. I was also heavily influenced by some fantastic mentors during my master's degree: Nathan Putman and Kyle Young shaped my interest in fish migration into a deep curiosity for the underlying sensory mechanisms. I am grateful for this, and for their guidance during my first rodeo in the field of magnetoreception.

I greatly appreciate the time and effort invested by my committee, Catherine Lohmann, Keith Sockman, John Bruno, and Joel Fodrie. Their wisdom, feedback, and support have helped me immensely during the course of my studies. Joel, I would like to especially thank you for your hospitality at the Institute of Marine Sciences (IMS); many of my dissertation chapters would not have been possible without your insight and the resources you so openly allowed me access to. In fact, I would like to thank a number of folks from IMS for their assistance over the years. These include Creed Branham, Savannah Paliotti, Shayla Biraff, Andrew McMains, Jeff Plumlee, EmmaLi Tsai, Lesley Arroyo, Navindah Bahl, Cori Lopenzawski, Owen Mulvey-Mcferron, Stacy Davis, Phil Herbst, and Kerry Irish.

Kerry Irish deserves a special acknowledgement for her support; it is to her that I owe much of the media exposure my work received.

During my six years of graduate school I have benefitted tremendously from friendships and collaborations with other scientists. During my first field season I was fortunate to build some lasting connections with a number of folks at the Oregon Hatchery Research Center. Of these, I thank Michelle Scanlan and Joseph O’Neil for their assistance with experiments and fish husbandry. David Noakes, who sadly passed away last year, was a strong advocate of my research. David welcomed me to OHRC with open arms, letting me use his personal living quarters and granting me access to his impressive fisheries library; he was a true gentleman and a scholar and will be sorely missed.

My latest research endeavours would also not have been possible without strong research ties and support from Jim Morley at ECU and Jeff Buckel at CMAST – I am extremely appreciative for their guidance, and for their encouragement during every aspect of this project.

I close by thanking my family, to whom I reserve my utmost love and gratitude. To my parents, Gary and Hayley, my siblings Solei and Fletcher, and my grandparents Barry and Wendy, and Raymond and Joan. Each of you played an integral part in shaping me into the person I am today. It is through your support and encouragement that I had the fortitude to pursue my aspirations and for that I am forever grateful. Finally, to my fiancé Eva, I cannot express how instrumental your love has been; you have supported me through my trials and tribulations and have been there to rejoice during my successes. With boundless love, thank you.

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CHAPTER 1: MAGNETORECEPTION AND MAGNETIC NAVIGATION IN FISHES: A HALF CENTURY OF DISCOVERY¹

Summary

The first evidence for magnetoreception in fishes emerged in the early 1970s, following behavioral investigations with iconic migrants such as salmon and eels. Since these early investigations, evidence for magnetoreception has accumulated in taxonomically diverse fishes, and has implicated magnetoreception in guiding wide ranging behavioral tasks. The last decade in particular has seen a substantial increase in the number of publications related to fish magnetoreception (Figure 1.1) and has highlighted the need for a synthesis of the literature.

The following chapter comprises a literature review summarizing the evidence presented for magnetoreception in fishes since the establishment of the field over 50 years ago. The review begins with a brief description of Earth's magnetic field and the characteristics that make it useful in navigation. Next, it describes the approaches to investigating magnetoreception in fishes and summarizes the evidence for magnetoreception in the two largest groups of fishes, the bony fishes and the cartilaginous fishes. Finally, it discusses the possible mechanism(s) of magnetic field detection in fishes, highlights groups that have not yet been studied in the context of magnetoreception, and concludes by discussing how future work on fishes is likely to expand the horizons of magnetoreception research.

¹ This chapter was previously published as:
Naisbett-Jones, L.C. and Lohmann, K.J. (2022) Magnetoreception and magnetic navigation in fishes: a half century of discovery. *J Comp Physiol A* (2022).
<https://doi.org/10.1007/s00359-021-01527-w>

Introduction

Aquatic environments are among the most challenging habitats on the planet for navigation. Animals migrating underwater confront a world in which visual cues are often limited or absent, while currents continuously displace swimming animals from their paths. Despite the inherent difficulties of navigating in the aquatic realm, numerous fishes routinely complete astonishing long-distance journeys. Among these are: (1) the transoceanic migrations of great white sharks (*Carcharodon carcharias*), which travel some 10,000 km between Australian and South African waters (Bonfil et al. 2005); (2) the homing of Pacific salmon (*Oncorhynchus sp.*) to their natal rivers from oceanic feeding grounds in the Pacific after a multi-year absence (Quinn 2018); and (3) the seasonal reproductive migrations of bluefin tuna (*Thunnus thynnus*) between feeding areas in the Atlantic Ocean and spawning grounds, either in the Gulf of Mexico or the Mediterranean Sea (Block 2001; Aranda et al. 2013).

Fishes and other long-distance marine migrants exploit a variety of sensory cues to guide their movements, including visual, auditory, and olfactory cues, as well as cues from waves and water movements (Lohmann et al. 2008a). An additional source of information, present in all environments that fish inhabit, is Earth's magnetic field. The first investigations into whether fish might sense the geomagnetic field were conducted in the early 1970s (Branover et al. 1971; Rommel and McCleave 1973). The work was inspired, in part, by reports of magnetic sensitivity in several invertebrates (e.g., Brown et al. 1960; Becker 1964) and the discovery of a magnetic compass sense in birds (Wiltschko and Merkel 1966). A half century later, phylogenetically diverse fish are now known to detect Earth's magnetic field and use it to guide movements over a variety of spatial scales.

Properties of Earth's magnetic field

Earth's magnetic field represents an extremely reliable, omnipresent sensory cue. It is present during all times of day and exists at all locations on the planet, from the depths of the ocean to the uppermost part of the atmosphere. Thus, the geomagnetic field is present throughout the aquatic realm, making it arguably the most pervasive cue available to fishes and other aquatic animals. The ubiquity of the geomagnetic field might be the principal reason why diverse aquatic animals have evolved the ability to use it in orientation, including molluscs (Cain et al. 2005), crustaceans (Lohmann et al. 1995; Ugolini and Pezzani 1995; Lohmann and Ernst 2013), sea turtles (Lohmann et al. 2012) and diverse fishes. In this section, we provide a brief overview of the geomagnetic field (Figure 1.2a) focusing on the properties that make it useful for animal navigation.

Animals can extract two types of information from the geomagnetic field: (1) directional, or “compass” information; and (2) positional or “map” information. Animals with a magnetic compass sense use the direction of magnetic field lines to maintain a consistent heading in a particular direction such as north or south (Lohmann 2010). By contrast, animals with a magnetic map sense rely on regular spatial features of the geomagnetic field to derive positional information, in effect using the field to determine where they are (Lohmann et al. 2007). Several geomagnetic field elements vary predictably across the surface of the earth (Skiles, 1985; Figure 1.2b). Two of these parameters, inclination angle and total intensity, vary in different directions over much of the globe, so that the two form a large-scale bi-coordinate grid over many oceanic regions (Lohmann et al., 1999; 2007). Evidence indicates that several fishes, as well as sea turtles and possibly other animals, exploit this pattern of magnetic variation as a kind of magnetic map (Lohmann et al. 2007, 2012; Putman et al. 2014c; Naisbett-Jones et al. 2017; Keller et al. 2021).

In addition to these global patterns of magnetic variation, concentrations of magnetic minerals in Earth's crust create additional, localized magnetic spatial patterns that animals might, in some cases, use in orientation and navigation. These finer-scale variations (or anomalies) in magnetic topography are far more complex than Earth's main dipole field. Although these localized anomalies typically only account for less than 1% of the total magnetic field, the gradients associated with anomalies can be significant and they can create distinctive magnetic patterns (McElhinny and McFadden 1999). For instance, at the ocean-basin scale, seafloor spreading creates somewhat linear magnetic hills and valleys that run parallel to mid-ocean ridges; this magnetic topography has been proposed to be used by some marine migrants (Klinowska 1985; Kirschvink et al. 1986; Walker et al. 1992). Geological formations such as seamounts also have unique magnetic properties which might make them useful as navigational landmarks for fishes and other marine animals; for example, scalloped hammerhead sharks (*Sphyrna lewini*) have been proposed to navigate using local, fine-scale magnetic anomalies associated with seamounts and the sea floor (Klimley 1993).

Methods of studying responses of fishes to magnetic fields

Observational studies, such as tracking fish through the wild and determining migratory paths, can provide a useful starting point for considering the navigational cues that might be available *en route*. Studies of this nature are especially valuable for organisms that are too large for laboratory-based experiments. In some cases, analysis of movement trajectories has provided valuable insight into cues that might underlie navigation (Klimley 1993; Alerstam et al. 2001; Azumaya et al. 2016). Because animals in their natural habitat usually have access to numerous sensory cues, however, inferring which are used to guide an animal along a given path is often challenging (Lohmann et al. 2008a).

Compelling evidence that an animal can detect a given sensory cue can often be acquired from experiments in which the cue is manipulated in some way and a response of the animal to the altered cue is observed. In the case of magnetoreception, altering magnetic fields has traditionally been achieved using two main approaches, each of which has advantages and limitations. Studies using various types of electromagnetic coil systems to carefully control magnetic field conditions have provided the strongest and most direct experimental evidence for magnetoreception in fishes and other animals (Wiltschko and Wiltschko 1995). While a number of magnetic coil configurations exist, they each serve the same purpose in behavioral experiments: to enable researchers to expose animals to carefully controlled magnetic fields, which often resemble the natural magnetic field of the earth.

In most studies, magnetic coil systems have been used to control the ambient field throughout an arena where an animal moves. If coils are miniaturized, however, then they can be placed on animals to alter the magnetic field in a small part of the body (Walcott and Green 1974). In one field study with salmon (Yano et al. 1997), small magnetic coils placed on the heads of fish were used in an attempt to disrupt the ability of fish to sense magnetic fields in the natural environment. No clear effects of the coils were observed, but the small sample size ($n = 4$) and availability of multiple cues in the environment make interpretation of the results difficult. To our knowledge, such studies have not been attempted with other fishes, likely due to logistical constraints; for example, most species lack a hard external surface to which devices can be attached and many fishes move over long distances, making it difficult to monitor movements.

An alternative approach to changing the ambient field with magnetic coil systems is to change it with magnets. Although simpler, the technique of using magnets is not without caveats. Magnets typically generate magnetic fields that are significantly stronger than earth strength; they also generate steep magnetic field gradients that do not exist in nature. For

these reasons, results of studies in which animals are exposed to magnets can sometimes be difficult to interpret. Nevertheless, magnets have proven to be a useful tool for demonstrating sensitivity of fishes to magnetic cues under some conditions (e.g., Newton and Kajiura 2017).

Quantifying magnetic orientation behavior

Determining how an animal orients in response to different magnetic field conditions is a key element of most magnetic behavioral experiments. Normally, animals are released within arenas and their directional tendencies are recorded. While this might at first glance appear to be a simple and straightforward task, it can be quite challenging. A set of experimental conditions must be created that results in clear responses. The researchers must then determine how to systematically quantify the behavior, a problem compounded when it is difficult to predict how an animal is going to behave. Indeed, even the responses of a single individual to the same stimulus can vary depending on the animal's behavioral state at the time of testing (e.g., whether it is motivated to mate, migrate, or forage), its health, and what other cues are available.

These considerations make behavioral investigations into any sensory modality challenging, but when magnetic fields are involved, the situation becomes even more difficult. Accumulating evidence suggests that the use of magnetic cues is likely restricted to certain behavioral scenarios; in addition, the magnetic sense may be inherently noisy, so that some degree of temporal averaging is required for the processing of magnetic cues (Wiltschko and Wiltschko 1991; Johnsen et al. 2020). Researchers can increase the likelihood of acquiring meaningful behavioral data by choosing test species that adapt readily to the conditions of laboratory studies. In fishes, favorable traits include high physiological tolerance to abiotic parameters (e.g. temperature, salinity, and dissolved oxygen levels) and solitary (non-schooling) behavior that allows testing of individuals in isolation. Odds of

success may also be improved by testing fish at or near life history stages in which oriented movements are critical for survival (e.g., Putman et al. 2014c; Naisbett-Jones et al. 2017; O'Connor and Muheim, 2017), although the discovery that some fish spontaneously align with the magnetic field (e.g., Kalmijn 1978; Hart et al. 2012) suggests that at least some species use magnetic information continuously throughout their lives.

Another factor that makes behavioral experiments on magnetic orientation challenging with fishes is that no universal testing apparatus exists, in part because different species move in diverse ways that often change through ontogeny. For example, modes of locomotion include the sinuous, lateral waves of anguilliform swimming characteristic of eels (Wootton 1990), the slow-moving “benthic walking” behavior of flatfish (Fox et al. 2018) and the continuous swimming of some sharks and bony fishes that are obligate ram ventilators (Roberts 1975). Each of these locomotory modes poses different challenges for researchers attempting to monitor orientation behavior. As a result, a number of different experimental arenas have been designed, ranging from simple circular arenas (Putman et al. 2014c) to more elaborate arenas and mazes (Nishi et al. 2018; Newton and Kajiura 2020a). The need to develop an arena that matches the behavior of each species of fish – and sometimes each life-history stage – stands in sharp contrast to magnetic orientation studies with birds, most of which rely on a standard experimental arena that takes advantage of the migratory restlessness characteristic of many songbirds (Emlen and Emlen 1966; Wiltschko and Wiltschko 1995).

Evidence for magnetoreception in fishes

Despite the challenges associated with testing behavioral responses to magnetic fields, considerable evidence for magnetoreception has accumulated in fishes, especially in two main groups: the bony fishes (Osteichthyes) and the cartilaginous fishes (Chondrichthyes). In

this section, we summarize evidence for magnetoreception derived from diverse approaches, including experiments focusing on magnetic compass orientation, experiments investigating the use of magnetic maps, studies of spontaneous alignment with the ambient magnetic field, studies using conditioning techniques, and correlative studies relating fish movements to magnetic field parameters.

Magnetoreception in bony fishes

Salmon (*Salmonidae*)

In the family *Salmonidae*, research has primarily focused on Pacific salmon from the genus *Oncorhynchus* and, to a lesser extent, salmon from the genus *Salmo*, which includes the Atlantic salmon *Salmo salar* (Table 1.1). Although non-migratory forms of many salmon exist, the majority are anadromous; in other words, they migrate from freshwater habitats where they hatch, to distant ocean foraging grounds, before returning to their natal streams to spawn and die (Quinn 2018). For these reasons, salmon have long been popular subjects for studies on the sensory basis of navigation in fishes. Indeed, they have shaped much of the current understanding of magnetoreception in fishes today.

Quinn (1980) reported the first unequivocal evidence for a magnetic compass sense in a fish. Sockeye salmon fry (*Oncorhynchus nerka*) from two populations were collected during their freshwater phase as they migrated from the gravel beds in which they hatched to the lakes where they feed and grow. Fish were released in an orientation arena and could exit through one of four equally spaced arms around the perimeter. The directions chosen by the young salmon were consistent with the hypothesis that they were orienting towards their respective lake habitats. Rotating the horizontal component of the ambient magnetic field by 90° resulted in a corresponding shift in the directional preference of the salmon fry, demonstrating that the orientation was based on magnetoreception. At night, the orientation persisted regardless of whether external celestial cues were occluded. During the day,

magnetic compass orientation was only observed under conditions in which the fish were deprived of celestial cues. Thus, the results were consistent with the interpretation that celestial cues take precedence over magnetic cues during daytime migrations in sockeye salmon. Interestingly, during a subsequent study in which a different type of arena was used and night-time celestial cues were again occluded, salmon displayed bimodal magnetic orientation instead of orienting in a single direction as they had previously (Quinn and Brannon 1982). Although the reason for the different outcomes is unclear, one possibility is that the different arenas used in the two studies might have affected the responses of the fish (Quinn and Brannon 1982).

Evidence exists for two different functional types of magnetic compasses in animals. Some animals rely on the polarity of the horizontal component of the geomagnetic field to determine the direction of magnetic north, in much the same way that a human compass does (Lohmann et al. 1995; Kimchi and Terkel 2001). Other animals, however, possess a magnetic compass that relies in part on the inclination of the field (Wiltschko and Wiltschko 1972; Light et al. 1993). An animal with an inclination compass defines ‘poleward’ as the direction along the earth’s surface in which the angle formed between the total field vector and the gravity vector is smallest (Wiltschko and Wiltschko 1972). The diagnostic test for determining whether an animal possesses a compass based on the polarity or inclination of the magnetic field involves exposing animals to a field with an inverted vertical component and observing the response. To an animal with an inclination compass, such a field is reversed relative to the normal condition, but to an animal with a polarity compass, the two fields are the same (Wiltschko et al. 1993).

As a first step toward investigating the nature of the magnetic compass in fishes, Quinn et al. (1981) exposed sockeye salmon fry to a local magnetic field and a magnetic field in which the vertical component was inverted. Fish tested under the two magnetic fields

oriented in similar directions. Thus, the results suggest that salmon have a polarity compass, although further investigations using additional fields, such as those used with birds and turtles (Wiltschko and Wiltschko 1972; Light et al. 1993), are needed to confirm these results.

The open-ocean migrations of young salmon likely involve complex navigational processes that function to guide the fish to appropriate oceanic feeding areas. The discovery that young sea turtles inherit a magnetic map in which regional magnetic fields elicit changes in swimming direction at crucial points in the migration (Lohmann et al., 2001; 2012) stimulated a search for a similar navigational system in young fish. Juvenile Chinook salmon that had never been in the ocean were exposed to magnetic fields that exist at the northern and southern edges of their oceanic range, as well as to the unaltered ambient magnetic field of the test site in Oregon, USA (Putman et al. 2014c). Fish tested in the northern magnetic field oriented south, whereas fish tested in the southern magnetic field oriented north. In contrast, fish tested in the ambient magnetic field did not show a directional preference. The results indicate that young salmon derive positional information from Earth's magnetic field and thus have a magnetic map sense. The magnetic map appears to help fish remain within favourable ocean habitats and offers a possible mechanism by which stocks might segregate into broad oceanic areas (Putman et al. 2014c). Because these experiments were performed on fish that had never migrated and thus had no opportunity to learn about how magnetic fields vary in the ocean, the results imply that the responses of the fish to regional magnetic fields are largely inherited (Putman et al. 2014c).

To investigate the magnetic field parameters involved in this inherited magnetic map, salmon were further tested under two magnetic field conditions designed to determine whether they relied on magnetic field intensity alone, inclination angle alone, or a combination of the two (Putman et al. 2014c). Specifically, the intensity of the northern

magnetic field was paired with the inclination angle of the southern magnetic field, and vice versa. If the fish rely exclusively on intensity or inclination angle, then a response to one of these parameters should dictate in each case whether salmon perceive themselves to be north or south of their oceanic range. Instead, fish tested in either of these hybrid magnetic conditions oriented randomly, indicating that neither field intensity alone nor inclination angle alone is sufficient to determine position. Thus, the findings are consistent with the interpretation that salmon rely on combinations of magnetic field intensity and inclination angle to assess their position, as other ocean migrants such as sea turtles and lobsters also appear to do (Boles and Lohmann 2003; Lohmann et al. 2004, 2012).

Magnetic maps have now been demonstrated in a number of salmon species, suggesting that an ability to derive positional information from Earth's magnetic field is widespread among the family *Salmonidae* (Putman et al. 2014b; Scanlan et al. 2018; Putman et al. 2020; Minkoff et al. 2020). A study on pink salmon (*Oncorhynchus gorbuscha*) provides an interesting example of how positional information from Earth's magnetic field might be used during oceanic migrations (Putman et al. 2020). Young pink salmon that had never migrated were tested in two magnetic fields that exist at locations where the orientation adopted by the fish might indicate whether they were homing, orienting to the center of their range, or following their migratory route (Figure 1.3a). In both cases, the observed orientation of the fish matched the direction fish would be expected to adopt if they were following their migratory route (Figure 1.3b). These findings suggest that magnetic maps in young salmon, like those in young sea turtles (Lohmann et al. 2012), appear to be fine-tuned to the migratory routes of individual populations; in effect, the animals seem to inherit instructions that tell them what direction to swim when they encounter specific magnetic fields along a migratory pathway.

Another interesting aspect of these ‘inherited magnetic maps’ is that young fish and turtles both responded to magnetic fields that they would likely not encounter until months (or for turtles, even years) after first entering the sea. These results suggest that such maps, which are present before the animals migrate, are likely retained throughout an animal’s early life; nevertheless, it is also possible that the responses are modified through experience and/or provide the framework upon which more expansive learned maps are constructed as an animal gains experience with its magnetic environment (Putman et al. 2017; Lohmann et al. this issue). Given that most salmon migrate to the ocean only once and to areas they have not previously been, an innate navigation system that guides the initial migration appears advantageous (Putman et al. 2020). Determining how magnetic maps change at different life history stages of salmon, if indeed they do, remains an outstanding research challenge, one that is complicated by an incomplete understanding of the ocean migrations of many species.

In principle, salmon might use magnetic map information not only to navigate along open-sea migratory pathways, but also to help them return to their area of origin as adults (Quinn 1984). Indeed, recent findings provide evidence that young salmon, as well as young sea turtles, imprint on the magnetic field that exists in or near their area of origin and use this information to return to the natal area to reproduce (Lohmann et al. 2008b; Bracis and Anderson 2012; Putman et al. 2013). For salmon, geomagnetic imprinting might occur in parallel with olfactory imprinting (Lohmann et al. 2008b); thus, magnetic cues might bring fish back into the general area of a river mouth, close enough for chemical cues to guide fish to the final destination.

A novel analysis of fisheries data has provided strong circumstantial evidence that young salmon do indeed imprint on the magnetic field of their home area, and that magnetic navigation plays a role in natal homing (Putman et al. 2013). The analysis exploited the fact that Earth’s magnetic field changes gradually over time and that isolines of inclination and

intensity shift slightly each year (Skiles, 1985; Lohmann et al., 1999). Thus, the inclination and/or intensity existing at a particular location one year might drift northward the next year and possibly southward the year after that. Putman et al. (2013) examined how such variation influenced the homing migration of sockeye salmon that originated in the Fraser River of British Columbia, Canada. These fish typically spend two years at sea before making their homeward migration. To reach the mouth of the Fraser River from their open-ocean habitat, returning fish must detour around Vancouver Island to reach the river through one of two pathways, one of which lies to the north of the island and the other to the south. The existence of a long-term data set on the year-by-year proportion of salmon that used the northern or southern route provided a unique opportunity to test a central tenet of the imprinting hypothesis. If salmon imprint on the magnetic field of the area where they first enter the sea, then the number of fish that choose one route over the other might be influenced by subtle changes in Earth's magnetic field near Vancouver Island. Specifically, the route chosen by any given individual may depend on how closely the magnetic field at the entry to each passage resembles the magnetic field that the fish imprinted upon two years before when departing from the river.

Consistent with these predictions, analyses revealed that, when the magnetic intensity at the southern passage closely matched the field that existed at the mouth of the Fraser River when the fish departed, a greater proportion of salmon used the southern passage to return. Likewise, when the magnetic field intensity of the northern entryway closely matched what existed at the mouth of the Fraser River when the fish began their migration, a higher proportion of salmon chose the northern route (Putman et al. 2013). These findings provided evidence for geomagnetic imprinting in salmon, yet other environmental factors, notably sea surface temperature, also accounted for a considerable portion of the variation in return routes, consistent with previous studies (Quinn and Groot 1987). In a subsequent study with

both sockeye and pink salmon, however, variations in the local magnetic field near the Fraser River accounted for more of the variation in return route for both species than did sea surface temperature (Putman et al. 2014a). These results, combined with recent findings in sea turtles and sea birds, provide strong empirical evidence for geomagnetic imprinting and suggest that this process may underlie long-distance natal homing in diverse animals (Putman et al. 2013; Brothers and Lohmann 2015, 2018; Lohmann and Lohmann, 2019; Wynn et al. 2020).

Yet another use of magnetic cues by salmon occurs when young salmon fry first emerge from gravel and enter the water column (Putman et al. 2018). Chinook salmon (*Oncorhynchus tshawytscha*) ready to emerge were placed in vertical tubes filled with transparent substrate that replicated conditions within a nest while allowing observations of the fish. Fish were exposed to one of three magnetic field conditions: (1) the ambient magnetic field; (2) an ‘intensified’ field in which the strength of the vertical component was increased; and (3) a magnetic field in which the normal vertical component was inverted (Figure 1.4a). Fish tested in the normal ambient field moved upward significantly farther than did fish exposed to the inverted magnetic field condition (Figure 1.4b). By contrast, fish tested in the intensified magnetic field moved upwards only slightly, with the mean distance falling between the means of the other two groups. These results are consistent with the interpretation that the magnetic field is one of several factors that influences emergence from the gravel, possibly serving as an orientation cue that helps fish determine which way is up.

Eels (*Anguillidae*)

In contrast to salmon, eels are catadromous, meaning they hatch in the ocean and migrate to freshwater (or brackish water) habitats where they feed until the onset of sexual maturity, after which they return to the ocean and migrate back to their natal area to reproduce. In contrast to salmon, most eels are panmictic; in other words, they comprise a

single, randomly breeding population, with the larvae apparently transported to coastal areas largely by ocean currents (Aoyama 2009).

In an early study (Branover et al., 1971), adult European eels (*Anguilla anguilla*) were placed in an experimental arena and exposed to magnetic fields generated by an electromagnet. In the local magnetic field of the test site (Kaliningrad, Russia), eels showed bimodal orientation, but this orientation became random when eels were exposed to the imposed magnetic field. Several additional studies conducted during the same decade also demonstrated behavioral responses of eels to electromagnetic cues (McCleave et al. 1971; Rommel and McCleave 1973; Tesch and Lelek 1973; Tesch 1974), although responses were variable and sometimes difficult to interpret. With hindsight, the magnetic fields used in these early experiments were often unnatural and sometimes several orders of magnitude stronger than Earth's magnetic field, which may have contributed to inconsistent results (Wiltschko and Wiltschko 1995). Nonetheless, these studies provided initial evidence for a magnetic sense in eels and laid the foundation for future studies of fish magnetoreception.

Subsequent studies using more natural magnetic fields have confirmed that at least three species of anguillid eels are capable of magnetoreception (Table 1.1). In a study involving European eels at the resident yellow eel stage (Durif et al. 2013), eels tested in a funnel-shaped tank used directional information from Earth's magnetic field to guide their escape behavior, which took the form of brief movements up the sides of the funnel. The direction that the eels moved depended on the direction they were displaced from their holding tanks. This same magnetic compass response changed with ambient temperature; eels tested at lower temperatures exhibited unimodal orientation approximately perpendicular to the direction they were displaced, while individuals tested at higher temperatures exhibited bimodal orientation. The authors propose that temperature might influence the eels' motivation (e.g., whether they are sedentary or migratory).

During the spring, jelly-like glass eels enter estuarine environments, often in huge biomass. Unlike the open-sea migratory stages (e.g., eel *leptocephali* larvae and adult silver eels), glass eels are easily obtained by researchers. For this reason, glass eels have been the focus of many sensory studies, including some involving magnetoreception. In one study (Cresci et al. 2017b), laboratory experiments were combined with *in situ* behavioral observations. Glass eels tested in the laboratory under a series of rotated magnetic fields swam north during flood tides and south during ebb tides. By contrast, eels tested in floating arenas in the ocean did not differ in orientation between the different tides. Why the laboratory results and *in situ* results differed is not known, but one possibility is that glass eels in the ocean were influenced by sensory cues that were not present in the lab. Regardless, the laboratory results demonstrate that glass eels possess a magnetic compass sense, which might be linked in some way to the tidal cycle. Precedents for linkages between biological rhythms and orientation and/or swimming behavior exist (Cronin and Forward 1979; Lohmann and Willows 1987; Zhang et al. 2013). Further studies with glass eels are likely to be enlightening.

Although eels clearly have a magnetic compass, it is unlikely that the oceanic migrations undertaken by eels can be accomplished with a compass sense alone. Recent studies have revealed that young eels also exploit positional information in Earth's magnetic field and thus have a kind of magnetic map (Naisbett-Jones et al. 2017). European eels hatch in the Sargasso Sea and travel across the Atlantic Ocean to freshwater growth habitats along the coasts of Europe and North Africa, where they mature (Tesch 2003). To investigate whether young eels have a magnetic map sense, glass eels were subjected to magnetic fields replicating those found at several locations along the eel's migratory route (Naisbett-Jones et al. 2017). Eels tested in a magnetic field like one that exists near their spawning area swam southwest. Those exposed to a field that exists further along the migratory route and closer to

Europe swam northeast. Simulations carried out with an ocean circulation model revealed that swimming in the experimentally observed directions at the locations where the fields exist would result in increased entrainment in the Gulf Stream System, the oceanic current system that facilitates transport of young eels to European growth habitats. Thus, the results imply that young eels possess a magnetic map sense which can be used to distinguish among magnetic fields that exist in different oceanic regions; different magnetic fields along the migratory route elicit changes in swimming direction that presumably facilitate transport to appropriate destinations.

Numerous questions remain, however, about the eels' magnetic map; studies are challenging, in part, because working with key life history stages is difficult. For example, eel larvae (leptocephali) rarely survive in captivity and their behavior is nearly impossible to observe in the wild. Although adult eels can be obtained, eliciting consistent orientation responses from them has proven difficult (Karlsson 1985). While it seems likely that the magnetic map sense of young eels is retained throughout life and is used by adults during the spawning migration (Putman et al. 2017), this remains to be determined.

Magnetic sense in other bony fish

Although salmon and eels are the fishes that have been studied most extensively in the context of magnetoreception, magnetic field detection has also been demonstrated in a growing number of other bony fishes, primarily through conditioning techniques, orientation studies, or both (Table 1.1). One of the first studies to use magnetic conditioning techniques on a fish was carried out with juvenile yellowfin tuna (*Thunnus albacares*), which were trained in water-filled arenas surrounded by a wire coil that could be used to alter the ambient magnetic field (Walker 1984). Fish were conditioned to swim through a rectangular pipe frame. Some individuals received a food reward for passing through the frame when the

ambient magnetic field was altered, while others were rewarded for passing through the frame when the ambient field was unchanged. Fish quickly learned to discriminate between the two magnetic field conditions, although the exact parameter(s) of the field detected by the fish could not be determined. A similar approach was later used in studies with several additional species (e.g., Walker et al. 1997; Newton and Kajiura 2020a), demonstrating that magnetic conditioning is viable in diverse fishes. A somewhat different technique involving cardiac conditioning has provided evidence that rainbow trout can detect not only changes in magnetic field direction, but also large shifts in magnetic field inclination and intensity (Hellinger and Hoffmann 2009).

Captive zebrafish (*Danio rerio*) have also been conditioned to respond to imposed magnetic fields (Shcherbakov et al. 2005). Fish were placed in a tank and trained to swim to the opposite side when the field intensity was increased with magnetic coils. The behavior was reinforced by exposing fish that made the incorrect choice to weak electrical discharges. Interestingly, although zebrafish learned to discriminate between the different magnetic fields, they did not perform as well as migratory fish (Mozambique tilapia; *Oreochromis mossambicus*) trained and tested in the same apparatus (Shcherbakov et al. 2005).

Evidence for magnetoreception in zebrafish has also been acquired in orientation studies in which the horizontal and/or vertical components of the ambient field were altered (Takebe et al. 2012; Osipova et al. 2016; Krylov et al. 2016). Although inconsistencies exist among results from the different studies, one peculiar pattern that emerged repeatedly was a tendency of zebrafish to exhibit bimodal (or axial) orientation (Takebe et al. 2012; Osipova et al. 2016; Krylov et al. 2016). The alignment of this bimodal response appeared to shift in response to changes in the direction of the horizontal field. Details remain to be resolved, but the overall results are consistent with the interpretation that zebrafish have a magnetic compass. Little is known about how magnetic cues are exploited by zebrafish, though some

evidence suggests that magnetic field information might be used to orient rheotactic behavior when fish are part of shoals (Cresci et al. 2017a, 2018). Given that zebrafish are a genetic model organism, they represent a promising system for elucidating the mechanism(s) and genetic basis of magnetoreception.

Coral-reef fishes are another piscine group capable of magnetoreception. After hatching, coral-reef fish larvae often disperse tens of kilometers away from their natal reefs; then, after days to weeks in the open ocean, many return to their home reef and settle there (Brothers et al. 1983; Gerlach et al. 2007). Previous studies have suggested that olfactory cues play a role in helping larvae locate their reef once they are close (Gerlach et al. 2007), but the mechanism underlying the initial stages of this migration have only recently been studied. Cardinal fish (*Ostorhinchus doederleini*) collected shortly after settlement on a reef in Australia were tested for a magnetic compass sense (Bottesch et al. 2016). Fish tested under ambient magnetic field conditions during the day and night oriented southeast, a direction that may help the fish reach the home reef after currents displace them to the north and west (Figure 1.5). To investigate whether a magnetic compass plays a role in this orientation, a group of fish was tested at night in a magnetic field in which the horizontal component was rotated 120° clockwise. These fish showed a corresponding shift in orientation (Figure 1.5). The results suggest that a magnetic compass sense guides the nighttime swimming behavior of larval reef fish. Additional studies on damselfish (*Chromis atripectoralis*), another coral-reef species, have demonstrated that a magnetic compass sense is present in pre-settlement larvae and is used during daytime navigation (O'Connor and Muheim 2017). The results thus suggest that a magnetic compass helps guide movements in several reef fishes.

In addition to the species and groups already discussed, evidence for magnetoreception also exists in a number of other bony fishes (Table 1.1), including tilapia

(*Oreochromis mossambicus*) (Shcherbakov et al. 2005), roach (*Rutilus rutilus*) (Krylov et al. 2016), haddock (*Melanogrammus aeglefinus*) (Cresci et al. 2019b) and medaka (*Oryzias latipes*) (Myklatun et al. 2018). These findings imply that the ability to detect magnetic fields is widespread among bony fishes and perhaps even universal.

Magnetoreception in cartilaginous fishes

Investigations into the magnetic sense of chondrichthyan fishes have so far focused exclusively on sharks, skates, and rays from the subclass Elasmobranchii (Table 1.2). These ancient marine fishes possess an extremely sensitive electrical sense that allows them to cue in on the bioelectric fields of their prey (Kalmijn 1966, 1971). In principle, this same sense might indirectly provide the physical basis of a magnetic sense (Kalmijn 1973, 1982), although whether it does remains unknown (Johnsen and Lohmann 2005, 2008).

The first evidence for magnetic field detection in elasmobranchs came from behavioral studies on leopard sharks (*Triakis semifasciata*) (Kalmijn 1978). Observations of captive sharks revealed that each morning they tended to rest in a part of the tank that corresponded with magnetic north. Occlusion of visual cues had no effect on this behavior and relocating the tanks to a new location failed to disrupt it. By contrast, using a magnetic coil system to null Earth's magnetic field within the tank led to random dispersal of the fish, suggesting that they were using magnetic cues to orient. Magnetic conditioning techniques were subsequently used to investigate whether the round stingray (*Urolophus halleri*) can perceive magnetic fields (Kalmijn 1978). Two individuals were trained to feed on the eastern side of their enclosure, with a magnetic coil system being used to reverse the field in half of the trials. The stingrays soon learned to move toward the side of the arena that corresponded to magnetic east under both magnetic field conditions, providing additional evidence for magnetic sensitivity in elasmobranchs.

Since this early work, a number of additional behavioral studies have provided experimental evidence consistent with the hypothesis that elasmobranchs possess a magnetic sense. Yet the presence of a highly sensitive electric sense in elasmobranchs greatly complicates interpretation of results of experiments involving magnetic fields. The central difficulty is that changing a magnetic field, as is normally done in magnetoreception research, also unavoidably generates a transient electrical field (Faraday 1832). For animals that lack an electric sense – *i.e.*, the vast majority of species in the animal kingdom – this weak electric field is of no consequence. But for animals such as elasmobranchs that are exquisitely sensitive to electrical stimuli, disentangling whether fish are responding to magnetic or electric stimuli in a given situation is often challenging.

From an ecological perspective, there are good reasons to suspect that elasmobranchs are magnetically sensitive, inasmuch as many species undertake lengthy and highly oriented migrations across ocean environments where an ability to sense Earth's magnetic field would potentially be useful in navigation (e.g., Carey and Scharold 1990; Bonfil et al. 2005). At present, however, unequivocal demonstrations that elasmobranchs detect and exploit earth-strength magnetic fields in navigation have remained sparse, in part because of the inseparable nature of electric and magnetic fields. For example, in a laboratory conditioning experiment involving captive sandbar (*Carcharhinus plumbeus*) and hammerhead sharks (*Sphyrna lewini*), fish learned to approach an object in the center of the tank when a wire coil surrounding the tank was turned on, which increased the intensity of the magnetic field (Meyer et al. 2005). Sharks could clearly determine when the coil was turned on, yet in principle might have solved the discrimination task in one of two ways: either by detecting changes in the magnetic field, or by detecting and responding to the transient electrical fields that were produced each time the magnetic coil was activated (Johnsen and Lohmann 2005). Follow-up studies using the same conditioning technique suggested that the background

electrical environment at the test site was sufficiently ‘noisy’ that discriminating transient electrical signals from the coil might have been difficult, consistent with the hypothesis that the sharks detected the magnetic field (Anderson et al. 2017); at the same time, measurements of the transient electrical fields revealed that they were above the threshold that sharks can detect, so that the possibility of a response to electric fields cannot be entirely excluded. These findings highlight the challenges of studying magnetoreception in electrically sensitive animals.

Despite these obstacles, several experiments have provided strong evidence for a magnetic sense in elasmobranchs. In a study with yellow stingrays (*Urobatis jamaicensis*) (Newton and Kajiura 2020a), fish were placed in a T-shaped maze and conditioned to associate either the north or south arm of the maze with a food reward. A magnetic coil was then used to alternate the direction of magnetic north or south between the two arms. Fish learned to select the correct maze arm based on the polarity of the imposed magnetic field. As with earlier conditioning experiments with round stingrays (Kalmijn, 1978), the results are consistent with the interpretation that these fish have a magnetic compass sense.

Relative to teleost fishes, few studies have investigated whether elasmobranchs possess a magnetic map sense. A recent study with bonnethead sharks (*Sphyrna tiburo*), however, has provided strong evidence that sharks can indeed exploit positional information in Earth’s magnetic field (Keller et al. 2021). Juvenile bonnethead sharks were captured in the Gulf of Mexico near the Florida panhandle, in a location where land masses prevent long-distance movements to the north. Fish were tested in three different magnetic fields: (1) the local magnetic field of the capture site; (2) a magnetic field replicating one that exists in the ocean ~600 km south of the capture site; and (3) a magnetic field that exists ~600 km north of the capture site on the US mainland. Fish tested in the local field and in the northern magnetic field condition oriented in random directions. By contrast, fish tested in the

southern magnetic field condition oriented approximately northward, the direction they would need to travel to return to the capture site from the location where the southern magnetic field actually exists. Why sharks failed to respond to the northern magnetic field was unclear. An interesting possibility, however, is that sharks had no experience with fields that exist to the north because land prevented them from moving north from the capture site (Keller et al. 2021). Regardless, the results provide the strongest evidence to date of a magnetic map sense in sharks and suggest that bonnethead sharks might be a promising species for future studies.

In a related analysis, Keller et al. (2021) investigated whether the use of magnetic positional information in navigation might explain aspects of the genetic structure of bonnethead populations, as has been reported in sea turtles (Brothers and Lohmann, 2018). Specifically, if sharks imprint on the magnetic field of their natal area and return to the site partly by relying on magnetic navigation, then a relationship may exist between population genetics and the magnetic fields that exist in different locations where bonnetheads reproduce (Keller et al. 2020). For example, geographic areas with similar magnetic fields may be used by genetically similar sharks because sharks have difficulty distinguishing between the two locations. To test this hypothesis, the population structure of bonnetheads was analyzed in the context of spatial variation in the earth's magnetic field. Results revealed a relationship between genetic differentiation and the magnetic fields that exist at different reproductive sites. These findings bolster the evidence for magnetic navigation in bonnetheads. In addition, they complement earlier findings with sea turtles suggesting that geomagnetic imprinting and magnetic navigation are important drivers of population structure in some migratory animals (Brothers and Lohmann 2018; Lohmann and Lohmann, 2019).

Conditioning studies with yellow stingrays have also investigated whether elasmobranchs can detect parameters of Earth's magnetic field that might function in a

magnetic map. Stingrays were placed in a tank and trained to move across the central axis of the tank when the magnetic field within the tank was repeatedly changed (Newton & Kajiura, 2020b). Results implied that the fish could distinguish between a field that oscillated between two inclinations and a field that oscillated between two different intensities. Two different interpretations are possible (Newton and Kajiura 2020b). One is that the stingrays can detect magnetic inclination and intensity, features of the geomagnetic field that underlie the magnetic map sense in other fishes; another is that the fish detected and responded to the two different transient electric fields generated by the different field changes. Regardless, the findings are intriguing and warrant further investigation.

To date, nearly all studies involving magnetic maps in fishes have focused on use of magnetic parameters such as inclination and intensity, which vary predictably over large oceanic regions (Figure 1.2). A different form of magnetic navigation, based on fine-scale magnetic topography, has been proposed for scalloped hammerhead sharks (Klimley, 1993). Night-time tracking of scalloped hammerheads revealed that fish performed highly directional movements between foraging areas and seamounts, often through areas of variable ocean currents, bathymetry, and temperature (Klimley, 1993). Analysis of paths relative to the local magnetic field contours suggested that the trajectories of the shark's tracks could often be explained if sharks are attentive to local gradients in magnetic intensity associated with the seamounts. Further investigation is warranted.

A final line of evidence for magnetoreception in elasmobranchs comes from studies involving responses of sharks and rays to strong magnets. For unknown reasons, some elasmobranchs appear to spontaneously avoid magnets when they first encounter them (O'Connell et al. 2011a). In a conditioning study, however, yellow stingrays were trained to approach magnets buried in the sediment (Newton & Kaijura, 2017). Although all of these findings are consistent with the interpretation that the fish can detect magnetism – either

directly with a magnetic sense or indirectly with their electric sense – the relationship between detecting unnaturally strong fields under laboratory conditions, and detecting weaker natural fields in the environment, remains to be elucidated.

Mechanisms of magnetic field detection

Little is known about the mechanism or mechanisms that underlie magnetic field detection in fishes. In a group as large and diverse as fishes, it is possible that magnetoreception has evolved independently on multiple occasions, so that different mechanisms exist in different fish species. Indeed, it is even possible that at least two different mechanisms exist within the same species, with one mechanism underlying the magnetic compass and a different mechanism involved in detecting magnetic parameters associated with a magnetic map (Lohmann 2010).

Most recent research on magnetoreception mechanisms, both in fish and in other animals, has focused on three main hypotheses (Johnsen and Lohmann 2005, 2008; Nordmann et al. 2017). The first involves crystals of biogenic magnetite coupled to mechanoreceptors. The second proposes that electrically-sensitive animals such as elasmobranchs detect magnetic fields via electromagnetic induction (Kalmijn 1973), and/or that animals such as birds detect magnetic fields with an induction-based mechanism located within the semi-circular canals of the inner ear (e.g. Nimpf et al. 2019). The third hypothesis proposes a complex series of biochemical reactions that are modulated by earth-strength magnetic fields. All of these mechanisms are presently hypothetical, inasmuch as primary magnetoreceptors have not yet been identified unequivocally in any animal. Nevertheless, behavioral and histological studies have provided some initial indications about how fishes might sense magnetic fields.

For bony fishes, evidence consistent with the magnetite hypothesis has come from studies in which magnetic material (presumably magnetite) has been detected in magnetically sensitive species such as eels, salmon and tuna (Walker et al. 1984, 1988; Kirschvink et al. 1985; Ogura et al. 1992; Moore and Riley 2009). Although direct evidence that these magnetic crystals function in magnetoreception has not been acquired, efforts have been made to investigate possible links between putative magnetite-based receptors and the nervous system. For example, in a study with rainbow trout, Walker et al. (1997) used electrophysiological techniques to record from the ophthalmic branch of the trigeminal nerve while the fish were exposed to changes in the ambient magnetic field. Results provided evidence of enhanced neural activity in response to the field changes. Because the nerve branch from which the recordings were obtained innervates the nose of the fish, the results were consistent with the hypothesis that receptors for the magnetic sense in fish, and perhaps in other vertebrates, are located in or near the nasal area. In the same study, the authors identified potential magnetoreceptor cells in the olfactory lamellae; these structures were subsequently found to contain crystals of single-domain magnetite (Diebel et al. 2000). These findings are promising, yet additional studies are needed to establish a definitive link between the putative receptors and the magnetic sense, both in rainbow trout and in other fishes.

Additional evidence consistent with the magnetite hypothesis has been obtained in behavioral experiments in which animals have been exposed to strong magnetic pulses (e.g., Wiltschko et al. 2002; Holland 2010; Ernst and Lohmann 2016). Importantly, while this technique is capable of permanently altering the magnetic dipole moment of magnetite crystals, it should have no lasting effect on other proposed mechanisms of magnetic field detection (Shaw et al. 2015). Thus, magnetic pulses have been viewed by some as a behavioral diagnostic technique for magnetite-based magnetoreception (Kirschvink et al. 2001), although others have urged caution in interpreting results, given that such pulses

appear to elicit at least some changes in gene expression unrelated to magnetoreception (Fitak et al. 2017; Ernst et al. 2020).

In one study (Naisbett-Jones et al. 2020), juvenile Chinook salmon were exposed to a strong magnetic pulse, after which magnetic orientation behavior of the fish was compared to that of control fish under two magnetic field conditions: (1) the local magnetic field and (2) a magnetic field that exists near the southern boundary of the fish's range. In the local field, no differences were detected between pulsed and control groups. Interestingly, however, the orientation of the two groups was significantly different when tested in the magnetic field from the distant location. It is unclear whether the magnetic pulse affected the magnetic compass, map sense, or both, but the results are consistent with the hypothesis that at least part of a salmon's magnetoreception system is based on magnetite-based receptors. Analyses of gene expression in rainbow trout after exposure to a similar magnetic pulse revealed that 181 genes had altered expression (Fitak et al. 2017). Some were ferritin genes involved in the binding and trafficking of iron and might, in principle, function to repair or replace magnetite-based magnetoreceptors damaged by the pulse. Surprisingly, the technique of testing the effect of magnetic pulses on fish has only been used with species from the family *Salmonidae* (Fitak et al. 2017, 2020; Arniella et al. 2018; Naisbett-Jones et al. 2020). Thus, whether other fishes are also affected by a magnetic pulse remains unclear.

Magnetite is not the only mechanism that has been proposed to underlie magnetoreception in fishes. For species capable of electroreception, an alternative possibility is electromagnetic induction. Distilled to its simplest form, the electromagnetic induction hypothesis proposes that fish capable of electroreception use their electrical sense to sense magnetic fields indirectly. Specifically, as elasmobranchs such as sharks swim through Earth's magnetic field, a slight separation of charge presumably develops between the dorsal and ventral surfaces of the fish; the fish might then use their highly sensitive electroreceptors

to detect the voltage drop of the induced current that flows through the sea water around them (Kalmijn 1973; Johnsen and Lohmann 2008).

Whether elasmobranchs actually perceive magnetic fields in this way, however, is not known. Moreover, distinguishing between the magnetite hypothesis and the electromagnetic induction hypothesis has proven challenging. In principle, a critical test might involve attaching magnets to fish. If the magnet is stationary relative to electroreceptors, then it should not affect a mechanism based on induction, but it should affect a mechanism based on magnetite (Johnsen and Lohmann 2005).

Following this rationale, Walker et al. (2003) sought to test whether magnetoreception in short-tailed stingrays (*Dasyatis brevicaudata*) is based on magnetite or electromagnetic induction. Stingrays were first trained to discriminate between the presence and absence of a magnetic anomaly produced by a coil system. Next, small neodymium magnets or non-magnetic brass bars were implanted into the nasal cavity of the rays. Fish from the control group with the brass bars were able to successfully discriminate between the anomalies, but those with magnets were unable to do so. Similar results were subsequently obtained in experiments with sandbar sharks (Anderson et al. 2017). In both cases, the results were interpreted as evidence for a magnetite-based mechanism. A crucial question in all such tests, however, is whether the movements of the magnets precisely matched the movements of electroreceptors on the flexible bodies of the fish; if slight differences in motion occurred, then a magnetoreception system based on induction might inadvertently have been affected (Johnsen and Lohmann 2005). Indeed, studies modelling the movement of the fish's bodies relative to the attached magnets suggest that the movement of the magnet might have been sufficient to impair an induction-based mechanism (Molteno and Kennedy 2009). Future experiments with weaker magnets that are less likely to interfere with an induction-based

mechanism may help elucidate the mechanism of magnetoreception in elasmobranchs (Molteno and Kennedy 2009). For now, the question remains unresolved.

An additional mechanism that has been proposed to underlie magnetic field detection involves a complex series of chemical reactions that may involve photopigments known as cryptochromes (Ritz et al. 2000). Although evidence consistent with this hypothesis has been acquired in some animals, especially in insects and birds (e.g., Ritz et al., 2004; Gegear et al. 2008; Wan et al. 2021; Netušil et al. 2021), the idea has received little attention in fishes. Nonetheless, studies with zebrafish have revealed the presence of cryptochromes without a known function, leading to the suggestion that these play a role in magnetoreception (Balay et al. 2020). Further study is needed to investigate this hypothesis.

Given the potential involvement of photopigments in magnetic field detection, the finding that magnetic orientation behavior in some animals is light-dependent, and also that it is affected by specific wavelengths of light, has been interpreted as support for the chemical magnetoreception hypothesis (e.g., Phillips and Borland 1992; Wiltschko and Wiltschko 1999). Few studies have investigated the relationship between light and magnetoreception in fishes, although some evidence suggests that salmonids can detect magnetic stimuli in total darkness (Quinn, 1980; Hellinger and Hoffmann 2012), suggesting that magnetoreception in this group might occur independent of light. Whether this is a universal feature of magnetoreception in fishes remains to be explored.

Missing behavioral links in fishes

The existence of a magnetic sense in diverse groups of fishes suggests that magnetoreception has either been heavily conserved through evolution or has evolved independently in multiple groups. The astonishing diversity of fishes, and their evolutionary position relative to other vertebrates, provides a unique opportunity to investigate the origins

and evolution of magnetoreception. Although such investigations are currently impeded by the dearth of information on magnetoreception mechanisms, useful insights can potentially be gained from behavioral experiments. For example, one of the most significant knowledge gaps exists in the ancient lineage of jawless fishes from the superclass Agnatha (Figure 1.6). Two orders of agnathans, lampreys (Petromyzontidae) and hagfish (Myxiniiformes), are among the oldest existing vertebrates, having originated more than 500 million years ago (Volff 2005). Hagfish pose a challenging study system because they occupy deep benthic areas of the ocean and are difficult to acquire. Lampreys, on the other hand, are relatively common in aquatic ecosystems worldwide and represent an interesting candidate given the migratory life histories of some species (Beamish, 1979). Other knowledge gaps can be found in the lobe-finned fishes from the class Sarcopterygii. Extant members from this group include members of the lungfish order (Dipnoi), as well as the coelacanth (Coelacanthiformes). While coelacanth live in deep water and are difficult to acquire, lungfish are relatively common in shallow, freshwater ecosystems worldwide and can thus be readily collected. Lastly, within the Chondrichthyes there are two extant subclasses of cartilaginous fishes which represent the earliest stage in the evolution of the jawed vertebrates: the Elasmobranchii (sharks, rays and skates) and the Holocephali (chimaerids and elephant sharks). To our knowledge, no study has investigated magnetoreception in Holocephali fishes. In fact, very little information exists about the sensory ecology of this group.

Future directions

Over the last half century, a wealth of behavioral evidence has demonstrated that fishes can sense Earth's magnetic field and use it to guide their movements. This ability is not limited to iconic, long-distance migrants such as salmon and eels, but instead appears to be

phylogenetically widespread. Magnetic compasses are common among fishes and exist both in species that move over short distances and those that undertake trans-oceanic migrations. Magnetic maps have now been discovered in several groups of fishes including salmonids, European eels, and bonnethead sharks. In addition, growing evidence suggests that such maps, in combination with geomagnetic imprinting, assist some fish in returning to natal areas to reproduce.

Despite considerable progress in recent years, research on magnetoreception in fishes is still in its infancy. Numerous questions remain unresolved, including the mechanism(s) of magnetoreception, how fish use magnetic cues in their natural behavior, and how magnetic information is integrated with other sensory systems during migration. Indeed, even a baseline knowledge of which fishes are capable of magnetoreception has not yet been acquired, insofar as studies have not been carried out with a number of key taxonomic groups.

Magnetoreception is of interest not only from the perspective of basic research, but also from the standpoint of conservation and management. Although researchers have investigated how numerous factors affect fish stocks, little consideration has been given to the electromagnetic environment. Yet nowadays aquatic environments are increasingly awash in electromagnetic fields of anthropogenic origin, generated by sources as diverse as hydroelectric and hydrokinetic facilities, underwater electrical cables, oil platforms, shipboard radar, and coastal cell phone towers. Understanding the role of magnetic fields in the lives of marine animals, and the impacts of anthropogenic fields on their welfare, is an important area for future study (Albert et al. 2020; Klimley et al. 2021).

Finally, as the oldest and most diverse vertebrate group on the planet, fishes represent a particularly promising group for studies on magnetoreception and its evolutionary origins. Many species are relatively easy to acquire and maintain in captivity, making them amenable

to laboratory studies. In addition, the existence of fish model systems such as zebrafish and medaka (e.g., Lin et al. 2016; Teame et al. 2019; Hilgers and Schwarzer 2019) mean that a variety of modern genetic, molecular, and developmental approaches are feasible. Given these advantages, it appears likely that fishes will play a pivotal role in unravelling many long-standing mysteries of magnetoreception.

Figures

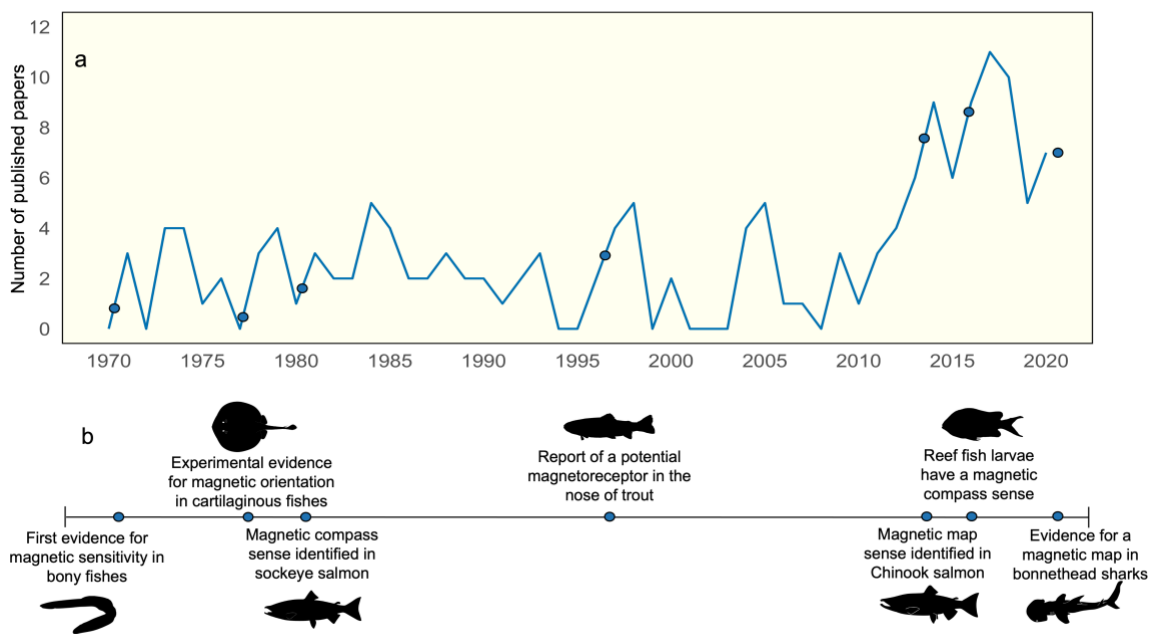


Figure 1.1 Number of papers published and timeline of significant advances in the field of fish magnetoreception.

(A) The number of published papers investigating magnetoreception in fishes since the first paper on the subject in 1971 (survey goes through 2020). (B) Timeline of some significant advances. Silhouette images were obtained under Public Domain courtesy of PhyloPic (<http://phylopic.org>).

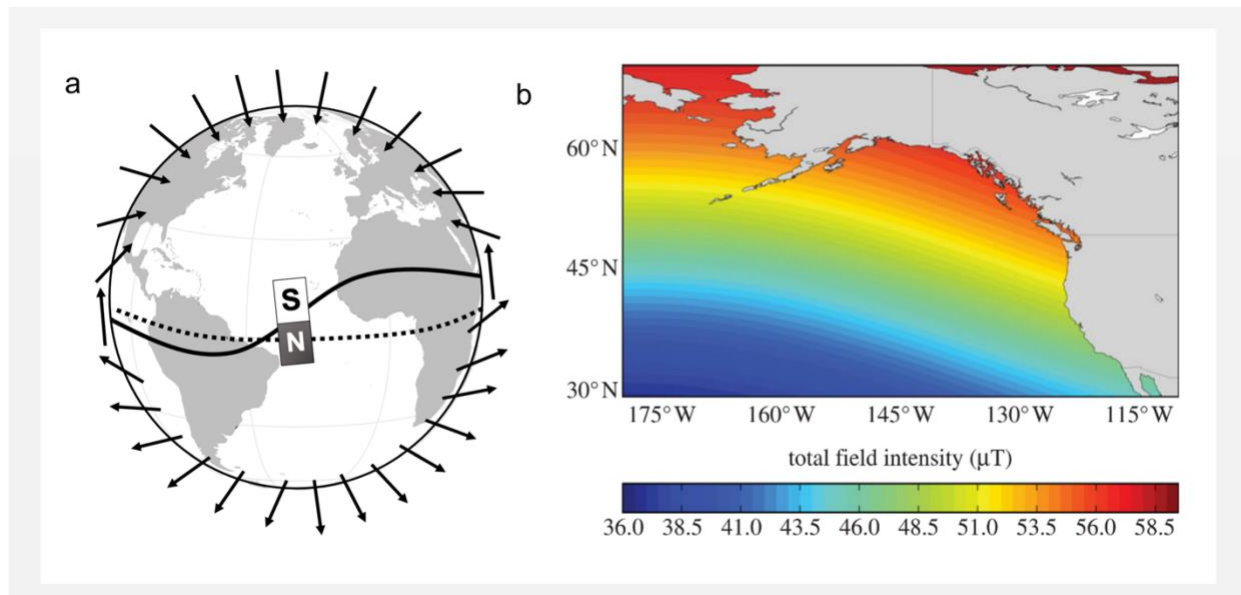


Figure 1.2 Diagrammatic representation of the earth's magnetic field.

(A) On a global scale, the geomagnetic field resembles the dipole field of a giant bar magnet (the north pole of the hypothetical magnetic is directed toward the southern hemisphere).

Magnetic field lines (represented by arrows) intersect the earth's surface in a predictable way across the globe. At the magnetic equator (solid curving black line) field lines are parallel to the earth's surface and the inclination angle is zero; at the poles field lines are perpendicular to the earth's surface and the inclination angle is 90 degrees. Dotted curved line represents the geographic equator. (B) Map of the western North Pacific. Like inclination angle, the magnetic field intensity also varies across Earth's surface but in a slightly different direction than does inclination angle; thus, different geographic areas have different magnetic signatures consisting of specific combinations of inclination and intensity (Modified from Putman et al. 2014a). A more detailed description of the geomagnetic field is provided by Skiles (1985).

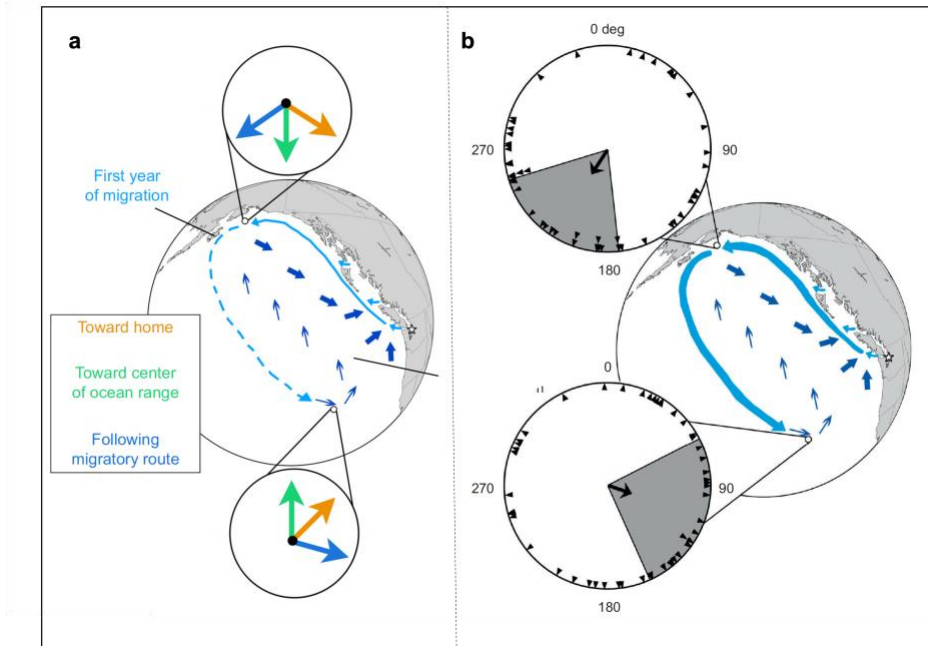


Figure 1.3 Hypothesized and observed orientation responses of juvenile pink salmon to magnetic map information.

(A) The migratory route of pink salmon and possible orientation responses of juvenile fish to magnetic map information. Light blue arrows show the migratory movements for the first year at sea (solid line = spring/summer, dashed line = autumn/winter). Dark blue arrows indicate the hypothesized movements during the second year in the ocean (thin arrows = movements during second spring/summer; thick arrows = homeward migrations in the second summer/autumn). Arrows within the circles show the direction that salmon might adopt if they use magnetic cues to assess their location and orient. (B) Circular graphs show the orientation of juvenile pink salmon to magnetic fields that exist at the northern and southern ends of their migratory route. The orientation of pink salmon tested in the northern and southern magnetic fields differed significantly, indicating that they distinguished between the two magnetic fields and responded by swimming in different directions. Triangles represent the mean heading of individuals and the central arrow and gray shading shows the population-level mean direction and 95% CI, respectively (modified from Putman et al. 2020).

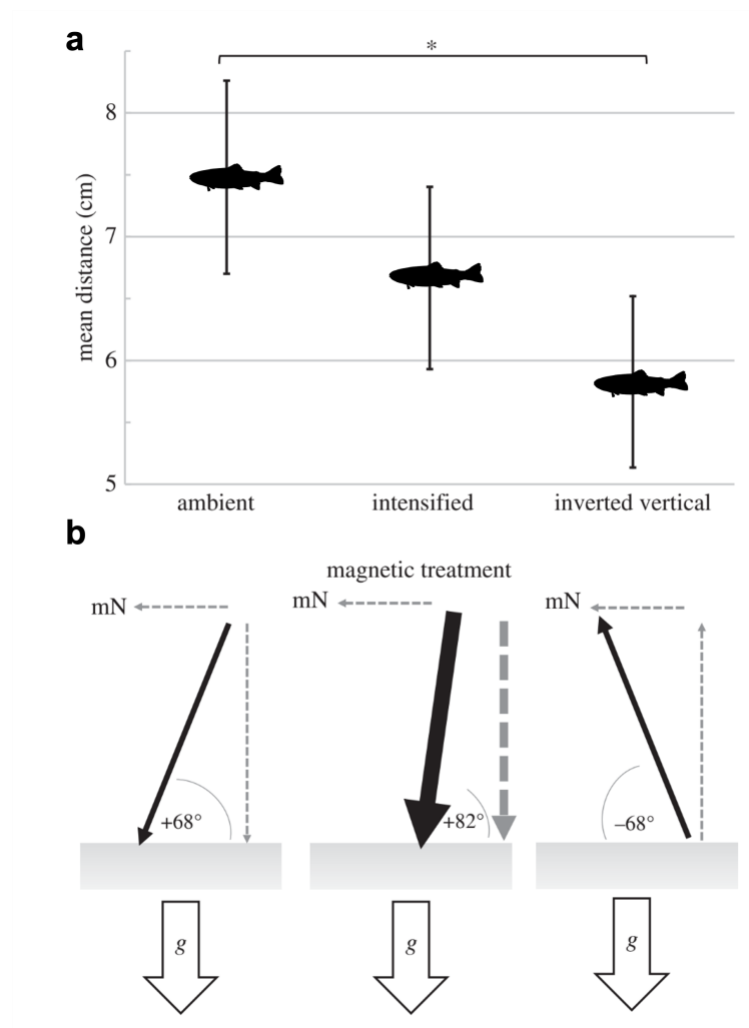


Figure 1.4 Response of young salmon to magnetic fields while emerging from their nests.

(A) Mean height of fish movement under three different magnetic field conditions. Asterix denotes significance at $p < 0.05$. (B) The features of each magnetic field. Horizontal and vertical components of the geomagnetic field are grey dashed arrows and 'mN' denotes magnetic north. The solid black arrow denotes the direction of the resultant field, with greater width indicating increased field intensity. White arrows at the bottom indicate the direction of the gravity vector (Modified from Putman et al. 2018).

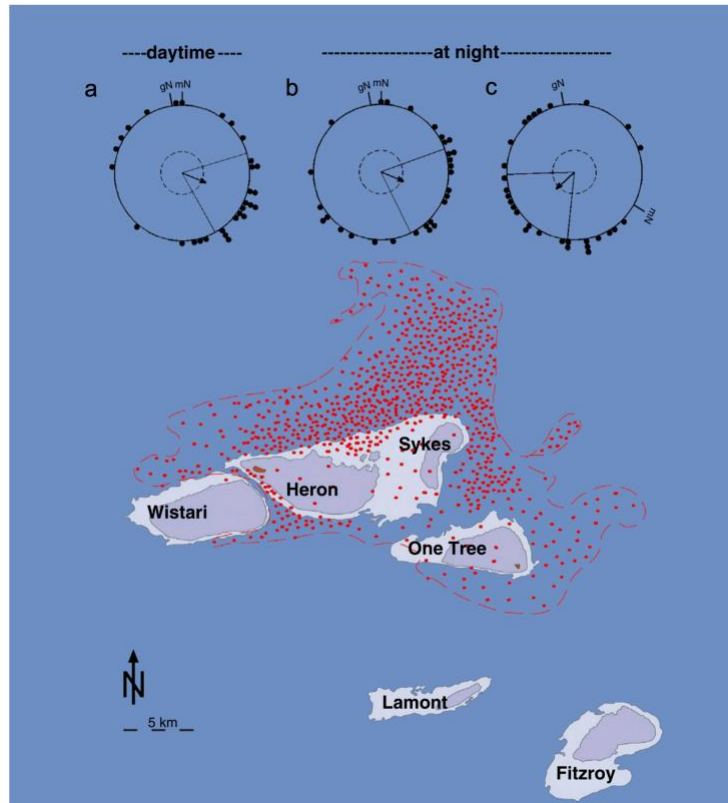


Figure 1.5 Map of Capricorn Bunker Reef Group, Australia, and results of orientation experiments.

Red dots indicate the expected distribution of passively dispersing particles released from One Tree Reef (see Bottesch et al. 2016 for details of hydrodynamic model). Purple patches indicate lagoons and white patches indicate reef slopes where the depth reaches 20 meters. Circular diagrams at the top of the figure indicate the orientation of fish tested: (A) under clear skies and natural magnetic field conditions during the day; (B) under natural magnetic field conditions at night; and (C) at night in a magnetic field in which the horizontal component was rotated 120 degrees clockwise. For each diagram, ‘mN’ indicates magnetic North and ‘gN’ indicates geographic North. Each black dot represents the mean direction of a single fish. Arrows within each circle indicate the mean direction of the group. Lines on either side of the arrows indicate the 95% confidence intervals for the mean angle. Dashed circles indicate the radius needed to achieve significance ($p < 0.05$) based on the Rayleigh test. Figure is modified from Bottesch et al. (2016).

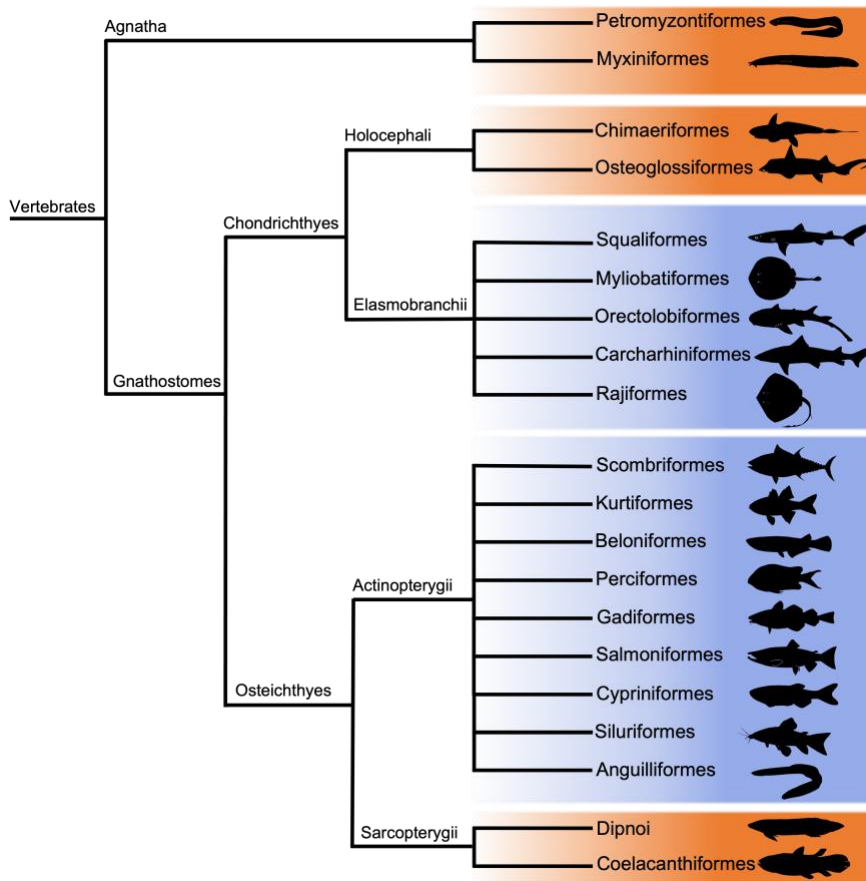


Figure 1.6 A cladogram showing simplified phylogenetic relationships among the main fish groups.

Clades highlighted in blue represent orders in which behavioral evidence for magnetoreception has been obtained; clades highlighted in orange show orders in which studies of magnetoreception have not, to our knowledge, been undertaken. Silhouette images were obtained under Public Domain courtesy of PhyloPic (<http://phylopic.org>).

Tables

Table 1.1 Experimentally demonstrated behavioral responses of bony fishes to magnetic stimuli¹.

| Class | Order | Family | Species | Citations | Types of responses |
|----------------|----------------|-------------|---------------------|---|---|
| Actinopterygii | Anguilliformes | Anguillidae | <i>A. anguilla</i> | (Branover et al. 1971; Tesch and Lelek 1973; Tesch 1974; Karlsson 1985; Tesch et al. 1992; Durif et al. 2013; Naisbett-Jones et al. 2017; Cresci et al. 2017b, 2019a) | map, compass, alignment, other |
| ” | ” | ” | <i>A. japonica</i> | (Nishi et al. 2004, 2005, 2018; Nishi and Kawamura 2005) | anomalous |
| ” | ” | ” | <i>A. rostrata</i> | (McCleave et al. 1971; Rommel and McCleave 1973; Zimmerman and McCleave 1975; Souza et al. 1988) | anomalous, conditioning, other |
| ” | Salmoniformes | Salmonidae | <i>O. nerka</i> | (Quinn 1980; Quinn et al. 1981; Quinn and Brannon 1982) | compass, other |
| ” | ” | ” | <i>S. salar</i> | (McCleave et al. 1971; Rommel and McCleave 1973; Varanelli and McCleave 1974; Scanlan et al. 2018; Minkoff et al. 2020) | map, conditioning, other |
| ” | ” | ” | <i>S. trutta</i> | (Formicki et al. 1997, 2004) | anomalous, alignment |
| ” | ” | ” | <i>O. gorbuscha</i> | (Putman et al. 2020) | map |
| ” | ” | ” | <i>O. keta</i> | (Quinn and Groot 1983) | other |
| ” | ” | ” | <i>O. mykiss</i> | (Chew and Brown 1989; Walker et al. 1997; Formicki et al. 1997; Haugh and Walker 1998; Hellinger and Hoffmann 2009, 2012; Putman et al. 2014b; Fitak et al. 2017, 2020) | conditioning, anomalous, map, alignment |

| | | | | | |
|---|---------------|-------------------------|--------------------------|---|---|
| ” | ” | ” | <i>O. tshawytscha</i> | (Taylor 1986; Putman et al. 2014c, 2018; Naisbett-Jones et al. 2020) | anomalous, other, compass, map |
| ” | Scombriformes | <i>Scombridae</i> | <i>T. albacares</i> | (Walker 1984) | conditioning |
| ” | Cypriniformes | <i>Cyprinidae</i> | <i>D. rerio</i> | (Shcherbakov et al. 2005; Takebe et al. 2012; Osipova et al. 2016; Krylov et al. 2016; Cresci et al. 2017a, 2018; Myklatun et al. 2018) | conditioning, other, alignment, compass |
| ” | ” | ” | <i>R. rutilus</i> | (Krylov et al. 2016) | compass |
| ” | ” | ” | <i>C. auratus</i> | (Becker 1974) | other |
| ” | ” | ” | <i>C. carpio</i> | (Hart et al. 2012) | alignment |
| ” | Beloniformes | <i>Adrianichthyidae</i> | <i>O. latipes</i> | (Myklatun et al. 2018) | other |
| ” | Kurtiformes | <i>Apogonidae</i> | <i>O. doederleini</i> | (Bottesch et al. 2016) | compass |
| ” | Perciformes | <i>Pomacentridae</i> | <i>C. atripectoralis</i> | (O’Connor and Muheim 2017) | compass |
| ” | Cichliformes | <i>Cichlidae</i> | <i>O. mossambicus</i> | (Shcherbakov et al. 2005) | conditioning |
| ” | Gadiformes | <i>Gadidae</i> | <i>M. aeglefinus</i> | (Cresci et al. 2019b) | compass |
| ” | Siluriformes | <i>Siluridae</i> | <i>K. vitreolus</i> | (Hunt et al. 2021) | other |

¹Types of responses: **Compass** = evidence that fishes use directional information in Earth’s magnetic field; **Map** = evidence that fishes use positional information in Earth’s magnetic field; **Alignment** = studies indicating spontaneous alignment of fishes relative to the axis of magnetic field lines; **Anomalous** = avoidance, attraction, or other responses of fishes to strong (greater than earth strength) anomalous magnetic fields produced by a magnet, solenoid, or magnetic coil; **Conditioning** = conditioning of fishes to magnetic field stimuli; **Other** = other evidence (not included in the previous categories) that suggests or demonstrates magnetic sensitivity.

Table 1.2 Experimentally demonstrated behavioral responses of cartilaginous fishes to electromagnetic stimuli².

| Class | Order | Family | Species | Citations | Types of responses |
|----------------|-------------------|-----------------------|-------------------------|---|----------------------------------|
| Chondrichthyes | Myliobatiformes | <i>Urotrygonidae</i> | <i>U. jamaicensis</i> | (Newton and Kajiura 2017, 2020a, b) | conditioning, compass, anomalous |
| ” | ” | ” | <i>U. halleri</i> | (Kalmijn 1978) | conditioning |
| ” | ” | <i>Dasyatidae</i> | <i>D. americanus</i> | (O’Connell et al. 2010, 2011b) | anomalous |
| ” | Rajiformes | <i>Rajidae</i> | <i>R. clavata</i> | (Smith and O’Connell 2014) | anomalous |
| ” | Carcharhiniformes | <i>Carcharhinidae</i> | <i>C. plumbeus</i> | (Meyer et al. 2005; Siegenthaler et al. 2016; Anderson et al. 2017) | conditioning, anomalous |
| ” | ” | ” | <i>C. leucas</i> | (O’Connell et al. 2014c) | anomalous |
| ” | ” | ” | <i>N. brevirostris</i> | (O’Connell et al. 2011a, 2014a) | anomalous |
| ” | ” | ” | <i>R. terraenovae</i> | (O’Connell et al. 2011b) | anomalous |
| ” | ” | ” | <i>C. limbatus</i> | (O’Connell et al. 2011b) | anomalous |
| ” | ” | ” | <i>C. tilstoni</i> | (Rigg et al. 2009) | anomalous |
| ” | ” | ” | <i>C. amblyrhynchos</i> | (Rigg et al. 2009) | anomalous |
| ” | ” | ” | <i>R. acutus</i> | (Rigg et al. 2009) | anomalous |
| ” | ” | ” | <i>G. glyphis</i> | (Rigg et al. 2009) | anomalous |
| ” | ” | <i>Sphyrnidae</i> | <i>S. mokarran</i> | (O’Connell et al. 2015) | anomalous |

| | | | | | |
|---|------------------|---------------------------|------------------------|----------------------------|-----------|
| ” | ” | ” | <i>S. lewini</i> | (Rigg et al. 2009) | anomalous |
| ” | ” | ” | <i>S. tiburo</i> | (Keller et al. 2021) | map |
| ” | ” | <i>Scyliorhinidae</i> | <i>S. canicula</i> | (Smith and O’Connell 2014) | anomalous |
| ” | ” | <i>Triakidae</i> | <i>M. canis</i> | (O’Connell et al. 2011b) | anomalous |
| ” | ” | ” | <i>T. semifasciata</i> | (Kalmijn 1978) | alignment |
| ” | Squaliformes | <i>Squalidae</i> | <i>S. acanthias</i> | (O’Connell et al. 2014b) | anomalous |
| ” | Orectolobiformes | <i>Ginglymostomatidae</i> | <i>G. cirratum</i> | (O’Connell et al. 2010) | anomalous |

²Types of responses: **Compass** = evidence that fishes use directional information in Earth’s magnetic field; **Map** = evidence that fishes use positional information in Earth’s magnetic field; **Alignment** = studies indicating spontaneous alignment of fishes relative to the axis of magnetic field lines; **Anomalous** = avoidance, attraction, or other responses of fishes to strong (greater than earth strength) anomalous magnetic fields produced by a magnet, solenoid, or magnetic coil; **Conditioning** = conditioning of fishes to magnetic field stimuli; **Other** = other evidence (not included in the previous categories) that suggests or demonstrates magnetic sensitivity.

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CHAPTER 2: IN FISHES: THE EFFECT OF MAGNETIC PULSES ON ORIENTATION OF JUVENILE PACIFIC SALMON¹

Summary

A variety of animals sense Earth's magnetic field and use it to guide movements over a wide range of spatial scales. Little is known, however, about the mechanisms that underlie magnetic field detection. Among teleost fish, growing evidence suggests that crystals of the mineral magnetite provide the physical basis of the magnetic sense. In this study, juvenile Chinook salmon (*Oncorhynchus tshawytscha*) were exposed to a brief but strong magnetic pulse capable of altering the magnetic dipole moment of biogenic magnetite. Orientation behaviour of pulsed fish and untreated control fish was then compared in a magnetic coil system under two conditions: (1) the local magnetic field; and (2) a magnetic field that exists near the southern boundary of the natural oceanic range of Chinook salmon. In the local field, no significant difference existed between the orientation of the control and pulsed groups. By contrast, orientation of the two groups was significantly different in the magnetic field from the distant site. These results demonstrate that a magnetic pulse can alter the magnetic orientation behaviour of a fish and are consistent with the hypothesis that salmon have magnetite-based magnetoreception.

¹ This chapter was previously published as:
Naisbett-Jones L.C., Putman N.F., Scanlan M.M., Noakes D.L.G., and Lohmann K.J. (2020) Magnetoreception in fishes: the effect of magnetic pulses on orientation of juvenile Pacific salmon. *Journal of Experimental Biology* 18:223. <https://doi.org/10.1242/jeb.222091>

Introduction

Diverse animals detect Earth's magnetic field and use it as a cue to guide their movements (Wiltschko et al., 1993; Kimchi and Terkel, 2001; Boles and Lohmann, 2003; Naisbett-Jones et al., 2017; Lohmann and Lohmann, 2019). Little is known, however, about the mechanism (or mechanisms) that enable animals to sense magnetic fields. Recent research has focused on two possibilities. The chemical magnetoreception (or radical pairs) hypothesis proposes that the detection of magnetic fields involves biochemical reactions that are influenced by the ambient magnetic field (Ritz et al., 2000; Rodgers and Hore, 2009). By contrast, the magnetite hypothesis proposes that crystals of the magnetic mineral magnetite (Fe_3O_4) underlie magnetoreception (Kirschvink et al., 2001; Shaw et al., 2015). It is possible that different animals have different mechanisms, that both mechanisms coexist in some animals (Johnsen and Lohmann, 2005; Lohmann, 2010), and also that magnetoreception is accomplished by a different biophysical process (e.g., Nimpf et al., 2019).

Two main lines of evidence are consistent with the magnetite hypothesis. The first is that magnetic material has been detected in many magnetically sensitive species (Lohmann, 1984; Kirschvink et al., 1985; Moore et al., 1990; Moore and Riley, 2009). The second is that strong but brief magnetic pulses alter magnetic orientation behaviour in several animals including lobsters (Ernst and Lohmann, 2016), turtles (Irwin and Lohmann, 2005), birds (Beason et al., 1995) and bats (Holland et al., 2008). The effect of magnetic pulses on behaviour is noteworthy because such pulses have the potential to modify the magnetic dipole moment of magnetite crystals, which in turn might alter magnetic information relayed to the brain by magnetite-based receptors (Wiltschko et al., 2002). Importantly, magnetic pulses should have no lasting effect on animals that rely on chemical magnetoreception (Shaw et al., 2015). For this reason, subjecting animals to strong magnetic pulses and monitoring subsequent changes in behaviour has often been described as a diagnostic test for

magnetite-based magnetoreception (Beason et al., 1995; Wiltschko et al., 1998; Holland et al., 2008).

Fish have played a prominent role in magnetoreception research (Putman et al., 2014a; Bottesch et al., 2016; Naisbett-Jones et al., 2017) and magnetite has been detected in several species (Walker et al., 1984; Kirschvink et al., 1985; Diebel et al., 2000). However, whether a magnetic pulse affects the orientation behaviour of fish is not known. Here we report such an experiment with Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum 1792), a migratory fish that uses Earth's magnetic field for orientation (Putman et al., 2014a; Putman et al., 2018) and is known to possess chains of single-domain magnetite particles that might function as magnetoreceptors (Kirschvink et al., 1985). The results indicate that a magnetic pulse alters subsequent magnetic orientation behaviour in young salmon, a finding consistent with the hypothesis that magnetoreception in salmon, and perhaps in other teleost fish, is at least partly based on magnetite.

Materials & methods

Animals and facilities

Chinook salmon from the Elk River, Oregon, were spawned in December 2016 from a mix of wild and hatchery adults (29 pairs). Fertilized eggs were incubated at the Elk River hatchery (Port Orford, Oregon, USA; 42.73°N, 124.44°W) and transported at the eyed stage to the Oregon Hatchery Research Center (Alsea, Oregon, USA; 44.40°N, 123.75°W) in January 2017. After hatching, fish were transferred into plastic, circular, outdoor holding tanks (0.9 m diameter). Holding tanks received a continuous supply of natural stream water. Water parameters varied with ambient conditions. Between June and July 2017, we tested a total of 432 stream-dwelling Chinook salmon parr (fork lengths ranged from 5 to 7 cm). All animal care and procedures were approved by the Institutional Animal Care and Use Committee of

Oregon State University (approval number 4761) and the University of North Carolina approval number 17-189).

Magnetic pulse protocol

Fish were randomly assigned to one of two treatment groups. One group of fish was treated with a strong magnetic pulse (85 mT) capable of realigning the magnetic dipole moments of single-domain biogenic magnetite crystals (Ernst and Lohmann, 2016). The second group of fish served as controls and were subjected to identical handling, but not exposed to a magnetic pulse.

The magnetic pulse was generated with a magnetizer (model 7515-G, Magnetic Instrumentation, Indianapolis, Indiana, USA). The magnetizer consisted of a bank of capacitors (425 V max) that discharged to a solenoid (Figure 2.1A). The solenoid (32 cm diameter, 20 cm length) was aligned with the magnetic north-south axis.

During the pulsing procedure, fish were individually placed into non-magnetic pulsing chambers (6×15×2.5 cm; Figure 2.1A). Each pulsing chamber was constructed of black acrylic and was filled with water to a depth of 5 cm. These chambers were designed to align fish along a single axis while preventing them from turning around. Salmon were placed into the solenoid facing north and pulsed in two groups of eight fish, one directly after the other (Figure 2.1A). Pulsed fish experienced a magnetic pulse directed antiparallel to the horizontal component of the geomagnetic field (i.e. toward magnetic south) (Figure 2.1B).

Testing procedure

We designed our experiment to provide two different contexts in which differential orientation might be expressed by pulsed and control salmon: (1) in the local magnetic field; and (2) during a “magnetic displacement” in which fish were tested in a magnetic field that

exists at a distant location near the southern border of the Chinook salmon oceanic range. In a previous study (Putman et al., 2014a), this field elicited northward orientation in Chinook salmon slightly older than the ones we tested.

Our orientation assay was similar to that used by Putman et al., 2014a. Following the magnetic pulse treatment, we tested the magnetic orientation behaviour of the fish inside a magnetic coil system (Figure 2.2A). Fish from the control and pulse groups were tested separately, with tests for the two groups alternated throughout the day. Prior to testing, each fish was placed into one of 16 opaque circular buckets (diameter: 30.5 cm; water depth: 20 cm) within the magnetic coil. The fish were then given a 5-min acclimation period in the local magnetic field (coil turned off), after which the orientation behaviour of fish in the same field was recorded for the next 5 min (see Figure 2.3 for detailed timeline). We then used the magnetic coil to generate the magnetic field that exists in the ocean at the southern limit of the Chinook salmon's range (Putman et al., 2014a). Salmon experienced this southern magnetic field for 10 min before the completion of the trial. Fish from both treatment groups experienced the same testing procedure within the magnetic coil. Each fish was tested only once and experienced the local ambient field before being exposed to the southern field (Figure 2.3). In total, 224 fish were tested in the control treatment and 208 were tested in the pulse treatment.

Magnetic field conditions

A triaxial fluxgate magnetometer (Applied Physics model 520A) was used to measure the magnetic fields fish experienced. Within the holding tanks, field intensity was 51.9 μT and the inclination angle was 67.0°. In the magnetic coil system, the local ambient magnetic field had an intensity 51.7 μT and an inclination of 66.3°. The magnetic field intensity of the southern treatment field was 44.1 μT (uniformity: $\pm 0.1 \mu\text{T}$) and the inclination angle 56.7°

(uniformity: $\pm 0.5^\circ$). This southern magnetic field replicated one that exists at a location (38° N, 145° W) near the southern border of the Chinook salmon range, as determined using the International Geomagnetic Reference Field (IGRF-11; Finlay et al., 2010) for June, 2017, when the experiment began.

Data collection and analysis

Two GoPro cameras positioned above the coil system (Figure 2.2B) were programmed to take photos at specific timepoints (shown in Figure 2.3) during both the 5-min test period in the local ambient field and the following 10 min in the southern magnetic field. This resulted in two experimental conditions that we considered separately; in other words, we compared orientation between the control and pulsed fish in the local magnetic field and also in the southern displacement field.

Orientation angles were measured using the image processing program ImageJ (ImageJ 1.52a) (<https://imagej.net/ImageJ>). Observers blind to which group fish belonged to analyzed the photos by recording the orientation of each fish. This was achieved using the angle tool in ImageJ to draw a line along the body axis of each fish, from the caudal peduncle to the snout (Figure 2.2C). The orientation angle relative to magnetic north was then recorded.

Using the orientation angles extracted from the photographs taken in the local field and in the southern (displacement) field (Figure 2.3), we used standard procedures in circular statistics (Batschelet, 1981) to calculate a mean angle representing the orientation of each fish in each of the two fields. Because 16 fish were tested in the coil at a single time, we then calculated a single mean angle for each trial, which represented the average direction of all the fish that were tested simultaneously. This step was taken to account for the possibility that fish tested in the same trial might not have been fully independent, inasmuch as ambient conditions (e.g. lighting, cloud cover, etc.) at the time of testing might have influenced the fish in a

similar way. This conservative analysis, which treated trials rather than individual fish as independent data points, resulted in a sample size of 14 for the control treatment group and 13 for the pulse group. To further explore the data, a second analysis treating each fish as an independent data point was also undertaken (Figure A1). The two analyses yielded qualitatively identical results (see Figure 2.4 and Figure A1).

Rayleigh tests were used to determine whether each treatment group was significantly oriented. The nonparametric Mardia-Watson-Wheeler test was used to determine whether pulsed and control groups differed in their orientation under each of the two magnetic field conditions. We used the statistical software R (Version 1.1.423, R Development Core Team, 2016) for analyses and to generate graphics.

Results

Under local magnetic field conditions fish from the control treatment group were significantly oriented with a mean angle of 338 degrees (Rayleigh test, $n=14$, $r=0.55$, $Z=4.17$, $P=0.01$; Figure 2.4A). In contrast, fish from the pulse group exhibited orientation that was statistically indistinguishable from random (Rayleigh test, $n=13$, $r=0.37$, $Z=1.73$, $P=0.18$; Figure 2.4B). No significant difference between the orientation of the control and pulse groups was observed (Mardia-Watson-Wheeler test, $W=2.69$, $P=0.26$; Figure 2.4A,B).

When exposed to a magnetic field that exists near the southern limit of the Chinook salmon range, control fish had orientation that was statistically indistinguishable from random (Rayleigh test, $n=14$, $r=0.13$, $Z=0.22$, $P=0.81$; Figure 2.4C). In contrast, pulsed fish were significantly oriented towards the east-northeast with a mean angle of 72 degrees (Rayleigh test, $n=13$, $r=0.51$, $Z=3.37$, $P=0.03$; Figure 2.4D). The orientation of control and pulsed fish differed significantly (Mardia-Watson-Wheeler test, $W=7.12$, $P=0.03$; Figure 2.4C,D).

Discussion

The results demonstrate that a strong magnetic pulse influences the subsequent orientation behaviour of juvenile Chinook salmon. Salmon from the pulse and control groups exhibited significantly different orientation when tested in a magnetic field that exists near the southern boundary of their oceanic range (Figure 2.4C,D). To our knowledge, these results are the first to demonstrate that a magnetic pulse affects orientation behaviour in fish. The findings are consistent with the magnetite hypothesis of magnetoreception, inasmuch as a magnetic pulse can potentially alter magnetite-based receptors, but should not exert any lasting effect on either chemical magnetoreception or electromagnetic induction (Wiltschko et al., 2002; Shaw et al., 2015).

Magnetic pulses have previously been demonstrated to affect magnetic orientation behaviour in a variety of terrestrial and aquatic animals including rodents (Marhold et al., 1997), bats (Holland et al., 2008), birds (Beason et al., 1995; Wiltschko et al., 1998; Holland and Helm, 2013), sea turtles (Irwin and Lohmann, 2005), and lobsters (Ernst and Lohmann, 2016). Interestingly, the effects of pulses on different species have been highly variable. In some cases, magnetic pulses led to increased dispersion in orientation bearings (Irwin and Lohmann, 2005). In others, the direction of orientation changed after a pulse (Holland et al., 2008) or the pulse elicited a directional preference in animals that previously lacked one (Ernst and Lohmann, 2016). The variability in responses may be due in part to methodological differences such as the strength and direction of the applied pulse, the recovery period after the pulse, and the way in which animals were handled. In addition, the outcome may be influenced by the navigational task that confronts the animal during the test conditions – for example, whether it is tested in a setting that encourages homing (Beason et al., 1997; Holland et al., 2008), migration (Wiltschko and Wiltschko, 1995a) or neither (Ernst and Lohmann, 2016). Regardless, a change in orientation behaviour following treatment with

a magnetic pulse has been interpreted as evidence for magnetite-based magnetoreception (Beason et al., 1995; Holland et al., 2008), although the possibility of a more general effect on the health or physiology of animals cannot be excluded with certainty (Ernst and Lohmann, 2016; Fitak et al., 2017).

Effect on magnetic compass or magnetic map?

In the present study, salmon subjected to a pulse did not differ in orientation from control fish when tested in the local magnetic field, but did differ significantly when tested in the magnetic field of a location near the southern periphery of their range (Figure 2.4 C,D). Interestingly, salmon are known to possess both a magnetic ‘compass’ that enables them to use Earth’s magnetic field as a directional cue (Quinn, 1980) and a magnetic ‘map’ that allows them, in effect, to assess their position within an ocean basin (Putman et al., 2014a; Putman, 2015; Scanlan et al., 2018; Putman et al., 2020). In principle, the mechanism underlying the compass, the map, or both might have been affected by the magnetic pulse.

The salmon magnetic compass detects the polarity of the ambient field (Quinn and Brannon, 1982), making it functionally different from the magnetic compasses of birds (Wiltschko and Wiltschko, 1972) and sea turtles (Light et al., 1993; Goff et al., 1998). Polarity compasses have properties consistent with magnetite but are incompatible with chemical magnetoreception (Johnsen & Lohmann, 2005; Rodgers and Hore, 2009). It is noteworthy that mole rats and bats also have polarity compasses (Marhold et al., 1997b; Wang et al., 2007) and that the orientation behaviour of these animals is also altered by a magnetic pulse. Thus, a possible interpretation is that salmon, mole rats, and bats all have magnetite-based magnetic compasses.

Findings with migratory birds, however, suggest that it is premature to conclude that magnetic pulses necessarily affected the salmon compass, inasmuch as similar magnetic

pulses are thought to primarily affect a map sense in birds (Wiltschko and Wiltschko, 1995b; Wiltschko and Wiltschko, 2003; Holland and Helm, 2013). In birds, juveniles making their first migration are thought to lack map information and guide themselves by maintaining a compass heading, whereas adults exploit a map acquired from previous migratory experience (Wiltschko and Wiltschko, 2003). Interestingly, the effect of a magnetic pulse was restricted to experienced birds that had already completed at least one migration, whereas naïve birds were unaffected by the same pulse (Munro et al., 1997; Wiltschko et al., 1998). For salmon, further studies will be needed to determine precisely what parts of the salmon magnetoreception and navigation system are affected by a magnetic pulse.

Comparison to previous salmon studies

In part of our study, juvenile Chinook salmon were exposed to a magnetic field that exists near the southern periphery of their oceanic range. In a previous experiment with Chinook salmon, this field elicited northward orientation (Putman et al., 2014a) but in the present study, control fish tested in this same field had orientation indistinguishable from random. The reason for this difference is not known. A possible explanation, however, is that fish used in this study were younger and originated from the Elk River, which enters the Pacific approximately 400 km south of the entry point of fish used previously (Putman et al., 2014a). Chinook salmon populations are known to vary in their oceanic distribution (Weitkamp, 2010) and thus presumably have different oceanic boundaries. An interesting possibility is that different salmon populations have different responses to magnetic fields, with each population responding most strongly to combinations of intensity and inclination angle that represent boundaries for that group (Putman et al., 2014a). A wider survey of magnetic orientation responses across Chinook populations and through ontogeny is required before firm conclusions can be drawn.

Another methodological difference between the present study and that of Putman et al. (2014a) is that all fish in our study, including controls, were briefly placed in a solenoid prior to testing in a magnetic coil. Although control fish were not exposed to a magnetic pulse, they were nevertheless exposed to an altered magnetic field with a different inclination and intensity immediately before testing. Fish in the solenoid experienced a change in field intensity of about 0.8 μT (about 1.5% of the local field), with the effect on inclination being difficult to measure. Whether this brief exposure to an altered field affected subsequent behaviour is not known, but longer exposures to stronger magnetic distortions reduce the ability of salmonids to respond with directed orientation to magnetic displacements (Putman et al., 2014b).

As noted previously, magnetic pulse experiments have been conducted using a variety of different animals and a number of different methodologies. One potential complication of such studies is that a magnetic pulse is inevitably accompanied by a transient electric field; thus, in principle, either the magnetic pulse or the electric field might produce an effect. Some studies have attempted to control for possible effects of the transient electric field by administering pulsed fields while the animal is in a strong ‘biasing’ magnetic field oriented in one of two directions (e.g., Holland and Helm, 2013; Holland et al., 2008; Wiltschko et al., 2002). By contrast, other studies have not used biasing fields (e.g., Beason et al., 1995; Ernst and Lohmann, 2016; Wiltschko et al., 1998; Wiltschko et al., 2007), including the present one. No obvious difference has emerged between studies using biasing fields and those that have not, inasmuch as pulsed fields affected subsequent orientation behaviour in both methodologies. Nevertheless, additional studies using a variety of experimental designs may be worthwhile in both fish and other animals.

Regardless of these considerations, the pulsed fish and control fish in the present study had significantly different orientation when tested in the magnetic field of a distant ocean

location (Figure 2.4 C,D). This study provides the first evidence linking a magnetic pulse to behavioural changes in fish, adding salmon to the growing list of taxa affected by magnetic pulses. The finding that magnetic pulses alter orientation behaviour of salmon is consistent with the hypothesis that magnetoreceptors in teleost fish are based on magnetite crystals. Further research will be needed to confirm or refute this hypothesis and to definitively characterize the mechanisms that underlie magnetoreception in animals.

Figures

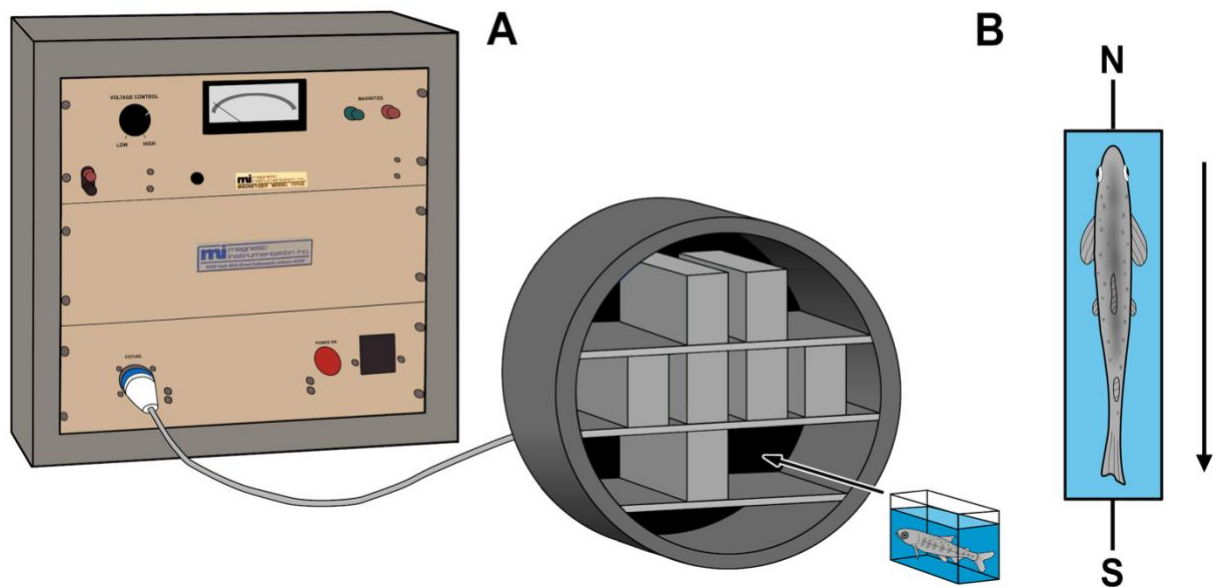


Figure 2.1 Magnetic pulse protocol.

(A) Magnetizer and solenoid. Diagram shows the positions of the eight pulsing chambers in which all fish were placed prior to being tested in orientation experiments. Fish in the pulse group were subjected to a magnetic pulse; control fish were not. (B) View of pulsing chamber from above. Fish were placed into the solenoid facing north. Arrow indicates the direction of the pulse with respect to ambient magnetic field conditions.

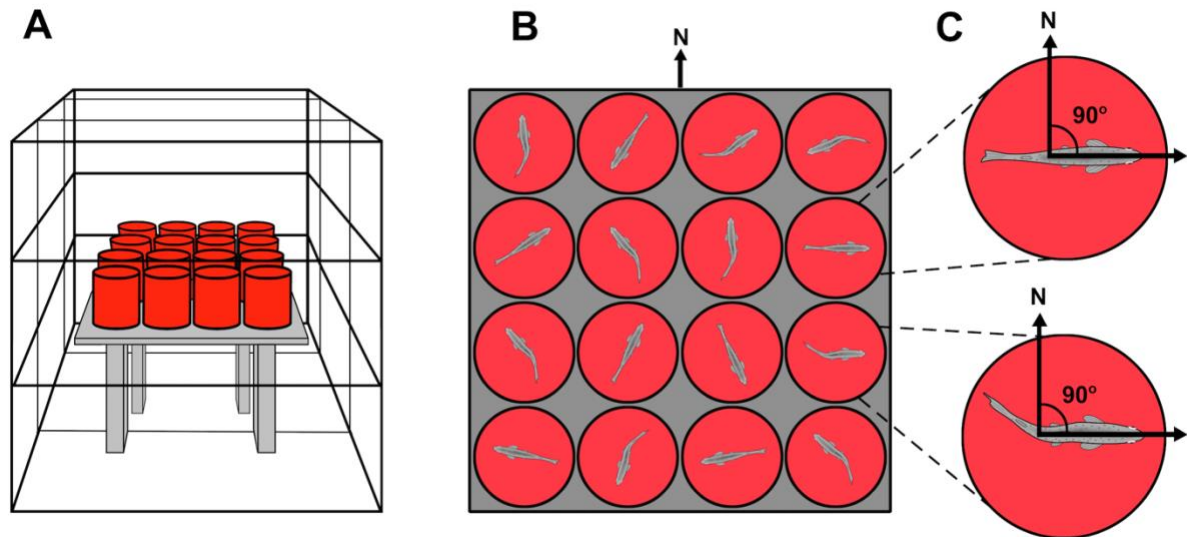


Figure 2.2 Magnetic coil system and orientation arenas.

A) Schematic of the magnetic coil , table, and 16 orientation arenas used in the study. The magnetic coil system consisted of two orthogonal Merritt 4-coil systems (Merritt et al., 1983). The outer, vertical coil side length was 3.32 m; the inner, horizontal coil side length was 3.05 m. Additional information about the coil is provided in Putman et al., 2014a. (B) Camera view from above the magnetic coil system showing the 16 fish in their individual arenas. (C) Examples of how fish orientation was measured. A line was drawn from the caudal peduncle to the snout to record the angle of orientation.

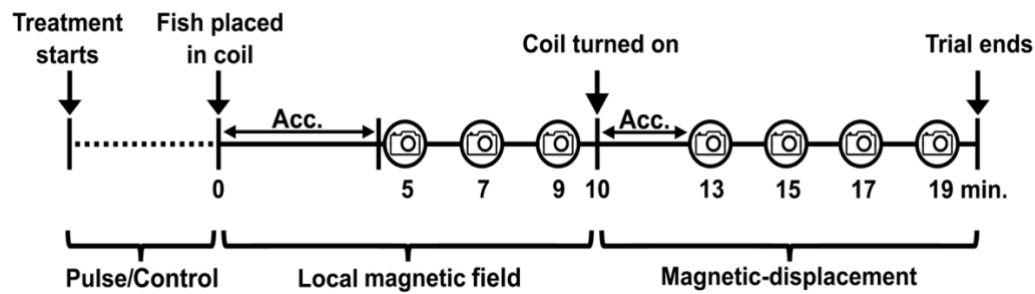


Figure 2.3 Timeline of the experiment.

After each group of fish was placed into the solenoid and subjected to either the pulse or control procedure (see text for details), fish were placed into the magnetic coil at time zero and given a 5-min acclimation period (Acc). Fish then experienced an additional 5 min in the local magnetic field conditions, during which several photographs (timepoints indicated by camera icons) were taken at 2-min intervals for the purpose of assessing orientation in this field (see text). The coil was then turned on and fish experienced a magnetic field that exists near the southern limit of the Chinook salmon range. After a 3-min acclimation period in the new field, several photographs were taken at 2-min intervals for the purpose of assessing orientation in the displacement field. Trials concluded after fish had been in the arena for a total of 20 min.

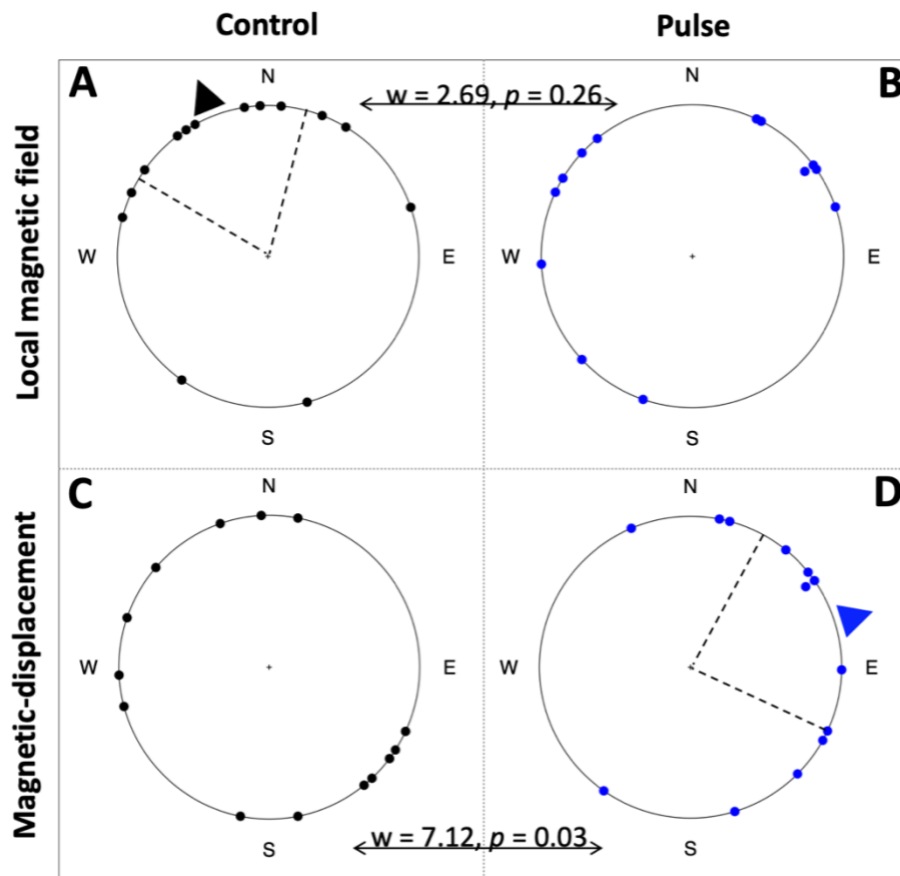


Figure 2.4 Orientation of salmon under two different magnetic fields.

(A) In the local magnetic field, fish from the control group were significantly oriented with a mean angle of 338 deg (Rayleigh test, $n=14$, $r=0.55$, $P=0.01$). (B) In the local magnetic field, salmon that experienced a strong magnetic pulse were not oriented as a group (Rayleigh test, $n=13$, $r=0.37$, $P=0.18$). (C) During a magnetic displacement to a southern ocean region, control fish were not oriented as a group (Rayleigh test, $n=14$, $r=0.13$, $P=0.81$). (D) During the magnetic displacement, salmon from the pulse group were significantly oriented with a mean angle of 72 deg (Rayleigh test, $n=13$, $r=0.51$, $P=0.03$). Each data point represents the mean angle of 16 fish that were tested in the coil simultaneously (see text). Arrow heads indicate the mean direction of each treatment group. Dotted lines represent the 95% confidence interval for the mean.

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CHAPTER 3: A GEOMAGNETIC MAP AND COMPASS IN A BARELY MIGRATORY FLATFISH¹

Summary

Diverse marine animals, including numerous species of fishes and sea turtles, undergo spectacular long-distance migrations that sometimes span entire ocean basins. For many such migrants, the ability to use Earth's magnetic field as both a compass (for maintaining direction) and a map (for determining position) is a central part of the navigational mechanisms that guide long-distance movements. Little is known, however, about whether the navigational mechanisms used by highly migratory species also exist in species that never travel far. The Gulf flounder (*Paralichthys albigutta*) is a bottom-dwelling marine flatfish with a largely sedentary lifestyle. Both young and adults only travel short distances (a few tens of km or less) between offshore and inshore habitats and are not known to exhibit site fidelity or undergo natal homing. Here we report evidence that the Gulf flounder nevertheless possesses both a magnetic compass sense and a magnetic map sense resembling those reported in salmon and eels. The existence of these dual sensory abilities in a fish that undertakes only modest movements implies that navigation based on a magnetic map and compass is not limited to iconic long-distance migrants, but may instead be present in diverse animals that move over a wide range of spatial scales.

¹ This chapter is in preparation for Current Biology.

Results and Discussion

Research on mechanisms of animal navigation has frequently focused on species that undertake long-distance migrations (Horton et al. 2011; Lohmann et al. 2012; Wynn et al. 2020). Among fishes, much of what is known has been derived from studies on iconic long-distance migrants such as salmon, eels, and sharks, all of which traverse vast expanses of open ocean and depend at least partly on a magnetic map and a magnetic compass for navigation (Naisbett-Jones and Lohmann 2022; Lohmann et al. 2022). These migratory specialists, however, represent only one extreme along the spectrum of diverse life-history strategies that exist in marine animals. Whether marine species that exhibit more limited migratory strategies have evolved similar navigation mechanisms has, until now, received little attention.

The Gulf flounder (*Paralichthys albigutta*) is a dorso-ventrally flattened teleost fish with extreme adaptations for a sedentary, benthic existence (Figure 3.1). Compared to more famously mobile marine fishes like salmon, which often migrate thousands of kilometres between foraging and reproductive areas, Gulf flounder are barely migratory, traveling distances of only 5 to 30 kilometres between offshore spawning areas and inshore environments. Moreover, in contrast to migrants such as sea turtles, salmon, and some sharks (Bonfil 2005; Lohmann et al. 2008), Gulf flounder are not known to engage in natal homing (defined as the ability to return to an area of origin after first migrating a long distance away) and they are not known to exhibit homing or foraging site fidelity (VanderKooy 2000; Fitzhugh et al. 2008). Thus, at first glance, nothing in the Gulf flounder's ecology or lifestyle suggests that it requires the same kinds of navigational mechanisms that exist in transoceanic migrants.

As a first step toward comparing the navigational mechanisms of fishes that move over vastly different spatial scales, we conducted behavioral experiments to investigate

whether Gulf flounder have a magnetic compass and magnetic map sense (Figure 3.2). To test for a magnetic compass sense, we exposed flounder to the local magnetic field of the test site (our control field) or to a magnetic field in which the direction of the horizontal field component was reversed using a magnetic coil system. Individuals tested in the local magnetic field oriented toward magnetic west, a direction consistent with their onshore migration (mean angle = 273° ; Rayleigh test, $n = 19$, $r = 0.48$, $p = 0.01$ Figure 3.3). By contrast, flounder tested in a reversed magnetic field oriented in approximately the opposite direction (mean angle = 83° ; Rayleigh test, $n = 21$, $r = 0.45$, $p = 0.01$; Figure 3.3). Orientation under the two conditions was significantly different (Mardia-Watson-Wheeler test: $W = 15.5$, $p < 0.001$). The results indicate that Gulf flounder can use Earth's magnetic field as a compass to maintain direction. Under natural conditions, this westward orientation may function in guiding flounder to suitable growth habitats in coastal areas, inasmuch as swimming west along the east coast of the U.S. will invariably lead flounder toward inshore habitats.

To investigate whether flounder possess a magnetic map sense, we used a 'magnetic displacement experiment' conceptually similar to one conducted previously with salmon (Putman et al. 2014; Putman et al. 2020). Flounder were tested in circular water-filled arenas surrounded by a magnetic coil system (Figure 3.2), so that fish could be exposed to magnetic fields that exist near the northern and southern boundaries of their range. Fish tested in a magnetic field replicating one found near the northern range boundary oriented significantly towards the south (mean angle = 147° ; Rayleigh test, $r = 0.28$, $p < 0.05$; Figure 3.4). By contrast, fish tested in the southern magnetic field had orientation indistinguishable from random (Figure 3.4). The two distributions were significantly different (Mardia-Watson-Wheeler test: $W = 7.0$, $p = 0.03$), implying that flounder can distinguish between magnetic fields that exist in different locations in the Atlantic Ocean. Because the Gulf flounder is a

subtropical species unable to survive in cold water (Gilbert 1986), swimming south in response to a magnetic field that exists near the northern boundary of the species range is likely adaptive. Thus, the results are consistent with the interpretation that Gulf flounder derive map information from the geomagnetic field.

In contrast to the southeasterly orientation of fish tested in a magnetic field that exists along the northern range boundary, flounder tested in a magnetic field that exists near the southern boundary trended northeast (mean angle 39 degrees) but were not significantly oriented as a group (Figure 3.4). Why fish responded more strongly to the northern field than to the southern field is not known. One possibility, however, is that flounder in North Carolina waters are considerably more likely to encounter fields near the northern boundary of their range, both because they are physically closer to this boundary, and because the prevailing oceanic current in the area (the Gulf Stream) displaces flounder predominantly northward. Under these conditions, flounder from North Carolina might seldom encounter the southern boundary of the species range; for this reason, natural selection might have failed to sculpt a consistent response to magnetic fields that exist there. Similar asymmetries, in which the magnetic field from one location elicits a directional response while the field from another location does not, have been reported in several animals (Henshaw et al. 2010; Putman et al. 2015; Naisbett-Jones et al. 2017; Keller et al. 2021).

Our results demonstrate for the first time that a benthic and largely sedentary fish (Figure 3.1) possesses magnetic sensing abilities functionally similar to those present in fish that are migratory specialists. These findings raise the interesting possibility that the ability to derive both directional and positional information from the geomagnetic field, instead of being a specialized sensory ability restricted to long-distance migrants, is instead a widespread and perhaps even universal ability among fishes that move over a variety of spatial scales. The discovery of a well-developed magnetic sense in a benthic fish is

consistent with the hypothesis that magnetoreception is particularly well developed among marine animals, perhaps in part because so few other directional cues are available to animals that live well below the surface. Indeed, the benthic environment that flounder inhabit may place an additional premium on magnetic field sensing because many inshore or estuarine benthic environments inhabited by Gulf flounder are frequently turbid (Walsh et al. 1999; Minello and Benfield 2018), so that visual and celestial cues are routinely obscured, and the geomagnetic field is among the only sensory cues reliably available.

Our findings give credence to reports of magnetic compasses in reef fish (Bottesch et al. 2016; O'Connor and Muheim 2017), as well as magnetic maps being used by lobsters and newts, two animals that also move over relatively short distances (Lohmann et al. 1995; Fischer et al. 2001; Boles and Lohmann 2003; Diego-Rasilla and Phillips 2021). While our results imply that flounder respond to large-scale changes in magnetic map information, whether flounder also derive high-resolution positional information for use in small-scale navigation, as has been suggested in newts (Diego-Rasilla and Phillips 2021), will require further investigation.

Much like that of esteemed long-distance migrants, our findings indicate that directional and positional information from Earth's magnetic field are a key component of the flounder's sensory repertoire and provide a plausible means by which flounder and other benthic organisms exploit key ocean habitats, as well as avoid drifting towards uninhabitable environments. The ability to integrate map and compass information to meet these goals has clear fitness benefits and likely acts as a strong selection force within populations and through ontogeny. Understanding the mechanism(s) that permit magnetoreception in animals, the underlying genetic controls, and how behavioral responses to magnetic information might determine population structure (Brothers and Lohmann, 2018) are outstanding research

challenges in the field. As the most diverse vertebrate group on the planet, fishes provide a rich number of opportunities to address many of these long-standing research questions.

Materials and Methods

Fish collection and housing

All animal care and procedures were approved by the Institutional Animal Care and Use Committee of the University of North Carolina (approval number 17-189). Juvenile Gulf flounder (*Paralichthys albiguttata*) were obtained by trawling seagrass beds in Back Sound, North Carolina, USA (34.68361° N, 76.5625° W) where juvenile flounder are abundant. All fish were collected during May–August of 2018 and 2019. Following capture, fish were transported to non-magnetic holding tanks located at the University of North Carolina’s Institute of Marine Science (UNC-IMS; 34.7234° N, 76.7522° W). Tanks received a constant supply of fresh seawater from the adjacent Bogue Sound. Tanks were covered with shade cloth to minimize fish stress and to help maintain suitable temperatures (range: 22-29 °C) and oxygen levels (~8.0 Mg/L). Flounder were fed a mixed diet of fish and shrimp daily. All individuals were tested and released within five days of capture.

Magnetic coil and orientation arenas

An outdoor magnetic coil system was used for both the magnetic compass and map experiments. The coil system consisted of two independent four-coil systems arranged orthogonally (Merritt et al. 1983). Both coils were controlled with a separate power supply (BK Precision Model 1550 DC) operating at constant current. The coil surrounded a center platform. Magnetic field uniformity across the center platform was $\pm 0.1 \mu\text{T}$ for total field intensity and $\pm 0.2^\circ$ for field inclination. All magnetic fields used in experiments were based

on magnetic parameters from the International Geomagnetic Reference Field (IGRF-12) (Finlay et al. 2010) and were verified using a FVM400 Vector magnetometer.

Four plastic orientation arenas were placed on the center platform of the magnetic coil. This platform was enclosed on all sides with two layers of 100% black-out material, removing all external visual cues. Each circular orientation arena was identical and measured 32 cm in diameter and 45 cm in height. Prior to the start of the experiments, each orientation arena was filled with fresh sea water to a depth of 20 cm

Magnetic compass experiments

To test for the presence of a magnetic compass sense, we used two experimental treatments: (1) the local magnetic field that existed at the test site in Morehead City (total field intensity $48.8 \mu\text{T}$, inclination angle 62.3°), or; (2) a magnetic field in which the horizontal component of the magnetic field was reversed using the magnetic coil system (Merritt et al. 1983). Importantly, the magnetic field with the reversed horizontal component had a total field strength and inclination that matched the local magnetic field (total intensity: $48.8 \mu\text{T}$, inclination: 62.3°). One juvenile flounder (mean total length = 17.8 cm) was randomly placed into each of the four orientation arenas on the center platform of the magnetic coil. Groups of fish remained in one of the two experimental treatments for two hours before trials concluded. A total of 40 fish (19 in the local field and 21 in reversed) were tested between the two experimental treatments.

Magnetic map experiments

During a second set of experiments, aimed to investigate whether flounder possess a magnetic map sense, we tested smaller juveniles (mean total length = 10.3 cm, standard deviation = 2.7 cm). These fish ($n = 100$) had recently recruited to coastal growth habitats,

and were easily obtainable in coastal areas close to our study site. We used the magnetic coil system to produce one of two magnetic field treatments: (1) a magnetic field that exists past the northern extent of their range in coastal New Jersey (intensity 51.1 μT and an inclination angle of 65.5°); or (2) or a magnetic field that exists at the southern extent of their range, near Jamaica (intensity 38.4 μT and an inclination angle of 54.6°). Locations near latitudinal boundaries like these have been shown to elicit map responses in some animals and were similarly chosen here to maximize the likelihood of a response (Putman et al. 2014; Putman et al. 2015). One fish was placed into each of the four orientation arenas and was recorded for a total of 1.5 hours under one of the two magnetic field treatments. This shorter duration was chosen because the younger flounder used in the map experiments were more active than the older fish tested previously. We were thus able to collect a comparable amount of data in a shorter time.

Behavioral assay and data analysis

All experiments were conducted at night between 21:00 and 03:00, a time when juvenile flounder are active (Miyazaki et al. 1997). Fish were recorded using an infrared (IR) adapted GoPro Camera and IR illuminators. The first 10 minutes of each trial was used as an acclimation period for the fish and was not included in the analyses.

To determine flounder orientation during experiments, we first assessed baseline swimming behavior of flounder under normal conditions. We observed that while flounder were typically inactive for prolonged periods of time (common for most flatfish), fish would exhibit intermittent periods of swimming during which they moved up the sides of the arenas. These “active swimming periods” were used as the behavioral assay for both our map and compass experiments. Similar approaches confining analysis to periods of increased animal activity have also been used in sea turtles and birds (Wiltschko and Wiltschko, 1995).

During the onset of each active swimming period, the direction the fish's head was pointing relative to magnetic north was recorded. For each fish we took angles during their first 10 active swimming periods only, since we observed that these movements were representative of the trial as a whole. Fish that failed to move, or fish movements that did not result in the fish leaving the bottom of the arena, were not recorded.

All angles were recorded using Image-J (version 1.44) and were analyzed by observers blind to the experimental treatments. A mean angle for each fish was calculated using the statistical software Oriana V.4 (Kovach Computing). Rayleigh tests were used to assess whether each group of fishes was significantly oriented. Comparisons between groups were done with Mardia-Watson-Wheeler tests. Post hoc analyses of temporal variables such as tidal cycle and year were conducted to confirm that the observed differences among experimental treatments did not result from non-magnetic conditions. Neither tidal cycle nor year had an effect on flounder orientation in our map and compass experiments (Figures A1 and A2).

Figures



Figure 3.1 A juvenile Gulf flounder (*Paralichthys albiguttata*), camouflaged to the sediment.

A peculiarity of flounder is that, during metamorphosis from pelagic to benthic life, one eye migrates 180 degrees so that both eyes come to be located on the left side of the head.

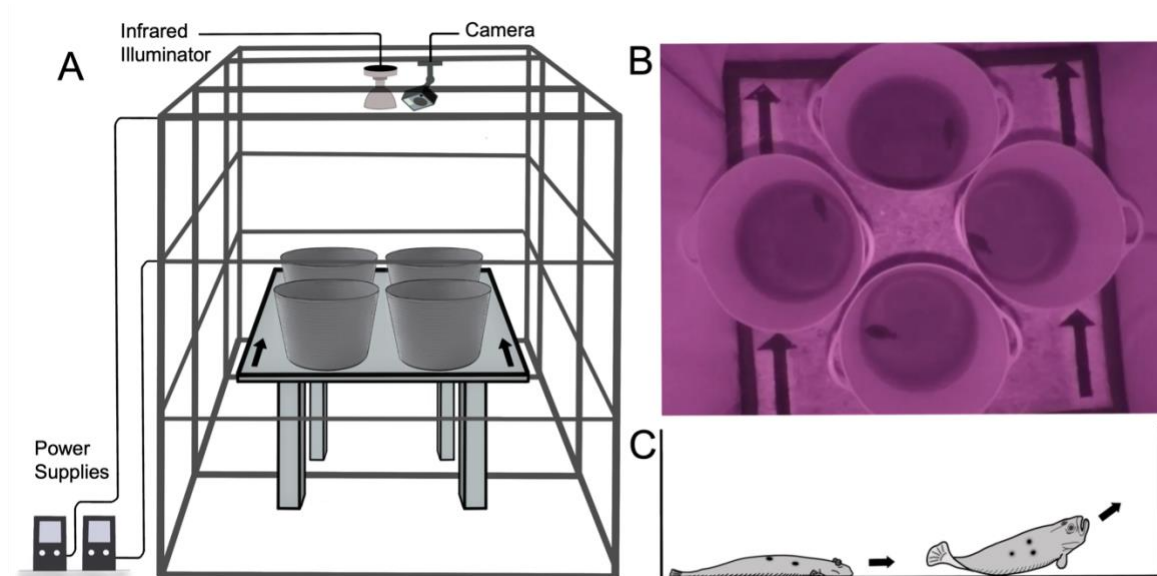


Figure 3.2 Experimental apparatus used to monitor orientation responses of juvenile flounder to magnetic fields.

(A) Two Merritt 4-coil systems were constructed and arranged orthogonally around a center platform which housed four orientation arenas. This center platform was encompassed by two layers of 100% blackout material (not pictured). (B) Sample view inside the enclosed platform showing flounder viewed under an infrared camera. Arrows indicate direction of magnetic north. (C) A diagram of flounder swimming behavior used in our analysis.

Following periods of rest on the bottom of the arena, fish actively swim up the sides of the arenas before settling down again.

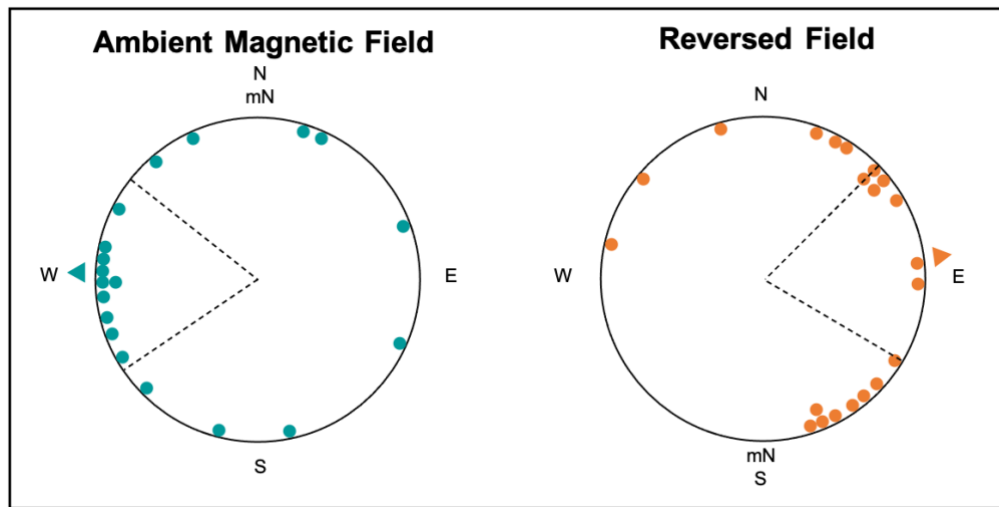


Figure 3.3 Evidence for a magnetic compass in flounder.

(A) Circular graph showing orientation of flounder in the ambient, control field (mean heading = 273° ; Rayleigh test: $n = 19$, $r = 0.48$, $p < 0.05$). Dots at the periphery of the circles mark the mean headings of individual fish, the arrow heads represent the mean heading of each group, “mN” denotes the direction of magnetic north, and the dotted lines represent the 95% confidence interval. (B) Circular graph showing orientation of flounder in the reversed magnetic field (mean heading = 83° ; Rayleigh test: $n = 21$, $r = 0.45$, $p < 0.05$; conventions as in (A)).

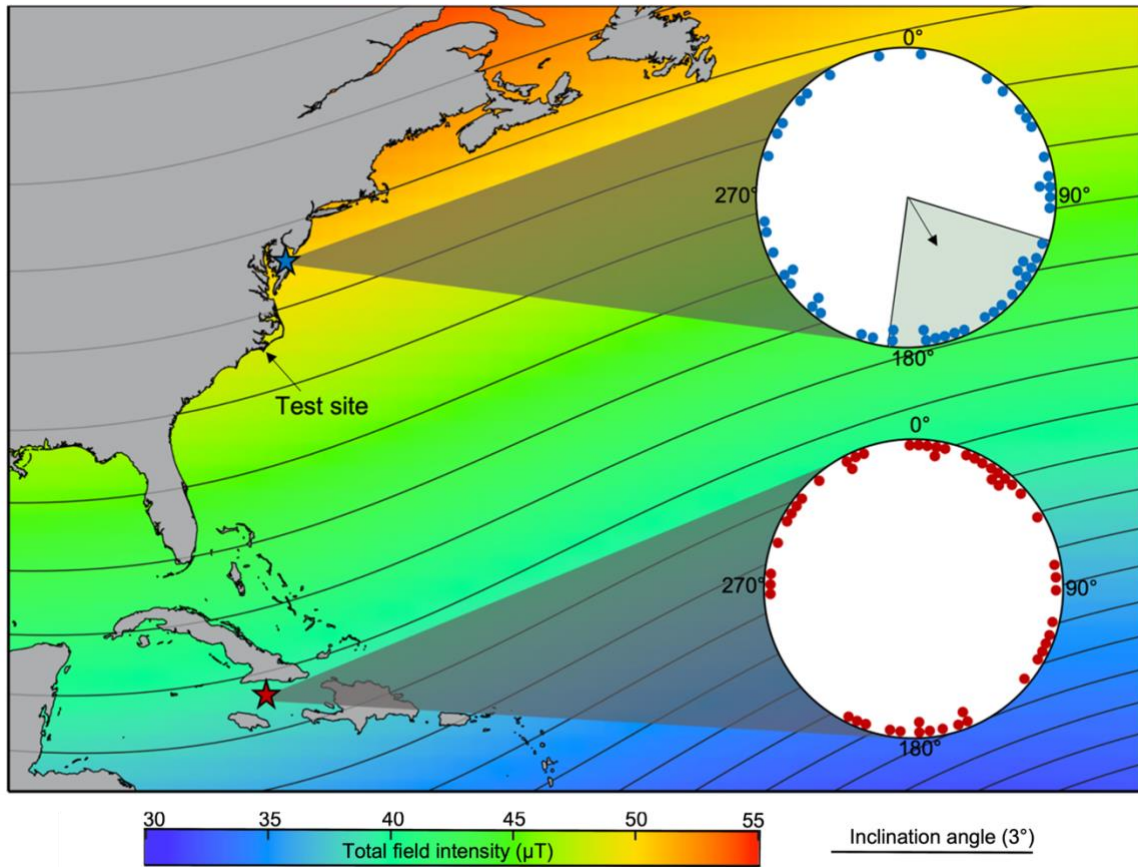


Figure 3.4 Evidence for a magnetic map in flounder.

Circular histograms show the orientation of flounder exposed to magnetic field locations that exist north and south of the test site. Each dot on the circular diagrams represents the orientation of a single fish, tested at night, and only once. Arrow heads indicate the mean direction of the group, grey shaded areas the 95% confidence interval for the mean. Blue star and associated histogram indicated the magnetic displacement location and orientation of fish exposed to this northern magnetic field (mean heading = 147° ; Rayleigh test: $n = 49$, $r = 0.28$, $p = 0.02$). Red star and associated histogram indicated the magnetic displacement location and orientation of fish exposed to the southern magnetic field (mean heading = 39° ; Rayleigh test: $n = 51$, $r = 0.19$, $p > 0.05$). The intensity of the total magnetic field is represented by the color bar, and the inclination by 3-degree contours.

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CHAPTER 4: FUNCTIONAL PROPERTIES OF THE FLOUNDER MAGNETIC COMPASS SENSE

Summary

Previous experiments have demonstrated that flounder have a magnetic compass sense, but the functional properties of the magnetic compass have not yet been investigated. In all animal species studied so far, the magnetic compass has fit into one of two categories: a polarity compass that detects the polarity of the earth's field in much the same way that a human hand-held compass does, or an inclination (or axial) compass that does not detect polarity, but instead defines "poleward" as the direction along the earth's surface in which the angle formed between the total field vector and the gravity vector is smallest. To investigate the functional properties of the flounder magnetic compass, we tested the orientation of fish in three magnetic field conditions that have been used to distinguish between polarity and inclination compasses in other taxa: (1) the unaltered local magnetic field; (2) a reversal of the vertical component of earth's field; (3) a reversal of the horizontal and vertical components of earth's field. Consistent with prior experiments on flounder, fish exhibited a strong westward compass response in the local magnetic field. In contrast, orientation of fish tested in the two diagnostic fields was random and did not conform to the expected behavior of an animal with the functional properties of either an inclination or polarity compass. This outcome implies that the magnetic compass of flounder has characteristics that differ from those of magnetic compasses described previously in other animals.

Introduction

Growing evidence has demonstrated that diverse fishes can orient using Earth's magnetic field (Naisbett-Jones and Lohmann 2022). Findings with salmon show that young fish possess a magnetic compass sense that is used to find appropriate growth habitats, and a magnetic map sense that aids fish from straying into unfavourable ocean areas (Putman et al. 2014; Putman et al. 2020). Similarly, coral reef fish appear to use a magnetic compass sense to orient towards their natal reefs (Bottesch et al. 2016; O'Connor and Muheim 2017), and evidence in bonnethead sharks suggests that fish use a magnetic map in homing (Keller et al. 2021). Recent lab studies have added the Gulf flounder (*Paralichthys albigutta*) to this list of migrants by illustrating that flounder possess a magnetic map and compass sense (Chapter 3).

Although many fishes are capable of orienting to Earth's magnetic field, the functional characteristics of their magnetic compass sense has received little investigation. Evidence has been reported for two main functional types of magnetic compasses. Birds, sea turtles, and some insects appear to use an inclination compass (Wiltschko and Wiltschko 1972; Beason 1989; Light et al. 1993; Vácha et al. 2008). In the case of an inclination compass, an animal does not distinguish the polarity of field lines, but instead defines "poleward" as the direction along the earth's surface in which the angle formed between the total field vector and the gravity vector is smallest (Wiltschko et al. 1993). Thus, for an animal with an inclination compass, inverting the vertical component of the local magnetic field elicits the same behavioral effect as reversing the horizontal component (Wiltschko and Wiltschko 2005).

In contrast, polarity compasses, which are present in salmon, lobsters, and mole rats, determine north using the polarity of the horizontal field component, in much the same way that a human technical compass does (Quinn et al. 1981; Lohmann et al. 1995; Marhold et al. 1997). For animals with a polarity compass, inverting the vertical component of the local magnetic field does not alter the direction a polarity compass perceives as north, but changing

the direction of the horizontal field component does (Wiltschko and Wiltschko 2005).

Interestingly, some amphibians have been shown to possess both types of functional compasses, which appear to be used to guide different behavioral tasks (Phillips 1986; Johnsen and Lohmann 2005).

Whereas the functional characteristics of magnetic compasses in several other vertebrate groups have received considerable attention, comparable investigations with fishes are lacking. A single study provides some evidence for a polarity compass in salmon (Quinn et al. 1981), but whether this is a universal feature in fishes remains to be determined. In the present study, we investigated the functional characteristics of the magnetic compass in the flounder. Specifically, flounder were exposed to a magnetic field with an inverted vertical component, and also to a field with horizontal and vertical components both inverted. Traditionally these two magnetic field conditions have led to opposing orientation of animals that rely on inclination and polarity compasses (Figure 4.1), and thus these conditions have been used as a behavioral diagnostic test for the functional characteristics of the magnetic compass (Wiltschko and Wiltschko 2005). Consistent with a previous investigation, we report that flounder in the local magnetic field oriented approximately west (Chapter 3). This orientation became random in the two test fields. These results differ from results obtained in other animals tested in similar magnetic fields and imply that flounder possess a compass that differs in its functional characteristics from magnetic compasses previously described in other animals, including salmon.

Materials & Methods

Fish collection and housing

All animal care and procedures were approved by the Institutional Animal Care and Use Committee of the University of North Carolina (approval number 17-189). Juvenile Gulf flounder (*Paralichthys albiguttata*) were obtained by trawling seagrass beds in Back Sound, North Carolina, USA (34.68361° N, 76.5625° W). All fish were collected between May–August of 2019. Following capture, fish were transported to non-magnetic holding tanks located at the University of North Carolina’s Institute of Marine Science (UNC-IMS; 34.7234° N, 76.7522° W). Tanks received a constant supply of fresh seawater from the adjacent Bogue Sound. Tanks were covered with shade cloth to maintain suitable temperatures (range: 22-29 °C) and oxygen levels (~8.0 Mg/L). Flounder were fed a mixed diet of fish and shrimp daily.

Magnetic coil and magnetic fields

An outdoor magnetic coil system was used for the experiments (Figure 4.2). The coil system consisted of two independent four-coil systems arranged orthogonally (Merritt et al. 1983). Both coils were controlled with a separate power supply (BK Precision Model 1550 DC) operating at constant current. The coil systems were used to produce two magnetic field treatments, identical to those used to investigate the functional properties of the magnetic compass in other animals (Wiltschko and Wiltschko 1972; Light et al. 1993; Wang et al. 2007). Importantly, these two magnetic field treatments varied in the polarity of the horizontal and/or vertical components but all fields had a total field strength and inclination that matched the local magnetic field (total intensity: 48.7 μ T, inclination: 61.7°).

In the first treatment, the Merritt coil was used to invert the vertical component of the local magnetic field (total intensity: 48.7 μ T, inclination: -61.7°). In the second magnetic field treatment, both the vertical and horizontal components were reversed (total intensity: 48.7 μ T, inclination: -61.7°). Each of these magnetic fields was verified using a recently calibrated FVM400 vector magnetometer. Measurements of the local magnetic field closely matched local magnetic field parameters reported by the International Geomagnetic Reference Field (IGRF) model (Finlay et al. 2010). Magnetic field uniformity across the center platform of the coil (Fig 4.2) was ± 0.1 μ T and $\pm 0.2^\circ$ for total field intensity and field inclination, respectively.

Orientation arenas and data acquisition

Magnetic compass experiments followed procedures previously outlined in Chapter 3. Briefly, four plastic orientation arenas were placed on a level platform in the center of the coil system. This platform was enclosed on all sides with two layers of 100% black-out material, removing all external visual cues. Each circular orientation arena was identical and measured 32 cm in diameter and 45 cm in height. Prior to the start of the experiments, each orientation arena was filled with fresh sea water to a depth of 20 cm. Next, we used the magnetic coil system to produce one of the three randomly chosen magnetic field treatments: (1) the local magnetic field; (2) a field with an inverted vertical component; and (3) a field with inverted vertical and horizontal components (Figure 4.2). One juvenile flounder was then placed into each of the four orientation arenas and was recorded for a total of two hours. All experiments were conducted at night between 21:00 and 03:00, a time when juvenile flounder are thought to be most active (Miyazaki et al. 1997). Fish were recorded using an

infrared (IR) adapted GoPro Camera and IR illuminators. A total of 70 fish were tested among the three experimental treatments.

Behavioral and data analysis

Fish were given a 10-minute acclimation period at the start of each experiment. Following acclimation we analysed fish behavior during periods of active swimming in the arena (behavioral assay described in detail in Chapter 3). These active swimming periods were recorded by measuring the direction in which the fish's head was pointing (relative to magnetic north) at the onset of movement. For each fish we took angles during their first 10 active swimming periods only, since we observed that these movements were representative of the trial as a whole. A mean angle for each fish was calculated based on the active swimming periods recorded during the experiment. Fish that failed to move, or fish movements that did not result in the fish leaving the bottom of the arena were not recorded.

All analyses of recorded experiments were carried out by observers blind to the experimental treatments. All angles were recorded using Image-J (version 1.44). Rayleigh tests were used to determine whether each treatment group was significantly oriented. The nonparametric Mardia-Watson-Wheeler test was used to determine whether differences in orientation existed among groups of fish tested in the three magnetic field conditions. Random distributions were further explored for axial bimodality using Rayleigh tests, as previously described (Beason et al. 1995; Deutschlander et al. 2003).

Post hoc analyses of temporal variables such as tidal cycle and year were conducted to confirm that the observed differences among experimental treatments did not result from non-magnetic conditions. Neither tidal cycle nor year had an effect on flounder orientation. Data generated in this experiment was compared to previously collected data (Chapter 3) investigating the response of flounder to a magnetic field in which the horizontal component

was inverted (Figure 4.3). We used the statistical software Oriana V.4 (Kovach Computing) for analyses and to generate graphics.

Results

Under local magnetic field conditions fish were significantly oriented with a mean angle of 245 degrees (Rayleigh test, $n=22$, $r=0.52$, $Z=6.0$, $P<0.01$; Figure. 4.4). In contrast, fish tested under conditions in which the vertical component of the geomagnetic field was inverted were not oriented (Rayleigh test, $n=26$, $r=0.21$, $Z=1.1$, $P=0.3$; Figure. 4.4). Similarly, fish tested in a magnetic field in which the vertical and horizontal components of the geomagnetic field were inverted had orientation indistinguishable from random (Rayleigh test, $n=22$, $r=0.14$, $Z=0.4$, $P=0.7$; Figure. 4.4). No significant bimodal orientations were identified ($Z<1.1$, $r<0.23$, $p>0.33$, in all cases).

The orientation of fish tested in the local magnetic field differed significantly from fish tested in a field in which the vertical and horizontal components were inverted (Mardia-Watson-Wheeler test, $W=6.49$, $P<0.05$; Figure. 4.4). Likewise, orientation in the local field differed significantly from orientation of fish tested in the inverted vertical magnetic field (Mardia-Watson-Wheeler test, $W=6.54$, $P<0.05$; Figure. 4). No significant differences between the orientation of fish in the inverted vertical and the inverted vertical/horizontal magnetic field was observed (Mardia-Watson-Wheeler test, $W=0.43$, $P=0.8$; Figure 4.4).

Discussion

To investigate the functional properties of the magnetic compass of flounder, fish were exposed to the local magnetic field, as well as to two additional magnetic fields that have been used previously with other animals to differentiate between magnetic compasses based on polarity and those based on field inclination. As in previous studies (Chapter 3),

flounder tested in the local magnetic field oriented nonrandomly toward the west (Figure. 4.4), a direction that may lead fish toward inshore habitats. By contrast, fish tested in a magnetic field in which the vertical component was reversed had orientation that was indistinguishable from random (Figure. 4.4). Similarly, flounder tested in a magnetic field with the vertical and horizontal components both reversed had orientation indistinguishable from random (Figure. 4.4). These results were unexpected, inasmuch as they did not conform to predictions for an animal with either a polarity or an inclination compass. Instead, the results suggest that the flounder compass differs in its functional properties from the magnetic compasses that have previously been described in other animals.

Comparison to evidence in other animals

Behavioural evidence in other taxa suggests that most animals rely on one of two functional types of magnetic compass to orient. Birds, sea turtles, and some insects appear to have inclination or axial compasses that are not sensitive to the polarity of the field but instead determine the ‘poleward’ direction on the basis of angles formed between the magnetic and gravity vectors (Wiltschko and Wiltschko 1972; Light et al. 1993; Goff et al., 1998; Vácha et al. 2008). For animals with an inclination compass, inverting the vertical component of the magnetic field has the same effect as reversing the horizontal component of the field, but reversing the vertical and horizontal components together has no effect.

By contrast, lobsters, mole rats, and bats have magnetic compasses that detect the polarity of the field and, in particular, the polarity of the horizontal field component (Lohmann et al. 1995; Marhold et al. 1997; Wang et al. 2007). For animals with a polarity compass, inverting the vertical component of the magnetic field has no effect on orientation behavior, but reversing the vertical and horizontal fields together leads to a reversal in orientation direction.

The results with the flounder differed from those obtained previously in other animals. Both of the manipulated magnetic fields – the one with the inverted vertical component and the field with the horizontal and vertical components both reversed – resulted in random orientation instead of either orientation identical to that of controls or orientation reversed in direction from that of controls.

Until now, investigation into the functionality of the compass sense in fishes has been limited to one species of salmon. Quinn (1980) reported that the orientation of young sockeye salmon changed with a 90-degree shift in the horizontal component, thus demonstrating that salmon possess a magnetic compass sense. In a follow up experiment, a different group of fish were tested in a local magnetic field and a magnetic field in which the vertical component was inverted (Quinn et al. 1981). Because fish tested under the two magnetic fields oriented in a direction similar to that observed in previous experiments, the results suggest that salmon have a polarity compass. However, additional experimental conditions used in other taxa, such as magnetic fields with both inverted vertical and horizontal components were not used in these early experiments. Given that a field with an inverted vertical component disrupted the orientation of flounder (Figure. 4.4) but had no apparent effect on salmon (Quinn et al. 1981), the new findings suggest that the magnetic compasses of different teleost fishes may differ in their functional characteristics.

Why might the flounder compass differ?

Why the magnetic compass of flounder differs from the magnetic compasses of other animals is not known, but several explanations are plausible. One possibility is that flounder possess both an inclination and a polarity compass that are normally used for different navigational tasks. If so, then fish exposed to our treatment fields might have received conflicting compass cues that led to the random orientation we observed (Figure 4.4). There

is a precedent for an animal possessing more than one type of magnetic compass. Red-spotted newts (*Notophthalmus viridescens*), when orienting in a magnetic direction that they had learned to associate with land, reversed their direction of orientation in a magnetic field with a reversed vertical component, implying they use an inclination compass to perform this simple task (Phillips 1986). In contrast, newts actively homing were unaffected by the same treatment, but did respond to changes to the horizontal component of the field, a finding consistent with a polarity compass that may in some way be linked to the map sense (Phillips 1986). In our experiments, fish were of the same age and were tested in the same navigational context. Thus, it seems unlikely that the random orientation we observed resulted from individuals performing different behavioral tasks, although we cannot rule out this possibility with certainty. Perhaps more plausible is that flounder use both inclination and polarity compasses in conjunction with one another and became confused by the conflicting cues given to them in our experiments. Further studies will be needed to confirm or refute this hypothesis.

Another possibility is that flounder possess magnetoreceptors that differ from those of other animals. How flounder detect magnetic fields is not known, but magnetite has been detected in a number of fishes, and experiments with magnetic pulses suggest that this material might provide the physical basis for magnetoreception in fishes (Kirschvink et al. 1985; Walker et al. 1988; Naisbett-Jones et al. 2020). A number of theoretical models have been put forward for how a magnetite-based receptor might function in animals (Johnsen and Lohmann 2005). Although they differ in a number of respects, each model postulates that magnetite crystals are not firmly bound in place, but instead should to some degree be able to move in response to changes to the ambient magnetic field (Kirschvink and Gould 1981; Walker 2008; Winklhofer and Kirschvink 2010; Lohmann 2016). An interesting speculation is that inverting the vertical component of the geomagnetic field moves these crystals into a

configuration that causes flounder to perceive compass information incorrectly. Future investigation into the mechanism(s) underlying the flounder's compass sense are needed to better elucidate this possibility.

Flounder are known to possess a magnetic map sense, and it is also hypothetically possible that changes to the vertical component of Earth's magnetic field resulted in positional information that confused fish in our experiments (Chapter 3). However, we consider this unlikely for two main reasons. First, our magnetic field treatments differed in their horizontal and/or vertical components but not in total field strength. Second, no effects of this treatment on the map sense have been reported in similar experiments with other animals, including those known to possess a magnetic map (Light et al. 1993; Lohmann et al. 1995).

Mechanisms of magnetic field detection

Although evidence for magnetoreception has been accumulating, magnetic receptors have not been unequivocally identified in any organism. Nonetheless, three main magnetoreception transduction mechanisms have been proposed (Johnsen and Lohmann 2005; Nordmann et al. 2017). The first involves crystals of biogenic magnetite coupled to mechanoreceptors (Kirschvink et al. 2001). The second proposes that electrically-sensitive animals such as elasmobranchs detect magnetic fields via electromagnetic induction (Kalmijn 1973), and/or that some birds detect magnetic fields with an induction-based mechanism located in the inner ear (e.g. Nimpf et al. 2019). The third hypothesis proposes a complex series of biochemical reactions that are modulated by earth-strength magnetic fields (Hore and Mouritsen 2016). While some evidence exists for a magnetite-based mechanism in fishes (Naisbett-Jones and Lohmann 2022; Naisbett-Jones et al. 2020), the putative magnetoreceptors have not yet been identified with certainty. As a consequence, whether

polarity or inclination compasses rely on different transduction mechanism(s) has similarly not been determined (Johnsen and Lohmann 2005).

Some authors have suggested that the functional differences between polarity and inclination compasses may reflect properties intrinsic to the different underlying receptors (Lohmann et al. 1995). At the same time, these differences in compass responses may also arise as a result of higher-order neural processing (Johnsen and Lohmann 2005). In light of these complications, it is not possible to determine whether the flounder compass is based on a single receptor type with characteristics of both inclination and polarity compasses, whether flounder possess two independent sets of receptors (one that utilizes the polarity of the field and the other field inclination), or whether the receptors in flounder differ entirely from those that exist in other organisms.

Regardless of the underlying mechanism, fish exposed to a reversal of the vertical or the vertical and horizontal components together had significantly different orientation relative to that of our control group. To our knowledge, flounder represent the first animal with a magnetic compass in which the functional properties of the compass do not fall into the binary categorization of polarity or inclination compasses. Future work will be needed to determine if these characteristics are unique to flounder and to definitively characterize the mechanism(s) that underly the compass sense in flounder and other animals.

Figures

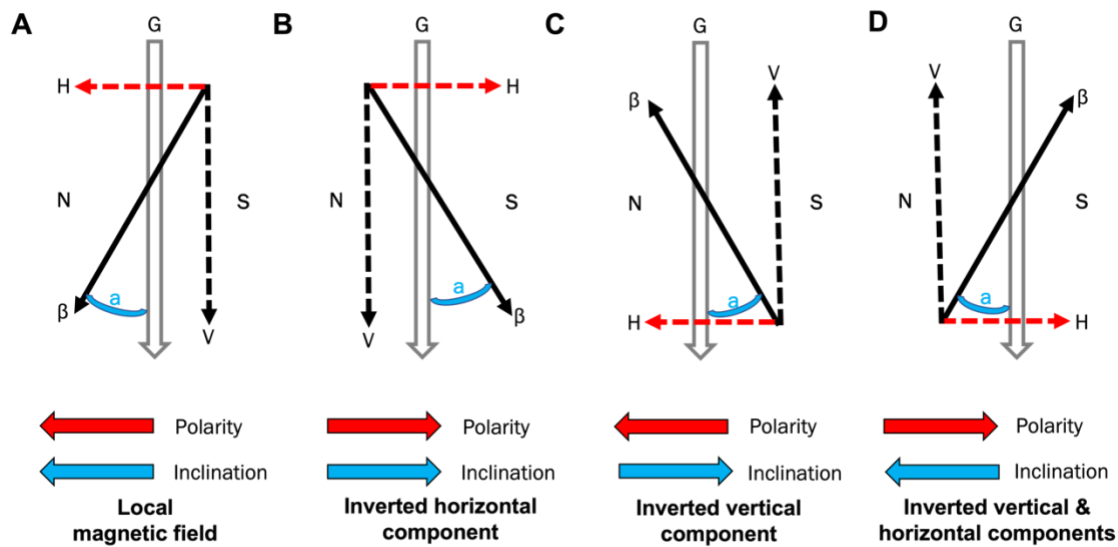


Figure 4.1 Diagrams of Earth's magnetic field in the northern hemisphere showing comparison of behavioral responses governed by an inclination compass and a polarity compass.

Geographic north and south are denoted by 'N' and 'S', respectively. 'G' gravity; 'H' horizontal magnetic field component; 'V' vertical magnetic field component; 'β' resultant magnetic field vector; 'a' the direction in which the magnetic field lines form the smallest angle relative to the force of gravity (as defined by an inclination compass). Shown are four magnetic field conditions: (A) the local magnetic field (total intensity: $48.8 \mu\text{T}$, inclination: 61.7°). (B) a magnetic field with an inverted horizontal component (total intensity: $48.8 \mu\text{T}$, inclination: 61.7°) as used in previous experiments. (C) a magnetic field with an inverted vertical component (total intensity: $48.8 \mu\text{T}$, inclination: -61.7°). (D) a magnetic field with inverted horizontal and vertical components (total intensity: $48.8 \mu\text{T}$, inclination: -61.7°). Red and blue arrows indicate the expected direction an animal with a polarity or inclination compass would adopt, respectively.

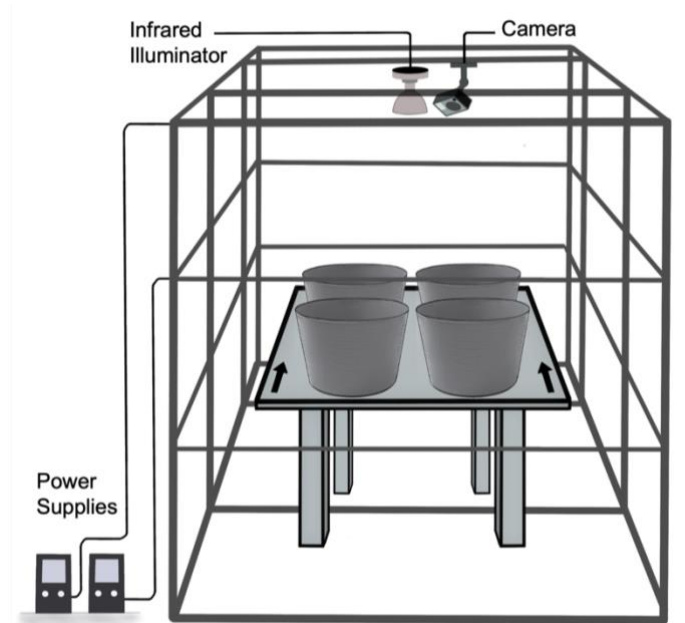


Figure 4.2 Experimental apparatus used to monitor orientation responses of juvenile flounder to magnetic fields.

A series of Merritt coils were constructed around a center platform which housed four circular arenas (Merritt et al. 1983). This center platform was enclosed by two layers of 100% blackout material (not pictured). Fish were placed into one of the four circular arenas inside the magnetic coil system and their behavior was recorded using an infrared camera and illuminator.

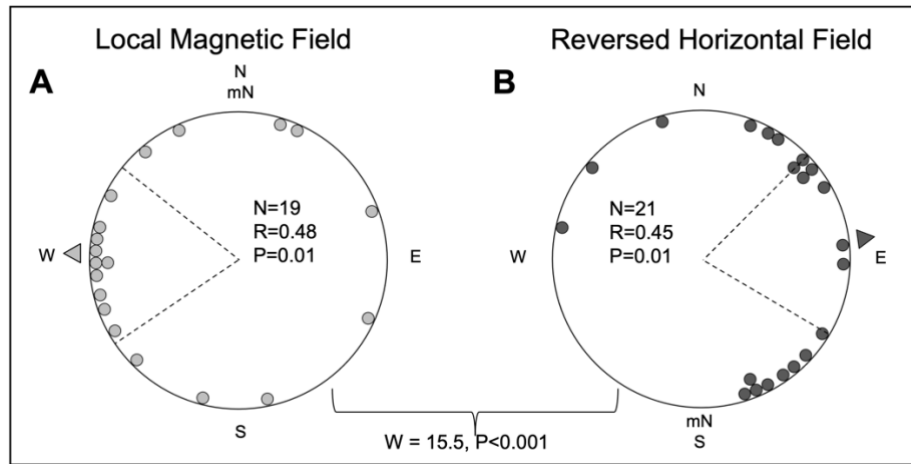


Figure 4.3 Previously reported magnetic orientation of flounder.

(A) the local magnetic field; (B) a field in which the horizontal component was inverted with a magnetic coil system (Chapter 3).

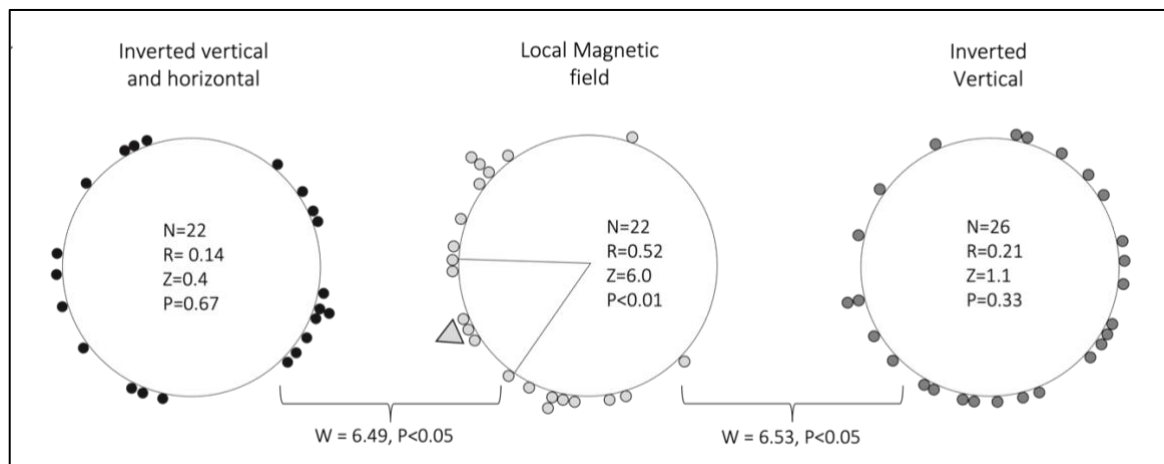


Figure 4.4 Orientation of flounder in three different magnetic fields.

(A) In a field in which the vertical and horizontal components were inverted with a magnetic coil system, fish were not oriented as a group (Rayleigh test, $n=22$, $r=0.14$, $Z=0.4$, $P=0.6$).

(B) In the local magnetic field, fish were significantly oriented with a mean angle of 245 deg (Rayleigh test, $n=22$, $r=0.52$, $Z=6.0$, $P<0.01$).

(C) In a field in which the vertical component alone was inverted, fish were not oriented as a group (Rayleigh test, $n=26$, $r=0.21$, $Z=1.1$, $P=0.3$). Each data point represents the mean angle of an individual fish. Arrow heads indicate the mean direction of the group. Lines represent the 95% confidence interval for the mean and brackets indicate significant Mardia-Watson-Wheeler pairwise comparisons.

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CHAPTER 5: A METHOD FOR ATTACHING SATELLITE TAGS TO SMALL MIGRATORY FISHES

Summary

Achieving long-term retention of pop-off satellite archival tags (PSATs) has proven difficult for all fishes but is particularly challenging for small migrants due to the large size of tags. In this study we tested the latest and smallest PSAT model on the market, the mark-report satellite tag (mrPAT), and developed a simple, cost-effective method of tag attachment on sheepshead (*Archosargus probatocephalus*) a small marine fish. During laboratory tests, our method of tag attachment outperformed existing methods with two ~40 cm fish maintaining their tags for three months (the duration of the study). During field deployments on 25 fish (37-50 cm fork length), data were successfully obtained for 17 of the 25 tagged fish. Of these, 14 tags (82%) remained on the fish until the pre-programmed release date resulting in tag retention times of up to 172 days (mean: 140 days). On average, tags collected daily temperature data for 67 days (SD=41.2) or during 45% of the deployment duration. Our investigation represents the first extensive study into the feasibility of PSATs for monitoring fishes in this size range. We demonstrate that our method of attachment, and this latest PSAT model, are feasible for ~5-month deployments on fishes that are less than 50 cm fork length. These results represent a significant advance in PSAT methodology. The method is likely applicable for a wide variety of fishes.

Introduction

Determining the movement patterns of fishes is a central component of fisheries conservation and management, and a key step toward a more complete understanding of species ecology (Secor 2015). Traditionally, empirical movement data has been acquired from fishing effort and conventional tagging techniques such as mark-recapture experiments (Thorstad et al. 2014). While these methods are valuable research tools, they fail to identify the full extent of fish movements. Moreover, because they require the recapture of tagged individuals, data are oftentimes biased to areas where fishing pressure (or researcher presence) are highest (Thorstad et al. 2014). The recent development of pop-off satellite archival tags (hereafter PSATs) has provided researchers with a fisheries-independent solution to examining the movement patterns and habitat use of fishes and other marine animals (Block et al. 1998; Swimmer et al. 2014). These external archival tags record data on environmental parameters; later, at a pre-programmed date, the tags detach from the animal, float to the surface, and transmit a summary of the stored data (including the final pop-off location) to ARGOS satellites orbiting overhead. Since tags do not need to be physically recovered, this approach is, to a large extent, fisheries-independent (Musyl et al. 2011).

Over the last few decades PSATs have been used to elucidate the movement patterns of many large pelagic fishes such as bluefin tuna (Block et al. 1998; Aranda et al. 2013), great white sharks (Bonfil 2005), swordfish (Braun et al. 2019), and ocean sunfish (Sims et al. 2009). While PSATs have advanced our understanding of the movements of many such migrants, the size of the first generation PSATs has largely prevented their use on many smaller fishes. Recent advances in PSAT technology have led to miniaturization of tags that permits their use on a wider range of fishes (Amilhat et al. 2016; O'Neill et al. 2018), but studies in which these tags have been used to track smaller fishes (<50 cm) remain sparse. One difficulty is that long-term attachment of satellite tags to smaller fishes has often been

problematic. For instance, in one of the only studies to use PSATs on small marine fishes (fork length ~44 cm), 7 out of 8 tags (88%) programmed for 3-12 month deployments surfaced within just 14 days (Rodgveller et al. 2017). One tag, however, did remain on an individual for 190 days, illustrating that long-term retention of PSATs on small fishes is possible under the right circumstances (Rodgveller et al. 2017).

In this study, we tested the latest development in PSAT technology, the mark-report satellite tag (mrPAT; Wildlife Computers Ltd, Redmond, Seattle; 2018). The mrPAT is currently the smallest PSAT on the market (~30% smaller) and the most cost-effective tag available in the PSAT suite (~70% cheaper). To date, only a few studies have tested the mrPAT and these have focused primarily on large to mid-sized fishes such as Greenland sharks (*Somniosus microcephalus*; mean fork length: 256 cm) (Hussey et al. 2018), Atlantic sturgeon (*Acipenser oxyrinchus*; mean fork length: 150 cm) (Hylton et al. 2018), and cobia (*Rachycentron canadum*; mean total length: 106 cm) (Jensen and Graves 2020). These studies have demonstrated the utility of mrPATs for fishes >100 cm, at least for relatively short tracking durations (40-80 days). Whether the smaller size of the mrPATs makes them suitable for smaller fishes, as well as for longer-term monitoring, has not yet been investigated.

Here, we evaluated the use of mrPATs on a small marine fish, the sheepshead (*Archosargus probatocephalus*). The sheepshead is an abundant species widely distributed from Nova Scotia to Brazil (Kells and Carpenter 2011). Sheepshead are primarily found in estuarine and coastal locations during warmer months and typically migrate offshore during cooler months for spawning (McDonough et al. 2011). Given their seasonal migrations, small size, and morphological similarities to many other perciform fishes, sheepshead make an ideal candidate for testing the efficacy of PSATs for small migrants. Additionally, due to their unique feeding ecology – grazing on sessile organisms – sheepshead have an affinity for

underwater structure, providing an opportunity to evaluate tag retention in settings that pose an unusually high risk of underwater tag entanglement, a common reason for early tag release (Økland et al. 2013).

Our objective was to develop a method for long-term attachment of PSATs to small marine fishes, as well as to test the performance of the newest mrPATs under field conditions. First, we developed a tag attachment method for sheepshead and compared our method to two attachment methods previously used on other fishes under laboratory conditions. Second, we determined the tag retention of mrPATs deployed on 25 fish in the field, as well as assessed the reliability of mrPATs for providing location and temperature data. To our knowledge, this study represents the first extensive investigation into the utility of these newly available mrPATs for monitoring small marine fishes.

Materials and Methods

Animal care

Animal collection was conducted under a scientific collection permit (#729210) granted by the North Carolina Division of Marine Fisheries. All animal care and procedures were approved by the Institutional Animal Care and Use Committee of the University of North Carolina (approval number #20-061.0).

Fish collection

All sheepshead used in the laboratory-based tag retention studies were collected in July, 2020. Fish used during the field investigations were collected between October and November, 2020. All individuals were obtained from inshore waters surrounding Morehead City, North Carolina (34.72 N, -76.72 W). The majority of fish (n=30) were caught using

conventional hook and line techniques, with collection taking place near hard structures (e.g. sea walls, docks, oyster reefs) where fish congregate. This approach is commonly used across most of the southeastern United States to target sheepshead (Schwartz 1990; Dutka-Gianelli and Murie 2001). Terminal tackle consisted of a Carolina rig constructed from 30 lb. fluorocarbon, a 1-2 oz egg sinker, and a single Owner Mosquito hook (Size: 1/0 - 5/0). Bait consisted of either a live fiddler crab (*Uca* sp.), mud crab (*Panopeus obesus*), or sea urchin (*Arabacia punctulata*). One additional fish was acquired from a local pound net fishery at Harker's Island, NC (Lat: 34.712, Lon: -76.481). All fish captured were carefully netted, measured (fork and total length in cm) and closely inspected. Only fish that were lightly hooked and in good body condition were used for subsequent satellite tagging.

Mark-report pop-off archival tag

The PSAT model used in this study was the newly developed mark-report pop-off archival tag (mrPAT; Wildlife Computers, Redmond, WA). The mrPAT is currently the smallest available PSAT on the market (127 x 28 mm, weight: 40g in air), and is presently the most cost-effective tag in the PSAT suite (~\$1200-1500 a tag). The mrPAT's internal battery provides ballast, offering stability while the tag is under tow and good keeling while floating and transmitting. During deployment, the mrPAT stores daily temperature (accuracy: $\pm 0.1^{\circ}\text{C}$) and tag orientation or "tilt" data (accuracy $\pm 2^{\circ}$). Both temperature and tilt are recorded every 10 minutes during each UTC Day. Tags report the daily temperature range (min and max values) and a single average tilt value to ARGOS satellites.

The mrPAT release mechanism involves a standard burn pin that separates the buoyant tag from its tether, allowing it to float to the surface. This tag release mechanism is initiated at a user-defined time and takes up to 12 hours to release from the tether depending

on external conditions. After releasing from the fish, the tag then floats to the surface and transmits a summary of the stored data and the final pop-off location to ARGOS satellites.

The mrPAT also possesses an auto-detect detachment feature that is designed to recognize instances in which tags surface prematurely (e.g. before the user-defined pop-off date). This auto-detachment feature is based on a wet/dry conductivity sensory which initiates tag release when the tag is 5% dry (e.g. at the surface or in low-salinity water). In this study, this mechanism was set so that the tag would release from the fish if the sensor determined it was dry for a six-hour period. This six-hour window encompassed the transition between flow and ebb tides and was designed to minimize premature release from fish that might reside in shallow or low-salinity waters for short periods.

Tag retention study in the laboratory

As a first step toward investigating the efficacy of mrPATs for sheepshead and other small fishes, we compared different tag attachment methods on fish housed in captivity. All fish used during our retention study were kept in indoor tanks (300 gallon) at the Institute of Marine Sciences (34.72 N, -76.75 W) in Morehead City, North Carolina, U.S.A. Tanks received a constant flow of fresh sea water from the adjacent Bogue Sound. Salinities varied with ambient conditions in the Sound (range: 30-37 ppm) and chillers were used to maintain water temperatures below 25 °C. Fish were fed a mixed diet of frozen crustaceans *ad libitum* and were given a one-week acclimation period prior to tagging.

For the tag retention study, we used non-functional mrPAT dummy tags that were identical in size and weight to functioning tags. Three attachment methods were chosen for our investigations: (1) a method in which a loop of monofilament line (250 lb) was threaded through the dorsal musculature and crimped to the PSAT (See Rodgveller et al., 2017 for details); (2) a method that consisted of attaching two ridged plastic plates on either side of the

dorsal fin with stainless steel wire (Adapted from Økland et al., 2013), and; (3) a method in which a loop of spaghetti tag material (Floy Tag and Manufacturing, Inc. Seattle, WA) was used for attachment to the dorsal musculature.

The third attachment method involved first inserting a 40-cm length of spaghetti tag (model: FT-4, Material: vinyl tubing) through the anterior portion of the dorsal musculature using a stainless-steel needle with a diameter equal to that of the spaghetti tag. The anterior portion of the dorsal fin was chosen because this is where the pterygiophores (and associated membranes) are most pronounced, thus reducing the likelihood of the tag pulling free (Fig 5.1a). The needle was then passed back through the musculature 4-6 cm posterior to the initial puncture location. This spacing helped minimize mechanical contact between the tag loop and the skin (Fig 5.1b). Lastly, the mrPAT tag was threaded onto the anterior end of the spaghetti tag and cinched with an overhand loop knot; the tag ends were then trimmed. Tying the mrPAT to the lateral side of the fish ensured that it did not inhibit protraction of the dorsal fin. In addition, it reduced the effects of the tag on swimming performance because the tag did not interfere with the dorsal or caudal fins. To minimize the likelihood of tag entanglement with underwater structures, the loop of spaghetti tag was kept as small as possible. Each spaghetti tag was printed with a unique identification number, the words “reward” and a toll-free phone number for reporting recaptures. This approach removed the need for a second identifying tag and reduced tagging procedure time, the risk of secondary infections, and overall fish stress. Moreover, the spaghetti tag – and contact information – remained on fish following the release of the mrPAT tags, leaving the possibility of additional data acquisition in the event of fish recaptures.

During the laboratory trials each attachment method was used to attach a tag to two different fish (mean total length: 40.6 cm). All tag attachment procedures followed standard aseptic techniques for fish tagging (as outlined by Wagner et al., 2011). Prior to tagging with

each method, fish were sedated in a solution of MS-222 (Tricaine methanesulfonate; 150 mg/l). During surgery, fish were positioned dorsal side-up onto a v-cut piece of foam and a lower concentration of MS-222 (50 mg/l) was pumped to the gills to maintain anaesthesia (Harms 2005). Following attachment of the tag, all puncture wounds were treated with an antiseptic ointment (5% Providine), applied liberally with a Q-tip. Each satellite tagging procedure was completed within approximately five minutes, after which fish were returned to separate holding tanks. All fish were monitored daily for signs of stress, tag loss, or illness. To evaluate the performance of each method in the presence of potential underwater entanglements, PVC pipes and concrete blocks were added to the tanks 45 days after the initial surgery (e.g. halfway through experiments). Specifically, two concrete blocks (19x19x39.5 cm) stood upright and two PVC pipes (30x25.4 cm) lay flat on the bottom of each tank. The openings of both the concrete blocks (12x12 cm) and the PVC pipes (25.3 cm) faced away from the tank wall and allowed fish to take refuge within each structure. Lab experiments concluded after 90 days, during which time we quantified the mean tag retention for each attachment method, as well as ranked the level of external trauma in cases where tags detached early.

Tag retention study in the field

Only the spaghetti tag attachment method (Fig. 5.1) – which performed best during laboratory investigations – was used during field tests. In total, 25 adult sheepshead (size range: 37 to 49 cm FL) were fitted with mrPAT's between October and November, 2020. Fish were observed for approximately 10-minutes post-surgery and released once equilibrium and rhythmic gill movements had returned. Release locations were in close proximity to the capture location for each fish. All were in inshore areas in the vicinity of Morehead City, North Carolina.

For field deployment the mrPATs were set to “auto start” mode, in which submersion in seawater activated the tag upon release. Satellite tags were programmed to detach so that the pop-off dates were staggered over a 2-week period in April, 2021, with planned deployment durations ranging from 140 to 165 days post-release. This timing was selected to correspond with the window of time thought to coincide with spawning in sheepshead, while also allowing us to assess the effectiveness of mrPATs over ~5-month field deployments.

Data and statistics

We evaluated the performance of each attachment method tested during lab trials based on three main criteria: (1) tag retention in days; (2) daily qualitative observations of fish health (e.g. fish appetite, lethargy, and buoyancy/swimming performance); and (3) signs of trauma either following the loss of tags, or at the conclusion of experiments. Only signs of trauma that occurred in areas within the immediate vicinity of the mrPAT attachment site were assessed, since signs of trauma outside of the tag radius most likely resulted from factors unrelated to the tagging procedure. Following established procedures for assessing tagging-related trauma (Runde et al. 2022), we classified fish trauma into four main levels (none, mild, moderate, severe) based on the size of the wound.

During field deployments we evaluated tag retention times, location accuracy, and the reliability of temperature data. For mrPAT location estimates we used an established method for determining the first reliable Argos satellite transmission in which the estimated error was <1500m (e.g. codes 3, 2, or 1; See Hussey et al. 2018).

Wildlife Computers estimates that the duration of the burn-pin sequence that detaches the mrPAT from fish can take up to 12 hours and varies with external temperature conditions in the water column. To account for potential differences between the programmed pop-off

date and the actual pop-off date that results from the timing of the burn pin, the estimated burn time was deducted from the initial pop off date.

A small number of tags were recovered after washing ashore following the programmed pop-off date. For these tags we quantified the extent of fouling on the external tag surface. This was achieved by counting and measuring the size (basal diameter in cm) of barnacles that settled on the tags.

Temperature data were visually inspected, and one tag was excluded from analyses due to suspected malfunction of the temperature/tilt sensors (e.g. wildly fluctuating and unrealistic temperatures). Additionally, data from three tags that surfaced prior to the pre-programmed pop-off date were excluded from analyses because data were only available for a short period of time following fish release. All temperature data were truncated in order to remove readings that occurred after the satellite tags popped up. Graphing and analyses were all conducted in R (R Core Team 2020).

Results

Tag retention in laboratory

No fish mortality occurred during the retention investigation, although several fish lost their tags prior to the study endpoint. Our attachment method using the spaghetti loop resulted in the greatest mrPAT retention time on our captive sheepshead. The two fish tagged using this method retained their tags for the entire 90-day duration of our lab tests (Fig. 5.2). All fish tagged with the rigid plate and monofilament loop methods lost their tags prior to the study endpoint. Fish tagged using the rigid plate method lost their tags at day 26 and day 34 post-surgery, prior to the addition of refugia to the tanks (mean retention: 30 days, n=2). Fish tagged with the monofilament loop method lost their tags at 46 and 51 days, shortly after the addition of refugia to the tanks (mean retention: 48.5 days, n=2).

Visual inspection of fish tagged with the spaghetti loop method revealed moderate trauma to the tag attachment site only (Figure A4). In the case of the rigid plate method, we observed severe levels of trauma on each lateral side of the fish (Figure A4). For fish tagged with the monofilament loop, we observed moderate trauma to the attachment site, but severe trauma to the dorsal fin likely resulting from tag abrasion (Figure A4). For all methods, daily observations of fish health revealed no noticeable differences in buoyancy, appetite, or lethargy between tag attachment methods.

Satellite tag field performance

Following the success with the spaghetti tag method during our lab trials, this method alone was used on fish released in the field. A total of 25 mrPAT's were deployed on 25 sheepshead caught in North Carolina waters in October–November 2020. Fish tagged ranged from 37 to 49 cm fork length (FL) (mean FL = 42.4 cm, Fig 5.3a). All fish were observed swimming away strongly following a quick (< 2 minute) surgery.

Data were successfully obtained for 17 of the 25 satellite tagged fish (68 %). Of the tags for which data was obtained, 14 (82%) remained on the fish until the pre-programmed release date. The other three tags floated to the surface prior to the programmed pop-off date at 20, 24, and 42 days and activated the auto-detect mechanism of the mrPAT. For those tags that did not transmit data to the ARGOS satellite system (n = 8), three communicated with satellites but did not transmit data (location, temperature or tilt data) and the remaining five tags failed to communicate with the ARGOS satellite network entirely.

The mean tag retention duration for the 17 tags that transmitted data to satellites ranged from 20 to 172 days (SD =53.8) and resulted in a mean tag retention of 140 days (Fig 5.3b). The exact time it took tags to surface and communicate with satellites varied between tags. Nine out of the 14 tags (64%) reported within ~1 day of the expected pop-off day. On

average, satellite tags surfaced within 1.14 days (SD=1.51) of the expected pop-off day. One tag communicated with satellites 5 days after the expected pop-off day (Table 5.1).

Three fish were recaptured by recreational fisherman following satellite tag pop-off 185, 213, and 456 days after fish were first release with tags. Inspection of images of the spaghetti tags provided by anglers (Figure A6) showed varying signs of trauma at the site where the spaghetti tag and nose cone remained attached. Considerable amounts of epifaunal growth were seen on the nose cones of two of the recaptured fish. One additional individual was recaptured 111 days after it was released and approximately one month prior to the pre-programmed pop-off date. This individual showed no signs of trauma and high levels of healing surrounding the tag attachment site (Figure A6).

In addition, six tags were recovered after popping-off fish and washing ashore. These tags had no data to retrieve, but inspection of the six tags revealed varying amounts of biofouling by barnacles (Figure A5).

Location data

The majority of tags (71%) transmitted accurate location estimates (<1500 meters) within 10 minutes of the initial pop-off transmission, with 88% of tags providing accurate location estimates within the first two hours after the first transmission. Two tags that surfaced close to shore took 9.5 and 8.4 hours to provide accurate location estimates (Codes 3, 2, or 1). The three tags that released early spent approximately six hours (the length of the auto-detect wet/dry mechanism) at the surface before connecting with satellites.

The number of location transmissions and the quality of location estimates received was highly variable among tags. On average tags transmitted 40 locations (range = 4 to 175). In terms of overall location quality, 60% of all received transmissions were for location

estimates with an estimated error of < 1500 m, with 32% of these providing location estimates within < 250 meters.

Temperature data

Fourteen of the 17 tags that transmitted pop-off locations to satellites also successfully transmitted daily minimum and maximum temperature data. The amount of temperature data received via ARGOS satellites varied considerably between tags. On average, tags collected daily temperature data for 67 days (SD=41.2) or during 45% of the deployment duration (Table 5.1). For a number of tags the temperature data received was highly discontinuous with some tags failing to provide data for multiple weeks at a time (Fig. 5.4)

Discussion

Although satellite tags have decreased in size considerably in recent times, their usefulness for studying smaller fishes has remained largely unexplored. To address this challenge, our goal was to design a method for long-term attachment of PSATs on small migratory fishes, using the smallest PSAT available on the market. Our simple and cost-effective spaghetti loop method for PSAT attachment performed well during our lab tests and emerged as the method with the highest tag retention. Subsequent testing of the spaghetti loop method during field trials resulted in a mean tag retention of 140 days, with 82% of tags lasting until our pre-programmed pop-off date. Thus, our results demonstrate that the spaghetti loop method is sufficient for long-term (~5 month) attachment of PSATs on sheephead of 37-49 cm fork length. In addition, the results imply that mrPATs represent a viable option for movement studies on small migrants.

We observed a high degree of variability in tag retention among the three PSAT attachment methods tested under lab conditions. A method using two rigid plates to attach tags resulted in the lowest tag retention. While this method has previously resulted in six-month retention of PSATs on European eels (*Anguilla anguilla*), the tendency of the plates to cause skin erosion and changes in fish behavior have previously been noted (Økland et al. 2013). Even with the addition of silicone pads, fish tagged in our study expressed severe levels of trauma that likely resulted in the loss of tags.

Previous studies using the monofilament loop method on Atlantic salmon (*Salmo salar*) and sable fish (*Anoplopoma fimbria*) have demonstrated the utility of this method for long-term deployments (Lacroix 2013; Echave 2016). Investigations with smaller fishes, such as the Blackspotted rockfish, however, have resulted in considerably lower tag retention (Rodgveller et al. 2017). In our study, fish tagged with this method also had low tag retention (Fig. 5.2). One difficulty associated with this method is the tendency of the monofilament loop to cause trauma to the dorsal fin. Additionally, the large size of the monofilament loop was observed to increase the tendency of tags to become entangled in structure. While this method is clearly applicable for salmonids (Lacroix 2013), it appears to be less effective for fishes with larger dorsal fins and for fishes like sheephead that possess a higher affinity for structure.

Our method using the spaghetti loop resulted in the highest tag retention and the lowest degree of fish trauma. Contrary to the monofilament method, the smaller loop and position of the tag on the lateral side of the fish appeared to minimize tag entanglement and interference with the dorsal fin. Studies using spaghetti tag material for the external attachment of acoustic tags have reported similar successes (Runde et al. 2022). Runde et al. (2022) compared six methods of external acoustic tag attachment and report that a method that involved passing a single loop of spaghetti tag through the dorsal musculature resulted in

high tag retention, and low detriment on fish welfare. While distinct differences exist in the way the spaghetti tag was used in our study and in the study by Runde et al. (2022), in both cases the spaghetti tag material (vinyl tubing) emerged as the material that caused the lowest detriment to fish welfare.

The success of the spaghetti tag method is further evident from our field investigations, in which 25 mrPATs were attached to sheephead smaller than 50-cm fork length. Results of these investigations resulted in a mean tag retention of 140 days, with a maximum retention of 172 days. Compared to a previous study on similar sized fish, our results represent a considerable improvement in tag retention (Rodgveller et al. 2017). Rodgveller et al. (2017) tagged eight Blackspotted rockfish, but all tags detached prematurely, with a mean tag retention of 35 days. Although rockfish are morphologically similar to sheephead, our tag retention was considerably higher. Given the high percentage of tags that remained on fish until the pre-programmed pop-off dates in our study, higher levels of retention could likely have been achieved with the programming of later pop-off dates. In addition, PSAT loss rates in this study (18 % early detachment) were generally lower than those reported in other studies on larger fishes (82% early detachment; see Musyl et al. 2011). The reasons for high tag retention and low rates of tag loss in our study can likely be attributed to the success of the spaghetti loop method, as well as the use of mrPATs which are appreciably smaller than many of the larger PSAT models used in earlier studies.

Studies on mrPATs are limited given that this particular PSAT model is a relatively new addition to the market. Nonetheless, mrPATs have been used in a small number of studies providing the opportunity for more direct comparisons of the effectiveness of mrPATs between species and attachment method. In one study on Greenland sharks, 100% of mrPATs (n=18) successfully reported data on the programmed pop-off date with a mean deployment duration of approximately 40 days (Hussey et al. 2018). Similarly, a study

deploying mrPATs (n=6) on Atlantic sturgeon achieved a mean tag retention of 82 days, again with 100% of tags successfully reporting on the programmed pop-off date (Hylton et al. 2018). More recently, a study on cobia deployed 26 mrPATs reporting a mean tag retention of 42 days with 90% of tags reporting, but only 4% of tags (a single tag) remaining on a fish for the entire deployment duration (180 days).

While our tag retention was considerably higher, our mrPAT reporting rate (68%) was lower than that previously seen in other mrPAT studies (Hussey et al. 2018; Hylton et al. 2018). Several factors may have influenced mrPAT failure during deployment, including tag malfunction (Hays et al. 2007), predation events (Kerstetter et al. 2004), and biofouling preventing tags from surfacing (Kneebone et al. 2014). High levels of predation have been reported in studies tagging relatively small anguillid eels (83-101 cm TL), implying that the risk of predation may be considerably higher for smaller fishes (Wahlberg et al. 2014; Westerberg et al. 2021). Given the extent of fouling we observed on recovered tags (Figure A5), however, fouling remains the most plausible explanation for the eight tags that failed to report. Similarly, fouling of tags, particularly in areas close to the external temperature sensor, may explain the irregularities in temperature data transmitted to ARGOS satellites. While the risk of fouling may have been heightened in our study given the close proximity of fish to inshore areas, fouling of tags remains a persistent complication for long-term field deployments for many species (Thorstad et al. 2014).

Regardless of the above considerations, our results demonstrate that the spaghetti loop method, and mrPATs, are a viable means for studying sheepshead over 5-month deployments. To our knowledge, this study represents the first investigation into the use of mrPATs on fishes of this size (<50 cm). Given the small size of fish we tagged and the proclivity of sheepshead for refugia, our results represent a significant advance in PSAT attachment methodology. We suspect the spaghetti loop method of mrPAT attachment will be

transferrable to many other marine migrants and will provide a key solution to obtaining fisheries-independent data for many important fishes globally.

Figures

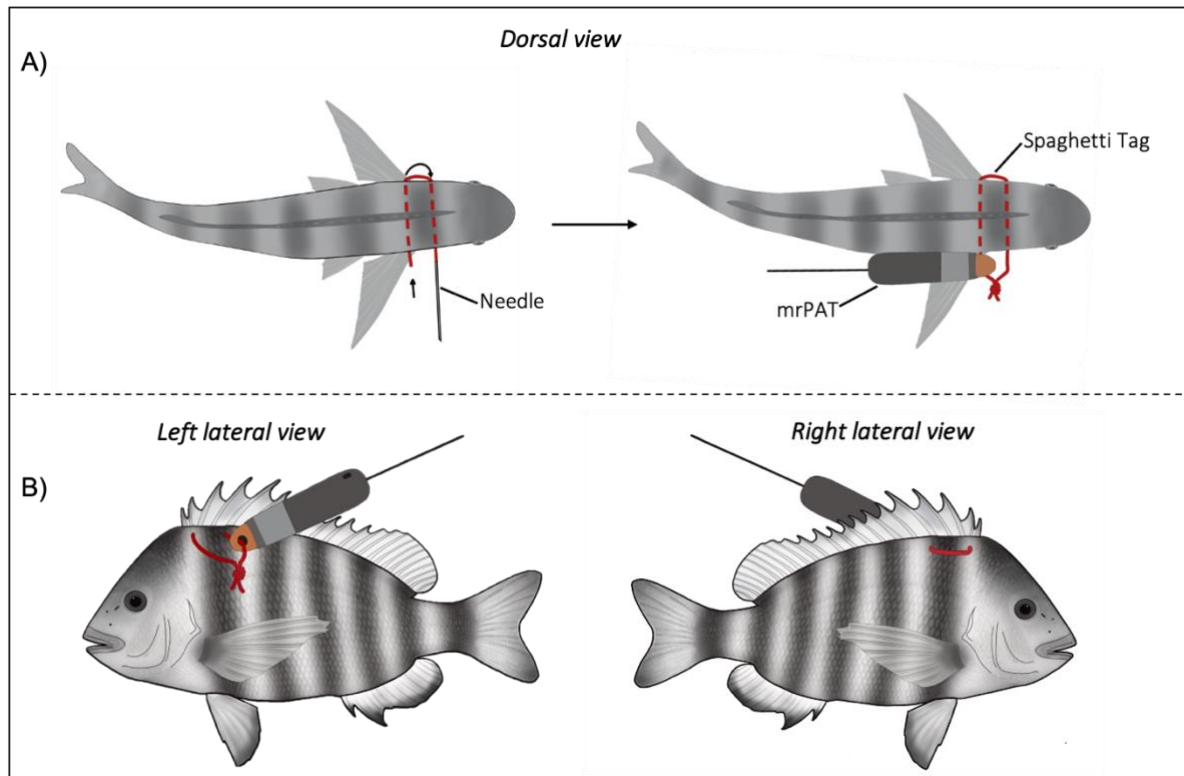


Figure 5.1 Diagram of the spaghetti tag method of PSAT attachment for sheepshead.

Diagram of the satellite tag attached method for sheepshead and other fishes. (A) dorsal view; the spaghetti tag (red) was threaded through the anterior portion of dorsal musculature in two separate places as denoted by the red dotted lines. (B) View of the left and right lateral sides of the fish showing the position of the needle puncture areas relative to the dorsal fin.

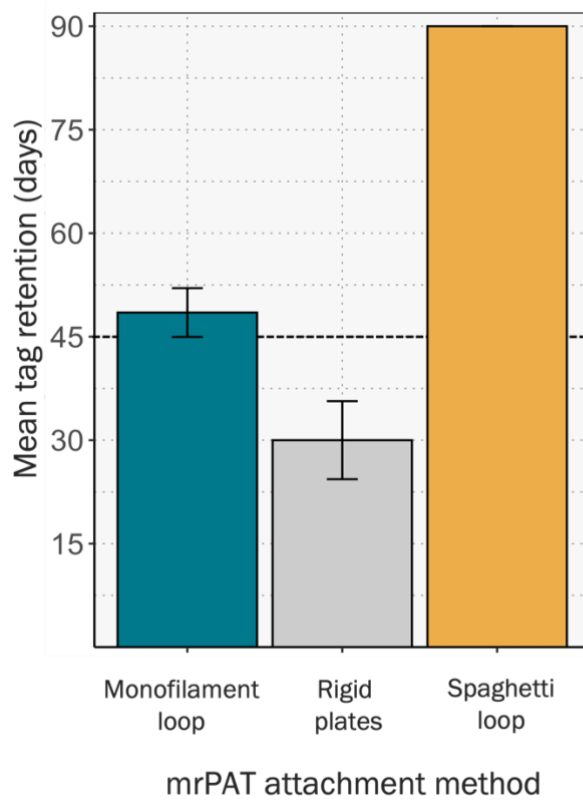


Figure 5.2 Results of the laboratory tag retention study.

Each bar represents the mean retention of mrPAT dummy tags for two tagged fish. Black dotted horizontal line denotes the point at which structure (PVC and concrete blocks) was added to the tank. Lab investigations concluded at the 90-day mark.

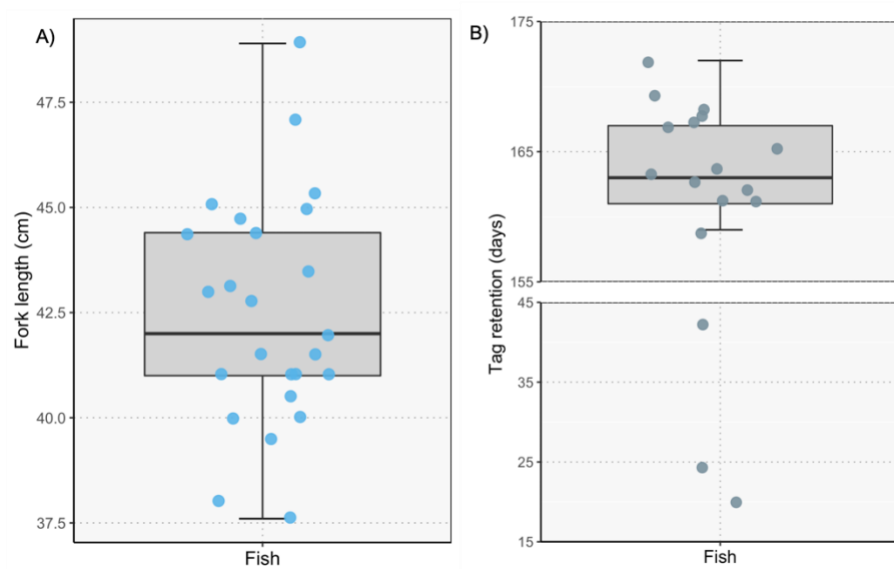


Figure 5.3 Sheepshad fork length and tag retention.

(A) Fork length (cm) for sheepshad ($n=25$) tagged with satellite tags. (B) The number of days each fish ($n=17$) retained the satellite tag before the tag first transmitted data to the ARGOS satellite network (axis break dashes). In each case, dots represent the raw values for a single fish. The solid horizontal black line represents the median, the upper and lower boundaries of the grey box represent the interquartile range and the whiskers denote the minimum and maximum data values.

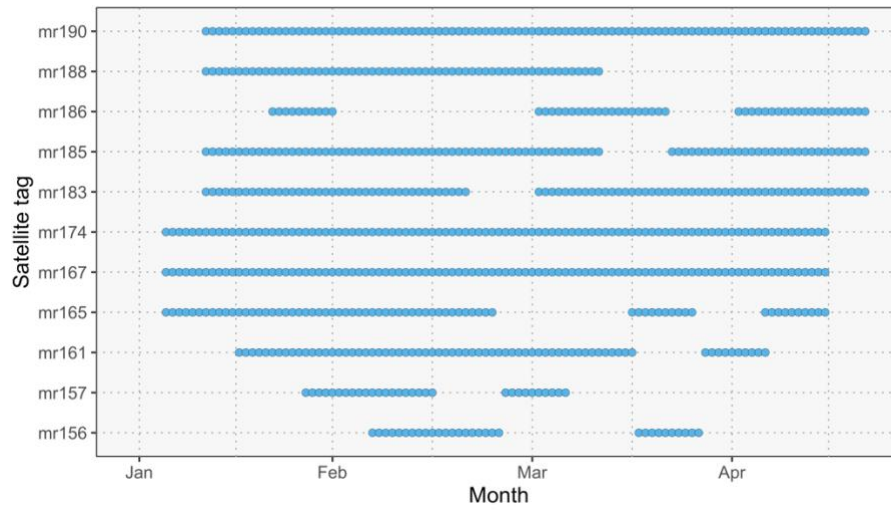


Figure 5.4 Abacus plot showing days when daily minimum and maximum temperature were reported.

Each line represents a single satellite tag, and each blue data point a day when temperature data was received.

Tables

Table 5.1 Summary data for 25 sheephead tagged with a mark report pop-off archival satellite tag (mrPAT).

TL = total length; FL = fork length; dates are in dd-mm-yyyy format.

| Fish | Satellite tag | FL (cm) | TL (cm) | Fish release date | Expected pop-off date | Actual pop-off date | Days +/- expected pop-off date | Tag retention (days) | Temperature data reported (days) |
|------|---------------|---------|---------|-------------------|-----------------------|---------------------|--------------------------------|----------------------|----------------------------------|
| 1 | mr154 | 41 | 44.5 | 27-10-2020 | 11-4-2021 | - | - | - | - |
| 2 | mr155 | 45 | 49 | " | 11-4-2021 | 11-19-2020 | -141 | 24 | 8 |
| 3 | mr156 | 43.1 | 48.1 | " | 11-4-2021 | 4-11-21 | 0 | 167 | 30 |
| 4 | mr157 | 41 | 45 | " | 11-4-2021 | 04-16-2021 | 5 | 172 | 30 |
| 5 | mr158 | 41.5 | 45.7 | " | 11-4-2021 | 04-13-2021 | 2 | 169 | 0 |
| 6 | mr159 | 44.7 | 54.7 | 04-11-2020 | 11-4-2021 | - | - | - | - |
| 7 | mr160 | 41 | 45 | " | 11-4-2021 | - | - | - | - |
| 8 | mr161 | 37.6 | 42.1 | " | 11-4-2021 | 4-11-21 | 0 | 159 | 70 |
| 9 | mr162 | 43 | 47.2 | " | 20-4-2021 | 12-15-2020 | -124 | 42 | 0 |
| 10 | mr163 | 40 | 44.2 | " | 20-4-2021 | - | - | - | - |
| 11 | mr164 | 45.1 | 50.7 | " | 20-4-2021 | 11-23-2020 | -146 | 20 | 7 |
| 12 | mr165 | 47.1 | 51.6 | " | 20-4-2021 | 04-20-2021 | 0 | 168 | 70 |
| 13 | mr166 | 42.8 | 47.5 | " | 20-4-2021 | - | - | - | - |
| 14 | mr167 | 43.5 | 48.1 | 11-11-2020 | 20-4-2021 | 04-20-2021 | 0 | 161 | 100 |
| 15 | mr174 | 40.5 | 45 | " | 20-4-2021 | 04-22-2021 | 2 | 163 | 100 |
| 16 | mr182 | 38 | 42.2 | " | 20-4-2021 | 04-20-2021 | 0 | 161 | 0 |
| 17 | mr183 | 42 | 45.6 | " | 26-4-2021 | 04-26-2021 | 0 | 167 | 90 |
| 18 | mr184 | 40 | 44.6 | " | 26-4-2021 | - | - | - | - |
| 19 | mr185 | 44.4 | 49 | " | 26-4-2021 | 04-27-2021 | 1 | 168 | 90 |
| 20 | mr186 | 45.3 | 50 | 13-11-2020 | 26-4-2021 | 04-26-2021 | 0 | 165 | 50 |
| 21 | mr187 | 41.5 | 46.6 | 17-11-2020 | 26-4-2021 | - | - | - | - |
| 22 | mr188 | 44.4 | 49.4 | " | 26-4-2021 | 04-27-2021 | 1 | 162 | 60 |
| 23 | mr189 | 39.5 | 44 | " | 26-4-2021 | - | - | - | - |
| 24 | mr190 | 48.9 | 54.3 | " | 26-4-2021 | 04-29-2021 | 3 | 164 | 160 |
| 25 | mr191 | 41 | 45 | " | 26-4-2021 | 04-28-2021 | 2 | 163 | 70 |

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CHAPTER 6: CONCLUSIONS

Almost half a century has passed since James D. McCleave conducted one of the first known investigations into magnetic field detection in fishes. At the time of this initial research, whether fishes could sense magnetic fields was not known. McCleave's seminal work on eels provided the first evidence of magnetic sensitivity in a fish. Over the following decades the idea that fishes can sense Earth's magnetic field travelled a path from ridicule to well-established fact.

My dissertation represents a multi-pronged investigation into the ecology, behavior, and underlying mechanism(s) of magnetoreception in fishes. Behavioral studies with Gulf flounder revealed for the first time that flounder can use directional ("compass") and positional ("map") information from the earth's magnetic field. Subsequent investigation into the functional characteristics of the flounder compass imply that the magnetic compass of flounder may differ in important ways from that of other animal groups. An investigation with chinook salmon provides evidence consistent with the idea that magnetic field detection in fishes may involve magnetite. And lastly, evaluation of state-of-the-art satellite tracking technology provides new advancements in methods for tracking the movements of many small migrants.

Chapter one constitutes an extensive literature review of the field of fish magnetoreception since its establishment over 50 years ago. The review shows how the number of empirical papers on fish magnetoreception has grown considerably over the last 50 years. It highlights how the past 20 years have been the most prolific and supports the notion that magnetic navigation in fishes is a rapidly developing field of research. It discusses the

widespread existence of both magnetic compasses and maps, providing the first detailed taxonomic breakdown of magnetoreception in different groups of fishes. In addition, the review discusses the advantages and limitations of different approaches to studying fish magnetoreception. A central conclusion is that, although many fundamental aspects of magnetic navigation are now understood, a number of substantive questions remain.

One such question relates to the mechanism(s) of magnetic field detection in fishes. Although behavioral evidence has established that diverse fishes sense magnetic fields, how they do so remains enigmatic. In Chapter two I explore one possible mechanism of magnetoreception with chinook salmon by exposing fish to a brief but strong magnetic pulse capable of affecting a magnetite-based magnetoreceptor. Although used as a diagnostic technique for magnetite-based magnetoreception in other taxa, this technique had not previously been tested on a fish. Results from this investigation implied that magnetoreception in salmon may in part rely on magnetite, thus lending credence to the identification of magnetite particles with no known function in salmon.

Studies on magnetic navigation have often focused on species that undergo the longest and most spectacular migrations. Among fishes, much of what has been learned focuses on migratory specialists such as salmon and eels – the only species known to possess both a magnetic map and compass. These iconic migrants, however, represent only a small proportion of a diverse array of marine life-history strategies. In the second and third chapters of my thesis I investigate the magnetic sense of the Gulf flounder, a largely sedentary flatfish known to travel only short distances between offshore and inshore habitats. In chapter two I provide the first behavioral evidence for a magnetic map and compass in flounder. These findings are significant for several reasons. Most notably, they suggest that navigation based on a magnetic map and compass is not limited to iconic long-distance migrants, but is instead present in fishes with diverse life history strategies that move over a range of spatial scales.

Evidence in other animal groups implies that the magnetic compass fits into one of two distinct categories: a polarity compass or an inclination compass. Little is known, however, about the functional characteristics of the compass sense of most fishes. In chapter three I capitalize on the behavioral assay I developed with flounder to investigate the functional properties of the flounder magnetic compass. Flounder were exposed to three magnetic fields that have previously been used to distinguish between polarity and inclination compasses in other taxa. The behavioral results of these experiments did not conform to predictions for animals that have either a polarity compass or an inclination compass. This outcome suggests that the flounder compass differs in its functional properties and/or underlying mechanism from that of other animals.

Chapters two through four all provide evidence that fishes can use Earth's magnetic field as a compass and/or map in navigation, and that these abilities are present in both long and short-distance migrants. Moreover, results add further evidence for the magnetite hypothesis in fishes, as well as demonstrate that the flounder compass may be unique in its functional characteristics. Taken together, these three independent lines of evidence provide important new insights into fish magnetoreception. This work provides a solid foundation for future studies on the behavior and underlying mechanisms of magnetoreception in fishes and other animals.

Much like the sensory cues underlying fish movements, the pathways travelled by many migratory fishes have similarly eluded researchers. One significant obstacle has been the lack of a method for attaching (and retaining) satellite tags on small fish as the fish undertake movements during multiple months at sea. In chapter five I test the latest and smallest satellite tag model on the market, and developed a simple, cost-effective method of tag attachment on sheepshead, a small marine fish. The method performed remarkably well during both lab and field tests and appears feasible for tracking movements of small fishes

over a period of months. This result represents a significant advance in tag attachment methodology and paves the way for future investigation on many understudied fishes in this size range.

Preliminary results from tracking studies with sheepshead suggest that these fish show site fidelity to specific inshore foraging areas, and that fish return to these areas after a multiple-month absence offshore. Elucidating these movements is only possible through telemetry techniques like those used here, and an understanding of the sensory cues that fish use to relocate these foraging areas will only be gained through future behavioural investigations like those presented in chapters one through four. Integrating studies on the in-situ movements of fishes with controlled ex-situ behavioural investigations represents a powerful tool for researchers to investigate many of the outstanding mysteries surrounding fish migration.

Migratory fishes (like eels and salmon) have been important to human civilizations for millennia. While mankind's fascination with migratory fishes has endured the test of time, so have many of the mysteries surrounding the movements and sensory underpinnings of fish migration. The evidence presented in my thesis represents a key step towards better understanding many of these elusive aspects of fish behavior. Through multiple lines of evidence I add to the emerging picture that information from Earth's magnetic field is an essential part of the sensory repertoire of many migrants. These results lend credence to accumulating evidence that magnetoreception is used to guide diverse types of animal movements and provide plausible means for the cues guiding the comings and goings of diverse fishes.

I conclude by expressing that there is cause for great optimism in the field. Advances in technology are likely to increase mankind's ability to track the movements of many fishes with increasingly higher accuracy, and further miniaturization of tracking technology will

only increase our capacity to study a wider variety of fishes. Likewise, as the search for the putative magnetoreceptors continue, future advances in genetic techniques and microscopy will likely provide key tools for cracking this age-old puzzle. Cross-disciplinary approaches that utilize these emergent technologies represent a rich testing ground for understanding fish movements and the sensory mechanisms that underpin them.

APPENDIX 1: AN ADDITIONAL APPROACH TO ANALYZING MAGNETIC PULSE DATA

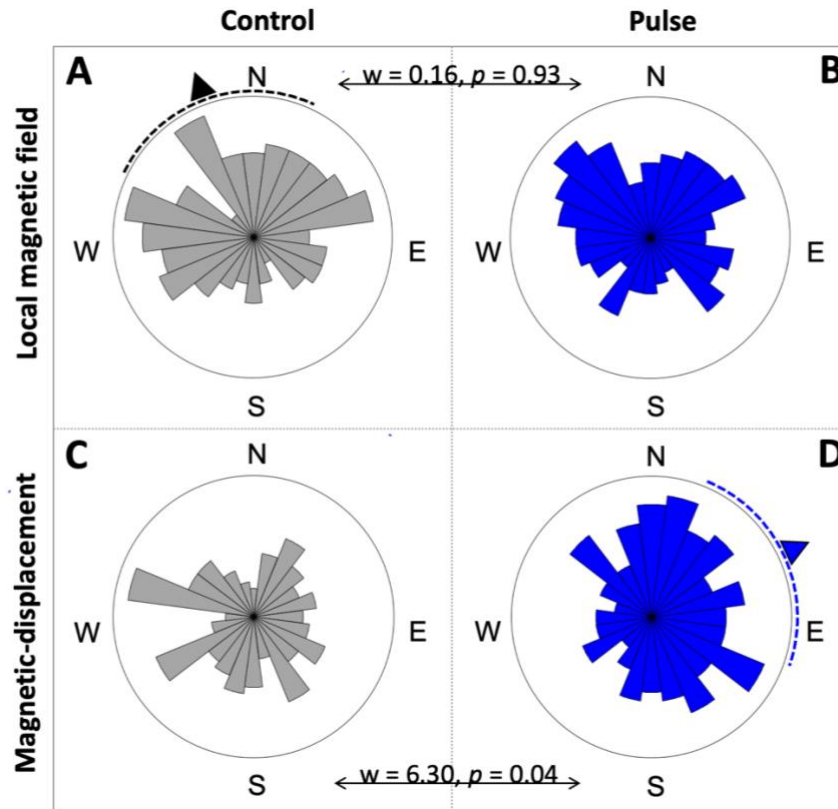


Figure A1 Analysis of data treating each fish as an independent data point.

(A) Under local magnetic field conditions fish from the control group were significantly oriented with a mean angle of 341 deg (Rayleigh test, $n = 208$, $r = 0.12$, $z = 3.20$, $p = 0.04$).

(B) Under local magnetic field conditions salmon that experienced a strong magnetic pulse were not oriented as a group (Rayleigh test, $n = 196$, $r = 0.12$, $z = 2.78$, $p = 0.06$). (C) During a magnetic displacement to a southern ocean region, control fish were not oriented as a group (Rayleigh test, $n = 216$, $r = 0.06$, $z = 0.72$, $p = 0.49$). (D) During the magnetic displacement, salmon from the pulse group were significantly oriented with a mean angle of

66 deg (Rayleigh test, $n = 204$, $r = 0.13$, $z = 3.30$, $p = 0.04$). The length of each bar indicates the number of fish that were oriented within each 15-degree range of directions. Arrow heads indicate the mean direction of each treatment group. Dotted lines represent the 95% confidence interval for the mean. Fish that we were unable to determine a clear angle of orientation for (due to glare in the photos) were omitted from the analysis, resulting in the slightly uneven sample sizes.

APPENDIX 2: FURTHER EXPLORATION OF FLOUNDER COMPASS DATA

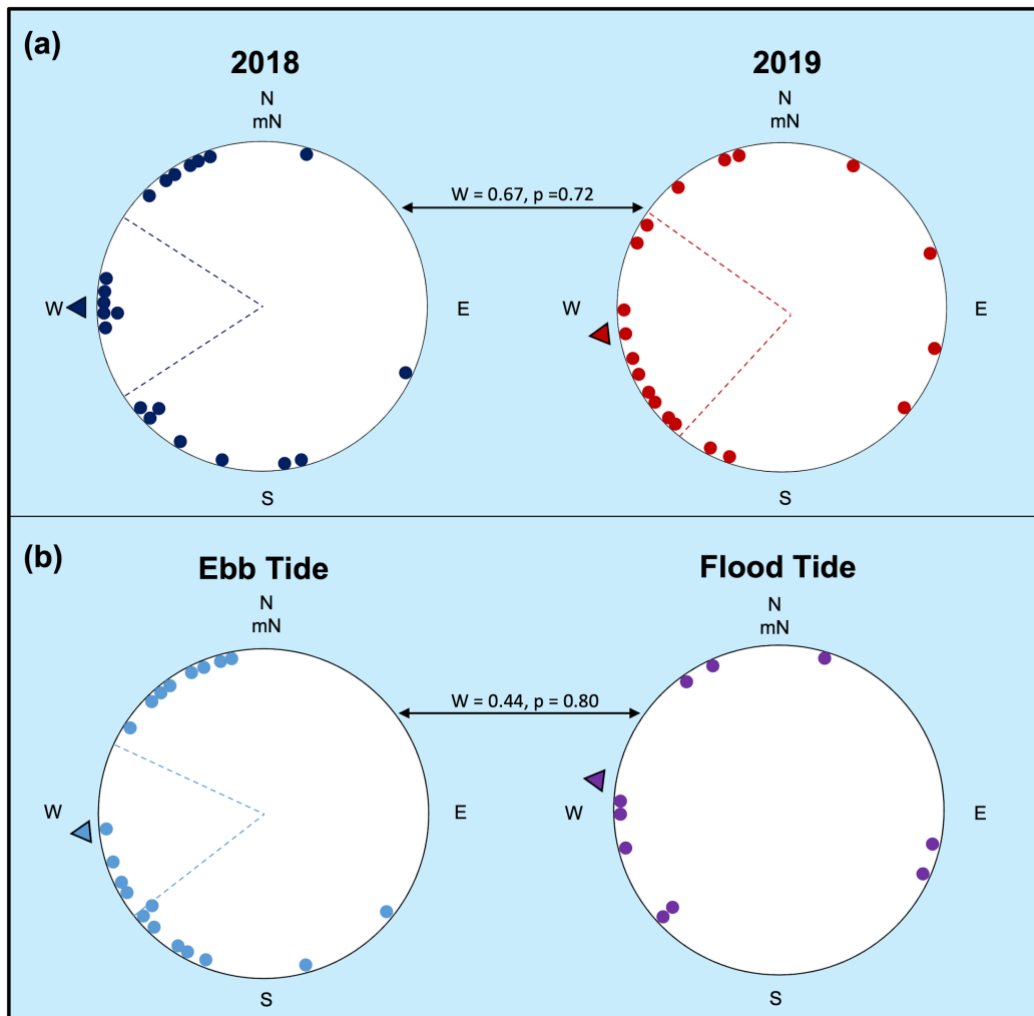


Figure A2 Compass orientation in response to tidal cycle and year.

(A) Circular graphs of fish orientation when tested in 2018 (mean heading = 270° ; Rayleigh test: $n = 21, r = 0.50, p = 0.005$) and 2019 (mean heading = 265° ; Rayleigh test: $n = 19, r = 0.42, p = 0.03$). Orientations of fish tested in 2018 and 2019 were not significantly different (Mardia-Watson-Wheeler test: $W = 0.67, p = 0.72$). (B) Circular graphs of fish tested during ebb tide (mean heading = 264° ; Rayleigh test: $n = 20, r = 0.53, p = 0.003$) and flood tide (mean heading = 278° ; Rayleigh test: $n = 10, r = 0.33, p = 0.35$). Data collected during flood and ebb tide were not significantly different (Mardia-Watson-Wheeler test: $W = 0.44, p = 0.80$). For all graphs, dots along the edge of the circular graph represent the mean heading of

an individual fish, arrowhead indicate the mean direction of the group, dashed lines indicate 95% confidence intervals for significant unimodal orientation, and “mN” denotes the direction of magnetic north. Data for all circular graphs are normalized such that magnetic north is the same as geographic north. Black two-way arrows show pairwise comparisons between two groups.

APPENDIX 3: FURTHER EXPLORATION OF FLOUNDER MAP DATA

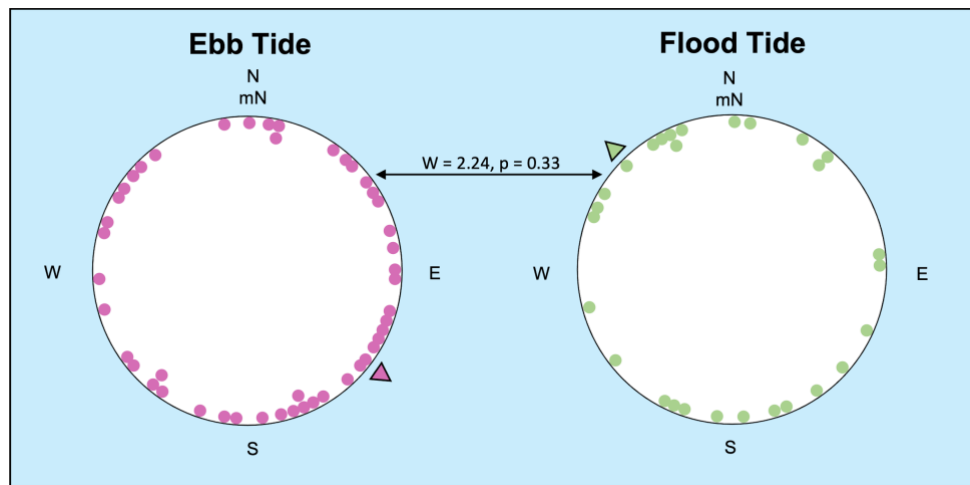


Figure A3 Flounder map orientations by tidal cycle

Circular graph to the left displays the mean orientation of fish tested during ebb tide (mean heading = 126° ; Rayleigh test: $n = 47$, $r = 0.15$, $p = 0.36$). Circular graph to the right displays the mean orientation of fish tested during flood tide (mean heading = 314° ; Rayleigh test: $n = 28$, $r = 0.09$, $p = 0.79$). Data collected during ebb and flood tide were not significantly different (Mardia-Watson-Wheeler test: $W = 2.24$, $p = 0.33$). For each graph, dots along the edge of the circle graph represent the mean heading of an individual fish. The mean heading of a group of fish can be identified with the colored triangle outside the circular graph, and “mN” denotes the direction of magnetic north. Black arrows connecting circular graphs represent pairwise comparisons between groups.

APPENDIX 4: EXTENT OF TRAUMA FROM PSATS

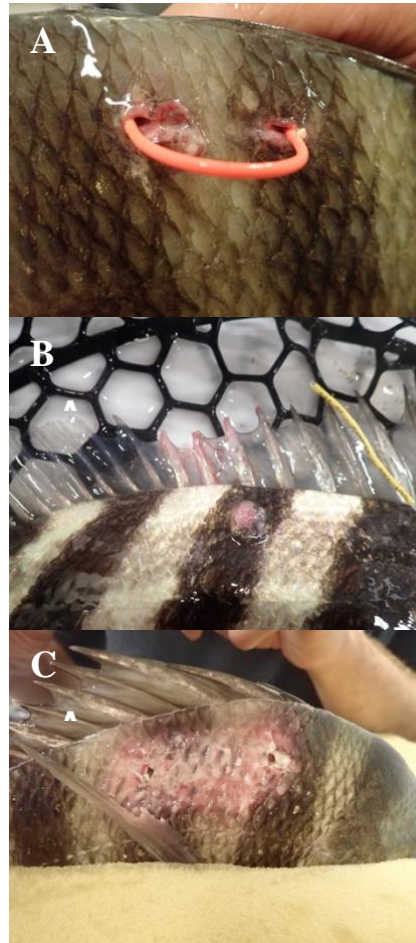


Figure A4 Comparison of trauma from three mrPAT attachment methods.

(A) the spaghetti loop method; (B) the monofilament loop method, and; (C) the rigid plate method.

APPENDIX 5: EXTENT OF FOULING ON PSATS

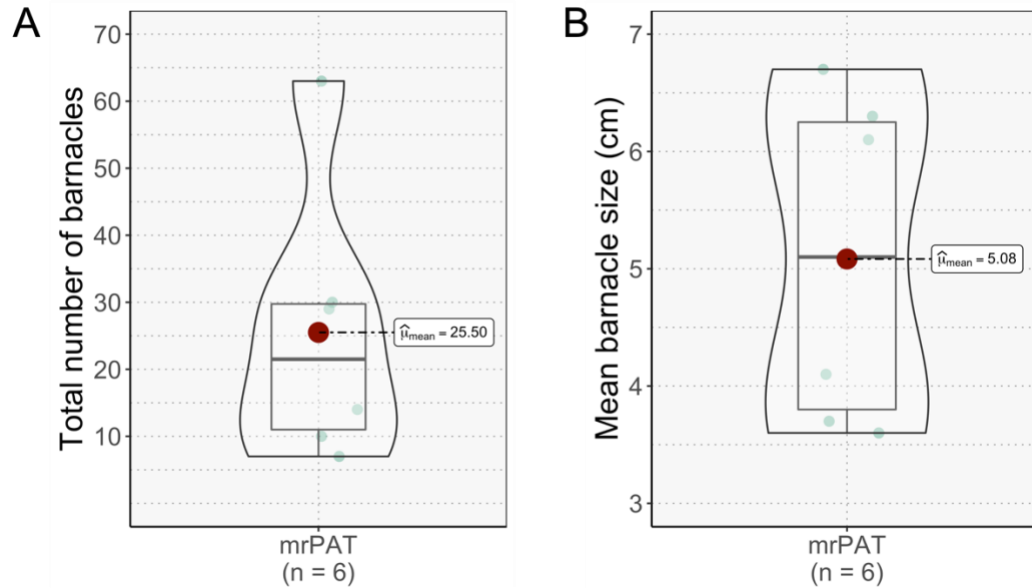


Figure A5. Degree of barnacle settlement on recovered mrPATs.

(A) Total number of barnacles that settled on six recovered mrPATs. (B) Mean basal diameter (cm) of barnacles that settled on six recovered mrPATs.

APPENDIX 6: EXTENT OF TRAUMA ON RECAPTURED FISH



Figure A6 Images of sheephead recaptured by anglers.

Shown above each image is the number of days between the original fish release date and the data upon which each respective fish was recaptured. Images provided by anglers varied in quality, but nonetheless provided a rare opportunity to inspect the attachment site following multiple months, or years in the field.