Life History Analysis and Identification of Potential Nursery Grounds for Juvenile Manta Rays

By

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April 24, 2020

Master's Project submitted in partial fulfillment of the requirements for the Master of Environmental Management degree in the Nicholas School of the Environment of Duke University.

Executive Summary

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Manta rays are some of the largest fish that inhabit our oceans, feeding primarily on zooplankton across tropical, subtropical, and temperate waters. There are currently two recognized species of manta – the Giant Oceanic Manta, *Mobula birostris*, and the recently resurrected Reef Manta, *Mobula alfredi* – as well as a putative third species, Mobula sp. cf birostris. Recent genetic comparisons resulted in the reabsorption of mantas into the Mobulidae family, but they are still commonly referred to as mantas, and will be referenced as such throughout this paper.

Manta rays are characterized by very conservative life history traits – late sexual maturity, relatively long lives, and exceptionally low fecundity – all of which increase their vulnerability to environmental stressors and substantially diminish their resiliency. Already listed as "Vulnerable" by the IUCN Red List, mantas are facing increasing anthropogenic pressure from targeted and bycatch fisheries that harvest their gill rakers for use in eastern medicine.

With decreasing populations, a complicated phylogenetic history, and a prevailing lack of knowledge regarding their fundamental biology, ecology, and life history, there is a pressing need for future research regarding these species. This is particularly true for juvenile manta rays, which are exceedingly rare in the wild, but are believed to be one of the most important life stages in terms of manta ray population growth and reproductive success.

Therefore, this project attempted to answer two main research questions:

1. What can the current body of manta ray research teach us about knowledge gaps and future research priorities, particularly related to the juvenile life stage?
2. Can common characteristics of known or believed manta ray nursery grounds be isolated, and used to identify other potential manta ray nursery sites?

To answer these questions, three research objectives were undertaken:

1. Primarily, to conduct a literature review of current manta ray publications, with emphasis on the juvenile life stage;
2. Secondarily, to use GIS to isolate characteristics of a recently discovered manta ray nursery in the Gulf of Mexico (Flower Garden Banks National Marine Sanctuary), and determine physical and environmental characteristics that could define a manta ray nursery ground;
3. Lastly, to use these physical and environmental characteristics to identify locations in the Gulf of Mexico with similar features that could potentially serve as yet undiscovered nursery grounds.

The literature review was conducted using a simple Google Scholar online search, followed by the Snowball Method – where selected papers were examined for additional research that could be incorporated into the review. This resulted in the compilation of 66 publications, revealing a widespread paucity of data including glaring gaps regarding sexual maturation and fecundity, lifespan, and the occurrence of and mortality rates associated with natural predation. These gaps, and many more, were especially prevalent within non-adult life stages. The majority of research to date has been conducted on *M. alfredi* (48 publications), which is understandable considering they tend to aggregate in coastal areas more frequently than *M. birostris* (33 publications). Furthermore, the bulk of the studies were in the Indian Ocean, Western Pacific Ocean, and Red Sea – 9 in Eastern Australia and 7 in Indonesia – as well as 15 that were done on a global scale.

From the literature review and the broader research conducted for this project, the preliminary characteristics believed to influence potential manta ray nursery ground locations are: (1) Water Depth, (2) Water Temperature – both seafloor temperature and sea surface temperature (SST), (3) Productivity, and (4) Distance to Deep Water. These traits were sampled by writing python scripts in a Jupyter Notebook and Spyder Script, importing the results into ArcGIS Pro for further analysis, and running both a generalized linear model (GLM) and Bayesian GLM in R Studio. All five characteristics were found to be statistically significant in both GLMs. However, these results should be cautiously interpreted due to a small sample size and the poor resolution associated with some of the data (SST in particular).

Unfortunately, even though these characteristics showed statistical significance, other, yet unidentified manta ray nursery grounds could be not identified in the broader Gulf of Mexico ecosystem. The global datasets used in this project are hosted online as imagery servers, and therefore cannot be manipulated to form the necessary probability rasters in ArcGIS Pro that would display areas with similar characteristics to Flower Garden Banks. That being said, the information gained from the literature review, knowledge gap analysis, and isolation and analysis of important nursery ground characteristics should not be ignored. Rather, this paper should serve as a basis upon which future research can build, with the ultimate goal of increasing effective protective measures and conserving global manta ray populations.

Abstract

Manta rays are zooplankton consuming filter-feeders that have a global distribution across tropical, subtropical, and temperate waters. There are two recognized species of manta – the Giant Oceanic Manta, *Mobula birostris*, and the recently resurrected Reef Manta, *Mobula alfredi* – as well as a putative third species, Mobula sp. cf birostris. Their conservative life history traits, including late sexual maturity, relatively long lives, and exceptionally low fecundity, increase manta vulnerability to environmental stressors and substantially diminish their resiliency. Currently listed as "Vulnerable" by the IUCN Red List, mantas are facing increasing anthropogenic pressure from targeted and bycatch fisheries that harvest their gill rakers for use in eastern medicine. Despite their perception as charismatic megafauna, much is still unknown about the biology, ecology, and life history of mantas, particularly relating to the juvenile life stage. Therefore, this research synthesized knowledge from published literature and identified a widespread lack of data, including glaring gaps regarding sexual maturation and fecundity, lifespan, and the occurrence of and mortality rates associated with natural predation. These gaps, and many more, were especially prevalent within non-adult life stages. From the available data, the following characteristics were isolated and sampled from a known manta ray nursery ground (Flower Garden Banks): water depth, water temperature, productivity, and proximity to deep water environments. These characteristics were applied within the Gulf of Mexico with limited results, though should form the basis for future, more in-depth studies.

Introduction

Manta Rays are zooplankton-consuming filter-feeders that have a circumglobal distribution across tropical, subtropical, and temperate waters (Couturier *et al.*, 2012). They belong to the subclass *Elasmobranchii*, which also includes sharks and all other cartilaginous fish, and falls within the *Chondrichthyes* class. On the genus-level, mantas have a long and complicated phylogenetic history – originally residing in the genus *Mobula* until a reclassification created the distinct genus *Manta*, only to have a 2018 phylogenetic reshuffling result in their re-absorption into the *Mobulidae* family that also includes seven other batoid ray species (White *et al.*, 2018). Nevertheless, they are still commonly referred to as mantas, and will be referenced as such for the remainder of this paper to distinguish them from other Mobulid rays.

Once considered monospecific, there are currently two recognized species of manta (Marshall *et al.*, 2009) – the Giant Oceanic Manta Ray, *Mobula birostris* (Walbaum 1792); and the Reef Manta Ray, *Mobula alfredi* (Krefft 1868) – as well as a third putative species, Manta sp. cf birostris (Hinojosa-Alvarez *et al.*, 2016), that is believed to occur in the Caribbean Sea, Gulf of Mexico, and along the east coast of the United States. However, the Reef Manta Ray was only recently resurrected in 2009 (Marshall *et al.*, 2009), due to perceived morphological distinctions from the formerly all-inclusive *Manta birostris* species. The resurrection of a second species only adds to mantas' complicated phylogenetic history, and casts uncertainty on the validity and accuracy of all research conducted prior to 2009, as data may have been collected on either species, with results solely attributed to *Mobula birostris* (Couturier *et al.*, 2012).

M. birostris can grow to disc widths (DW) of up to 7m (Stewart *et al.*, 2018), and is generally regarded as the more pelagic of the manta species (Graham *et al.*, 2012). Meanwhile, *M. alfredi* is believed to frequent known aggregation sites in coastal waters (Braun *et al.*, 2015) and reach a maximum DW of about 5m (Stewart *et al.*, 2018). The two species can also be distinguished based on their dorsal coloration, as *M. birostris* typically have a "T" shaped pattern, while *M. alfredi* have more of a "Y" shaped pattern (Marshall *et al.*, 2009). On an individual level, mantas can be identified using their unique ventral spot patterns (Town *et al.*, 2013), which are thought to remain unchanged throughout their lives, and have been used to study their distribution (Couturier *et al.*, 2011), residency and site affinity (Couturier *et al.*, 2014), and generate preliminary population estimates (Marshall *et al.*, 2011).

Elasmobranchs as a whole are characterized by conservative life history traits, and mantas are no exception – their late sexual maturity, relatively long lives, and exceptionally low fecundity are extreme even among elasmobranchs. Individuals can live for more than 50 years, do not mature until between the ages of 8 – 10 years, and normally give birth to a single pup once every two to five years after a 12-month gestation period (Deakos, 2012). Despite mantas being a seemingly K-Selected species, studies have noted unusually limited post-birth parental investment in offspring (Marshall & Bennett, 2010). These characteristics makes mantas considerably more vulnerable to anthropogenic and environmental stressors, and substantially diminish their resiliency in the face of increasing threats (Deakos *et al.*, 2011).

Already listed as "Vulnerable" by the IUCN Red List, which is just one step below "Endangered," it is widely believed that populations of both manta species are in decline, signaling the potential for an endangered listing in the next volume of the IUCN Red List. In addition to the aforementioned life history traits that allow for minimal reproductive capability, manta rays are also facing increasing anthropogenic pressure from expanding fisheries *(Lewis et al.*, 2015). Targeted fisheries and illegal harvesting of manta gill rakers (gill plates or branchial filaments) are on the rise, with their gill rakers being sold for use in traditional eastern medicine, despite a lack of historical use (Croll *et al.*, 2016). Bycatch in other fisheries is also of increasing concern, especially in Southeast Asia where many small-scale and artisanal fisheries are not as strictly regulated (Acebes *et al.*, 2016). Though the meat is generally considered to be worth very little (White *et al.*, 2006), the FAO reported a dramatic increase in mobulid catches from 900 to 3300 tonnes between 2000 – 2007. Furthermore, this reporting does not even consider other factors of mortality, like unreported catches, incidental bycatch, boat strikes, entanglement, or natural predation (O'Malley *et al.*, 2013).

Perhaps one of the most dangerous, albeit unseen, threats facing manta rays today is the simple paucity of data. Manta rays are maybe the best studied of the *Batoidea* superorder, which is comprised of over 600 species and 26 families, yet there is still very little known about the fundamental biology and ecology of these elusive animals. This lack of data could even provide a partial explanation as to why mantas are "only" listed as vulnerable, although more research may suggest an endangered listing to be more appropriate. Even less is known about juvenile manta rays, which are extremely elusive and rarely seen in the wild (Stewart *et al.*, 2018). Meanwhile, the importance of the juvenile life stage should not be understated, particularly for a species like the manta, which remains an immature juvenile for an extended period of time.

Smallegange *et al.* 2016 found that, when modeling the effect of increased annual manta survival rates on population growth rate, mean lifetime reproductive success, and cohort generation time, an increase in the juvenile survival rate always had a larger impact than the same increase in newborn or adult survival rates (Smallegange *et al.*, 2016). This emphasizes the importance of the juvenile life stage in manta rays, supporting the idea that improved protection for young individuals will benefit the population as a whole. Moreover, since juvenile mantas, along with juvenile elasmobranchs in general, are believed to inhabit nursery grounds, these create ideal areas to implement strengthened conservation measures. This is especially true when comparing juveniles to adults, which are typically believed to be more migratory and occupy much larger home ranges (Kessel *et al.*, 2017). Therefore, juveniles should be considered vital, and their effective protection is crucial to sustaining healthy manta ray populations.

With this in mind, the two major research questions addressed by this project were:

1. What can the current body of manta ray research teach us about knowledge gaps and future research priorities, particularly related to the juvenile life stage?
2. Can common characteristics of known or believed manta ray nursery grounds be isolated, and used to identify other potential manta ray nursery sites?

Within this paper, the primary research objective was to conduct a literature review that undertook a life history and knowledge gap analysis of current manta ray research, with emphasis on the juvenile life stage. Secondarily, I aimed to isolate characteristics of a recently discovered manta ray nursery in the Gulf of Mexico (Flower Garden Banks National Marine Sanctuary, United States), and used information gleaned from the literature review, to determine physical and environmental characteristics that could define a manta ray nursery ground. Lastly, I hoped to use these physical and environmental characteristics to identify locations in the Gulf of Mexico with similar features that could potentially serve as yet undiscovered *nursery* grounds.

Methods

Location/Scale

The literature review portion of this project was conducted on a global-level in order to engage with as much current manta ray literature as possible, so that gaps could be identified at both a global and regional scale, depending on conservation needs. Meanwhile, the nursery ground identification portion focused on Flower Garden Banks National Marine Sanctuary (27.8841 °N, 93.8147 °W) in the Gulf of Mexico.

Life History & Knowledge Gap Analyses

The life history and knowledge gap analyses were completed by conducting a literature review of current manta ray publications, beginning with a simple search on Google Scholar. General search terms included "Manta Ray," "Mobula Ray," "Manta birostris," "Manta alfredi," "Mobula birostris," "Mobula alfredi," and "life history," as well as juvenile specific terms such as "juvenile," "newborn," "baby," "young," and "neonate." Once the searches had been exhausted, the relevant papers were analyzed and the "Snowball Method" was applied to expand the search – selected papers had their literature cited sections examined for additional publications that could be incorporated into the literature review.

Each paper was subsequently reviewed and certain aspects were noted, including author, year of publication, title, study site, species studied, and life stage studied. Additionally, each paper was categorized into at least one of five broad categories, as well as at least one corresponding subcategory:

1. Life History
    a. Population Structure
    b. Population Size
    c. Lifespan
    d. Sexual Maturity
    e. Size Measurements
    f. Fecundity
    g. Predation

2. Habitat Use
    a. Nursery Ground
    b. Site Affinity
    c. Mating
    d. Feeding
    e. Range Expansion
    f. Migration
3. Phylogeny
    a. Morphology
    b. Genetics
4. Human Use
    a. Ecotourism
    b. Targeted Fishery
    c. Fishery Bycatch
5. Synthesis
    a. Synthesis

Many papers covered numerous topics and/or multiple species; in which case they were noted for each. This categorical breakdown was done so that knowledge gaps could be readily quantified and pinpointed in order to inform future research priorities. Within the context of the literature review, there were a few restrictions that served to limit the number of papers which were included. The papers had to be (1) peer-reviewed publications in academic journals, to which access was obtainable, (2) conducted on manta rays in the wild (i.e. not in captivity), and (3) published in English.

Identification of Potential Nursery Grounds

There is currently only one confirmed manta ray nursery ground in the published literature – an *M. birostris* nursery at Flower Garden Banks (FGB) National Marine Sanctuary in the Gulf of

Mexico (Stewart *et al.*, 2018). From the paper by Stewart *et al.*, other publications used for the literature review, as well as some that were not included (for example, articles on general elasmobranch nursery grounds), the following preliminary characteristics were hypothesized to be preferable for mantas, particularly juveniles, and indicative of a possible nursery ground: (1) Water Depth, (2) Water Temperature – Seafloor Temperature and Sea Surface Temperature (SST), (3) Productivity, and (4) Distance to Deep Water.

Once the characteristics had been identified, an online search was conducted to find appropriate data that could be manipulated and analyzed with the hopes of finding areas with similar traits. ESRI provided many of the datasets, including data on bathymetry, which was used to determine water depth (https://oceans2.arcgis.com/arcgis/rest/services/Seafloor_Bathymetry/ImageServer), seafloor temperature (https://oceans2.arcgis.com/arcgis/rest/services/Seafloor_Temperature/ImageServer), sea surface temperature (https://earthobs2.arcgis.com/arcgis/rest/services/REMSS_SeaSurfaceTemp/ImageServer), and chlorophyll levels, which were used as a proxy for productivity (https://earthobs3.arcgis.com/arcgis/rest/services/Chlorophyll/ImageServer). Areas of deep water were determined by the presence of certain seafloor geomorphology features – canyons, basins, shelf valleys, and slope – with data provided by Blue Habitats (http://www.bluehabitats.org/?page_id=58).

Since most of the available data were in the form of global data sets that are hosted online as imagery servers, they could not be manipulated and analyzed in ArcGIS Pro as initially intended. Instead, python scripts were written in a Jupyter Notebook so that the data could be visualized as the code was written to ensure proper functioning. Then the code was transferred to a Spyder Script so that the script could be incorporated into an ArcGIS Pro model upon completion. The script was responsible for creating polygons that encompassed Flower Garden Banks from the boundary data that was provided by the National Oceanic and Atmospheric Administration's

(NOAA) National Centers for Environmental Information (https://service.ncddc.noaa.gov/website/google_maps/FGB/download_data.htm).

Next, 300 random points were generated within these FBG polygons and used as proxies for confirmed juvenile manta ray sightings, as demonstrated by the Stewart *et al.* paper. 5,000 random points were generated outside of these polygons, but still within the Gulf of Mexico, and used as proxies for habitat where juvenile manta rays were absent. The 300 FGB points and the 5,000 Gulf of Mexico points were sampled for water depth, water temperature (seafloor and sea surface), and productivity, with the respective data exported to two, separate excel spreadsheets. Next, all the points were added to a map in ArcGIS Pro and joined with the excel sheets so that each point contained its sampling data for water depth, water temperature, and productivity. Distance to the nearest deep-water geomorphology feature was calculated in ArcGIS Pro and added to each point, before exporting the complete data into two new excel spreadsheets.

Statistical Analyses

These new spreadsheets were then imported into R Studio and joined together before running both a generalized linear model (GLM) and a Bayesian generalized linear model, in order to determine the statistical significance of each environmental characteristic. In addition to the p-vale of each covariate, the AIC scores of the different models were compared to identify the model that best fit the data.

Results and Discussion

Life History & Knowledge Gap Analyses

In total, the literature review compiled and analyzed 66 papers concerning manta rays – 48 of which dealt with *M. alfredi*, and 33 that researched *M. birostris*. A full literature cited of these papers can be found in **Appendix 1**. There were a number of papers that dealt with both *M. alfredi* and *M. birostris*, as well as one that focused exclusively on the third putative manta species in the Gulf of Mexico, which explains why the number of papers for each species (48, 33, and 1, respectively) do not add up to the total number of papers from the literature review (66). As shown in **Figure 1**, 89% of the publications reviewed were published since 2010, though a sharp drop-off should be noted in 2017. I hypothesize that this distinct drop in publications in 2017 may have resulted from the political and administrative changes occurring in the United States at the end of 2016, and reduced funding for scientific research, though this claim should be explored further.

Only 7 reviewed papers were published prior to 2010, with multiple years having zero publications (2004, 2005, and 2007, as well as the years 2000 – 2002 which were omitted from **Figure 1**). The trend shown by the increasing number of annual manta ray publications is promising and could indicate that mantas have become more of a research priority over the past decade. The true cause for this rise in publications is unknown and beyond the scope of this project, but suggests an increased emphasis on mantas, and could represent increased interest in rays in general, or perhaps even elasmobranchs as a whole.

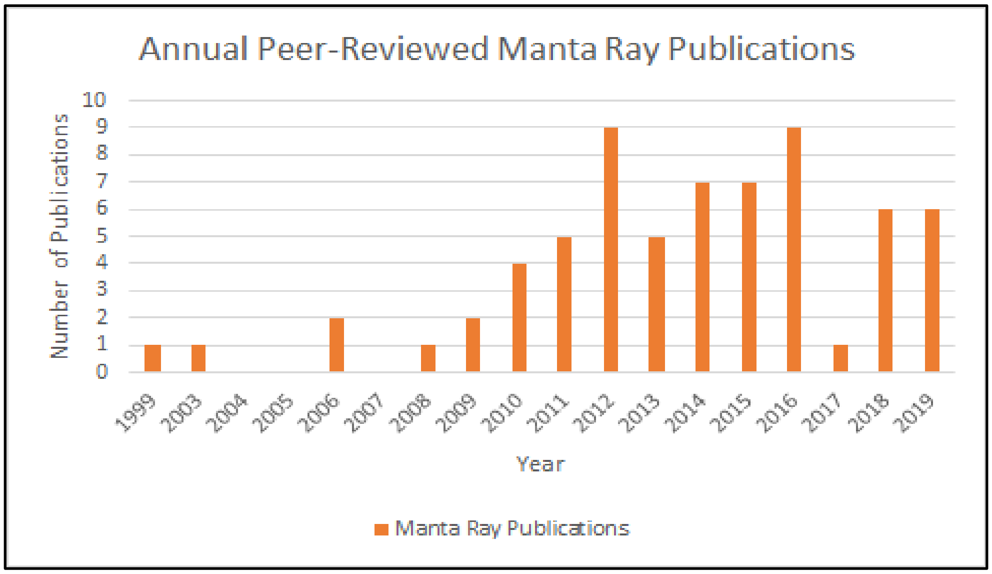

Figure 1. Graph showing the annual number of peer-reviewed manta ray publications, from 1999 – 2019.

Geographically, a majority of manta research has been conducted in the Indian Ocean and Western Pacific Ocean, with only a limited number of studies around the Americas, as seen in **Figure 2**. To date, the studies conducted off the coast of the Americas have primarily focused on *M. birostris*, other than one study off the coast of Costa Rica and a few further out in the Pacific Ocean, near Hawaii. This could indicate that there is a cut-off in the range of *M. alfredi*, and perhaps they have not historically existed east of Hawaii due to a lack of connecting coastal habitat between Hawaii and North and South America that would allow them to travel from one location to another. This theory is supported by the Arauz *et al.* 2019 paper which represents an opportunistic encounter of *M. alfredi* off the coast of Costa Rica, and suggests the possibility of either an accidental migration to the area, or a range expansion for the species (Arauz *et al.*, 2019). Moreover, the open water distance between Southeast Asia/Australia and Hawaii is also significant, meaning substantial travel would be necessary for any *M. alfredi* individual to reach the Pacific Islands in the first place, and indicates long distance travel by this species is feasible.

For the most part, and even in areas where both species of manta have been studied, *M. alfredi* is the predominantly studied species. The only exception to this is in Indonesia, where the second-highest number of studies have been conducted (7), and four of those studies concerned *M. birostris*. The east coast of Australia is the geographic area with the highest number of studies (9), though it is very skewed towards *M. alfredi*, with only one paper concerning *M. birostris*. There have also been a number of studies of *M. alfredi* in the Red Sea (4), indicating the potential for a resident population in the area. This is worthy of future research to determine if the population is stable, or if there is significant and consistent immigration and emigration. If the population is truly stable and relatively isolated, then genetic studies should be conducted to determine whether speciation is occurring (or has already occurred), as this population could form the basis for a fourth manta species, similar to the formation of the putative third species believed to reside in the Gulf of Mexico.

The large half-blue and half-orange circle situated above the map represents the 15 papers that had a global scale. For those papers, the split is nearly even, with 13 focusing on *M. birostris* and 14 studying *M. alfredi*. These global studies were primarily synthesis papers than aggregated data from other field studies, although a few did take samples in multiple locations around the world, primarily for genetic comparisons.

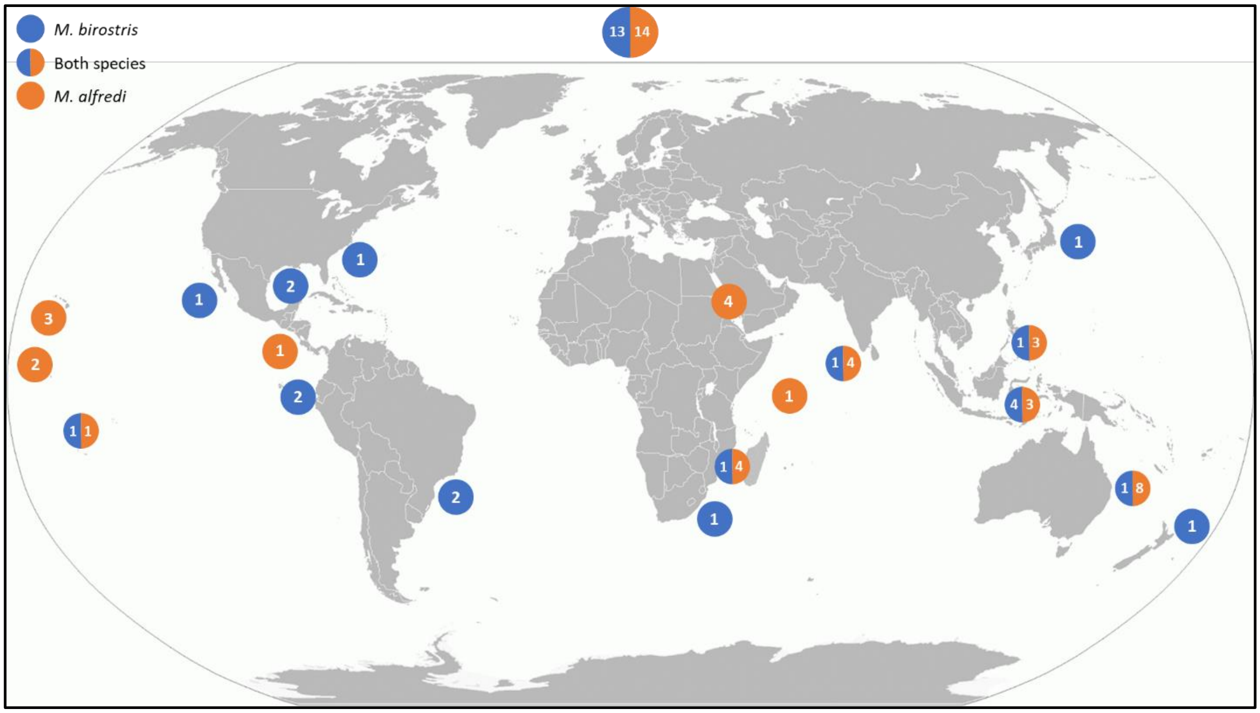

Figure 2. Map displaying geographic study sites for the 66 papers reviewed for this project. Blue circles correspond to sites where *M. birostris* has been studied, orange to sites where *M. alfredi* has been studied, and half-blue half-orange to sites where both species have been studied.

The overall results from the literature review categorization can be seen in **Table 1**. Again, the numbers do not add up to 66 (the total number of papers reviewed in this study) because many papers dealt with numerous topics and were therefore counted in multiple categories. The data overwhelmingly shows that *M. alfredi* is the more studied of the two species, which can also be seen in **Figure 3**, where *M. alfredi* are shown by the orange bars. This could be surprising considering the resurrection of *M. alfredi* only occurred in 2009, and all research prior to then was attributed to *M. birostris*. However, the vast increase in publications over the last decade has seemingly offset this initial phylogenetic error. Furthermore, *M. alfredi* is believed to be the more coastal species with known aggregation sites, making it easier to study than the more pelagic *M. birostris*.

The most common broad topic identified by the literature review was Habitat Use. Site Affinity and Foraging acted as the most commonly researched subcategories, accounting for 62.5% of the research conducted on manta ray habitat use and reinforcing the idea that there are fundamental unknowns regarding manta ray life history. Additionally, the most apparent knowledge gaps are found in the Life History category – Lifespan, Fecundity, and Predation – all three of which are completing lacking for either the Reef or Oceanic Manta.

		Adult		Juvenile (Both)	
		M. birostris	*M. alfredi*	*M. birostris*	*M. alfredi*
Life History Traits	Population Structure	3	12	2	6
	Population Size	1	4	0	2
	Lifespan	1	0	0	0
	Sexual Maturity	4	7	3	5
	Size Measurements	3	7	3	5
	Fecundity	0	2	0	2
	Predation	0	2	0	0
Habitat Use	Site Affinity	6	14	1	4
	Mating	2	5	0	3
	Foraging	5	10	1	1
	Migration	1	4	0	0
	Range Expansion	4	3	2	0
	Nursery Ground	1	1	2	1
Phylogeny	Genetics	5	4	0	0
	Morphology	2	4	1	1
Human Impacts	Ecotourism	1	3	0	2
	Targeted Fishery	4	4	0	0
	Fishery Bycatch	4	4	2	1
Synthesis	Synthesis	6	7	2	3

Table 1. Table showing the complete classification of all 66 papers reviewed in this study for adults and juveniles of both species, broken down by category and subcategory. Papers that included minimal data on at least one juvenile manta, in addition to adults, were included under the juvenile column.

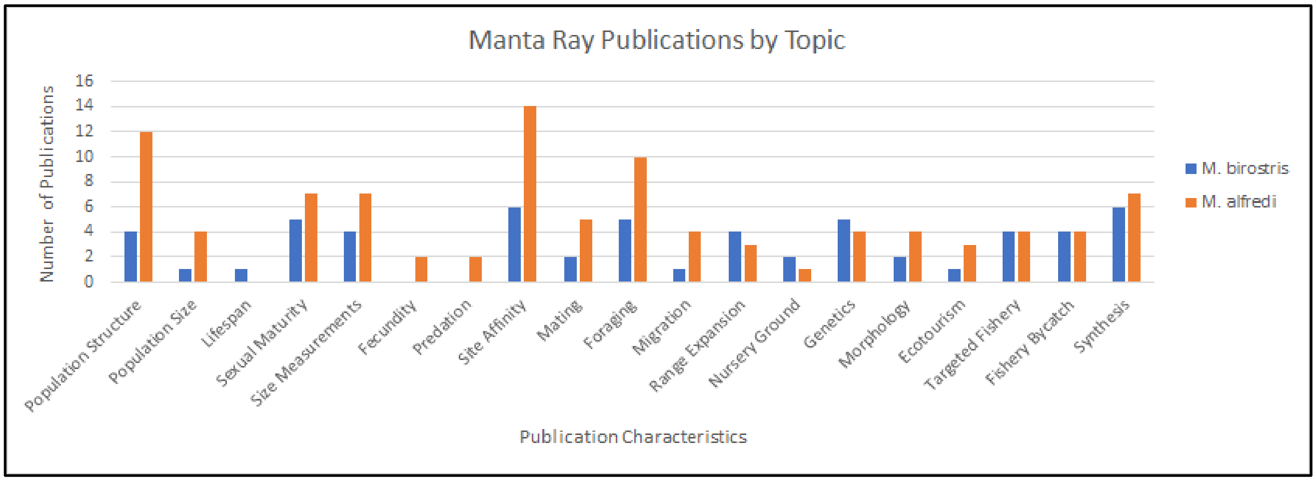

Figure 3. Graph displaying the breakdown of peer-reviewed manta ray publications, separated by species, for each subcategory used in the literature review. Blue bars show the number of publications related to *M. birostris*, and orange bars show the number of publications for *M. alfredi*.

There is also a clear knowledge gap surrounding nursery grounds, with the juvenile column in **Table 1** being somewhat misleading and showing slightly inflated numbers due to the characterization of papers based on the life stages studied. In fact, the juvenile stage has had almost no research done on it, partially due to the highly elusive nature of juveniles in the wild. There were 15 papers that reported data on juveniles, even though this was not the primary focus of their study, and they typically resulted from an unintended and opportunistic juvenile encounter. Conversely, there was only a single publication that principally studied juveniles – the Stewart *et al.* paper confirming the existence of an *M. birostris* nursery ground at FGB. Furthermore, even this research came about due to chance sightings of a high number of juvenile manta rays during a separate study that was being conducted at the location.

In addition, Heupel *et al.* defined nursery ground criteria for sharks such that (1) juveniles are *more commonly encountered* in the area than other areas, (2) juveniles have a *tendency to remain or return to the area* for extended periods, and (3) the area or habitat is *repeatedly used across* *years* (Heupel *et al.*, 2007). These criteria have been widely adopted as general standards that should be met for any elasmobranch nursery ground. Yet, Stewart *et al.* acknowledge the limitations of their work, confirming the presence of a nursery ground while simultaneously stating that they cannot meet the second criterion due to their reliance on historical data. Even the one study to focus on juvenile manta rays cannot fully support their claims of a nursery ground, highlighting the lack of research on this topic.

Identification of Potential Nursery Grounds

As a result of the literature review, and the broader research conducted for this project, a few characteristics were deemed fundamental in the potential identification of manta ray nursery grounds: (1) Water Depth, (2) Water Temperature – Seafloor and Sea Surface Temperature (SST), (3) Productivity, and (4) Distance to Deep Water. All five characteristics were found to be statistically significant in both the generalized linear model, **Figure 4**, and Bayesian generalized linear model, **Figure 5.**

```
Call:
glm(formula = Species ~ Depth + SFT + SST + Chlor + DWDist, family = binomial(link = "logit"),
    data = sp.pa)

Deviance Residuals:
     Min        1Q    Median        3Q       Max
-3.09859  -0.00012   0.00000   0.00000   1.87458

Coefficients:
              Estimate Std. Error z value Pr(>|z|)
(Intercept)  1.134e+02  8.949e+00  12.677  < 2e-16 ***
Depth        9.402e-02  1.148e-02   8.193 2.55e-16 ***
SFT         -1.257e+00  2.030e-01  -6.193 5.90e-10 ***
SST         -3.019e+00  2.404e-01 -12.555  < 2e-16 ***
Chlor       -7.280e+00  1.335e+00  -5.453 4.96e-08 ***
DWDist      -2.038e-04  1.671e-05 -12.199  < 2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 2305.70  on 5299  degrees of freedom
Residual deviance:  499.54  on 5294  degrees of freedom
AIC: 511.54

Number of Fisher Scoring iterations: 15
```

Figure 4. Results of the generalized linear model, summarizing the effect of water depth (Depth), seafloor temperature (SFT), sea surface temperature (SST), productivity as shown by chlorophyll levels (Chlor), and distance to deep water (DWDist) on the presence or absence of juvenile manta rays in the Gulf of Mexico. The statistical significance of each covariate is also provided.

```
Call:
bayesglm(formula = Species ~ Depth + SFT + SST + Chlor + DWDist,
    family = binomial(link = "logit"), data = sp.pa)

Deviance Residuals:
     Min        1Q    Median        3Q       Max
-3.02187  -0.00027   0.00000   0.00000   1.82119

Coefficients:
              Estimate Std. Error z value Pr(>|z|)
(Intercept)  1.045e+02  7.684e+00  13.604  < 2e-16 ***
Depth        8.217e-02  9.986e-03   8.228  < 2e-16 ***
SFT         -1.069e+00  1.796e-01  -5.952 2.65e-09 ***
SST         -2.872e+00  2.187e-01 -13.131  < 2e-16 ***
Chlor       -6.470e+00  1.171e+00  -5.526 3.27e-08 ***
DWDist      -1.925e-04  1.537e-05 -12.527  < 2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 2305.7  on 5299  degrees of freedom
Residual deviance:  500.9  on 5294  degrees of freedom
AIC: 512.9

Number of Fisher Scoring iterations: 25
```

Figure 5. Results of the Bayesian generalized linear model, summarizing the effect of water depth (Depth), seafloor temperature (SFT), sea surface temperature (SST), productivity as shown by chlorophyll levels (Chlor), and distance to deep water (DWDist) on the presence or absence of juvenile manta rays in the Gulf of Mexico. The statistical significance of each covariate is also provided.

The generalized linear model was preferred over the Bayesian generalized linear model, although it performed only slightly better. The lower residual deviance and AIC scores indicate a better fit, though the difference is marginal at best. Furthermore, even though the provided covariates were mentioned numerous times throughout the literature review and in papers concerning general elasmobranch nursery grounds, I hesitate to simply accept the statistical results at face value. I acknowledge that they likely have some degree of importance when it comes to defining nursery grounds, but question that both models returned extremely low p-values, indicating high statistical significance, for each covariate.

For example, the resolution of the SST data was poor compared to the other data, and within the FGB boundaries, the 300 random samples returned a limited number of different SST values. SST likely did not vary widely throughout the Gulf of Mexico, at least compared to the other variables, suggesting that perhaps it should not have been found statistically significant. This potentially false significance could have resulted from a small sample size for locations with juvenile manta rays present, and/or the possibility that 5,000 samples for the entire Gulf of Mexico was not enough to accurately portray absence.

Similarly, there were additional characteristics that repeatedly came up in the literature review that were beyond the scope of this project, typically due to data unavailability, so their importance could not be determined. Diel cycle (Braun *et al.*, 2014), lunar cycle (Anderson *et al.*, 2011), and current energy (Anderson *et al.*, 2011) could all factor into manta ray habitat use, though their effects on juveniles in potential nursery grounds cannot currently be quantified. Had these factors been included in the statistical analyses, they may have proven significant, and the significance of other covariates may have been altered as well. Additionally, other factors such as MPA designation, prevalence of natural predators, and other unknown factors should be accounted for, if possible. In the same way that salmon return to a specific river, or turtles return to a certain beach, it is also important to recognize that there may be innate and unquantifiable factors which play a role in the location of manta ray nursery grounds.

Unfortunately, even though the isolated characteristics – water depth, water temperature, productivity, and distance to deep water – were statistically significant in the course of this project, other, yet unidentified manta ray nursery grounds could be not identified. The global datasets are hosted online as imagery servers, and therefore cannot be manipulated to form the necessary probability rasters in ArcGIS Pro that would display locations with similar characteristics to FGB. Even though this is a disappointing result, the information gained from the literature review, knowledge gap analysis, and isolation and analysis of environmental characteristics important to manta ray nursery grounds should not be ignored. Rather, this paper should serve as a basis upon which future research can build, with the ultimate goal of increasing effective protective measures and conserving global manta ray populations.

Conclusions

Conducting the literature review and knowledge gap analysis of current manta ray publications served a number of purposes, with the principle being to highlight the simple paucity of data. 66 papers were identified for further review, which is a mere fraction of the number of papers that have been published on many other species, both terrestrial and aquatic. This is especially important when you consider the increasing fishing pressure that mantas, and all rays, are facing – both targeted and as bycatch. Moreover, mantas may be the best studied of all the rays, indicating a more widespread knowledge gap in elasmobranch research than previously believed.

The literature review also identified a concerning number of gaps in the basic biology, ecology, and life history of manta rays, specifically regarding lifespan, fecundity, and natural predation. The need for preliminary research on life stages other than sexually mature adults may be the most pressing concern, as management decisions cannot be properly informed without a fundamental understanding of manta ray life cycles and corresponding behavior. Likewise, the review helped isolate certain physical and environmental characteristics, all of which proved statistically significant, that could help identify juvenile manta ray nursery grounds in the future. The existence and any possible locations of these nursery grounds have the potential to direct protective measures that could prove crucial to the recovery and increased resilience of these species in the face of declining populations.

Even though the isolated environmental characteristics could not be used to identify potential manta ray nursery grounds within the scope of this study, it has laid the foundation for future research to do so. The literature review also revealed numerous other locations that are believed to be manta ray nursery grounds, including:

1. Wayag Lagoon, Raja Ampat, Indonesia (Beale *et al.*, 2019)
2. Manta Bay, Nusa Penida, Indonesia (Germanov *et al.*, 2019)
3. Paranaguá Estuarine Complex, Brazil (Medeiros *et al.*, 2015)

These sites should be visited and researched further to determine if they can be confirmed as manta ray nursery grounds. If confirmed, the physical and environmental characteristics – beginning with water depth, water temperature, productivity, and distance to deep water – of these three locations should be compared to those at FGB so that statistical analyses can be rerun to determine if all five covariates are still significant. This has the potential to create a stronger model that can better predict other locations with similar features. However, it should be noted that only *M. birostris* is confirmed to have nursery grounds at FGB, and Manta Bay is believed to be a nursery ground for *M. alfredi*. Though very closely related, *M. birostris* and *M. alfredi* are distinct species and may have differing environmental preferences when it comes to juvenile habitat, and these distinctions should be kept in mind when developing future habitat models.

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