AN ABSTRACT OF THE THESIS OF Julie Ann Hartup for the Master of Science in Environmental Science presented November 23rd, 2016.

Title: Manta rays (*Manta alfredi*) target surgeonfish (*Acanthurus* spp.) spawning aggregations to feed on fish spawn.

Approved: ____
Alexander M. Kerr, Chairman, Thesis Committee

Fish spawning aggregations often attract oophagous or pisivore predators that take advantage of the opportunity of this food source. This study documents Guam reef manta rays, *Manta alfredi*, targeting three species of surgeonfish spawning aggregations to feed on fish spawn. The three aspects of this ecological interaction this study focused on are population size dynamic and structure; *Acanthurus triostegus*, *A. guttatus*, and *A. lineatus* spawning aggregations and the relationship between astronomical and oceanographic variables that predict the probability of spawning; and the relationship between the three species of surgeonfish spawning events and manta rays present to feed at spawning events.

Spawning aggregations were found to be seasonal with all species statistically found that lunar phase, tidal height are a predictor of spawning. The environmental factors tidal height and lunar phase were correlated and therefore when considered together in an analysis one affect would mask the other affect. Best-fit model *Acanthurus triostegus* and *A. guttatus* had season and tidal height as predictors for spawning, whereas *A. guttatus* has season and moon phase as predictors for spawning. Photoperiodicity for *Acanthurus triostegus* and *A. guttatus* in relationship to sunset was a predictor for spawning while sunrise for spawning *A. lineatus* spawn was not significant.

Relationship between *Acanthurus triostegus*, *A. guttatus,* and *A. lineatus* spawning and Guam manta rays present to feed were found to be significant with increased sightings of manta rays followed spawning patterns of *Acanthurus* spp. Out of 142 spawning aggregations manta rays were present feeding for all except 25 events. From 189 surveys at the site when no fish were spawning only 19 times had manta rays present without spawning. This study shows that the Tumon Marine Preserve multi-species spawning aggregation is not only important for Guam fisheries but for Guam manta rays. Documentation of this ecological inter-relationship needs to be explored further with special enforcement that this site remains protected.

TO THE OFFICE OF GRADUATE STUDIES

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MANTA RAYS (*MANTA ALFREDI*) TARGET SURGEONFISH (*ACANTHURUS* SPP.) SPAWNING AGGREGATIONS TO FEED ON FISH SPAWN

BY

JULIE ANN HARTUP

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Chapter 1 Introduction

1.1. Background

Manta rays or "devil rays" are the largest rays in the family Mylobatidae. Walbaum first described the genus *Manta* in 1792. For some time, *Manta* was considered monospecific, with *Manta birostris* as the sole member (Walbaum, 1792). A recent taxonomic revision (Marshall *et al.*, 2009) recognized two distinct species, as well as a possible third species *Manta* sp. cf. *birostris* in the western Atlantic Ocean and Caribbean Sea. Differences between the first two species are morphological and behavioral (Marshall *et al.*, 2009), see Figure 1.

. The larger *Manta birostris* or "oceanic manta ray" is pelagic and migrates long distances into cold waters (Marshall, 2008; Marshall *et al.*, 2009). In contrast, the "residential manta ray", *Manta alfredi* (Krefft 1868), is commonly found around rock and coral reefs of tropical and subtropical coastlines (Couturier *et al.*, 2011). Publications prior to Marshall *et al.* (2009) revision mainly reference *Manta birostris*; however, some of these instances may actually be referring to *Manta alfredi* (Homma *et al.*, 1999; Dewar *et al.*, 2008). *Manta alfredi* is circumglobal and usually found on continental shelves in tropical and subtropical regions (Last & Stevens, 2009; Marshall *et al.*, 2009). The residential manta ray is often sighted feeding in habitats of high productivity: island groups, bays, channels, seamounts, pinnacles, and shallow coastal rocky and coral reefs (Deware *et al.*, 2008; Luiz *et al.*, 2009). Manta rays are curious and easy to approach and therefore often encountered by divers (Marshall *et al.*, 2011). Manta rays are generally found within 30 degrees north and south of the equator in the Pacific, Atlantic, and Indian Oceans (Marshall *et al.,* 2009). More studies of *Manta alfredi* need to be conducted to gain knowledge of their biology, ecology, and behavior. As well, increased knowledge of manta rays is needed to establish management policies addressing increased threats, such as overfishing.

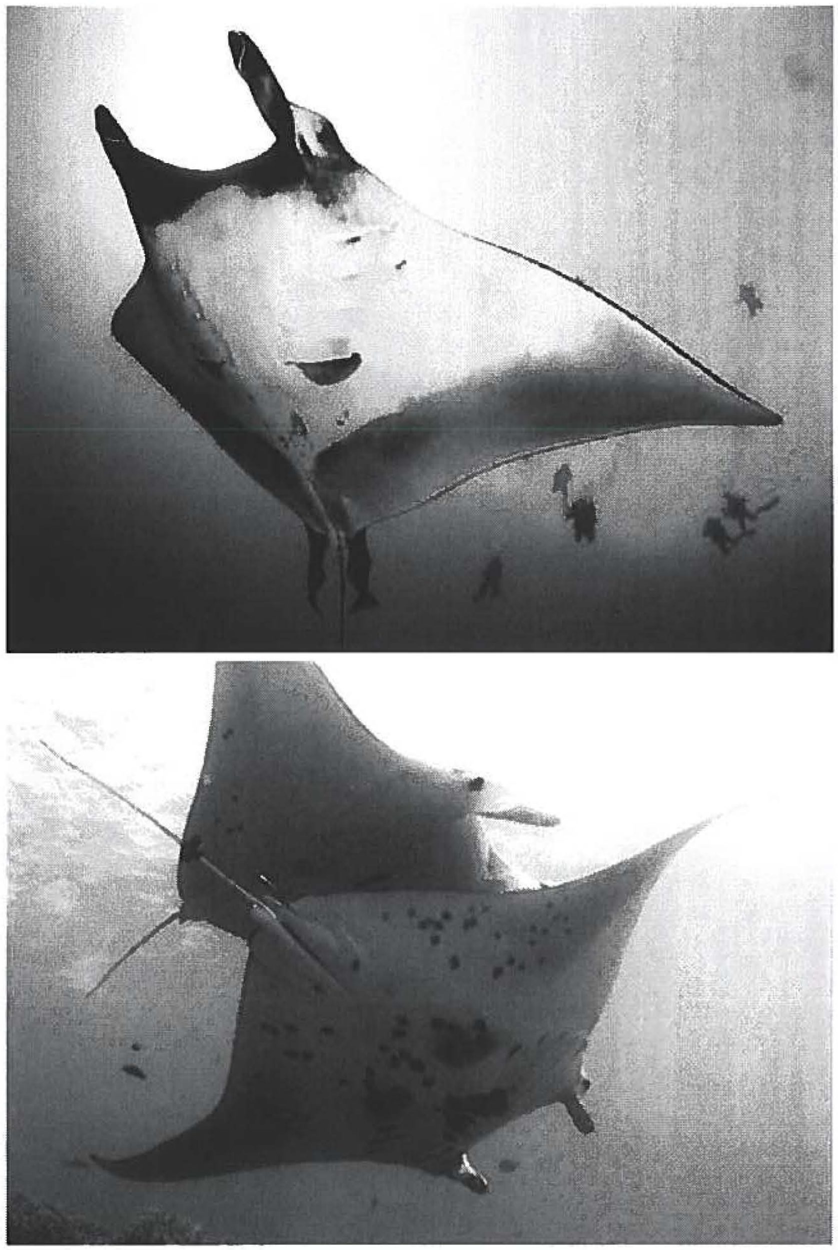


Figure 1. ***Manta birostris*** **(top; photo courtesy of Guy Stevens) or pelagic manta rays and** ***Manta alfredi*** **(bottom) the reef manta ray.**

Some manta-ray populations are resident year-round, such as the populations surrounding Hawaii, Mozambique, and French Polynesia, while other populations are seasonal, such as the population inhabiting the Maldive islands (Dewar *et al.,* 2008). In the Maldives, manta rays travel from one side of a chain of atolls to the other side, following plankton blooms caused by monsoon currents (Anderson *et al.*, 2008; Homma *et al.*, 1999). Near the main islands of Yap, Federated States of Micronesia, manta rays alternate between different channels during the season's winter or summer (Clark, 2001). Southwest of Yaeyama Okinawa; part of the manta ray population is resident year-round while the remainder migrates to a different island, Kerama (Homma *et al.,* 1999). Manta rays frequently migrate diurnally and usually between feeding and cleaning sites (Homma *et al.,* 1999).

1.2. Scope and Objectives

This study will gain insight into the Guam manta ray population, structure, and significant habitats, with particular emphasis in the first documentation of manta rays targeting multi-fish spawning aggregations as a food source. In the sections below I will 1) focus on manta-ray feeding behavior; 2) provide background on spawning aggregations; 3) explain how feeding aggregations form as a result of fish spawning aggregations; 4) define general methods used to collect information; 5) statistical analysis used to answer my questions; 6) report results found of data analyses; and 7) discuss findings of my study.

Chapter 2 Literature Review

2.1. Manta Feeding Behavior

Manta rays are planktivorous with a primary diet of zooplankton confirmed through stomach analyses. Their diet consists of euphausiids, copepods, mysids, decapod larvae, fish spawn, and possibly shrimp (Whitley, 1936; Bigelow & Schroeder, 1953; Clark, 2001; Last & Stevens 1994; Couturier *et al.*, 2012; Jaine *et al.*, 2012; Hartup 2013). Manta rays feed by a process called ram-jet feeding (Sanderson & Wassersug, 1990; 1993, Cortes *et al.*, 2008). Mantas unfurl their paddle like cephalic fins into optimal positions to funnel plankton-rich water toward the mouth and over the gills. Rakers, located on the five pairs of gills, strain the water and capture food (Figure 2). Most information on manta ray feeding comes from observations on pelagic individuals. Observational accounts of feeding behaviors vary; however, it is likely that feeding behavior alters or evolves depending on prey density and foraging effectiveness (Law, 2010). Consistently, reef mantas have been observed feeding on zooplankton along tidal slicks (Jaine, 2012). Within the slick, mantas travel back and forth within the current line feeding in the densest areas of zooplankton (J. Hartup, pers. obs.).

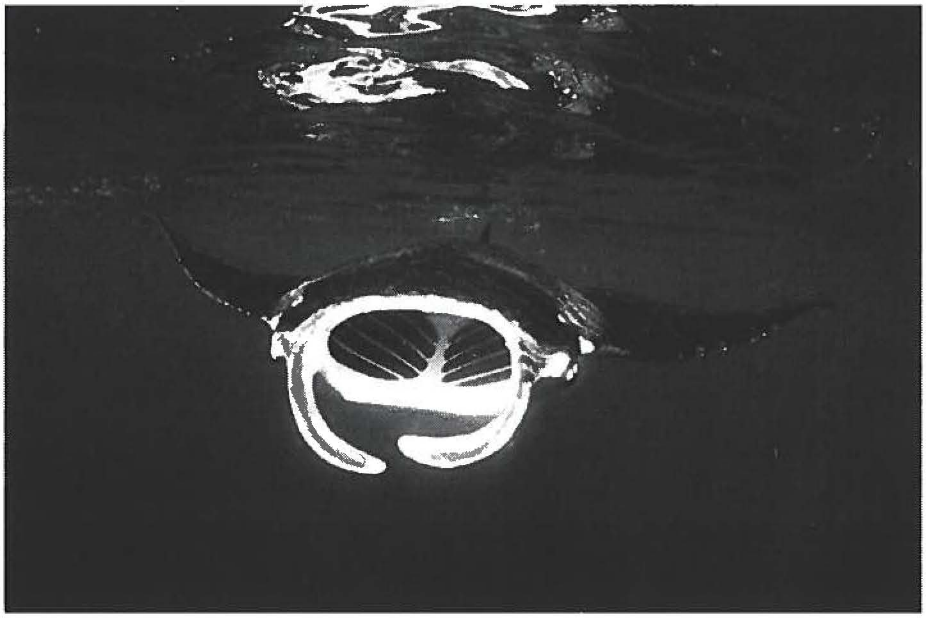


Figure 2. Guam *Manta alfredi* current line feeding on plankton.

Mantas have been observed somersaulting backwards (Figure 3) in a looping motion or skimming the substrate when feeding, demonstrating adaptation to varying prey dispersal modes (Deakos, 2010; Osada, 2010). In the Maldives, aggregated mantas perform a feeding behavior called chain feeding where individuals feed in a line. When over 50 individuals are present, the first manta in line joins up with the last manta, creating a circle and a mode of feeding called cyclone feeding. Mantas continue circling while the assembly moves from the surface downward, creating a feeding cyclone. Organized cyclone feeding ceases when more than 100 mantas are present, triggering a frenzy of unorganized feeding of individuals (Law, 2010).

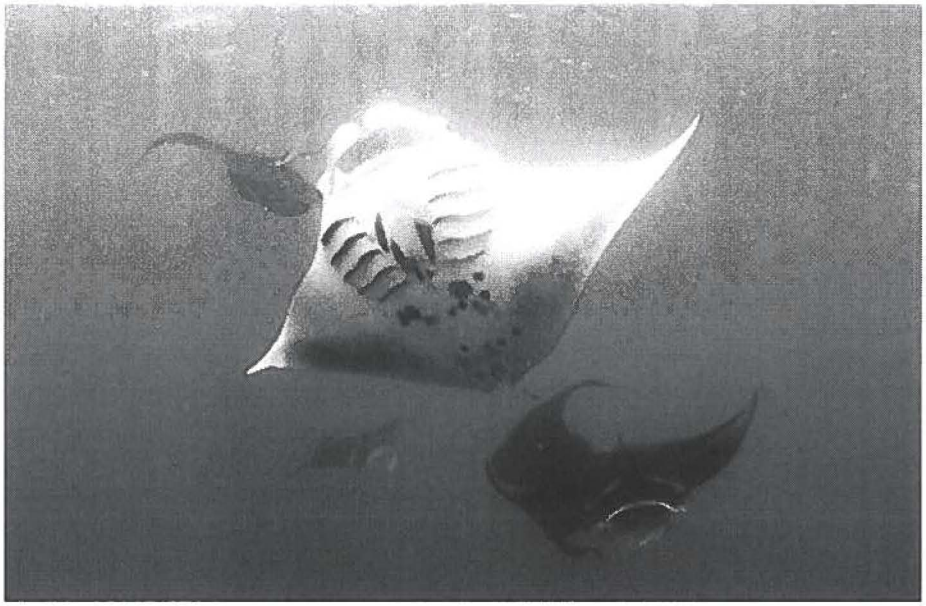


Figure 3. Manta ray somersault feeding (Photo courtesy of Steve Lindfield).

Information collected during this study has already resulted in the first documentation of the Guam residential manta rays, *Manta alfredi* targeting teleost fish spawning aggregations to feed off fish spawn (Hartup *et al.*, 2013). To accurately document and understand this novel behavior of manta rays, it is fundamental to examine and study the fish spawning aggregations for each species.

2.2 Fish Spawning Aggregations

Domeier & Colin (1997) defined a spawning aggregation as "a repeated concentration of conspecific marine animals, gathered for the purpose of spawning, that is predictable in time and space. The density/number of individuals participating in a spawning aggregation is at least four times that found outside the aggregation. The spawning aggregation results in a mass point source of offspring" (Sadovy de Mitcheson and Colin, 2012). Native fishermen have known for centuries of fish spawning aggregations (*e.g.*, Johannes 1978, 1981). Spawning aggregations because of their predictability in space and time are often targeted by fisherman and are under intense fishing pressure (Domeier, 1997). There are several hypotheses as to why marine animals form spawning aggregations. These include the optimization of egg and larval dispersal (Barlow, 1981), increased probability of local recruitment (Johannes, 1978; Lobel, 1978; Lobel & Robinson, 1988), predator satiation (Johannes, 1978), coordinating spawning to lunar, tidal, or solar phases (Lobel, 1978; Colin & Clavijo, 1988; Colin & Bell, 1991), increased probability of food while feeding in patchy environments by settling larvae (Doherty *et al.*, 1985), increased genetic material (Johannes, 1978), or decreased predation by adults (Shapiro *et al.*, 1988; Claydon, 2004).

There are two different types of spawning aggregations, resident and transient. Resident spawning aggregations are sites that are close to the individuals' home territory (Figure 4). Spawning usually occurs at a specific time lasting only a few hours for several days or can occur daily over an extended periotic time throughout the year or can occur yearlong. Conversely, transient spawning aggregation is made up of individuals that travel longer distances from their adult home range (Sadovy de Mitcheson & Colin, 2012). Typically, most fish species that form spawning aggregations produce pelagic eggs and larva. However, in the family Balistidae and Siganidae aggregated fish produce benthic eggs having a pelagic larval stage (Thresher, 1984). Depending on fish species and environmental conditions, larval stages can remain in the water column for hours to several weeks (Leis & McCormick, 2006).

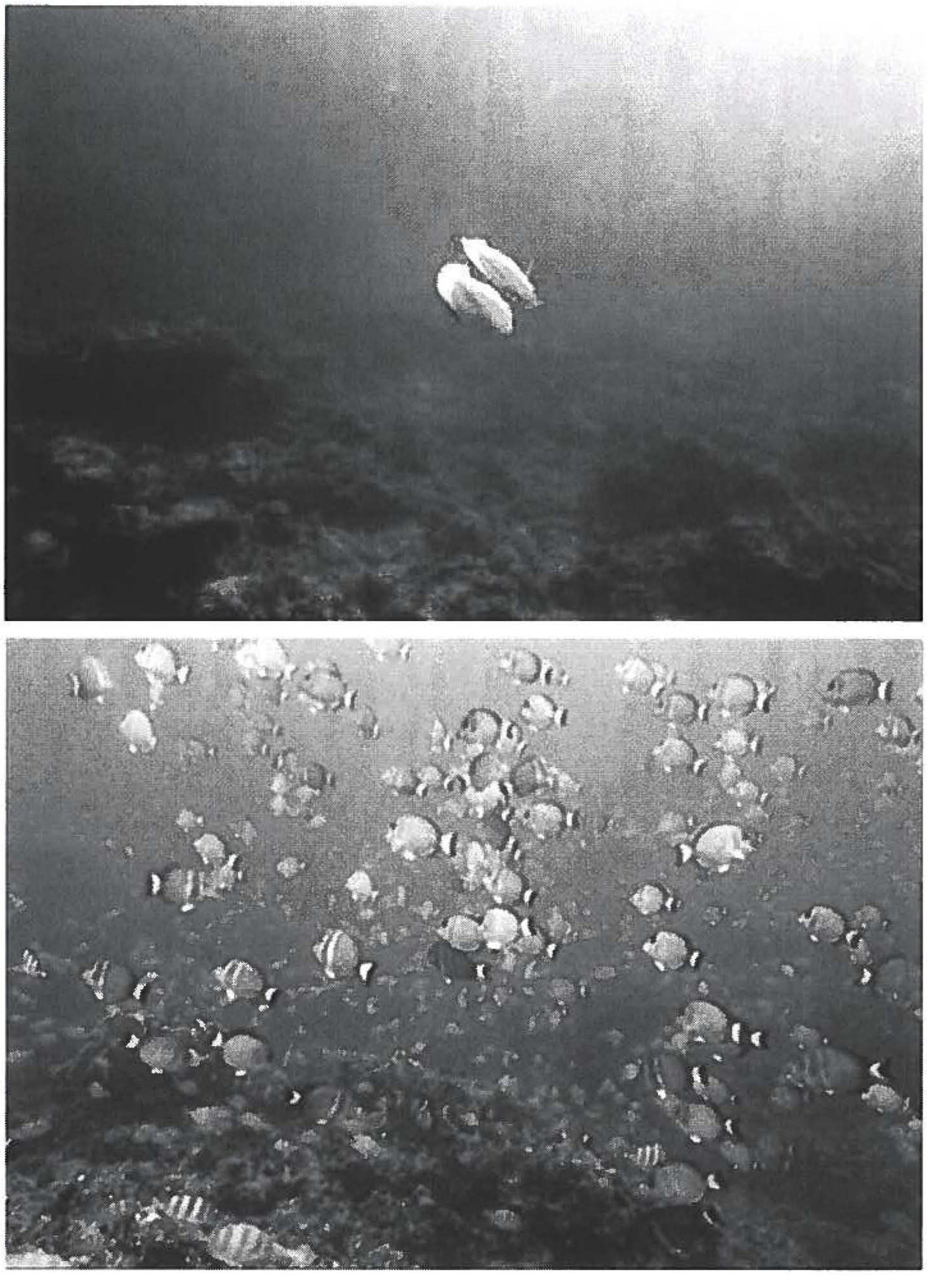


Figure 4. ***Bothus Mancus*** **Pair-Spawning (top) and** ***Acanthurus guttatus*** **Spawning Aggregation (bottom), Tumon Bay Marine Preserve in Guam.**

Of the six genera of surgeonfish (Acanthuridae), *Acanthurus* is the genus with the most species forming spawning aggregations. *Acanthurus triostegus* exhibits pair and group spawning whose timing varies geographically (Sadovy de Mitcheson & Colin, 2012). Randall (1961) documented *A. triostegus* (Figure 5) pair and group spawning in Hawaii from about December through July between the 4th and 17th days of the lunar month. Johannes (1981) observed *A. triostegus* spawns in Palau, Micronesia, around the 4th–10th day of the lunar months in the evenings between May through August. *Acanthurus triostegus* in Guam was found to have year-round spawning and no lunar pattern or lunar pattern of spawning from gonad maturity study (Davis, 1985). In Aldabra Atoll, Seychelles Islands, *A. triostegus* spawned between November through December four times, three of which were mass spawnings from midday to dusk (Robertson, 1983). Off the tip of India at Minicoy Atoll, Lakshadweep, *A. triostegus* indicates a year-round spawning with a peak in monsoon periods based on gonad maturity (Mohan, 1988). Spawning of *A. triostegus* in American Samoa occurred year-round at sunset, although no lunar or tidal effects were analyzed (Craig, 1998). The species *Acanthurus guttatus* was also found spawning at the same site and time year-round (Craig, 1998). A diver's account of *A. guttatus* in the Cook Islands indicates that spawning occurs in the late afternoon (Kuiter & Debelius, 2001).

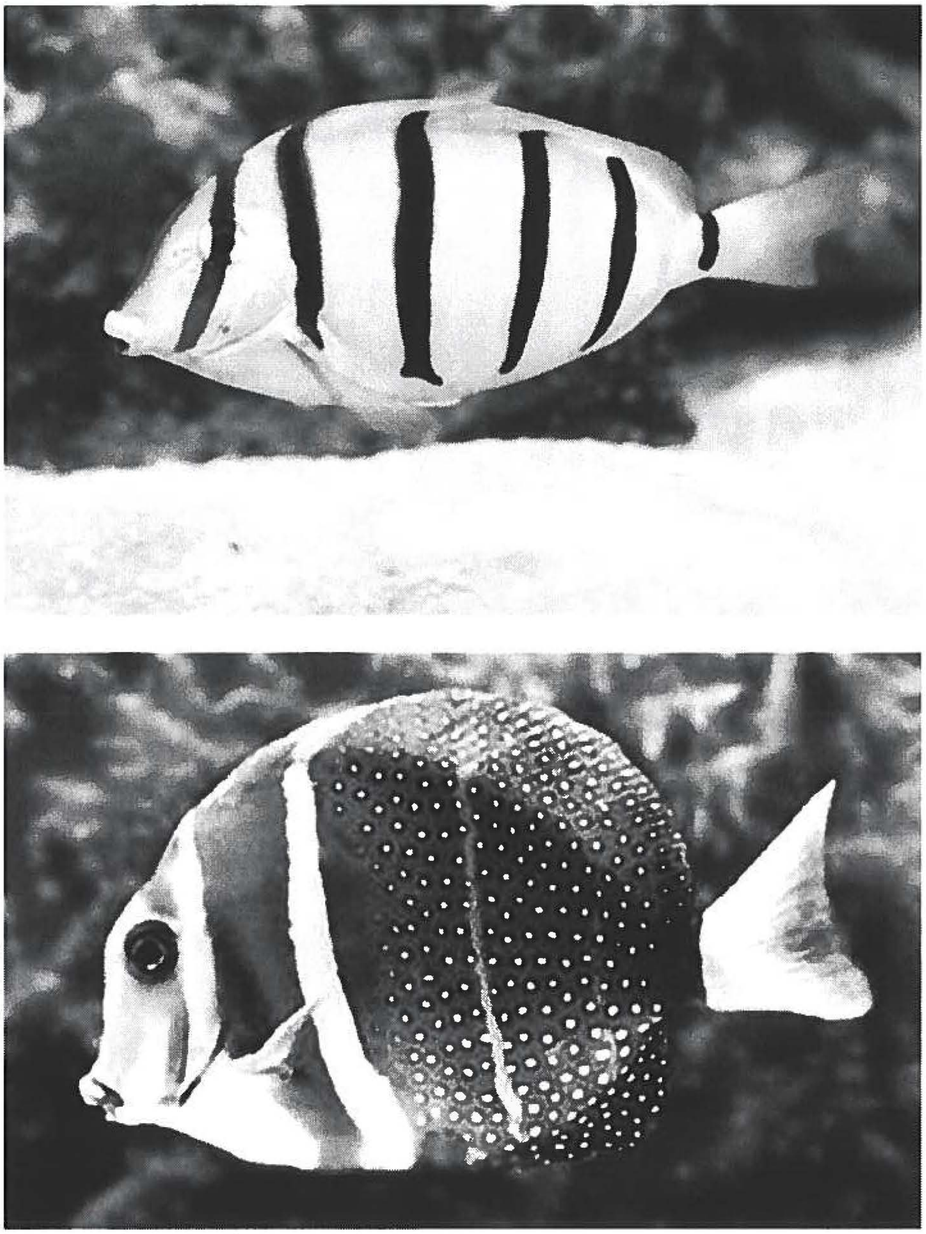


Figure 5. ***Acanthurus triostegus*** **(top) and** ***Acanthurus guttatus*** **(bottom) species.**

Spawning events for *Acanthurus lineatus* have been documented several times. First observations of group spawning of *A. lineatus* (Figure 6) came from Johannes (1981) in Peleliu, Palau during the month of April, at new moon around 0645 h. Additionally, in Palau off shallow fore reefs, Robertson (1993) observed *A. lineatus* spawning several days before full moon participating in group spawning between the months of February and April within one to three hours of ebb tide. Spawning occurred on 9th–13th lunar cycles with the possibility of spawning on 24th–27th lunar cycles. Robertson documented one spawn event in Australia off Escape Reef. All observations of group spawning events of *A. lineatus* occur in the morning hours (Sadovy de Mitcheson & Colin, 2012). From a gonad maturity study in Guam, *A. lineatus* was said to have year-round spawning and no lunar pattern (Davis, 1985).

Figure 6. ***Acanthurus lineatus*** **species.**

Knowledge of the ecology and the behavior of fish is essential in creating ecosystem-based management (Garcia *et al.*, 2003). The migration movement of aggregating fish, habitat use, and interspecific interactions between fish species play an ecological component within the ecosystem. Migration pathways from home range to temporary spawning sites creates provisional peaks in fish biomass, with increased feeding, defecation, piscivore predation of adults, predation on eggs and sperms, and reproduction at aggregations potentially affect food web dynamics and the transmission of energy (Sadovy de Mitcheson & Colin, 2012). Nemeth (2012) categorized the effect that aggregating species might have on food web dynamics in four groups: 1) feeding by aggregating fish on animals and plants residing at spawning aggregation sites or along migration pathways, 2) predation on piscivores on migrating or spawning adults, 3) egg predation, and 4) other trophic linkages (Sadovy de Mitcheson & Colin, 2012).

2.3 Predators Targeting Spawning Aggregations

A spawning aggregation is a "conspicuous phenomenon," visible due to increased fish density, and many times mating behaviors exhibited by the spawning fish are ornate and ostentatious (Clifton & Robertson, 1993). In addition, the nature of spawning aggregations is predictable in time and space. Spawning aggregations attract two main types of predators, oophagous species that feed on eggs, and piscivores that feed on spawning fish (Molloy *et al.*, 2012).

Intense fishing pressure can quickly disrupt and eliminate spawning aggregations (Sala *et al.*, 2001; Sadovy & Domeier 2005). Once a spawning aggregation is eliminated, it is not known to recover and heavily fished areas lack spawning aggregations (Sadovy *et al.*, 2008). Therefore, spawning aggregation sites are an indicator of long-term ecological stability (Domeier, 2012). Ecosystems that contain spawning aggregations that are predictable in time and space, that includes feeding aggregations targeting the spawning adult fish, or mass release of eggs can conceivably be an indicator of long-term stability of the spawning aggregation. Fish spawning aggregations must periodically form for a substantial amount of time before attracting predators that target such an event (Domeier, 2012).

Temporary fluctuations in number of fish along migration pathways and fish spawning aggregations may affect food web dynamics and energy transfer of fish. These peaks of fish biomass can affect the habitat through feeding, defecation, predation, and reproduction (Nemeth, 2012). Zooplankton in Palau consisted of 90% fish eggs from spawning aggregations comprised mostly of surgeonfish, parrotfishes, and wrasses during ebb tides (Hamner *et al.*, 2007). Increased numbers of spawning fish and eggs could attract a wide diversity of species taking advantages of this food source (Nemeth, 2012).

Piscivores targeting spawning adult fish included bluefin trevallies, jobfish, sharks, marlin, tunas, wahoo, snappers, moray eels, and bottlenose dolphins (Sancho *et al.*, 2000b; Nemeth, 2005; Hayman & Kjerfve, 2008; Colins, 2012). In total, known predators comprise 22 different species from 13 families (Nemeth, 2012). Resident spawning aggregations are described of having higher numbers of predation than transient spawning aggregations due to extended spawning seasons and spawns during daylight hours, typically exhibited by residential aggregations. However, observations of transient aggregations are often limited by a short spawning season, lower light conditions such as sunset or evening spawns, complex site locations found typically in deeper waters, and adult spawning fish that are typically larger bodied, limiting predation to larger predators (Colin *et al.*, 2003). Fishing at spawning aggregations can increase and induce predatory species and behavior; by hooking a fish from the aggregation present to spawn causes remaining fish to react by admitting chemicals or physical cues that lure in predatory species (Olsen & LaPlace, 1978; Nemeth 2005; Matos-Caraballo *et al.*, 2006; Heyman &

Kjerfve, 2008; Nemeth, 2012). Recent studies suggest that predation on spawning adults occurs much less often than predation on newly released eggs, but varies among locations and species (Robertson, 1983: Moyer 1987, Craig, 1998; Sancho et al., 2000a; Claydon, 2004; Nemeth, 2012).

Egg predation of recently spawned fish has been documented from both quantitative and observational studies. A total of 35 species within 13 families were egg predators (Sadovy de Mitcheson and Colin, 2012). The majority of observations of such events were similar, beginning first of newly released fish eggs and sperm, creating a visible gamete cloud. Egg predators would swim rapidly into the gamete cloud while picking at eggs or ram-jet filter feeding (Colin, 1976; Moyer, 1987; Sancho *et al.*, 2000a; Heyman *et al.*, 2001). The whale shark *Rhincodon typus* (Figure 7) targets fish spawning aggregation of snappers *Lutjanus cyanopterus* and *Lutjanus jocu* in Belize to feed off fish spawn (Heyman, 2001). Whether spawning aggregations are used as a temporary food source for large planktivores in unknown. Very few large planktivores live in tropical and subtropical waters due to these oceans usually being oligotrophic. Whale sharks, some mobulids species, and mantas rays are large planktivores forging within these boundaries. Most forage in higher latitudes where prey density is higher. Spatial movement and targeting of planktivores to feed at spawning aggregations reveals a new dimension and component to these ecosystems. The significance this temporary food source to the egg predators' diet and overall ecology is unknown (Nemeth, 2012).

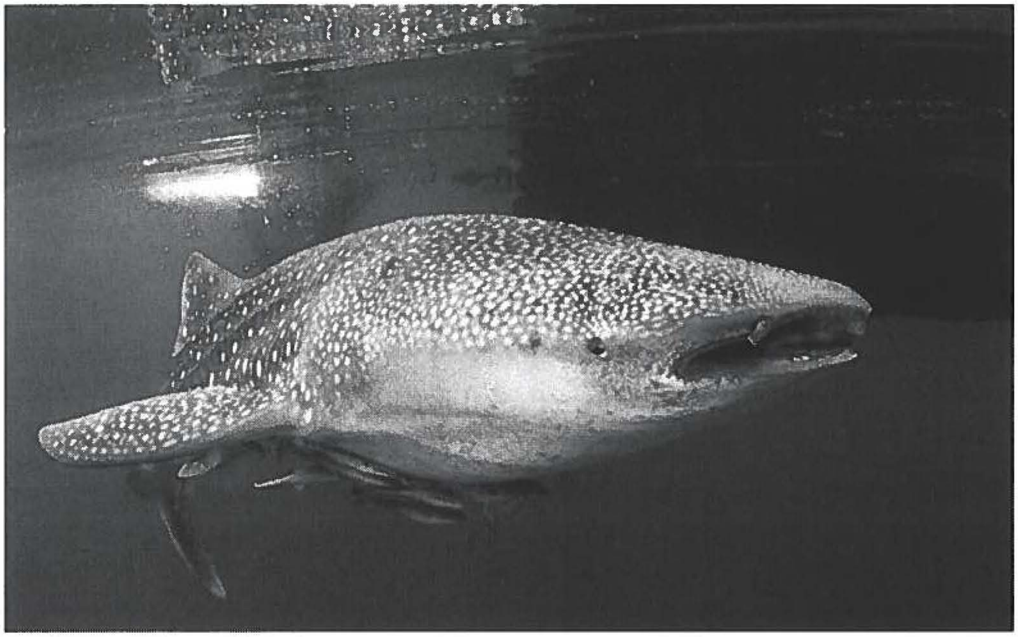


Figure 7. The whale shark *Rhincodon typus.*

This study will bring insight into reef manta rays targeting fish spawning aggregations as a food source, social structures that exist within the Guam manta ray population, time and duration of three surgeonfish species spawning aggregations and associated environmental cues, and interconnection between fish spawning and predator feeding at spawning aggregation as an indicator of ecological stability.

Chapter 3 Methods

This section describes how specific sites, designated dates, and times for surveys were selected, techniques used to collect data, and information gathered specifically statistical analysis used in this study. Study was conducted on the island of Guam, located in the western Pacific Ocean with the GPS coordinates of 13° 26' 39.4944" N and 144° 47' 37.4352" E. Guam shaped like a hanging sock is 50 km long and 6-9 km wide. Part of the Marians Islands it is the southernmost island. Guam is a territory of the United States of America.

3.1 Site Selection

Two sites were chosen based on the information gathered from manta-ray sightings. Anecdotal reports of Guam's manta rays date back at least 20-30 years from sightings by snorkelers and divers. Manta-ray sightings are usually along shallow reefs and rocky coastal areas. One particular site, Gun Beach is known to have regular sightings of manta rays around the month of March and April. Also in 2010, snorkelers on two separate occasions observed manta rays feeding at fish-spawning aggregations of two species of fish at the same site located in Tumon Bay (A. Marshell, pers. comm.; P. Carlson, pers. comm.), see Figure 8. These two sites formed the basis of my hypothesis that reef manta rays were targeting reef surgeonfish spawning aggregations to feed off spawn (Hartup *et al.*, 2012).

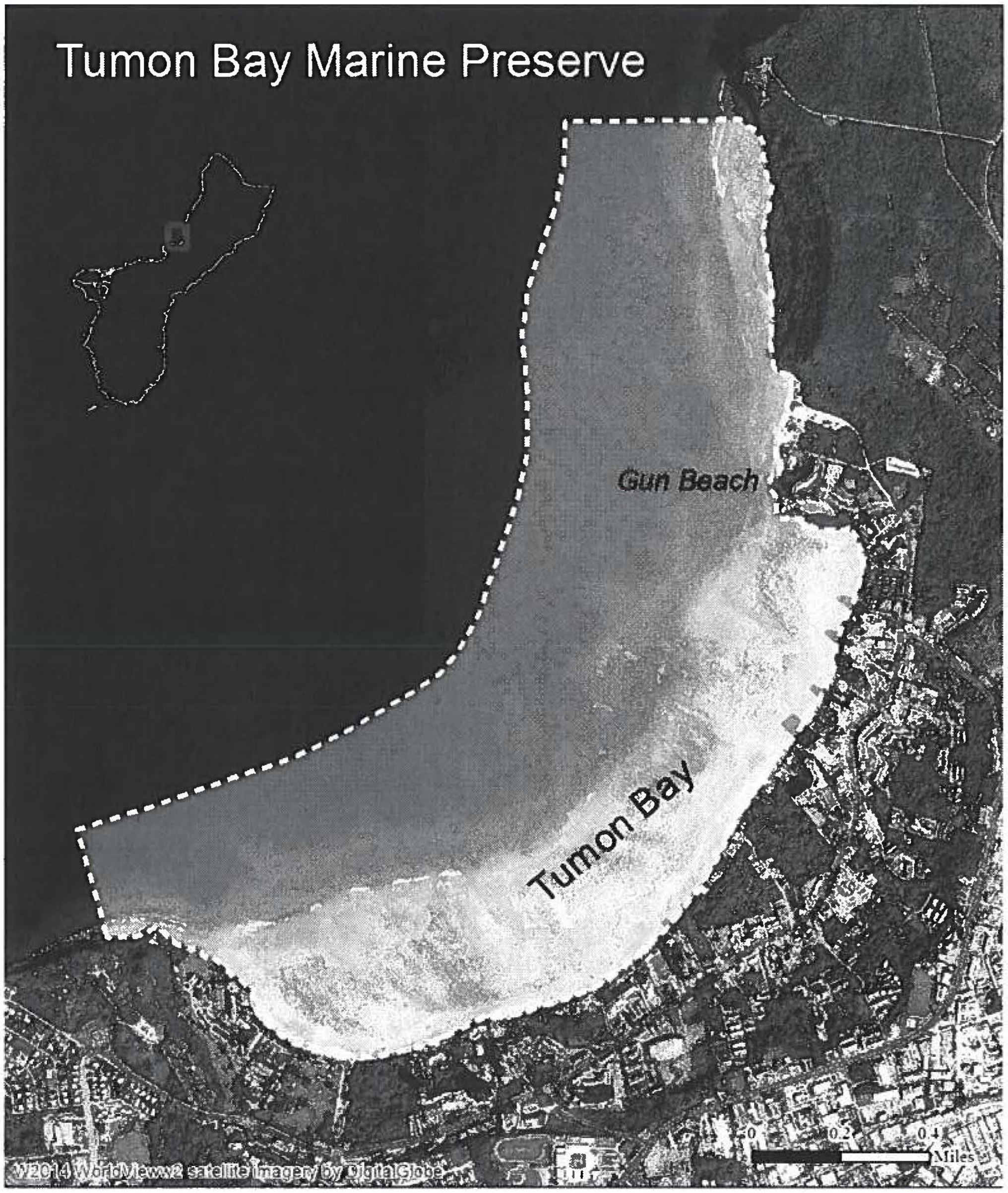


Figure 8. Map of the Tumon Bay Marine Preserve.

3.2 Date Selection

Dates of surveys were based on moon phases when fish species were thought to spawn. First, spawning times for each species of fish were documented. The identification of the spawning fish species was verified from videos and photographs as *Acanthurus* *guttatus* and *A. lineatus*. Moon phases for each observation dates were determined. From these two observations in 2010, two target dates were set for possible spawning and manta feeding events in 2012. In January 2013 and 2014, predicted spawning aggregation dates were determined from previous observations and corresponding moon phases. Spawning target dates for *A. triostegus* and *A. guttatus* begin two days before the full moon nearest the end of January in 2013 and middle of January 2014. One or two days before each spawning aggregation, an observation snorkel was done to determine if fish are aggregating and sightings of manta rays were recorded.

3.3 Data Collection

Beginning in December 2011 thru August 2016 snorkel surveys were usually conducted by myself and one other individual with a max of eight people and minimum of two. Surveys lasted between 31 to 161 minutes but on average 120 minutes. Main study site is located adjacent to a channel, at a depth of 5–13 meters on the reef slope of Tumon Bay Marine Preserve. Surveys were scheduled one or two days before spawning events were thought to begin, to record fish and manta ray activity. Subsequent surveys recorded date, time of day, photoperiod, lunar phase, lunar illumination, tidal cycle, spawning present/absent, species of fish, size of spawning aggregation, number of mantas present, record manta individuals, sex of mantas. After each survey still photographs were analyzed to determine actual number of manta rays present for each spawning event. Unique spot patterns on the ventral side verify a particular manta ray. To identify an individual, the area between the gills and the abdomen was examined to compare ventral markings with other photographs as seen in Figure 9. (Luz *et al.*, 2009; Kitchen-Wheeler, 2010; Couturier *et.*

al., 2011; Deakos *et al.*, 2011; Marshall & Bennett, 2011; Marshall & Pierce, 2012). Photographs were separated based on documentation of scars, markings, and distinguishing characters and categorized as either 'new' or 'repeat' sightings as seen in Figure 10 (Marshall & Bennett, 2010; Deakos *et al.*, 2011; Marshall & Pierce, 2012).

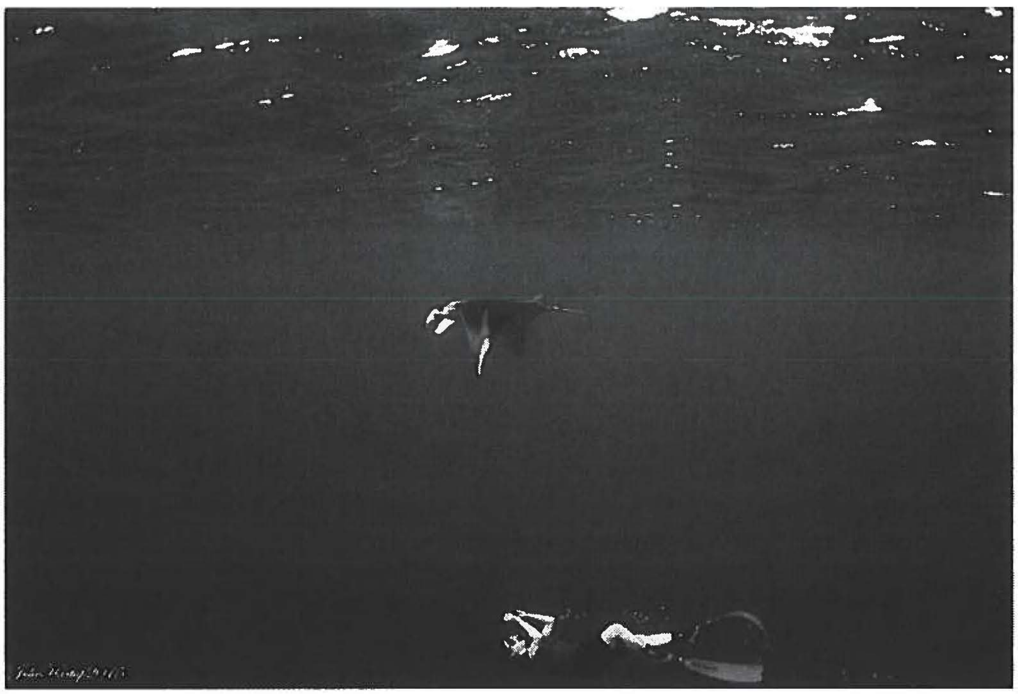


Figure 9. Taking an identification picture of a juvenile male manta ray in Guam.

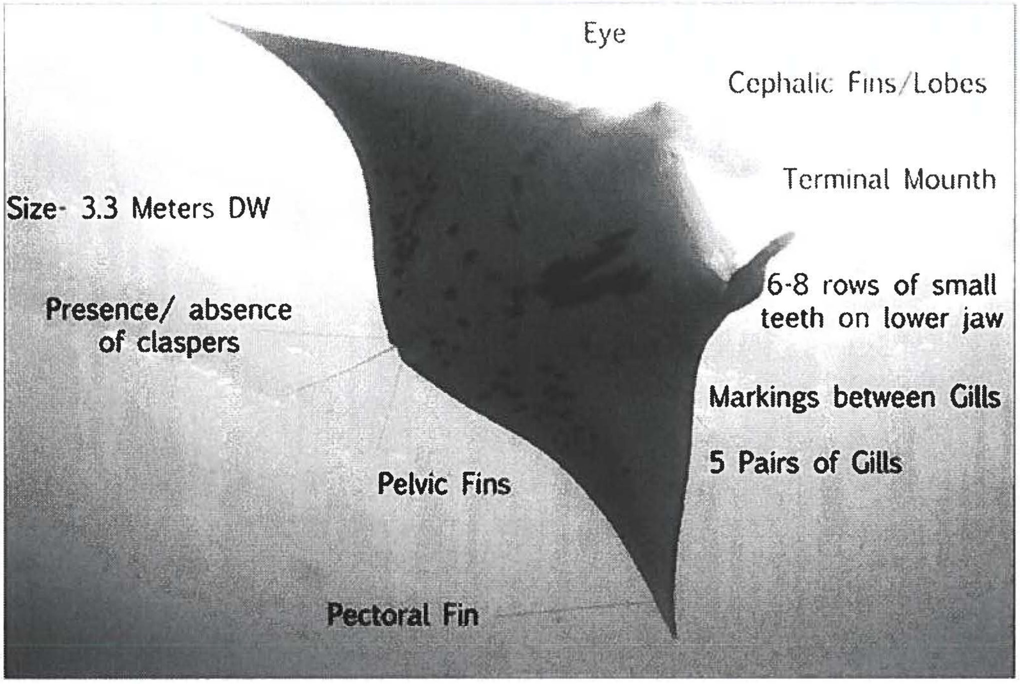


Figure 10. Guam manta ray (*Manta alfredi*). Description of manta ray appendages and defining markings for identification.

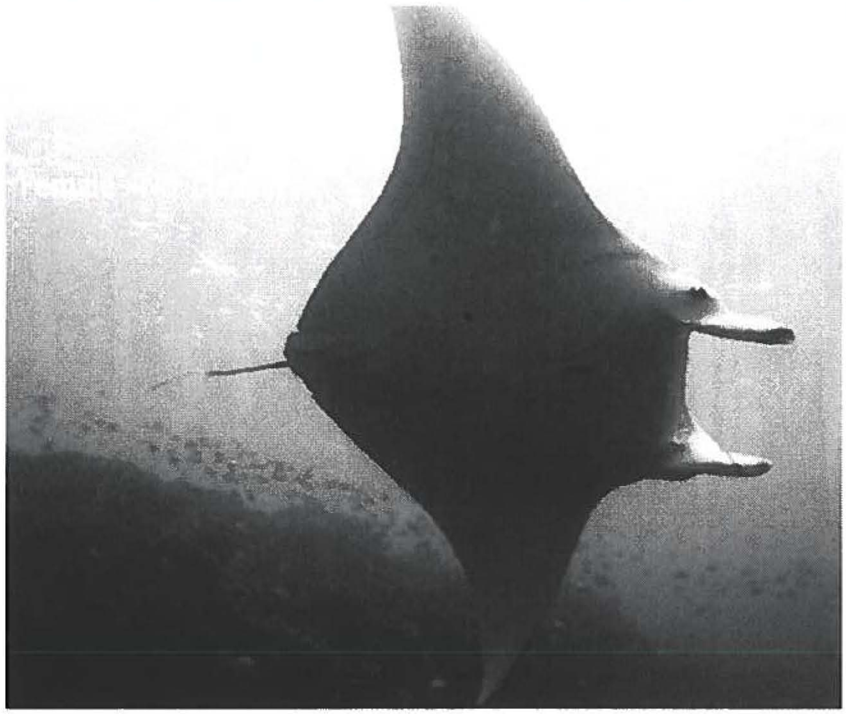
Manta ray size was measure by stereo-video (Klimley & Brown, 1983). Starting in January 2015, manta rays were videoed by a stereo-video system and were measured by using the computer software program EventMeasure from SeaGIS. Each individual manta was measured several times to insure accuracy by eliminating bias from parallax.

During each survey, fish spawning aggregations were observed and categorized in size class from very small to extra-large. Starting April 21, 2012, video cameras recorded most events to estimate fish quantities in order to acquire a numerical scale for each categorical category. On each survey one to four GoPros (™San Mateo, CA.) were set on the reef in specific areas to record videos of fish aggregations size, spawning, and manta rays and used to estimate categorical sizes. The number of GoPros deployed depended on weather conditions and expected size of fish spawning aggregation. GoPros were attached to a two-pound weight by screw and tripod mount. One GoPro was mounted to the top of a camera in a waterproof housing to assure that manta identifications marks were recorded along with spawning events (Figure 11). Videos were turned on and allowed to run throughout the survey. If placement of video was not optimal for picking up desired footage, GoPro was placed at a different location on reef. A picture was taken of GoPro placement along with accompanying GPS location.

Figure 11. GoPro set on reef to video spawning aggregations of *Acanthurus triostegus* and *A.guttatus* and *A. lineatus*.

In March 2013, a GPS unit was attached to a weight belt by a nylon cord. This allowed the recording of swimming tracks, while allowing the diver to free-dive down to obtain pictures of individual mantas, and record behaviors. A picture was taken of the GPS time and coordinate prior to each survey. On return photographs and GPS tracks were uploaded into the program Geo-Spatial Corporation (™Sarver, PA.). The program connected where photos were taken along the GPS recorded track, giving an approximate location to each photograph.

Upon encountering a manta rays ray a ventral picture of the individual was taken. If sex of the manta rays was not completely obvious from the picture a designated hand single denoting the sex was taken a picture of to aid in analysis of photographs later. Sex of manta rays was determined by looking at the pelvic fins of the manta to see presence or absence of clasper (Figure 12). Additional attention to smaller-sized mantas was taken to ensure underdeveloped claspers were not overlooked.



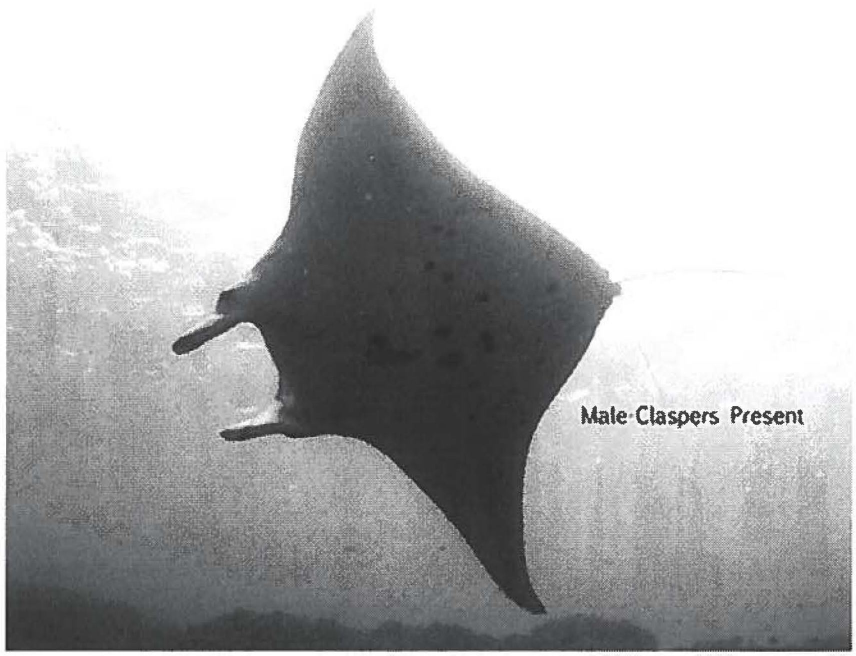


Figure 12. Guam female manta rays (top) and male manta ray (bottom) with presence of claspers.

Chapter 4 Hypotheses and Statistical Analysis

Hypotheses for the study are presented along with types of statistical analysis used to evaluate each question. First, I will examine Guam manta ray population size and structure. Second, I will look at the spawning aggregations seasonality, associated factors that periodicities of spawning fish. The final hypothesis specifically addresses manta rays targeting fishing spawning aggregations to feed.

4.1 Manta Ray Population Size

An understanding of species-basic biology and ecology is important for management and conservation. Knowledge of a species population size is necessary in comprehending anthropogenic and natural threats along with impacts affecting the ecology of the species (Heyman, 2001, Deakos, 2011, Sadovy de Mitcheson & Colin, 2012). To determine if the number of individuals of manta rays sighted on all surveys is a true representation of the population, each manta ray encounter resulted in an individual record and photo-ID and entered into a database. To determine if the number of individual manta rays is representative of the overall population, the number of new manta rays identified were plotted against the overall number of surveys, thus creating a discovery curve or species of accumulation curve graph.

4.1.1 Manta Ray Population Structure

To examine sex ration structure, manta rays were categorized into two groups, male and female. Then chi-square test was performed using sex as factor, number of individuals as counts, with an expected value of .5.

4.1.2 Manta Ray Population Age Structure

To investigate if the ratio of adult manta rays is equal or different then juvenile manta rays the number of adult manta rays each manta rays was classified as adult or juvenile. Individual manta ray photo ID and size of each manta ray was documented and categorized by sex and age class. Adults' DW size was estimated and recorded for each encounter. Male manta rays were determined by the presence of calcified claspers while adult females were determined by comparable size of adult males, presence of mating scars, and pregnancy. Juvenile manta rays were obvious by their smaller DW size. Juvenile males have claspers that are shorter than pelvic fins and are not calcified. Juvenile females are classified if their size was equivalent to the size of juvenile male mantas (Deakos, 2011; Marshall & Pierce, 2012). The sizes of six manta ray sizes were measured using the pair-laser system by Deakos (2011) to establish a scale to assist in size estimates of remaining manta rays. The number of adult manta rays was compared to juvenile manta rays.

To examine juvenile to adult structure, manta rays were categorized into two groups, sex and maturity. Then chi-square test was performed using sex and mature as factors and number of individuals as counts.

4.2. Spawning Aggregations

The ecology of spawning aggregations is flexible and changes depending on location, timing, and influential environmental factors (Sadovy de Mitcheson & Colin, 2012). Environmental factors that influence fish spawning aggregations are mostly likely a combination of atmospheric and oceanographic environments along with the fish biological ecology (Sadovy de Mitcheson and Colin, 2012). Environmental factors such as lunar moon phase, photoperiod time, and tidal stages influence the spawning of fish aggregations of *Acanthurus triostegus*, *A. guttatus*, and *A.lineatus*. *Acanthurus triostegus* and *A. guttatus* spawning data were combined and modeled together since results for each day were exactly the same, expect for photoperiod analysis species were studied separately, and then together. *Acanthurus lineatus* was modeled independently. A 95% confidence interval was determined around data and the best-fit model was chosen by comparing BIC numbers and choosing lowest number indicating the best statistical analysis.

4.2.1 **Acanthurus triostegus, A. guttatus** *and* **A. lineatus** *Spawning Aggregations*

In this study, I looked at the spawning periodicities of *Acanthurus triostegus*, and *A. guttatus* spawning aggregation and *A. lineatus* morning spawning aggregations to determine which moon day and which day of the year spawning is most likely to occur. Other factors such as moon phase, tidal height, and photoperiod that can affect spawning probability were tested to indicate any relationships. Surveys were conducted multiple times for multiple years (2012–2016) to record the presence or absence of spawning aggregations.

4.2.2 Peak Moon Age for Spawning

Acanthurus triostegus ***and*** **A. guttatus** ***Moon Age for Peak Spawning***

To determine when spawning is most likely to occur for *Acanthurus triostegus* and *A. guttatus*, moon age, a logistic regression model was fit that included a quadratic effect of moon age. After this model was fit, the moon age that yielded the highest probability of spawning was found. To account for the uncertainty in the estimate of that moon age, the method of bootstrapping (Efron,1979) was applied to find a 95% confidence interval surrounding that estimate. Bootstrapping is a statistical technique that involves fitting the same model a large number of times with resampled data to get a large number of new estimates. The number of resamples done in this instance (and future instances when needed) was 500. Moon age was measured day away from new moon, where day 15 was full moon a scale of 0-30 where 15 is a full moon and 30 is a new moon. Anything greater than 15 was subtracted by 15. To ensure there was no statistical difference of spawning patterns between full and new moon a variable called waning moon was included in the model.

Acanthurus lineatus ***Moon Age for Peak Spawning***

To determine when spawning is most likely to occur for *Acanthurus lineatus* for moon age, a similar method was used as in the above section. A logistic regression model was fit that included a linear effect of moon age that included a quadratic effect of moon age. After this model was fit, the moon age that yielded the highest probability of spawning was found. To account for the uncertainty in the estimate of the peak moon age, bootstrapping (500 resamples) was applied to find the 95% confidence intervals surrounding that estimate. Moon age was measured day away from new moon, where day 15 was full moon a scale of 0-30 where 15 is a full moon and 30 is a new moon. Anything greater than 15 was subtracted by 15. To ensure there was no statistical difference of spawning patterns between full and new moon a variable called waning moon was included in the model.

4.2.3 Day of the Year Peak Spawning

Acanthurus triostegus ***and*** **A. guttatus** ***Day of the Year for Peak Spawning***

A similar method was used to determine which day of the year spawning is most likely to occur for *Acanthurus triostegus* and *A. guttatus*. A logistic regression model was fit with a quadratic term for day of the year. By fitting the model, the day most probable day of spawning was found. To account for the uncertainty in the estimate of that peak spawning day of the year, bootstrapping was applied to find the 95% confidence interval surrounding that estimate.

Acanthurus lineatus Day of the Year for Peak Spawning

To determine which day of the year spawning is most likely to occur for *Acanthurus lineatus* a logistic regression model was fit with a quadratic term for day of the year. By fitting the model, the day most probable day of spawning was found. After this model was fit, the day of the year that yielded the highest probability of spawning was found. To account for the uncertainty in the estimate of that peak spawning day of the year, bootstrapping was applied to find the 95% confidence interval surrounding that estimate.

4.2.4 Environmental Factors of Spawning

Environmental Factors of **Acanthurus triostegus** *and* **A. guttatus** *Spawning*

To test if certain atmospheric and oceanographic factors relate to the predictability of *Acanthurus triostegus* and *A. guttatus*, spawning, a multiple logistic regression model was run. Variables were used to accounted for seasonal and lunar effects. The seasonal effect was measured by using the number of days before or after the day of the year that yielded the highest probability of spawning using the method described above in section 4.1.2. The lunar effect was measured by using the number of days before or after the moon day on which spawning was most probable, as described above in 4.1.1. The multiple logistic model included these two variables, as well as the average tidal height over the course of spawning.

Environmental Factors of **Acanthurus lineatus** *Spawning*

To test if certain atmospheric and oceanographic factors relate to the predictability of *Acanthurus lineatus*, spawning, a logistic regression model was run. Variables were created that accounted for seasonal and lunar effects. The seasonal effect was measured by using the number of days before or after the day of the year that yielded the highest probability of spawning using the method described above in section 4.1.2. This variable was named days away [season]. The lunar effect was measured by using the number of days before or after the moon day that yielded the highest probability of spawning, as described above in 4.1.1. A variable was named days away [moon age]. The multiple logistic model included these two variables as well as the average tidal height during spawning.

4.2.5 Photoperiodicity of Spawning

Acanthurus triostegus

To test if photoperiod affects the probability of spawning, of *Acanthurus triostegus* a best fix polynomial regression was run with time of spawn in minutes in relationship to sunset time in minutes. To check the relationship of spawning time differed between species I examined the 95% Confidence Interval to see if they are different when compared of the two best fix lines.

Acanthurus guttatus

To test if photoperiod affects the probability of spawning, of *Acanthurus guttatus* a linear regression statistical analysis was run with time of spawn in minutes in relationship to sunset time.

Photoperiodicity of* Acanthurus triostegus *and* A. guttatus *Spawning

To test if photoperiod affects the probability of spawning differently for *Acanthurus triostegus*, and *A. guttatus* a regression was run with time of spawn in minutes to sunset time in minutes. This was done by including variables for sunset time, species, and interactions between the two. Our model considered how the sunset time affected differently *A. triostegus*, and *A. guttatus* spawning.

Photoperiodicity of **Acanthurus lineatus**

To test if photoperiod affects the probability of spawning, of *Acanthurus lineatus* a regression statistical analysis was run with time of spawn in minutes in relationship to sunset time in minutes. A 95% confidence interval was placed around spawning times.

4.3 Manta Ray Targeting Spawning Aggregation to Feed

Fish spawning aggregations attract oophagous and piscivorous predators. To determine if the predatory behavior of manta rays targeting fish spawning aggregations to feed off fish spawn was significant, surveys were conducted throughout the year. Presence or absence of fish spawning aggregations and manta rays feeding at a site were documented. A chi-square test was run to test the association of number of manta rays present at number of spawning aggregations. A Yates' continuity correction was applied.

4.4 Frequency of Manta Rays Targeting Fish Spawning Aggregations

To assess if the frequency of manta ray sighting changes throughout the fish spawning season, a logistic regression assuming Poisson-distributed residuals was used with mean of the number of mantas as the response variable and months away from the peak month for spawning as a variable.

4.5 Manta Ray Sex Ratio at Targeting Fish Spawning Aggregations to Feed

Manta Rays at **Acanthurus triostegus** *and* **A. guttatus**

To examine the different between sexes of manta rays targeting fish spawning aggregations for *Acanthurus triostegus* and *A. gutttatus* a chi-square analysis was used with males and females as factors, with number of individuals as counts, with an expected value of .5.

Manta Rays at **Acanthurus lineatus**

To examine the different between sexes of manta rays targeting fish spawning aggregations for *Acanthurus lineatus* a chi-square analysis was used with males and females as factors, with number of individuals as counts, with an expected value of .5.

Chapter 5 Results

5.1 Manta Ray Population

An understanding of species-basic biology and ecology is important for management and conservation. Knowledge of a species population size is necessary in comprehending anthropogenic and natural threats along with impacts affecting the ecology of the species (Heyman, 2001, Deakos, 2011, Sadovy de Mitcheson and Colin, 2012). To determine if the number of individuals of manta rays sighted on all surveys is a true representation of the population, each manta ray encounter resulted in an individual record and identified from photos and entered into a database. To determine if the number of individual manta rays is representative of the overall population, the number of new manta rays identified were plotted against the overall number of surveys, thus creating a discovery curve or species of accumulation curve graph (Figure 13). A total of 44 individual manta rays were sighted throughout all 264 surveys. At the beginning of the study, the steep curve is the result in high number of new identifications. Towards the end of the study, the slope over time decreases, greatly, but still does not reach an asymptote. The discovery curve or species-accumulation curve indicates that the population is slightly larger than the total mantas recorded and that the final accumulated number is 45-and 50. recorded number is reaching a point a representation of the true population (Chao & Shen, 2004; Deakos, 2011).

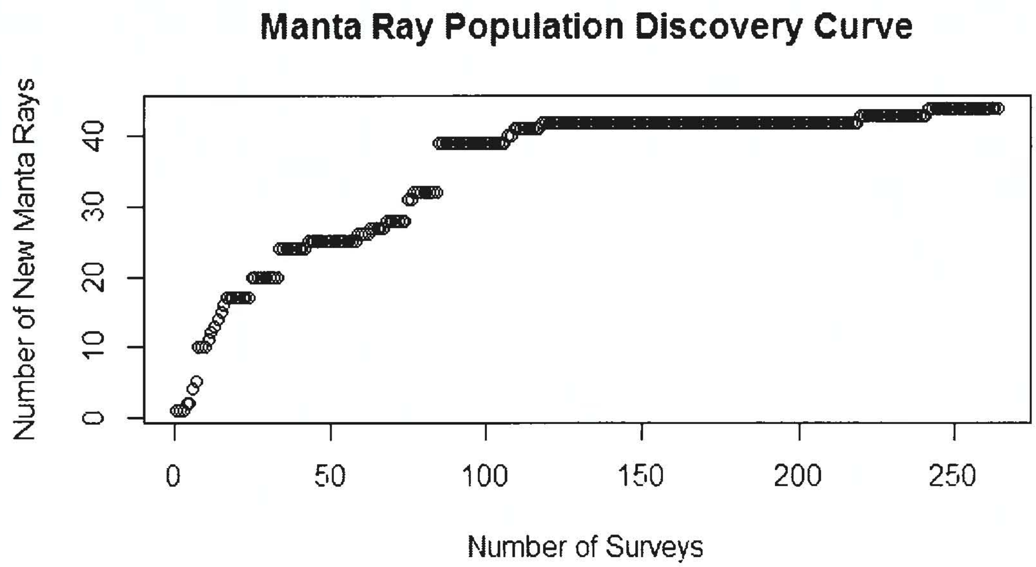


Figure 13. Discovery Curve from the population of Guam manta rays as cumulative of new individuals to each survey completed.

5.1.1 Manta Ray Population Structure

A chi-square test was performed using male and female as factors and number of individuals as counts (Table 1). The Population structure consists of 47% males, 53% female and not significant between sex gender. One manta ray's sex was undisguisable and was not used in the analysis.

Table 1. A Chi-square test with number of male to female counts.

	Number of Manta Rays
Male	20
Female	23
X squared = 0.20947, df = 1, p-value=0.6472	

5.1.2 Manta Ray Population Age Structure

Adult manta rays account for 93% of the population, while 7% are juveniles (three different individuals). The population consists of more adults then juveniles. Since the population consisted of only three juveniles, a statistical analysis was not necessary.

5.2 Spawning Aggregation

5.2.1 Peak Spawning for Moon Age

Acanthurus triostegus ***and*** **A. guttatus** ***Moon Age for Peak Spawning***

As seen in Figure 14, an estimate of the moon day where peak spawning would most likely occur could not be accurately determined. Even though this estimate was inconclusive, the most likely moon day was found to be day 11 and 26, four days before full and new moon as noteworthy. The variable waning moon was added to the linear regression and did not affect the results and the BIC level, suggesting there is not a difference in the probability of spawning between days before a full moon and days before a new moon, allowing the moon days to be scaled 0-15 together. Spawning was observed on the 8^{th} –15^{th} and 21^{st}–30^{th} lunar days.

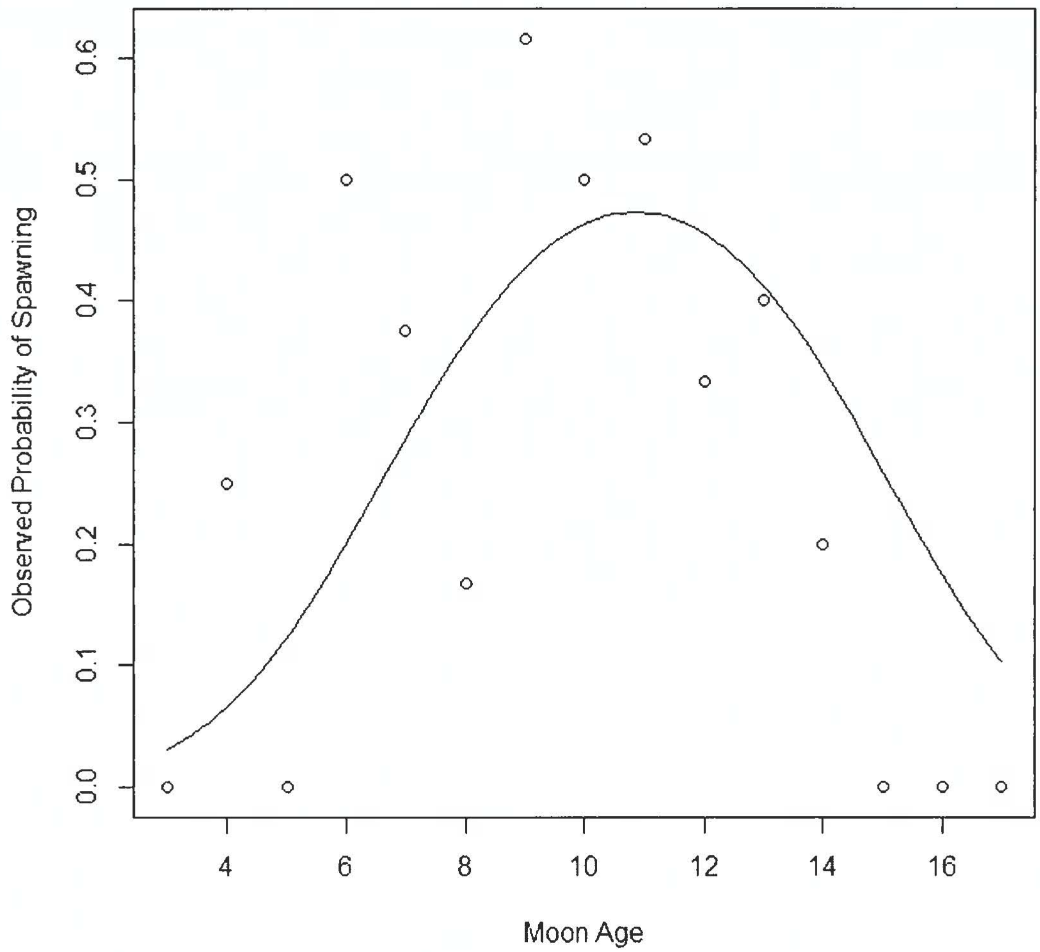


Figure 14. Moon age compared to the log odds of *A. triostegus* and *A. guttatus* spawning, moon age was scaled 0-15, with 15 equal to full/new moon.

Acanthurus lineatus *Moon Age for Peak Spawning*

Figure 15 estimates the moon age for the maximum fish spawn on the 13.74 day, one day before full and new moon, with a 95% confidence interval of 13.08 to 14.60 moon days. The variable waning moon was added to the linear regression and did not affect the results and the BIC level, suggesting there is not a difference in the probability of spawning between days before a full moon and days before a new moon, allowing the moon days to be scaled 0-15 together. Spawning was observed the 11th –19th and 25th –5th lunar months.

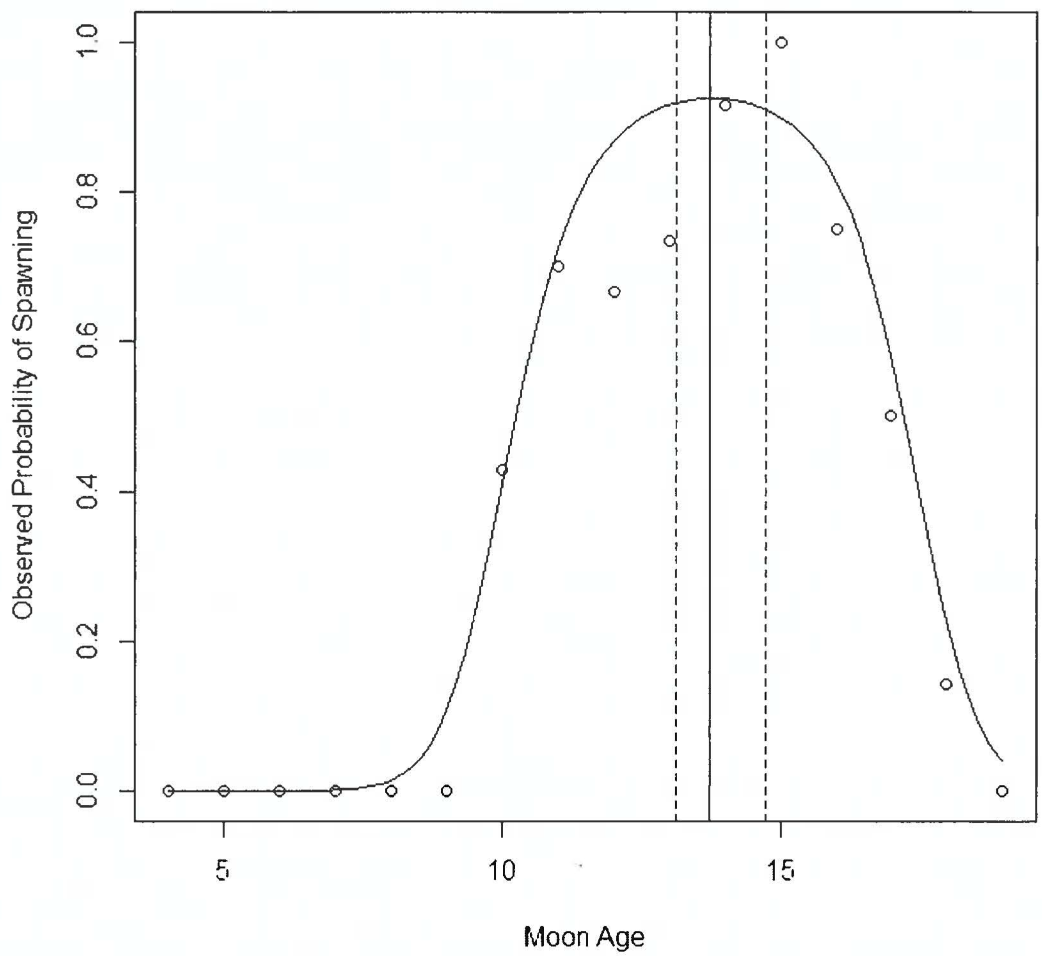


Figure 15. The observed probability of spawning of *A. lineatus* spawning compared moon age is scaled 0-15, with 15 equal to full/new moon. Moon day of peak spawning was 13.74 with 95% confidence intervals from moon days 13.08 to 14.60.

5.2.2 Day of the Year Peak Spawning

Acanthurus triostegus ***and*** **A. guttatus** ***Peak Day of the Year for Peak Spawning***

Day 56.9 (February 26th) was the most probably day for *A. triostegus* and *A. guttatus* spawning with a 95% confidence range between 48.7, and 162.7 days, (February 18th through March 16th calendar days), as seen in Figure 16.

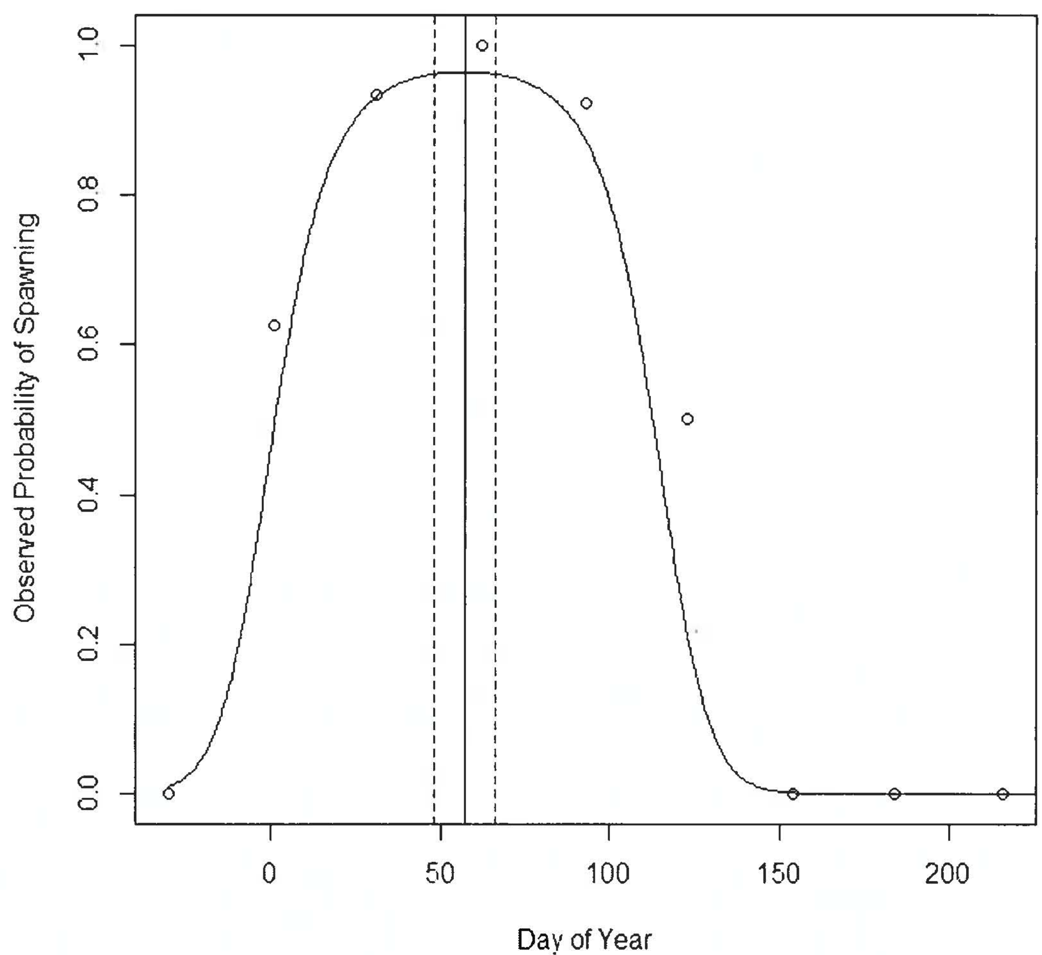


Figure 16. The observed probability of spawning compared to day of the year mostly likely to get peak spawning with a 95% CI between 48.7-62.7.

Acanthurus Lineatus ***Day of the Year for Peak Spawning***

The maximum probability of *Acanthurus lineatus* spawning on a given day, of a given year was found to be on the 145.3 day, calendar day May 25th with a 95% confidence range between 126.5, and 162.7 days, May 6th through June 16th calendar days (Figure 17). To 95% confidence interval was determined by the method bootstrapping.

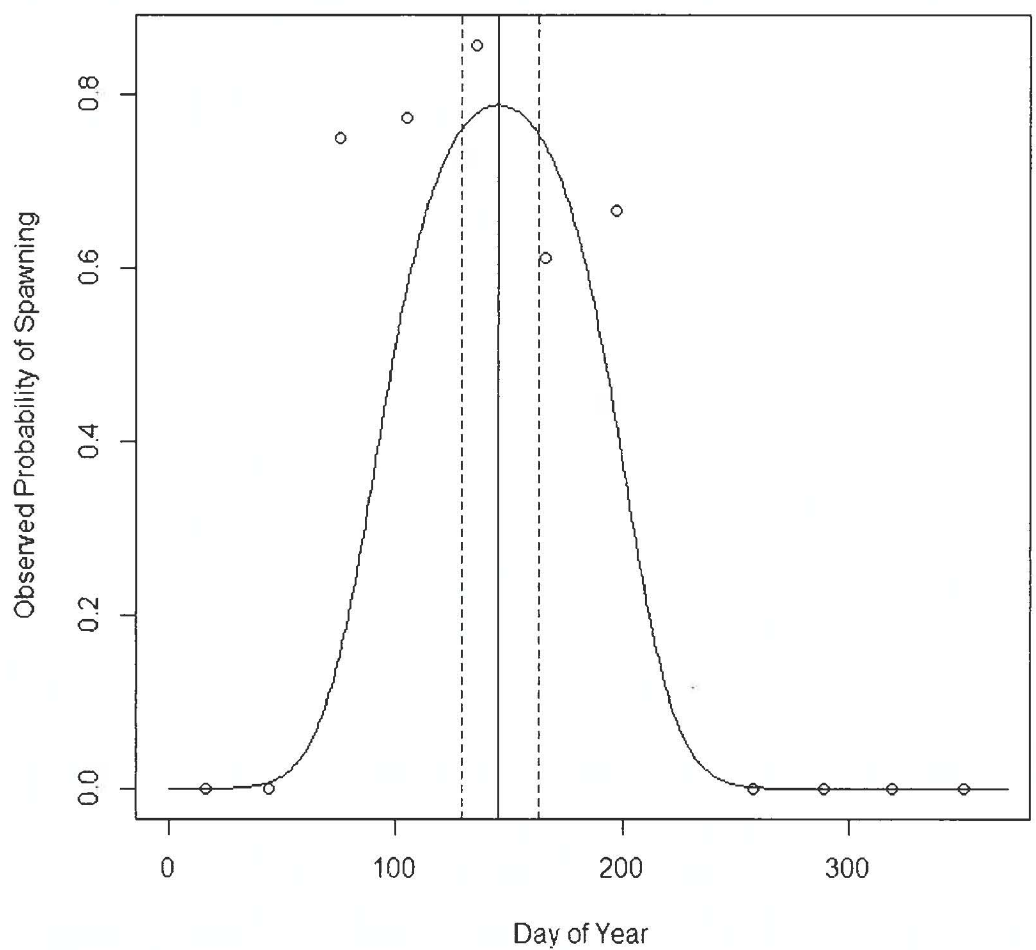


Figure 17. Day of the year compared to the observed present or absent of *A. lineatus* spawning. Day for peak spawning for year was 145.3 with a 95% CI between 126.5 to 162.7.

5.2.3 Environmental Factors for Spawning

Environmental Factors of Acanthurus triostegus and A. guttatus Spawning

The best-fit multiple logistic regression model (Table 2) found that two different variables are the strongest predictor for *A. triostegus*, and *A. guttatus* spawning: days away from February 26th, and tide depth. The further one gets away from February 26th the more the probability of spawning decreases (p = 0.00043) as seen in the predicted model for spawning 2015 (Figure 18). There is also an interaction of tide suggesting that the future you get away from February 26th the relationship between spawning and tide decreases (Figure 19). The statistical significance of tidal height indicates that as the average tidal height during spawning decreases so does the probability of *A. triostegus*, and *A. guttatus* spawning. Tide was found to be a stronger predictor of spawning then moon age (Table 2). Back transformation from the log odds scales, given the tide depth was 0 and the date was February 26th, we expect the odds of spawning to be 0.24 with a 95% confidence interval of 0.033 to 1.69. This equates to a 0.19 probability of spawning, with a confidence interval of 0.03187 to 0.6279. The two effects observed below in Table 2 (tide depth and the interaction of tide depth and season) were both significant. Moon age and tidal phase factors were correlated (r = 0.62). When both tidal and moon age were used jointly in the model, moon age was not significant; when moon age is used in the model without tide depth, it is significant (Table 3).

Table 2. Best-fit multiple logistic regression model using days away from peak spawning February 26th, and average tidal height as variables. With z = -3.52 and a p = .00049 significant to average tidal height BIC of 39.7.

	Estimate	Std. Error	*z*	*p*
Intercept	-1.45	1.00	-1.44	0.15
Tide Depth (meters)	26.91	7.723	3.48	0.00049
Tide Depth (meters) * Days Away	-0.39	0.110012	-3.52	0.00043

Table 3. Best-fit multiple logistic regression using adjusted moon age and days away from February 26th as variables.

	Estimate	Std. Error	z	p
Intercept	11.94	3.456	3.45	0.00055
Days Away (moon)	-1.768	0.68	-2.59	0.0095
Days Away	-0.18	0.053	-3.43	0.00060
Days Away (moon) * Days Away (Season)	0.023	0.0096	2.36	0.018

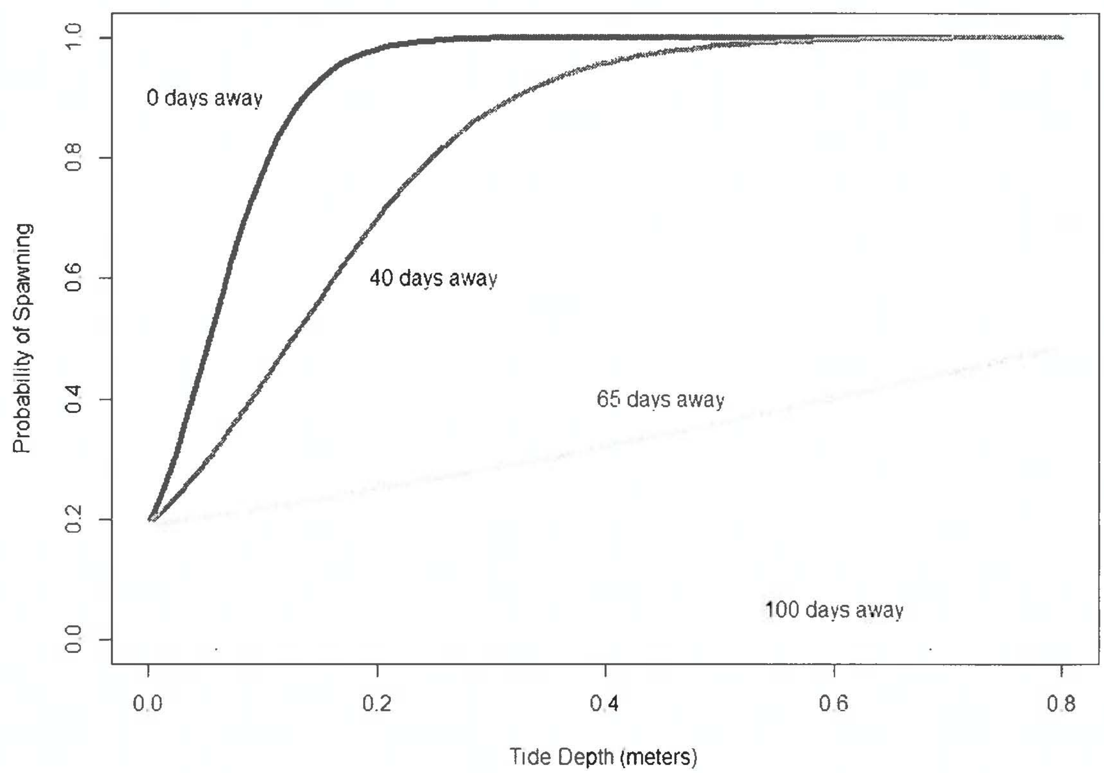


Figure 18. This graph indicates the interaction between average tidal height and days away from February 26th. The further you get away from February 26th the relationship between average tidal height decreases in predicting *Acanthurus triostegus*, and *A. guttatus* spawning.

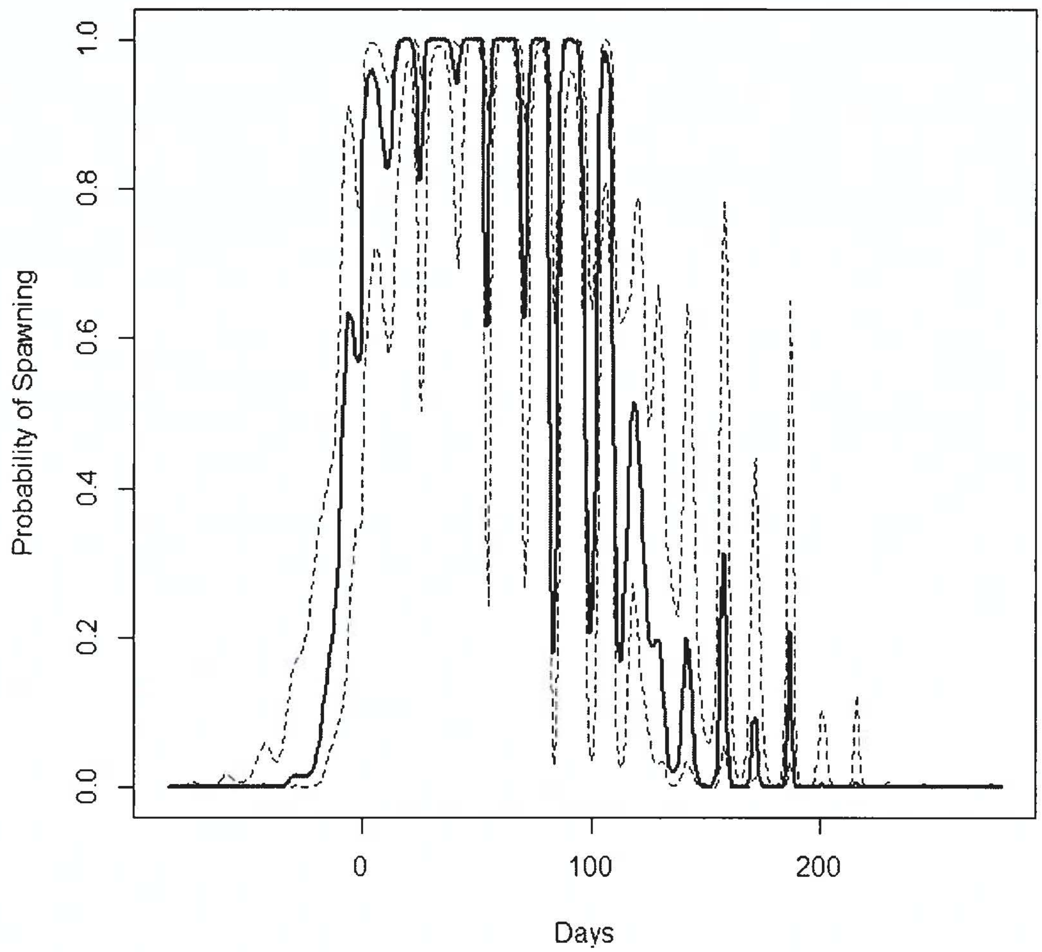


Figure 19. Predicted model for spawning for 2015 Acanthurus triostegus and A. guttatus, whereas 0 is February 26th with 95% CI around the estimates. The confounding effect lunar days creates a rutted line of estimates.

Environmental Factors of Acanthurus Lineatus Spawning

A multiple logistic regression model for Acanthurus lineatus was performed using the variables, spawn days away from May 25th, one day away from full/new moon (adjusted moon age), and average tidal height during spawn events. The best model to predict spawning was days away from May 25th plus adjusted moon age. Both were significant and an interaction was not detected. Adjusted moon age was significant with a p-value of 1.52E-05. The seasonal factor days away from May 25th was significant with a p-value of .00038 (Table 4). It was determined that there is a relationship between tide depth and moon age similar to the other surgeonfish having a correlation coefficient r=.81. Tidal height was not significant after accounting for the lunar effect. This model is saying that the further one gets away from adjusted moon age (one day before full and new moon) the higher the probability of spawning goes down as seen in Figure 20. Back transformation for the log odds scale, given on one day before a full/new moon and on May 25th, the expected odds of spawning was 272.08 with a 95% confidence interval of 28.027 to 264.31. This equates to a .9963 probability of spawning, with a confidence interval of .9656 to .9996. The two effects observed above (moon age and season) were both found to be significant. Back transforming the moon age effect, we observe a decrease of the odds of spawning by a factor of .42 for every 1 additional day further away from the optimum moon age for *Acanthurus lineatus* (moon day 14 or 29) with a 95% confidence interval of .2838 to .6225. Back transforming the seasonal effect, we observe a decrease of the odds of spawning by a factor of .9491 for every 1 additional day further away from May 25th with a confidence interval of .9221 to .9768. Additionally, the further you get away from May 25th the less likely *A. lineatus* will spawn as seen in Figure 20. This can be illustrated from the predicted spawning for 2015 from Figure 21.

Table 4. Multiple logistic regression model for *A. lineatus* spawning aggregation of days away (1 day before full/new moon) from May 26th of peak spawning and adjusted moon day.

	Estimate	Std. Error	z	p
Intercept	5.61	1.16	4.83	1.34E6
Days Away	-0.87	0.20	-4.33	1.52E5
Days Away (from May 25th)	-0.053	0.015	-3.55	0.00038

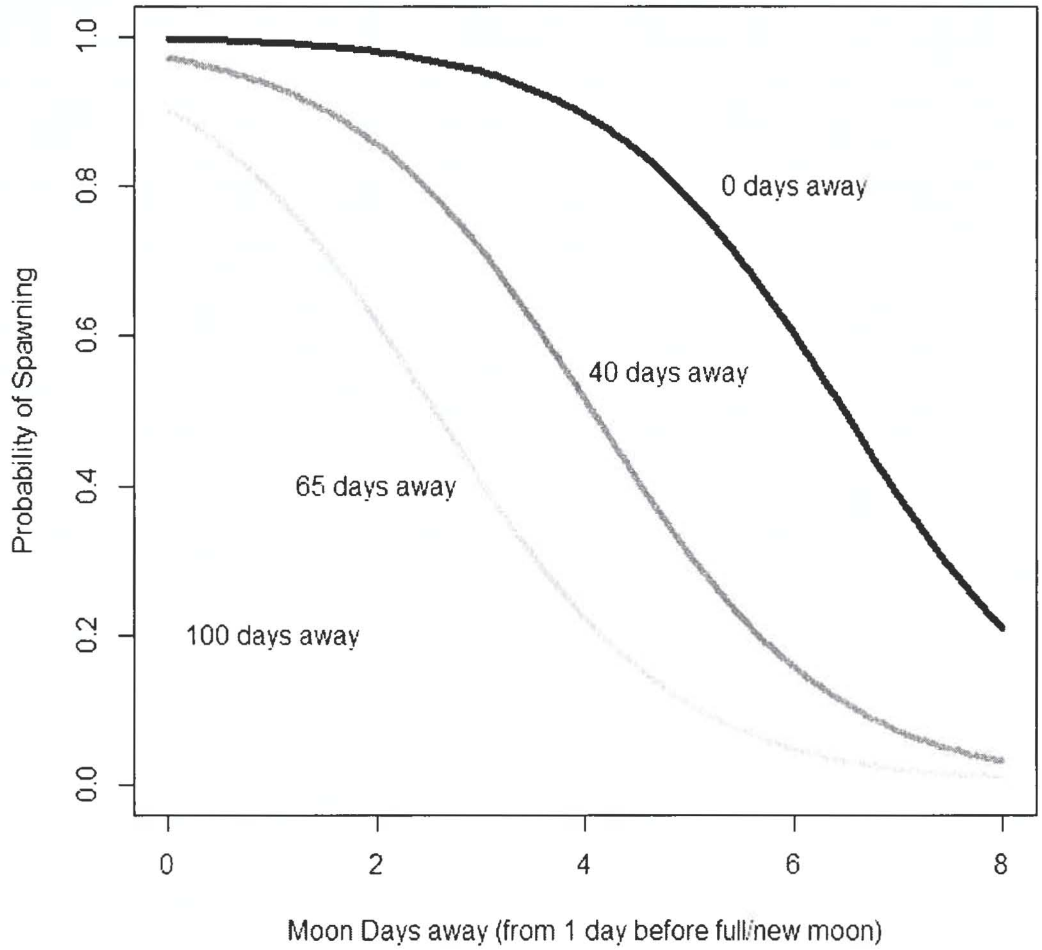


Figure 20. The probability of spawning the further you get away from full/new moon in relationship to the further you get away from May 25th (seasonal spawning time).

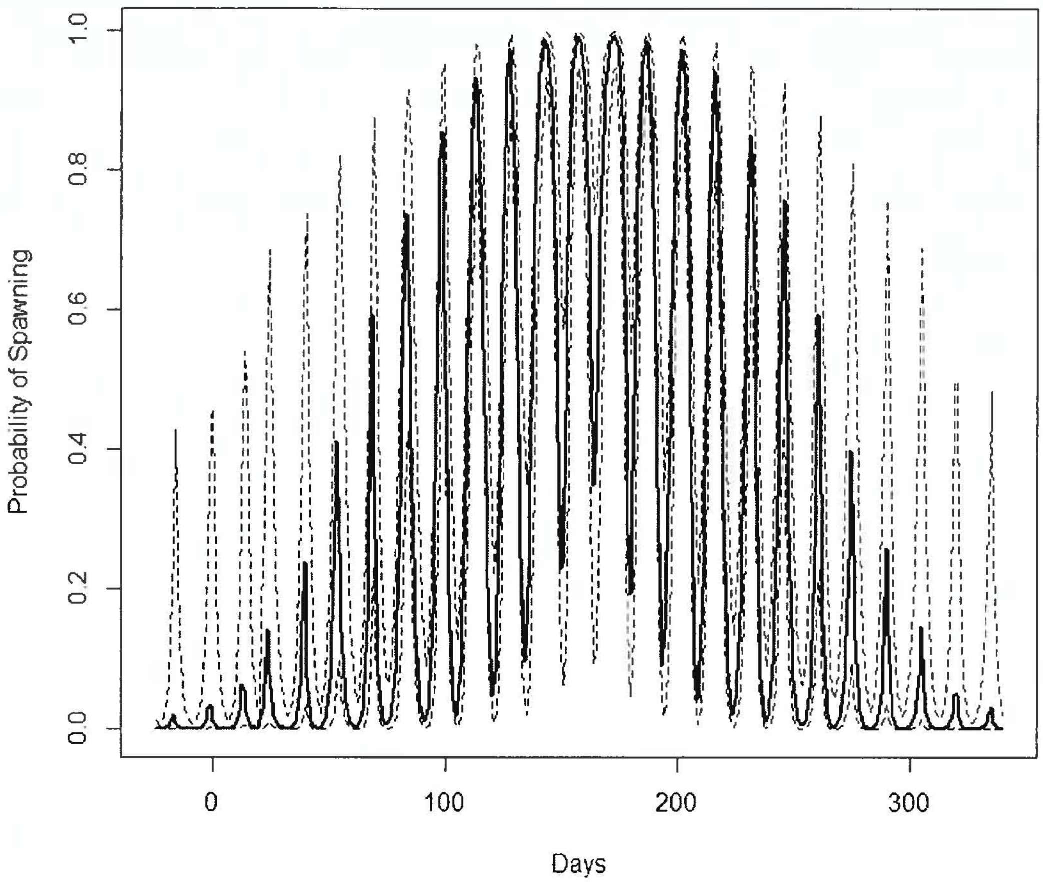


Figure 21. Predicted model for Probability of A, lineatus spawning for 2015, with 95% CI. The rutted lines of estimates for probability of spawning comes for the confounding factors between moon age and tidal height.

5.2.4 Photoperiodicity of Spawning Aggregations

Acanthurus triostegus

The probability of spawning, of *Acanthurus triostegus* the best-fit line with a quadratic relationship was a statistically significant with a p-value of 5.15E5, with times fish spawned in minutes, in relationship to sunset times in minutes (Table 5). The slops and the intercepts are greater than zero as indicated with the p-values less than .05. As sunset time increases so does the probability of spawning (see Fig. 4). The quadratic relationship illustrates that that there is most likely a decreasing relationship for *A. triostegus*, when sunset occurs earlier with a p of 5.11E5.

Table 5. Acanthurus triostegus spawning in minutes compared to sunset time in minutes. With a t = 4.55 and p = 5.15E5 with and interaction between spawning predictability as sunset gets earlier with a p = 5.11E5.

	Estimate	Std. Error	*t*	*p*
Intercept	91029.35	19779.93	4.60	4.36E5
Sun Set Time	-163.64	35.98	-4.54	5.15E5
(Sun Set Time)^2	0.07	0.01	4.55	5.11E5

Acanthurus guttatus

Acanthurus guttatus was statistically significant with a p-value of 0.00023 (Table 6) demonstrating that the time of spawning is correlated to the time of sunset. As sunset time shifts to later in the evening, so does the time *A. guttatus* spawns (Figure 22).

Table 6. Acanthurus guttatus spawning in minutes compared to sunset time in minutes with t = 4.044 and p = 0.00023.

	Estimate	Std. Error	*t*	*p*
Intercept	315.71	193.01	1.64	0.11
Sun Set Time (in minutes)	0.706	0.18	4.044	0.00023

Photoperiodicity of Acanthurus triostegus and A. guttatus Spawning

Results from statistically combining *Acanthurus triostegus*, and *A. guttatus* into the same model revealed a statistically significance interaction which signifies that the effect of sunset time on spawning times is different for *A. triostegus*, and *A. guttatus* (Table 7 and

Figure 22). To interpret this model, the p-value for *A. triostegus* is 8.25E6 indicating that the intercept of *A triostegus* and *A. guttatus* are different. The *p* value for Aca. Tri * Sun Set Time is 8.88E6 signifies that the linear relationship is different for how sunset time affects the fish spawning. By collectively looking at both these p-values it proves that the relationship is different even though we know both species of fish spawns in relationship to when the sun sets. Similarly, as seen in Table 7 it reveals a quadratic effect p-value of 8.88E6. There is curved relationship between *A. triostegus* and sunset time, meaning that while our model usually found a positive relationship between sunset time and spawning times for *A. triostegus*, and *A. guttatus,* there is most likely a decreasing relationship for *A. triostegus*, when sunset occurs earlier. In Figure 22, the black line is sunset and confidence bands were placed around spawning times for *A. triostegus*, and *A. guttatus*. You can see that *A. guttatus* spawning typically occurred before sunset while *A. triostegus* spawns after sunset.

Table 7. The best-fit linear regression model for times of spawning in minutes *A. triostegus* and *A. guttatus* compared to sunset times with a 95% confidence interval. With a *$t = 4.77$ and a $p = 8.25E6$.*

	Estimate	Std. Error	t	p
Intercept	315.71	201.28	1.57	0.12
Sun Set Time (in minutes)	0.70	0.18	3.88	0.00021
Aca. Triostegus	90713.64	19013.72	4.77	8.25E6
Aca. Tri. * Sun Set Time	-164.35	34.59	-4.75	8.88E6
Aca. Tri. * (Sun Set Time)^2	0.075	0.016	4.73	9.51E6

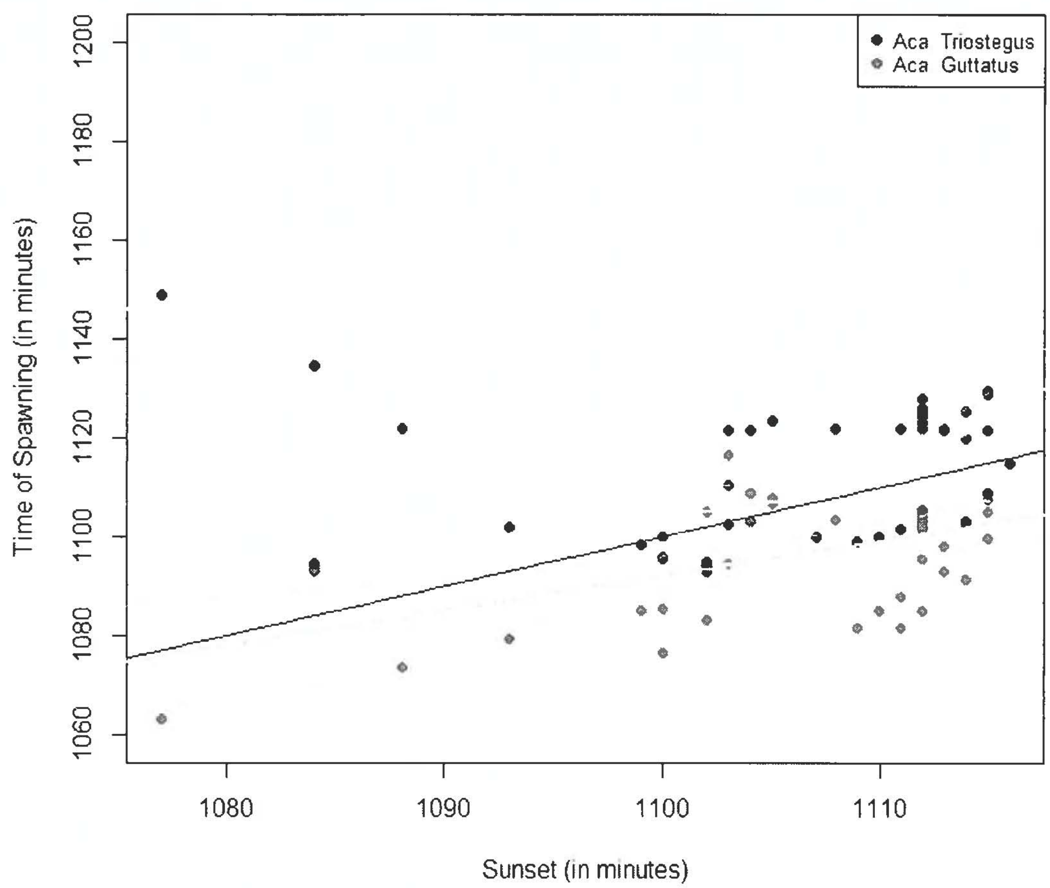


Figure 22. Time of spawning of *A. triostegus* and *A. guttatus* in relationship to sunset times in minutes with a 95% confidence bands around spawning times.

Photoperiodicity of Acanthurus lineatus *Spawning*

To test if photoperiod affects the probability of spawning of *Acanthurus lineatus*, a linear regression was run with time of spawn in minutes to sunrise in minutes (Figure 23).

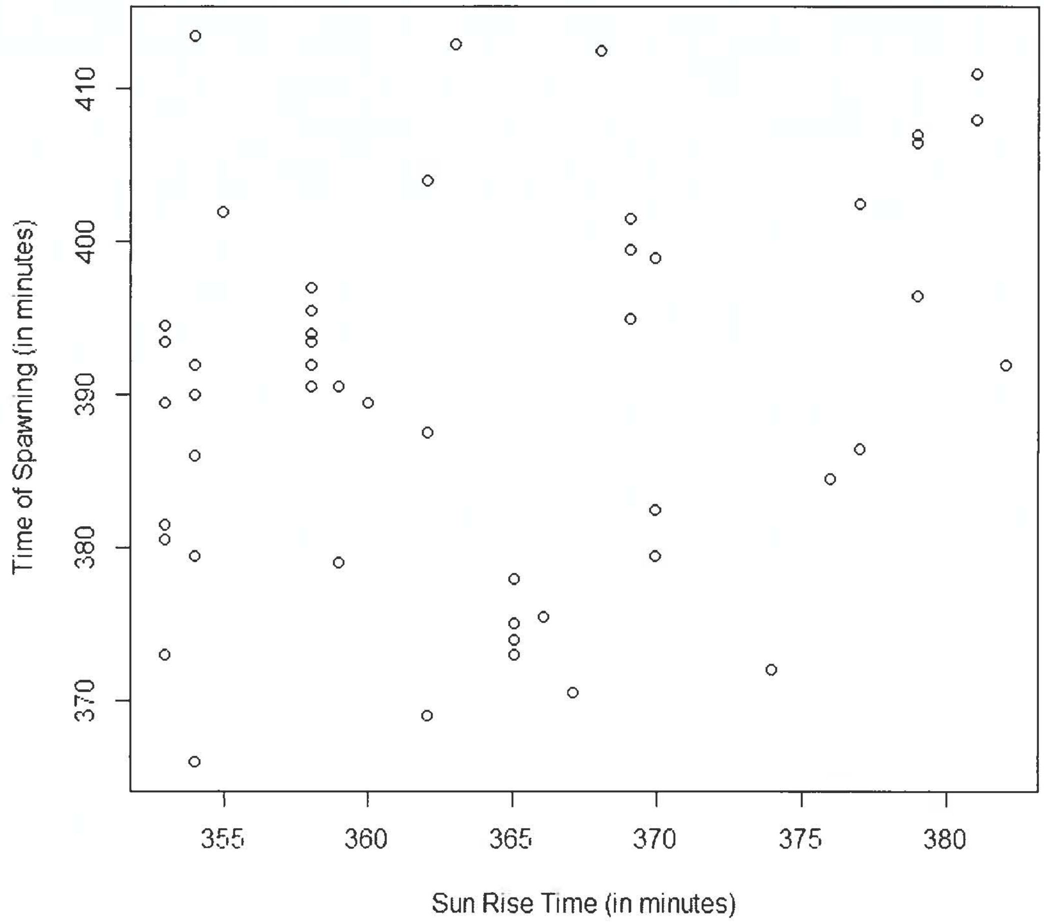


Figure 23. Sun rise times in minutes compared to time in minutes *A. lineatus* spawn.

Time of sunrise is not a predictor of *A. lineatus* having a *p* of .104 (Table 8). Interpretation of this model is that spawning time of *Acanthurus lineatus* is not correlated to time of sunrise.

Table 8. *Acanthurus lineatus* spawn times compared to sun rise times in minutes.

	Estimate	Std. Error	t value	p value
Intercept	274.7177	69.96136	3.926706	0.000275
Sun Rise Time (minutes)	0.318017	0.19225	1.654181	0.104616

5.3 Manta Ray Targeting Spawning Aggregation to Feed

Guam manta rays, *Manta alfredi* to be statistical significant targeting fish spawning aggregations to feed off fish spawn. With 142 spawning aggregations observed, 117 spawning aggregations had manta rays present to feed off fish spawn with a significant p-value of 2.2e-16 (Table 9).

Table 9. A Chi-square test with manta ray and spawning aggregation presence or absence, with a Yates' continuity correction.

	Fish Aggregations	
Manta Rays	absent	present
absent	170	25
present	19	117

Pearson's Chi-squared test with Yates' continuity correction
X-squared = 172.32, df = 1, p-value < 2.2e-16

5.4 Frequency of Manta Rays Targeting Fish Spawning Aggregations

The frequency of manta rays (mean number of manta rays) compared to the peak month of spawning by a logistic regression assuming Poisson-distributed residuals was found to be statistically significant with a $|z|$ of 1.33E16. The log number of manta rays we see in March is 5.579 and that for every one month away from March we'd expect the log number of mantas to go down by .73 (Table 10). Back transformation from the log scale we would expect to see 264.8 mantas in the month of March, and for every 1 month away from March we would expect to see a decrease of manta ray sightings to decrease by a factor of .482. It would suggest that in April we would except to see approximately 127.6 manta rays 61.5 manta rays in May and so on (Figure 24).

Table 10. A logistic regression assuming Poisson-distributed residuals of manta ray frequency months away from peak spawning month of March.

	Estimate	Std. Error	*z*	|z|
Intercept	5.58	0.05	110.2	0
Months away March	-0.73	0.029	-24.88	1.33E16

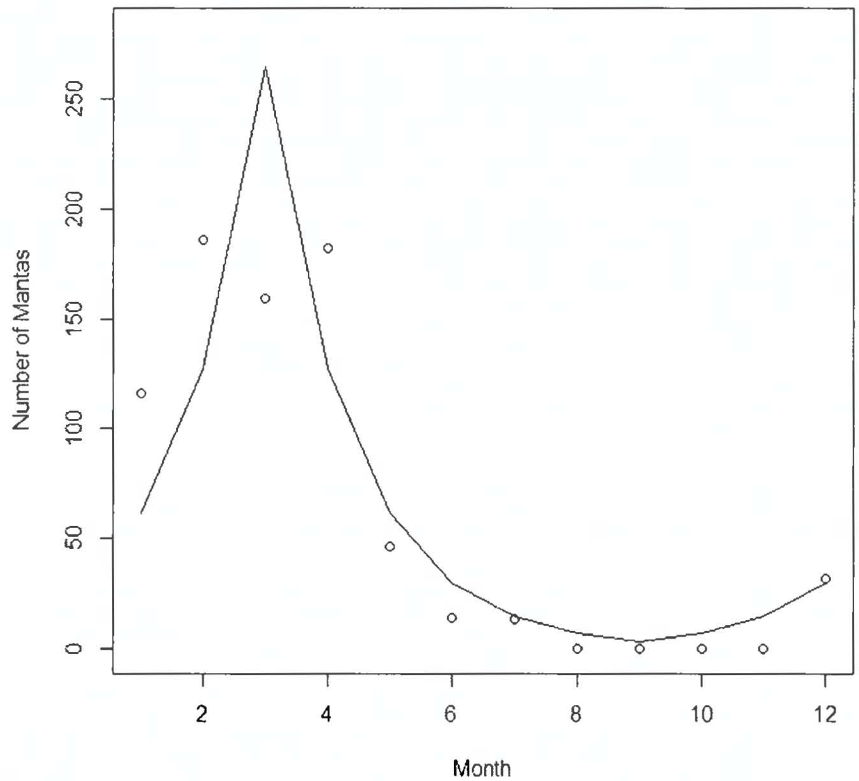


Figure 24. Number of manta rays, *Manta alfredi* when compared to months throughout the year.

5.5 Manta Ray Population Gender Targeting Fish Spawning Aggregations to Feed

Manta rays targeting fish spawning aggregations of *Acanthurus triostegus* and *A. guttatus* was found to be male bias present to feed. With 163 male manta rays compared to 122 female manta rays with expected frequency of .5 (Table 11). However, manta rays targeting fish spawning aggregations of *Acanthurus lineatus* to feed was found not significant with a *p* of 0.059 to differences of sex (Table 12).

Table 11. Chi-square test with number of male to female counts at *Acanthurus triostegus* and *guttatus* spawning aggregations feeding. With a p of 0.0245 it indicates a male manta ray bias feeding at evening spawn.

	Number of Manta Rays
Male	163
Female	122
X squared = 5.056, df = 1, p-value=0.025	

Table 12. A Chi-square test with number of male to female counts for *Acanthurus lineatus* spawning aggregatoins feeding.

	Number of Manta Rays
Male	100
Female	75
X squared = 3.57 df = 1, p-value=0.059	

Chapter 6 Discussion

In this section I will discuss observational and statistical findings for the three categories of hypothesis and results in this study: 1) Guam manta-ray population and how the results compare to other manta ray studies. 2) *Acanthurus triostegus A. guttatus* and *A. lineatus* fish species and the environmental and atmospheric factors that influence spawning aggregations. 3) Predatory behavior of manta rays targeting fish spawning aggregations to feed.

6.1 Manta Ray Population

The Guam manta-ray population consists of 44 individuals (Figure 25). This is the smallest population documented so for *Manta alfredi.* Several studies of manta ray populations have identified individual population numbers ranging between 320 in Maui, Hawaii (Deakos *et al.*, 2011), to over 1500 manta rays in the Maldives (Stevens, #). The discovery curve of mantas on Guam approaches an asymptote, indicating a population of about 45-50 manta rays (Figure 13). This small population therefore may be vulnerable to local extinction from natural or anthropogenic factors abetted by their life history; *e.g.*, late sexual maturity and low fecundity (Marshall *et al.*, 2011; Deakos *et al.*, 2011).

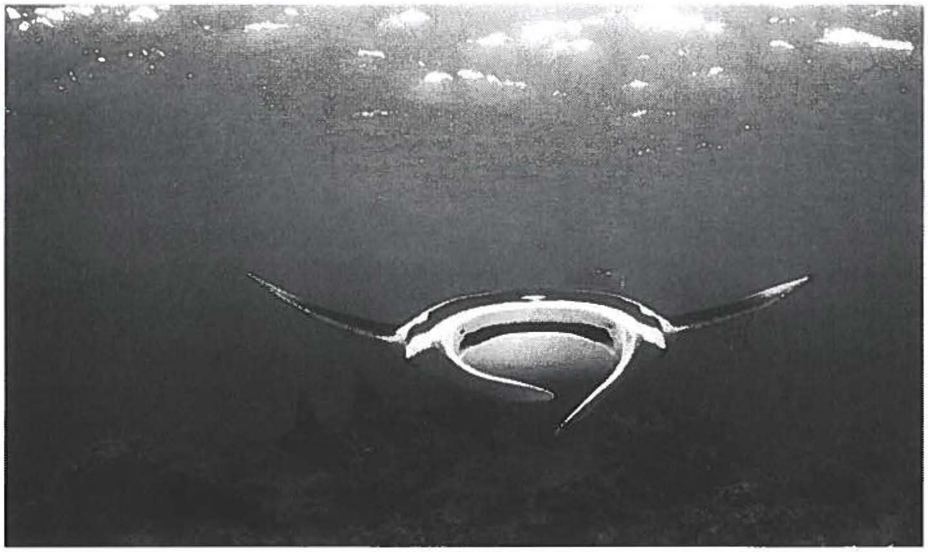


Figure 25. Guam manta ray at fish spawning aggregations.

Of the 44 manta rays observed on Guam, 47% were male and 53% were female, statistically indistinguishable from a 1:1 sex ratio (Table 1). Even sex ratios are also recorded in Maui where the population consists of 55% male and 45% females (Deakos *et al.*, 2011). In contrast, biased sex ratios are only seen in manta rays recently decimated by natural and anthropogenic factors, such as shark bites, boat strikes (Figure 26), and commercial fishing, both targeted fishing and by-catch (Deakos *et al.*, 2011; Marshall *et al.*, 2011; Couturier *et al.*, 2012). In Mozambique, females made up 78% of the population (Marshall *et al.*, 2011). There, targeted fishing has reduced the population by 96% since 2008, due to the popularity of their gill rakers as medicine in East Asia (Figure 27) (Marshall *et al.*, 2011). In Yap, Micronesia, the manta-ray population is comprised of only 33% females, where there is a large by-catch of mantas by tuna fishing industry (Julie Hartup, pers. obs.). The lack of sex bias in Guam and Maui's populations suggests that the level of threats both natural and anthropogenic are currently less than in other regions

(Marshall *et al.*, 2011; Deakos *et al.*, 2011). However, this can change quickly, given Guam's proximity to Asia and its convenience for shipping catch to market by air.

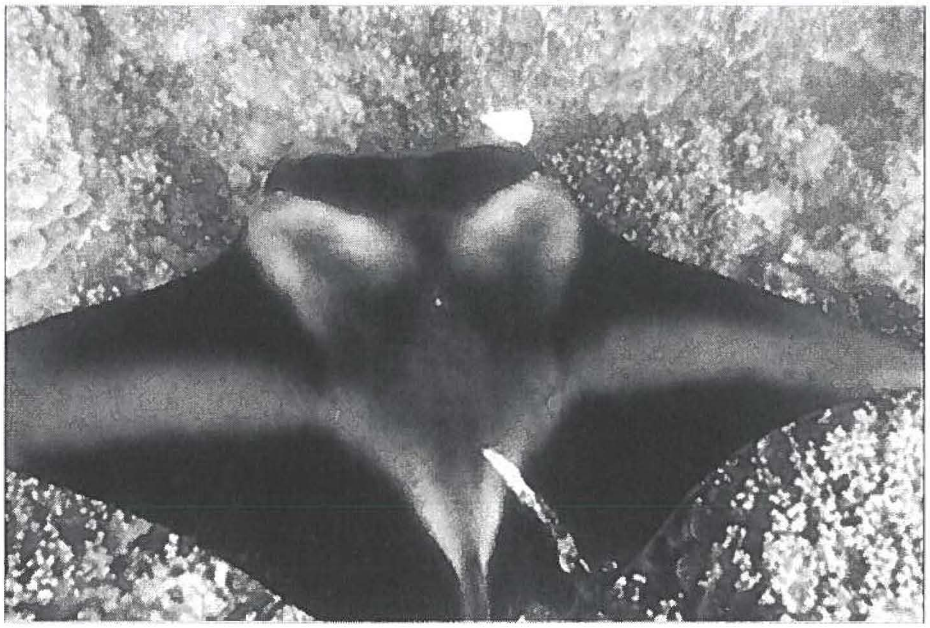


Figure 26. Picture of Guam manta rays from boat strike.

Figure 27. A mobula in southern Asia, fished for its gill rackers (Photo courtesy of Tom Taucher).

6.2 *Spawning Aggregations*

Even though the genus *Acanthurus* has the most species that form spawning aggregations, the three species *Acanthurus triostegus*, *A. guttatus*, and *A. lineatus* have never been comprehensively observed at spawning aggregations over a five-year period. Prior to this study, *Acanthurus triostegus* (Figure 28) and *A. lineatus* in Guam were thought to have year-round spawning and no lunar patterns (Davis, 1985), as seen in Samoa for *A. triostegus* (Craig, 1998). However, this study found that *Acanthurus triostegus* and *A. lineatus* in Guam spawn seasonally with lunar periodicity (Table 3, Table 4, Table 5, and Figure 19, Figure 21). Similar spawning patterns were noted for *A. triostegus* in Hawaii (Randall, 1961) and in Palau, Micronesia (Johannes, 1981). Additionally, Molina (1983) documented localized increased abundances of acanthurids in Guam between September and December. She suggested the increase was due to either concentration of spawning adults or genuine population growth by recruitment. Since spawning aggregations of the three acanthurids species studied here occur in December to May (Table 2,Table 3, Table 4 and Figure 18, Figure 20), the increases observed by Molina (1983) are almost certainly due to recruitment.

Seasonal spawning is widespread amongst *Acanthurus* species, the number of spawning days per lunar month in Guam is higher than previously recorded. *Acanthurus triostegus and guttatus* spawn at least 16 days per lunar month, while A. lineatus spawns' 18 days over this period (8^{th}–15^{th} and 21^{st}–30^{th} lunar days) with peak spawning on day 11^{th} and 26^{th} lunar days (Table 2, Table 3, Table 5 and Figure 18). *Acanthurus triostegus* in Hawaii spawn for 13 days before new moon based on gonadal dissections (Randall, 1961). In Palau, Robertson (1993) observed *A. lineatus* spawn four days before full moon and speculated they also spawned three days before new moon. There is no other spawning information on spawning days for other *Acanthurus* species.

Figure 28. Spawning Aggregation of *Acanthurus triostegus*.

This documentation of *Acanthurus guttatus* spawning aggregations in relationship to environmental factors of tidal height and moon phase are the first for this species *Acanthurus guttatus* spawning aggregations in American Samoa (Craig, 1998) was the only scientific paper to document spawning aggregations for this species, having year-round spawning just prior to sunset, with no attempts to look at lunar or tidal environmental effects. *Acanthurus guttatus* spawning aggregations in Guam (Figure 29) similarly spawn before sunset and changes over time in relationship to the sun setting later (Table 6, Table 8 Figure 22). Both in Guam and Samoa *A. guttatus* spawning aggregations co-occurs with

A. triostegus (Crain, 1998; Sadovy de Mitcheson and Colin, 2012). *Acanthurus guttatus* spawning aggregation in Guam is different from Samoan spawning aggregation by having seasonal spawning pattern (Table 2, Table 3, and Figure 18). In Guam, environmental factors such as tidal height, moon phase, and photoperiod in relationship to sunset were found to predict spawning.

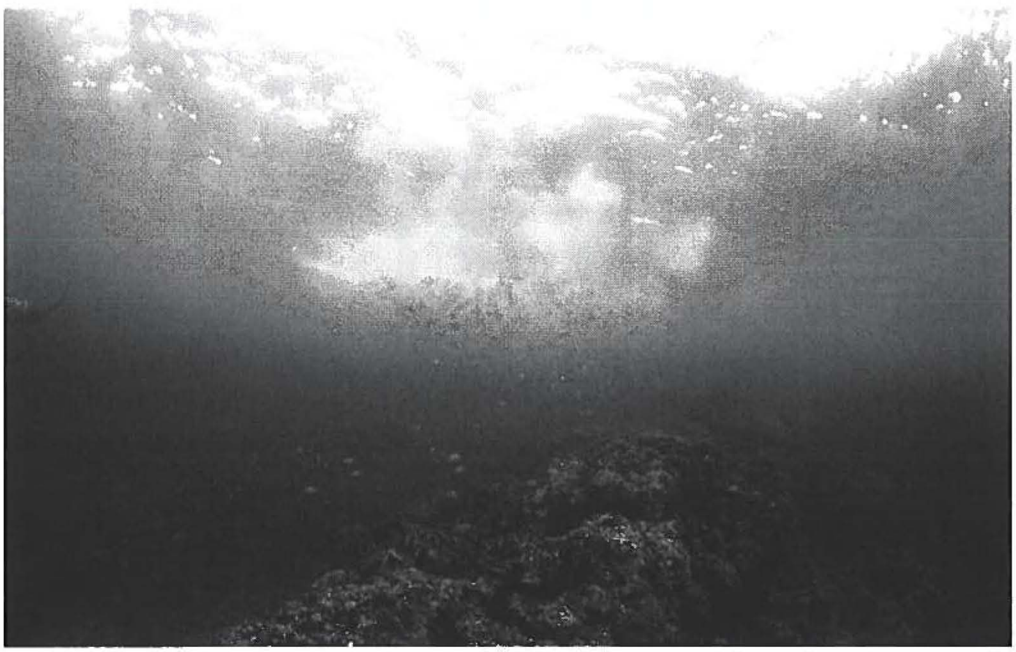


Figure 29. ***Acanthurus guttatus*** **extra** ***small*** **spawning aggregation.**

Spawn time for *Acanthurus lineatus* for all studies including this occurs in the morning, around sunrise (Johannes, 1981; Robertson, 1993; Sadovy de Mitcheson and Colin, 2012). Since *A. lineatus* is an herbivore and strongly territorial, it is thought best to spawn in the morning. By leaving its territory unguarded during the morning, when competing herbivores have low feeding rates it minimizes the loss of food (Craig, 1983; Robertson 1993, 1991; Kohda, 1988). Even though *Acanthurus lineatus* spawned in the mornings (Figure 30) when looked at statistically in relationship to time of sunrise, it was found not significant (Table 8Table 9). Spawn times varied in the morning over the course of the lunar cycles for new and full moon in relationship to sunrise (Figure 22). Spawn cycles would start closer to sunrise for the first two days but during later parts of the spawn cycle would often times spawn almost 45 minutes after sunrise. Strong exposure to solar radiation from the sun is known to have damaging effects on fish eggs and embryos (Robertson, 1983; Ferraro, 1980). Spawning later after sunrise increases the possibility of damage to the eggs and therefore it is unclear to why time of spawning changes throughout the morning sunrise. Time of spawning may change do to the presence of manta ray predation at aggregation (Table 10) and increasing egg survival out-weighs the effects of solar radiation damage. *Acanthurus lineatus* were present on arrival at the site, aggregating and therefore on days' spawns occurred later in the morning territorial home areas were left unguarded much longer than on other days. Possible predation threats in mornings hours could be much lower for longer periods then thought (Table 2, Table 3 and Figure 22).

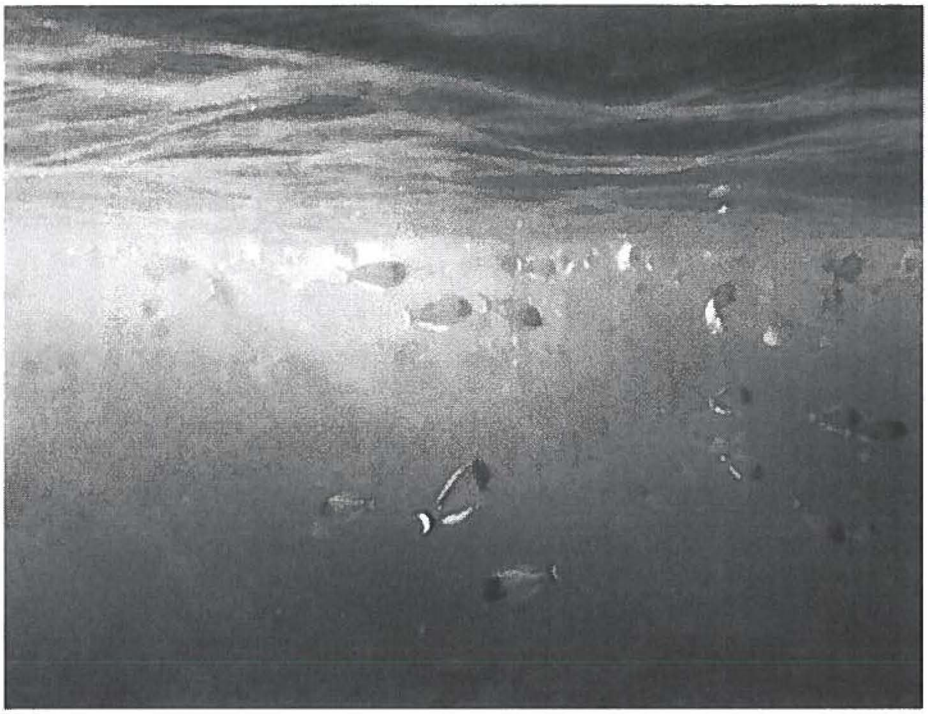


Figure 30. ***Acanthurus lineatus*** **spawning aggregation, showing color changes during events.**

Spawning aggressions are known to form in areas of outgoing or strong currents, and spawning during ebb tidal phase, increasing pelagic egg dispersal (Robertson, 1991; Sadovy de Mitcheson and Colin, 2012). All three *Acanthurus* spawning aggregations described here indicated tidal height as a predictor of spawning. However, the best-fit model for *Acanthurus triostegus* and *A. guttatus* showed tidal phase was a better predictor of spawning than moon phase, while *A. lineatus* best-fit model indicated moon phase was a better predictor of spawning then tidal height (Table 2, Table 3, Table 5 and Figure 18, Figure 20). Throughout the surveys Tumon channel even on an incoming current continually has an outgoing current. Due to the size of Tumon Bay (approximately 2.41 k long and depth ranging from 0.5-1m), tides and waves displace water creating a continual outgoing current. Larger waves and surf would enhance the amount of displaced water, increasing an outgoing current. That creates an ideal location for a spawning aggregation location by enhancing larval dispersal.

Even though *Acanthurus triostegus*, *A. guttatus* and *A. lineatus* are closely related, and each species spawned at the same site, each responded differently to environmental conditions. *Acanthurus triostegus* and *A. guttatus* best-fit model included tidal height as a predictor for spawning, while for *A. lineatus* moon age was a better predictor of spawning time (Table 5, Figure 20). Sunset predicted spawning for *Acanthurus triostegus* and *A. guttatus,* yet the functional relationship of spawning time to sunset differed between the two species (Table 8). In contrast, sunrise was not a significant factor for *A. lineatus* (Table 9). One might assume that because of their close phylogenetic relationship, these species would not differ greatly. Differences may be due to natural selection for spawning times that maximize larval survival and dispersal.

The site for the multi-species fish spawning aggregation is near a densely populated human area (Figure 7). Since it has been suggested that marine protected be specifically designed to protect spawning aggregations it is frustrating not to know what came first in Guam, the spawning aggregations or marine preserve. The presence of predation behavior by manta rays at these fish spawning aggregations does suggest that the aggregation has been established for a longer time (Sadovy de Mitcheson and Colin, 2012). The marine preserve was established without intending the protection of a multi-species fishing spawning aggregation and a site frequented by manta rays.

Due to Guam's small manta ray population, they should be protected under The Guam's Endangered Species Act of Guam under authority of the Department of Agriculture by 5 GCA, Section 63205. Even though *Manta alfredi* is considered threated globally, Guam's tiny population should be considered endangered and protected accordingly. It is also my suggestion that the list of endangered species for Guam be updated on an annual basis. The last update was from 2009. The University of Guam Marine Laboratory should make this a priority to ensure all marine species under threat are included in those updates.

6.3 Manta Ray Targeting Spawning Aggregation to Feed

This is the first study of this novel behavior of manta rays targeting reef fish spawning aggregations to feed on fish spawn (Table 9, Table 10 and Figure 24). In Belize, *Rhincodon typus* another large planktivore migrates large distances to a feed a spawning aggregations of snappers *Lutjanus cyanopterus* and *Lutjanus jocu* (Heyman, 2001). Guam manta rays targeting fish spawning aggregations (Figure 31) is probably not unique to Guam by evidenced by observations in the Cook Islands (Michael Domeir, pers. comm.) where manta rays feed near spawning *Acanthurus triostegus* (Sadovy de Mitcheson and Colin, 2012). Dr. Andrea Marshall (pers. comm.) has observed fish spawning without any interest from manta rays swimming in the close vicinity in Mozambique. Spawning aggregations of reef fish were observed by Dr. Mark Deakos (pers. comm.) close to a manta-ray cleaning stations without manta rays feeding on spawn. Some populations might have different feeding behaviors due to the overall lower levels of productivity in the water, needing to supplement their diet to maintain a particular energy level.

Figure 31. Picture of manta rays feeding at spawning aggregations of *Acanthurus lineatus*.

Manta rays targeting *Acanthurus triostegus and A. guttuatus* spawning aggregations were found to be male bias with 166 males compared to 122 females (Table 11). Manta rays targeting Acanthurus lineatus aggregations to feed were not sex biased but could be suggested with a p of 0.059. As mentioned site affinity for manta rays is thought to be possible due to feeding, sexual reproduction, and migratory stopping stations (Couturier, 2011). Difference between male and female manta ray counts could be due to male attracted for reproducing with females (Figure 32). Observations of mating behavior and trains have been documented at fish spawning aggregations.

Figure 32. Manta mating train of six with female manta ray at the front with six male manta rays following behind.

As we continue to study this ecological interaction between reef fish spawning and manta rays feeding on Guam, we gain a deeper understanding of the resident population distribution but also their social dynamic and genetic connectivity.

Chapter 7 Conclusion

In conclusion, this study looked at the populations of Guam manta rays and the ecological interaction of manta rays targeting a multi-species fishing spawning aggregations of *Acanthurus triostegus*, *A. guttatus,*and *A. lineatus* to spawn. Site of the multi-species aggregation is located in the Tumon Bay Marine Preserve and the predatory oophagous behavior of manta rays feeding behavior of fish spawn.

A total of 43 Guam manta rays was documented and re-sighting data suggested that population numbers should not reach more than 45-50 adults. Guam manta ray population is not a gender bias and consists of mostly adults. This study showed that the Guam manta ray population is endangered because of its small size and so far, is the smallest on record.

All fish species exhibited a seasonal pattern of group spawning but were slightly different in calendar months. *A. triostegus*, and *A. guttatus* spawn December to May, while *A. lineatus* spawns March to August or September. All three fish species were influenced by the atmospheric and environmental factors of tidal height and moon age. However, *A. triostegus* and *A. guttatus* found tidal height to be more of a factor in spawning, whereas moon age had a greater effect on *A. lineatus. The environmental factor,* photoperiod for *A. triostegus* and *guttatus* spawning was significant in relationship to sunset but did differentiate in strength of the relationship with *A. triostegus* having a stronger affiliation. Photoperiod for *A. lineatus* was not a predictor of spawning in relationship to sunrise. Manta rays target all three species of fish spawning aggregation to feed off fish spawn. March was found to be the highest number of manta ray sightings, correlates to the month most spawning aggregations were observed. Manta rays did show male bias at *Acanthurus triostegus* and *A. guttatus* spawing aggregation likely due to productivity as well as feeding.

Guam manta ray small population, multi-species spawning aggregation and this unique ecological interaction of predation by manta rays, needs protection, enforcement of the Tumon marine preserve, and additional future study. Amount of potential knowledge that could be gained by a long-term study of all three aspect could increase science understanding drastically and assist in global and Guam fisheries management.

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