

2020

Sensing Symbiosis: Investigating the Symbiotic Magnetic Sensing Hypothesis in Fish Using Genomics

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SENSING SYMBIOSIS: INVESTIGATING THE SYMBIOTIC MAGNETIC
SENSING HYPOTHESIS IN FISH USING GENOMICS

by

ELIZABETH BOGGS

A thesis submitted in fulfillment of the requirements
for the Honors Undergraduate Thesis Program in Biological Sciences
in the College of Sciences
and in the Burnett Honors College
at the University of Central Florida
Orlando, Florida

Fall Term, 2020

Thesis Chair: Robert Fitak, PhD

ABSTRACT

The mechanism behind magnetoreception – the ability to sense magnetic fields for orientation and navigation – still remains one of the most difficult questions to answer in sensory biology, with fish being just one of many taxa known to possess this sense. Characterizing a magnetic sense in fish is crucial for understanding how they navigate their environment and can inform on how increasing anthropogenic sources of electromagnetic fields in aquatic environments may affect threatened fish species. This study examined the hypothesis put forth by Natan and Vortman (2017) that magnetotactic bacteria (MTB), bacteria that create their own chains of magnetic particles for navigational use, act in symbiosis with their animal host to convey magnetic information about their surroundings. Utilizing existing, publicly available datasets of raw genomic sequences, this study demonstrated the presence of MTB within a diverse array of fishes and identified differences in species diversity of MTB between freshwater and marine species of fish. Future research aimed at identifying MTB in specific fish tissues, such as the eye and other neural tissues, will be necessary to provide support for this hypothesis and to further examine the relationships that MTB may have with magnetically sensitive animals.

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LIST OF ABBREVIATIONS

MTB – Magnetotactic Bacteria

MAI – Magnetosome Island

IMG/M – Integrated Microbial Genomes & Microbiomes

BLAST – Basic Local Alignment Search Tool

NCBI – National Center for Biotechnology Information

SRA – Sequence Read Archive

STAT – SRA Taxonomy Analysis Tool

INTRODUCTION

Magnetoreception as a Sense

Magnetoreception, or the ability to sense magnetic fields, has been observed as a critical sense in a variety of animals. Using this sense to align with Earth's geomagnetic field, animals are better equipped to successfully navigate long distances and even to detect prey with increased accuracy (Červený et al., 2011). However, the mechanism underlying this sense remains largely unknown and is considered one of the most pressing questions in sensory biology (Johnsen, 2017). Current research on magnetoreception is based on three main hypotheses: (i) radical pair chemistry (a biochemical reaction involving free electron pairs; often studied in birds), (ii) electromagnetic induction (electrical charges created by moving a conducting rod in a magnetic field; studied in elasmobranchs), and (iii) biogenic magnetite (permanently magnetized iron oxide particles; often studied in fish, reptiles, and arthropods) (reviewed by Formicki et al., 2019).

The hypothesis of radical pair chemistry posits that the geomagnetic field alters the spin state of light-dependent electron radical pairs, causing a biochemical signal cascade that transmits magnetic information to the animal (Nordmann et al., 2017). Cryptochromes, a set of flavoproteins that play a role in the circadian rhythm of plants and animals, are of particular interest to this hypothesis. They are light-sensitive proteins that are expressed in the organs and tissues, acting as photoreceptors, and have been linked to magnetoreception in several animal taxa, such as monarch butterflies (Gegear et al. 2010) and in the retina of migratory garden warblers (Mouritsen et al. 2004). One study also found a role of cryptochromes in the mediation

of fruit flies' circadian rhythm when exposed to magnetic stimuli (Yoshii et al. 2009).

A second hypothesized mechanism of magnetoreception, based on electromagnetic induction, relies on Faraday's law – a familiar concept, as this is the same mechanism that generates electricity in power plants and in car alternators. As an animal moves inside a magnetic field, like the earth's geomagnetic field, an electrical stimulus is induced that the voltage-sensitive channels in their sensory cells can detect as a change in electric potential (Nordmann et al., 2017). This hypothesis is most commonly suggested as the mechanism behind elasmobranchs' electrosensory system in the ampullae of Lorenzini, but it has also been proposed as the mechanism for magnetoreception in pigeons, occurring within semicircular canals of the inner ear (Nimpf et al., 2019). The ampullae of Lorenzini act as sensitive, efficient electrical conductors of the induced current as the animal moves in the geomagnetic field. However, empirical studies of sharks and rays, which utilize electrical fields to hunt prey, have also shown their ability to discern magnetic fields even when their electrical sensory system is impaired by strong neodymium magnets. This has led to the hypothesis that they have a separate mechanism for magnetoreception in addition to induction – one possibly based on small magnetic particles (Anderson et al. 2017, Newton and Kajiura 2017).

A third, and perhaps most well-studied, hypothesis for magnetoreception is based on the presence of naturally magnetic iron oxide crystals, particularly magnetite (Fe_3O_4). These ferromagnetic crystals are permanently magnetized and may act as tiny compass needles if they have a role in magnetoreception. These crystals can accumulate within the body, specifically in the skull and olfactory lamellae in fish (reviewed by Formicki et al. 2019). In order to function as

a magnetoreceptor, magnetite must be able to transmit magnetic stimuli to the animal's nervous system. Johnsen and Lohmann (2005) proposed that as magnetite crystals attempt to align with Earth's magnetic field, they may put mechanical pressure on secondary receptors or may open/close ion channels, but this has not yet been directly observed.

Magnetite particles have been identified in several tissues of different animal species, such as the nasal region of trout (Walker et al. 1997) and the lateral line of eels and salmon (Moore and Riley 2009; Moore et al. 1990). In addition, a variety of other animals known to use the geomagnetic field for orientation, such as sea turtles and honeybees, have demonstrated responses to magnetic fields consistent with a magnetite-based mechanism (reviewed by Kirschvink et al. 1985). As there are differing sizes of magnetite particles, which also differ in their magnetic moment, most systems of magnetite-based magnetoreception are based on single domain magnetite particles (e.g., a refrigerator magnet), which have a stable magnetic moment (Wiltschko and Wiltschko 2013). Multi-domain magnetite particles (larger than single domain) will have no net magnetization and, thus, will not confer a magnetic sense. Because of this, it is crucial to determine the size of magnetite crystals found within animal tissues when connecting them to a magnetic sense.

Magnetoreception in Fish

The dominant hypothesis supported by current research on magnetoreception in fish is that of biogenic magnetite particles, but a specific pathway for how magnetite serves as a magnetoreceptor has not yet been identified (Natan et al. 2017; Nordmann et al. 2017). Many

studies examining magnetoreception in fish have used rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792) to demonstrate their ability to discern and respond to magnetic fields (summarized by Arinella et al. 2018). Rainbow trout, a salmonid, have been reported to contain single-domain magnetite particles (Diebel et al. 2000). Studies examining the effects of an experimentally applied magnetic pulse on gene expression in the brain and retina of rainbow trout have identified several candidate magnetoreception genes (Fitak et al. 2017; 2018). Among them, the iron-storage protein *ferritin* is upregulated in the brain after exposure to the pulse and is of particular interest for a role in magnetoreception. Relative to the observed effects in brain tissue, a magnetic pulse produces little effect on gene expression in the retina, suggesting a magnetite-based mechanism within trout. Shcherbakov (2005) reported a magnetic sense within the fish model zebrafish (*Danio rerio*), which inhabits freshwater. Another study demonstrated a magnetic compass orientation in European eel (*Anguilla sp.*), a catadromous fish, which migrate from freshwater to the Sargasso Sea to spawn (Durif et al. 2013). These observations suggest that magnetoreception could be utilized for both long-range and short-range navigation and may also be present in freshwater fishes; otherwise, studies examining freshwater fish magnetoreception are limited.

In elasmobranchs, it has been suggested that there may be more than one mechanism responsible for sensing magnetic fields. For example, Anderson et al. (2017) behaviorally conditioned sharks to respond to magnetic stimuli using a food reward. However, when neodymium magnets were attached to the sharks, their conditioned responses were weakened, indicating impairment of their magnetic sense. Yellow stingrays (*Urobatis jamaicensis*) were

also behaviorally conditioned to associate a magnetic stimulus with a food reward, further demonstrating elasmobranchs' use of magnetic cues for navigation and locating resources (Newton and Kajiura 2017). The results from these studies are consistent with a magnetite-based mechanism that may be acting independently from electromagnetic induction in elasmobranchs.

Studying magnetoreception has become one of the most difficult tasks in sensory biology for a variety of reasons. The magnetic sense is not intrinsically familiar to humans, as other senses like sight and smell are, which leads to a fundamental lack of knowledge on the magnetic information being conveyed to an animal that utilizes this sense. In addition, magnetic fields are able to pass freely through an animal's body tissues, making the location for a magnetoreceptor challenging to identify. Most studies still address one of the three aforementioned putative mechanisms. However, Natan and Vortman (2017) recently proposed a new mechanism for magnetoreception: one based on symbiotic magnetite-generating bacteria residing within animals and conferring a magnetic sense to the host.

Magnetotactic Bacteria

Perhaps the most well-studied mechanism of magnetoreception is that of magnetotactic bacteria (MTB). These bacteria are diverse, environmentally ubiquitous bacteria with a functioning organelle responsible for magnetoreception, aptly called the "magnetosome." By synthesizing their own chains of magnetic particles in the magnetosome (Figure 1), MTB can mechanically align with Earth's magnetic field, utilizing magnetoreception to move about in their environment. Unique to MTB are the approximately 30 genes responsible for biogenesis of

magnetite, which are found on the magnetosome island (MAI), within a single chromosomal region (Uebe and Shuler 2016). MTB are found across several Gram-negative phyla (e.g., Proteobacteria and Nitrospirae), exhibiting a variety of cell morphologies (Uebe and Shuler 2016; Faivre and Shuler 2008). Primarily found within sediments, MTB navigate the oxic-anoxic interface and prefer to settle within anoxic environments (Bazylinski and Lefèvre 2013). Their established presence in marine and aquatic sediments could allow for frequent interactions between MTB and fish, giving way to a tentative origin of a symbiotic relationship.

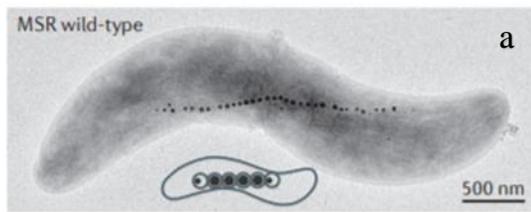
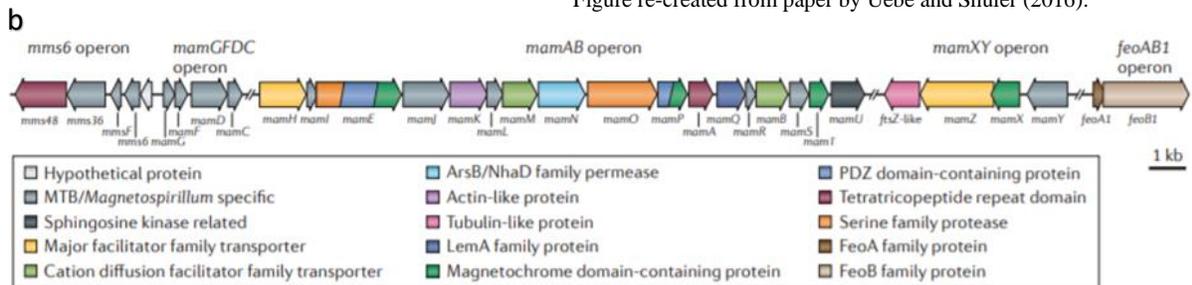


Figure 1. **a** | A transmission electron micrograph of the wild-type *Magnetospirillum gryphiswaldense* MSR-1 (MSR), a species of MTB in the Alphaproteobacteria class commonly used for MTB laboratory studies. **b** | Diagram of the magnetosome island (MAI) organized into five distinct operons. Figure re-created from paper by Uebe and Shuler (2016).



A Symbiotic Magnetoreception Mechanism

One of the possible mechanisms put forth by Natan and Vortman (2017) suggests that MTB could be located in the Harderian or lacrimal glands in vertebrates that are associated with the ophthalmic nerve, facilitating cell-to-cell communication with the host's nervous system by

aggregating in specific locations of the gland based on the animal's position at a given time. A second proposed mechanism involves MTB moving along the cornea to be visually detected by the animal; this means the MTB could act in tandem with the radical pair chemistry hypothesis, as light can also affect the orientation of MTB. Before identifying the exact mechanism involved in this proposed symbiosis, it can first be determined if MTB are, in general, present in the microbiota of magnetically sensitive animals.

Limited research has been published that explores this relationship between MTB and putative hypothetical hosts. In addition to being commonly found in marine and aquatic sediments, MTB have also been found residing within the gill epithelial cells of a marine bivalve (*Thyasira cf. gouldi*; Dufour et al. 2014) and forming biofilms that cover the surfaces of a marine protist (Symbiontida, Euglenozoa; Monteil et al. 2019). Thus, it is possible that MTB also live symbiotically within fish, where they may serve as the source of detectable magnetite and potentially communicating pertinent geomagnetic information to the host. Given the lack of information on how magnetoreception may differ in fish that inhabit different environments (marine, freshwater, or species that inhabit both at some point in their lives), obtaining data on MTB in a variety of fish is the first step to investigating the magnetic sensing hypothesis in this incredibly diverse group of organisms. This study aimed to identify evidence for the presence of MTB in fishes by mining three separate databases for existing genomic and transcriptomic data and determine any potential differences in MTB species diversity and abundance between marine, freshwater, and anadromous/catadromous fishes.

METHODOLOGY

IMG/M Data Collection

The Integrated Microbial Genomes & Microbiomes (IMG/M) system (<https://img.jgi.doe.gov/>) hosts the microbial genome and microbiome datasets sequenced at the Department of Energy's Joint Genome Institute, in addition to providing support for external microbial data to be uploaded for annotation and analysis (Markowitz et al. 2012). Using the IMG Genome browser tool, all microbiome datasets labeled as 'host-associated' and linked to a fish host specifically were identified. The IMG/M database provides a taxonomic breakdown of all DNA sequences in a project that uses a successive percent identity to display the BLAST hit distribution of the genome sample. Known species of MTB from the phyla Proteobacteria and Nitrospirae with BLAST hits were identified manually and then extracted from this distribution and assembled into a table containing their phylum, class, species, and the host species.

MGNify Data Collection

A second microbiome database, MGNify (<https://www.ebi.ac.uk/metagenomics/>), is a repository of microbial data from publicly uploaded datasets and provides a pipeline for users to upload and analyze their own microbial and environmental DNA sequencing data (Mitchell et al. 2020). Using the text search function on MGNify, all samples categorized as 'host-associated' with fish were downloaded for analysis. Samples that contain known MTB were identified and extracted from this dataset and counts of MTB within each sample were normalized to counts per 10,000 reads.

NCBI SRA Experiment Data Collection

The National Center for Biotechnology Information (NCBI; <https://www.ncbi.nlm.nih.gov/>) hosts a variety of genomic and biomedical databases and tools for analyzing and collecting genomic data. The Sequence Read Archive (SRA) Experiments database is a repository of high-throughput sequencing data (including raw sequencing data, metagenomic data, and environmental survey data) available for download and analysis (Leinonen et al. 2011). The SRA database also provides taxonomic assignments of all submitted raw sequencing data using the SRA Taxonomy Analysis Tool (STAT). STAT uses precomputed k-mer dictionary databases to match reads to a taxonomic hierarchy in two steps, which allows users to see a taxonomic breakdown of individual sequencing runs on the SRA database and access these data programmatically.

Genomic and transcriptomic data of ray-finned fishes (class Actinopterygii, NCBI:txid7898) were queried from the NCBI databases through the SRA database. The model fish species zebrafish (*Danio rerio*, NCBI:txid7955) was excluded from the search to maximize results representative of wild fish species. Using the advanced search feature on the SRA database, the search query

“((txid7898[Organism]) NOT txid7955[Organism]) AND "illumina"[Platform]”

was used to collect all the SRA submissions from Actinopterygii (ray-finned fishes) performed using Illumina sequencing methods.

For cartilaginous fishes, the search query

“(Chondrichthyes[Organism]) AND "illumina"[Platform]”

was used to collect all the SRA submissions from Chondrichthyes performed using Illumina sequencing methods.

Using Google’s BigQuery big data analytics cloud engine (<https://cloud.google.com/bigquery>), the SRA runs identified above were queried against 181 known MTB taxonomic IDs to return any possible matches for MTB found within the runs based off of the SRA taxonomic breakdown (Figure 2). This query returns a table of matches, including the MTB genus, species and/or strain, host organism, experiment number, and counts of specific MTB matches in that sample. Data were extracted from this table to only include matches with a self-count ≥ 1 , as the self-count number indicates the most specific taxonomic assignment that can be made. Environment of the host organism – marine, freshwater, anadromous, catadromous, or amphidromous – was identified using FishBase (<http://www.fishbase.org/search.php>), the largest available database of fish species, then added to allow for testing of differences between host organism environment and MTB diversity. The Shannon diversity index was used to calculate the diversity of MTB within each category of environment.

```

SELECT meta.acc, meta.assay_type, meta.center_name, meta.consent, meta.experiment, meta.sample_name, meta.instrument,
meta.librarylayout, meta.libraryselection, meta.librarysource, meta.platform, meta.sample_acc, meta.biosample, meta.organism,
meta.sra_study, meta.releasedate, meta.bioproject, meta.mbytes, meta.loaddate, meta.avgspotlen, meta.mbases, meta.insertsize, tax.acc,
tax.tax_id, tax.rank, tax.name, tax.total_count, tax.self_count, info.total_spot_count, info.analyzed_spot_count, info.unaligned_spot_count,
info.identified_spot_count
FROM (`nih-sra-datastore.sra_tax_analysis_tool.tax_analysis` as tax
INNER JOIN `nih-sra-datastore.sra_tax_analysis_tool.tax_analysis_info` as info ON tax.acc = info.acc)
INNER JOIN `nih-sra-datastore.sra.metadata` as meta ON tax.acc = meta.acc)
WHERE tax.acc = 'SRR2146930' AND tax.tax_id IN (1191478, 2614439, 2614438, 2609294, 2609293, 2609292, 2496818, 2032652, 2032651,
2032650, 2032649, 2032648, 2032647, 2032646, 2026759, 2026758, 1852403, 1852402, 1852401, 1852400, 1852399, 1852398, 1852397,
1852396, 1852395, 1836675, 1836674, 1552955, 1552954, 1472297, 1472292, 1434232, 1407050, 1407041, 1407040, 1407039, 1407035,
1407033, 1407032, 1407029, 1407026, 1293623, 1293622, 1191479, 1191478, 1124597, 451514, 304587, 259280, 162171, 156889, 2681467,
2681466, 2681465, 2617991, 2053833, 1979370, 1895778, 1740607, 1680635, 1663591, 1639348, 1545836, 1503910, 1497550, 1497549,
1497548, 1486080, 1437059, 1430440, 1407051, 1407049, 1407048, 1407047, 1407046, 1407045, 1407044, 1407043, 1407042, 1407038,
1407037, 1407036, 1407034, 1407031, 1407030, 1407028, 1407027, 1407025, 1407024, 1407023, 1354753, 1285243, 1285242, 1244869,
1105283, 1007128, 908842, 889077, 889076, 611300, 590051, 590050, 572961, 572960, 503941, 431944, 425942, 402646, 354119, 342108,
304586, 304585, 304584, 304583, 272627, 267354, 267351, 267350, 267349, 259282, 106545, 100868, 84159, 55518, 31872, 13134, 188,
2685734, 1902596, 1902595, 1449797, 1449796, 1288970, 1182780, 549691, 2637623, 2024836, 1697223, 1697222, 1313115, 28181,
1455061, 511466, 430691, 430690, 40118, 29290, 1609970, 995730, 995729, 947515, 1304872, 1206767, 573370, 184917, 2739430, 2641025,
1509431, 1392875, 1141979, 1141978, 890399, 418099, 418098, 2677082, 1817379, 1817353, 1463558, 1141977, 1141976, 2682548, 43945,
2642280, 2006184, 1246637, 1246635, 1073250, 2479342, 71996, 947516, 170969)

```

Figure 2. Example search query used on BigQuery to search NCBI SRA runs for MTB matches. This query works by matching the 6-digit Taxonomy IDs of known MTB species to the completed taxonomical assignments within the SRA runs being searched.

RESULTS

IMG/M and MGnify Databases

In the IMG/M dataset, eight of the 12 total metagenomic analyses returned one or more known MTB present in the BLAST hit distribution. These MTB belonged to the phyla Proteobacteria and Nitrospirae, with four unique species detected. Three unique fish species were identified in the analyses with MTB: one freshwater (10 of the 19 MTB detections; two unique MTB species), one marine (one MTB detection; one MTB species), and one catadromous species (eight of the 19 MTB detections; four unique MTB species). The most common MTB species, *Magnetococcus marinus* (NCBI:txid1124597), was detected in all three projects (and, thus, all three aforementioned species), accounting for eight of the 19 MTB matches in total.

| Taxon OID | Sample Name | MTB Species Match |
|------------|---|--------------------------------------|
| 3300013941 | Epidermal mucus viral and microbial communities from European eel in Spain | Candidatus Magnetobacterium casensis |
| 3300013941 | Epidermal mucus viral and microbial communities from European eel in Spain | Candidatus Magnetoovum chiemensis |
| 3300013941 | Epidermal mucus viral and microbial communities from European eel in Spain | Desulfovibrio magneticus |
| 3300013941 | Epidermal mucus viral and microbial communities from European eel in Spain | Magnetococcus marinus |
| 3300014042 | Epidermal mucus viral and microbial communities from European eel in Spain - Ebro delta (0.22 um filter) | Candidatus Magnetobacterium casensis |
| 3300014042 | Epidermal mucus viral and microbial communities from European eel in Spain - Ebro delta (0.22 um filter) | Candidatus Magnetoovum chiemensis |
| 3300014042 | Epidermal mucus viral and microbial communities from European eel in Spain - Ebro delta (0.22 um filter) | Desulfovibrio magneticus |
| 3300014042 | Epidermal mucus viral and microbial communities from European eel in Spain - Ebro delta (0.22 um filter) | Magnetococcus marinus |
| 3300002512 | Gut microbiomes of Pimephales promelas (fathead minnow) during Triclosan exposure experiment - DOC9- baseline | Desulfovibrio magneticus |
| 3300002512 | Gut microbiomes of Pimephales promelas (fathead minnow) during Triclosan exposure experiment - DOC9- baseline | Magnetococcus marinus |
| 3300002513 | Gut microbiomes of Pimephales promelas (fathead minnow) during Triclosan exposure experiment - D14H1- High Triclosan exposure day 14 | Desulfovibrio magneticus |
| 3300002513 | Gut microbiomes of Pimephales promelas (fathead minnow) during Triclosan exposure experiment - D14H1- High Triclosan exposure day 14 | Magnetococcus marinus |
| 3300002520 | Gut microbiomes of Pimephales promelas (fathead minnow) during Triclosan exposure experiment - D14H2- High Triclosan exposure day 14 | Desulfovibrio magneticus |
| 3300002520 | Gut microbiomes of Pimephales promelas (fathead minnow) during Triclosan exposure experiment - D14H2- High Triclosan exposure day 14 | Magnetococcus marinus |
| 3300002521 | Gut microbiomes of Pimephales promelas (fathead minnow) during Triclosan exposure experiment - D14S2- solvent control exposure day 14 | Desulfovibrio magneticus |
| 3300002521 | Gut microbiomes of Pimephales promelas (fathead minnow) during Triclosan exposure experiment - D14S2- solvent control exposure day 14 | Magnetococcus marinus |
| 3300002517 | Gut microbiomes of Pimephales promelas (fathead minnow) during Triclosan exposure experiment - D14S4- solvent control exposure day 14 | Desulfovibrio magneticus |
| 3300002517 | Gut microbiomes of Pimephales promelas (fathead minnow) during Triclosan exposure experiment - D14S4- solvent control exposure day 14 | Magnetococcus marinus |
| 3300000349 | Turbot gut microbial communities from Qingdao, China | Magnetococcus marinus |

Table 1. Full list of positive MTB matches found within the host-associated fish microbial samples hosted on the IMG/M database. Taxon OIDs correspond to the taxonomy identifiers on IMG/M. The sample names seen here are the exact samples used on IMG/M and correspond to the different analyses conducted within each study. The third column contains the specific MTB species detected in that sample.

In the MGnify dataset, 1,791 analyses matched the criteria for inclusion. Of these, 173 analyses returned ≥ 1 matches to MTB (e.g., one or more MTB identified per run), with 27.8% of the individual matches (n = 48) having a normalized count ≥ 1 and 72.2% having a normalized count < 1 (n = 125). MGnify identified two unique MTB species (*Magnetococcaceae bacterium I 56m* and *Magnetovibrio blakemorei*), five unique MTB genera (*Candidatus Magnetobacterium*, *Magnetococcus*, *Magnetospira*, *Magnetospirillum*, and *Magnetovibrio*), one unique MTB family (Magnetococcaceae), and one unique MTB order (Magnetococcales) across the positive matches to MTB. Of the 1,791 analyses, just 33 are metagenomic data, while the remaining 1,758 contain amplicon data. All analyses that returned MTB matches were derived from amplicon data.

NCBI SRA Experiments Database

Within Actinopterygii, more than 157,000 SRA datasets matched the criteria for inclusion. From those sequencing datasets, a total of 627 matches to MTB were detected and 31 unique species or strains of MTB were identified. Of these, 410 matches were identified to the species level, 215 were identified to the genus level (9 of these listed as ‘unclassified *Magnetospirillum*’), and 2 were identified to the order level. Of the matches identified to the species level, 234 were identified to a specific strain, with strains from the genus *Magnetospirillum* (n = 140; 59.8%) accounting for a majority of the strain matches.

97 unique species of fish were detected to contain MTB, with freshwater species accounting for 54.6% (n = 53), marine species 29.9% (n = 29), and species that inhabit both environments at some point in their lifetime (anadromous, catadromous, and amphidromous

species) 15.5% (n = 15) of the fish identified.

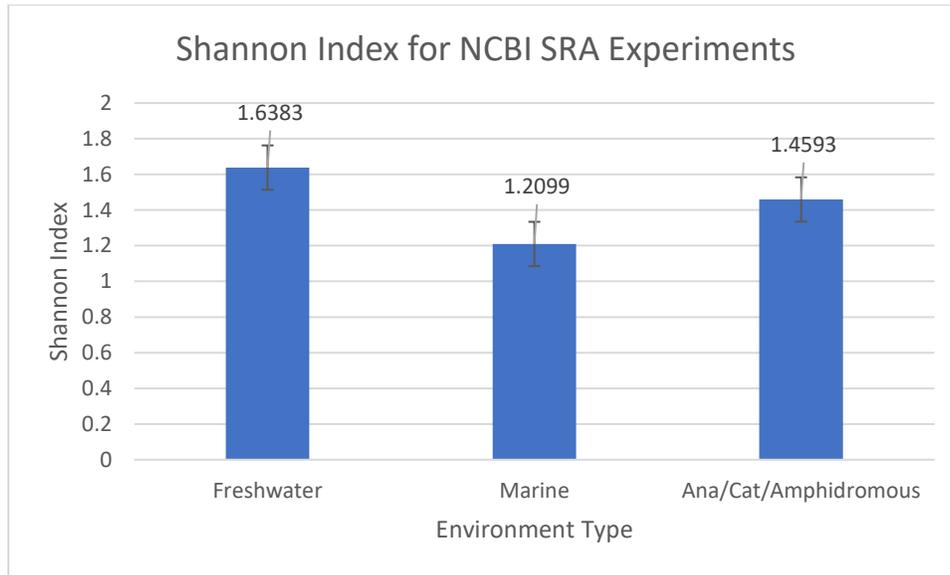


Figure 3. Bar graph depicting the normalized Shannon diversity index calculations of the MTB species within the Actinopterygii NCBI SRA Experiments data from three environments: freshwater, marine, and anadromous/catadromous/amphidromous species.

The normalized Shannon diversity index calculation, seen in *Figure 3* above, demonstrates that of the three environments tested, the freshwater fish species SRA data had the highest MTB species diversity, and the marine environment had the lowest relative to the other environments. Given that the majority of the SRA data originated in freshwater species, this is to be expected; however, the diversity index seen in multi-environment species is higher than the marine index, with the marine species accounting for double the amount of the data in the dataset compared to the multi-environment species.

In the Chondrichthyes dataset, 2,025 SRR accessions of cartilaginous fish that were sequenced using Illumina methods were searched for MTB matches. Of these, the BigQuery

search returned a single match – a transcriptomic sample taken from the retina of a blue-spotted stingray (*Neotrygon kuhlii*, NCBI:txid651721). This run contained 1 self-count match to the MTB species *Magnetospirillum gryphiswaldense* MSR-1 v2 (NCBI:txid1430440; a strain of *M. gryphiswaldense*).

DISCUSSION

While data on the presence of host-associated MTB within fishes is still limited, results from this study demonstrate that MTB can be found within fish samples from varying species, environments, and geographic locations. MTB were detected in both freshwater and marine species, in addition to fish that inhabit both at some point in their lifetime (where navigation between the two environments is key to their survival and reproduction).

The IMG/M database contained the smallest dataset out of the three databases, but one of the three studies that returned MTB matches is of particular interest – an experiment by Narrowe et al. (2015) examining the effects of triclosan, an antibacterial agent, on fathead minnows (*Pimephales promelas*), a type of freshwater teleost. This study exposed the fish to environmentally relevant levels of triclosan over a 7-day period, then extracted gut samples at four different times post-exposure: prior to exposure as a baseline, immediately following exposure, 1 week post-exposure, and 2 weeks post-exposure (in addition to a group exposed to methanol, a solvent, to act as the control). Two MTB species, *Desulfovibrio magneticus* and *Magnetococcus marinus*, were detected in three of the sample types: baseline, solvent, and 1 week post-exposure to triclosan. As triclosan is a strong antibacterial that kills a broad range of microorganisms, the consistent presence of MTB in the fish, even after triclosan exposure, is noteworthy. These two MTB species were also detected in the first project (Table 1), examining the epidermal mucus of European eel (*Anguilla sp.*), and *Magnetococcus marinus* was the one MTB species match returned for the third project (extracted from turbot, *Scophthalmus maximus*).

Given the magnitude of data available to analyze from the NCBI SRA database, several notable species containing MTB matches were found. One, the Japanese eel (*Anguilla japonica*, NCBI:txid7937), returned 17 SRR matches to MTB, with 15 matches identified to the species level (with eight different species detected) and two identified to the genus level of *Magnetospirillum*. This eel, a catadromous species in the same genus as the European eel identified in the IMG/M dataset, has demonstrated a magnetic sense in an experimental setting in which a magnetic field was applied (Nishi et al. 2004). This study suggested that a magnetoreceptor in the Japanese eel may be present in the olfactory region, so further research utilizing samples from the nares could be beneficial for examining the magnetic sense in anguillid eels.

Another species of interest is the Atlantic herring, *Clupea harengus* (NCBI:txid7950). This forage fish, which is an incredibly important species to fisheries, is unique in that it does not have a typical lateral line. The lateral line is an important organ present in fish, usually visible as a faint line running horizontally along the middle of the body, that detects movement and pressure in the water. In herring, the lateral line organ appears to be confined to the head region (Jørgensen 1985), which raises questions about how navigation and environmental sensing in herring may differ from other fish. The SRA database returned 5 individual matches to MTB in *C. harengus*, all within the *Magnetospirillum* genus. As herring is a major food fish, identifying the mechanism for magnetoreception in this unique fish could be useful in understanding their migration and movement patterns.

A singular match of Chondrichthyes that contained MTB was sourced from the retina of a

stingray; as the magnetic sensing hypothesis suggests that magnetoreception could take place in the ocular region, this match is in line with the proposed location of the mechanism. The majority of fish samples hosted in these databases are taken from other bodily tissues, such as the liver or muscle, which reduces the likelihood of detecting MTB within existing data if MTB do not confer the magnetic sense to its host through these tissues. While this highlights a limitation of this project – a lack of data containing samples from the specific tissues suggested to contain MTB in the magnetic sensing hypothesis – detecting MTB across many species and sample sources demonstrates the ubiquitous presence of MTB in marine and aquatic environments, including the microbiota found in fish.

Implications & Impact

As anthropogenic activity in the ocean increases, understanding magnetoreception in fish and the consequences of human-induced magnetic disturbances has become increasingly important (Forland and Sivle 2020). Few studies have examined the effects of electromagnetic surveys on animal behavior. However, these activities can generate magnetic fields within the detectable range of magnetoreceptive marine animals, possibly affecting the migration and orientation of these species, especially within elasmobranchs (Nyqvist et al. 2020).

While it remains unknown how exactly humans disturb electromagnetic fields in marine environments, one potential positive introduction is through the use of magnets as a bycatch reduction method (i.e., a magnetic repellent). Many species of migratory fish and sharks are threatened or endangered, with bycatch contributing to their increased mortality rates (Dapp et

al. 2016). Thus, the need for bycatch mitigation is vital to recovering these species. One promising example was the approximately 30% reduction in benthic shark bycatch in a trap fishery after permanent magnets were placed on traps (Richards et al. 2018). Other studies, such as those of longline and hook-and-line fisheries, have shown less successful results with no significant difference or even an increase in bycatch after introduction of magnets (Porsmoguer et al. 2015; Favaro and Côté 2015). Understanding the mechanisms responsible for the ability of fishes to discern these magnetic stimuli could provide a better basis for creating deterrents to reduce bycatch of magnetically sensitive marine species.

Elucidating the magnetic sense of animals is also crucial to strengthening our understanding of migration and navigation. Migration likely involves more than just one cue (e.g., temporal and olfactory cues), but the magnetic sense undoubtedly plays a role in navigating to the extremely specific locations that many migratory species travel to. As we discover more about the importance of host-associated microbiota across countless organisms (including humans), a symbiosis that relays information about the geomagnetic field to its host is gaining evidence.

Future Research

Studies investigating the relationships that fishes may have with MTB will need to employ methods that target MTB within specific tissues, such as neural or olfactory tissues. Developing a high-throughput genetic assay that utilizes conserved MTB genes found on the MAI as diagnostic genes may be an effective way to identify the presence of MTB and could

then be used to identify MTB in other organisms known to possess a magnetic sense.

Future studies could also compare the diversity and abundance of MTB within migratory and non-migratory species of fish. Given the importance of migration to many species of teleosts and elasmobranchs, there may be a difference in the taxonomic compositions and abundances of MTB found in fish that travel long distances, as opposed to fish that inhabit a single area for the duration of their lifetime. Identifying any differences (or lack thereof) may provide insight into the origins of magnetoreception in fish, especially if compared to the MTB abundance and diversity found in aquatic sediment samples from different geographic locations. The magnetic sensing hypothesis is still very new and will require much more research to provide support for its plausibility, but the results presented in this thesis and other recent works give it a promising future.

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