ECOLOGY AND SOCIAL BEHAVIOR OF A RESIDENT MANTA RAY (*MANTA ALFREDI*) POPULATION OFF MAUI, HAWAI‘I

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By

Mark H. Deakos

Dissertation Committee:

Karl A. Minke, Chairperson
Patricia A. Couvillon
Louis M. Herman
Adam A. Pack
Joseph R. Mobley, Jr

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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ABSTRACT

Late maturity, few offspring, and a residential nature, typical of *Manta alfredi*, make this species particularly vulnerable to localized anthropogenic threats, and much less likely to recover from depleted populations. Understanding the population characteristics and reproductive ecology of this species is critical for its successful management. Paired-laser photogrammetry, combined with photo-identification and active tracking were used to describe the population characteristics, demographics, habitat range and use, and reproductive ecology of a resident population of manta rays (*Manta alfredi*) in Hawai‘i. Paired-laser photogrammetry proved to be a simple, non-invasive, accurate (mean error of 0.39%), and precise (CV = 0.54%) method for sizing free-ranging manta rays. A total of 286 surveys were conducted between 2005 and 2010 at a known aggregation site off Maui, Hawai‘i. A total of 309 different individual manta rays were photo-identified. A discovery curve showed no asymptotic trend, indicating the number of individuals using the area was much larger than the total identified. Resights and manta follows revealed a home range spanning Maui County waters with visits to the four-islands but did not include the Big Island, suggesting there are independent, island-associated stocks. High resight rates within and across years at the study site provided strong evidence of site fidelity. Findings were consistent with a population of manta rays moving into and out of the Maui aggregation area, with a varying portion of the total population temporarily resident at any given time. Males, accounted for 53% of all individuals, and resided for shorter periods than females around the study site. Manta rays were usually absent at first light with numbers increasing throughout the day. Shark predation was evident in 24% of individuals, and 10% had an amputated or non-functional cephalic fin, likely caused by entanglement in monofilament fishing line. Repeated measurements on 154 different manta rays, produced a mean CV of 1.46%, providing further support for the paired-laser system. Sexual dimorphism was evident with the largest female (3.64 m DW) 19% larger than the largest male (3.05 m DW). Sexual maturity in females, based on evidence of pregnancy and mating scars, was conservatively determined to be 3.37 m DW. The DW at which 50% of the males were likely to begin maturation and accelerated clasper growth was between 2.70 and 2.80 m DW. The absence of individuals smaller than 2.50 m DW suggests age class segregation may be occurring in this population. Although

mating trains and late-term pregnant females were observed at all times of the year, they were more likely to occur during the winter months. Females seemed capable of ovulating multiple times during a year if their initial mating attempts are unsuccessful. Estrus may last at least several days based on repeated sightings of the same female in a mating train over several days. Sexual maturity appears delayed in both males and females until their body size exceeds 90% of their maximum size, an indicator that large body size provides a reproductive advantage. Larger females had higher pregnancy rates, and were more likely to reproduce in successive years. No direct physical competition was observed between males but evidence of mating trains lasting more than one day suggests endurance rivalry, during which larger males may benefit due to greater energy reserves. The Maui aggregation site appears to be an important staging area for breeding and parasite removal by members of this population. Late maturity, low fecundity, and the residential nature of individuals in this population make them particularly vulnerable to localized, anthropogenic threats. Implementing successful management practices to reduce the threat of entanglement and of unregulated “swim-with manta ray” programs is recommended.

1 GENERAL INTRODUCTION

1.1 Introduction

Elasmobranchii are cartilaginous fish that include sharks, skates, and rays, many of which are severely threatened by human activities (W. White & Kyne, 2010). Despite a growing interest in elasmobranch research, accurate life history and behavioral information of most species remain incomplete. Without an understanding of the basic biology and ecology of these species, effective management policies are difficult to implement (Bres, 1993). The research presented here examines one of the largest living elasmobranch species, *Manta alfredi*.

Because the behavior and biology of manta rays and other elasmobranchs are not commonly known, this dissertation begins with a brief overview of elasmobranch evolution followed by a summary of existing information on manta rays. Following this introduction, questions are raised addressing the gaps in our understanding of resident manta rays with a special emphasis on anthropogenic threats and effective management based on their life history and habitat use.

1.2 A Brief Evolutionary History of Elasmobranchs

Archeological evidence suggests that the first vertebrates emerged about 500 million years ago (mya) during the Cambrian period, and gave rise to two successful evolutionary lines of fishes 100 million years later, Chondrichthyes and Osteichthyes. Although Chondrichthyans retained the flexible, cartilaginous skeleton of their ancestors, Osteichthyans replaced cartilage with bone giving rise to present-day bony fish, comprising over 25,000 living species, the most abundant among vertebrates.

Today's living sharks, rays, and chimaeras, which include over 1100 species, can trace their common ancestry to early Chondrichthyans (for review see Leonard Compagno,

Dando, & Fowler, 2005). The chimaeras consist of only 40 species (4% of Chondrichthyans), making up the subclass Halocephali, and are characterized by a single gill slit covered by an operculum. The remaining 96% of Chondrichthyans belong to the class Elasmobranchii (sharks and rays) easily recognized by the five to seven paired gill openings on each side of the head. Sharks account for about 500 species and the rays or "batoid" fishes (superorder Batoidea), include over 600 species. The first sharks appeared about 400 mya while the first rays did not appear until about 150-200 mya.

Rays can be thought of as flat-bodied sharks, with large, modified pectoral fins, a likely adaptation to their bottom dwelling existence (Holmgren, 1940). The pectoral fins have become fused to the side of the head forming a flattened disc and the anal fin is no longer present. Ray propulsion is no longer done with the trunk and tail but with their large, expanded pectoral fins (Rosenberger, 2001). The 5-7 pairs of gill slits sit below the pectoral fins unlike sharks whose gills slits are positioned above the pectoral fins. Six orders of living rays are recognized and include the Pristiformes (sawfishes), Rhiniformes (sharkfin guitarfishes or wedgefishes), Rhinobatiformes (guitarfishes), Torpedoniformes (electric rays), Rajiformes (skates), and Myliobatiformes (stingrays).

The largest rays belong to the Myliobatiforme order, and more specifically to the family *Mobulidae*, also known as “devil rays.” The eleven species of devil ray all possess a characteristic pair of cephalic fins that protrude out from the front of the head. These unique structures aid in guiding food and water into their mouths and are furled during travel giving the appearance of a pair of horns. Devil rays represent two distinct genera, *Mobula,* composed of nine species, and *Manta* composed of two species. Mobula rays have their mouths positioned ventrally while manta rays have their mouths projecting forward instead of downward.

1.3 Manta Rays

Manta rays are unique in that they have evolved to take advantage of large abundances of zooplankton that inhabit the open water. Their large, rectangular mouths project forward

instead of downward to facilitate feeding. The spiracles, (a pair of small vestigial gill slits), although still present, are no longer used. Instead, water enters through the manta ray's mouth while they swim, passes over the gills, providing oxygen to the blood. They have evolved large pectoral fins that are used like wings to propel themselves through the water. Their skin is covered with dermal denticals (small tooth-like structures) much like their shark cousins. A mucus coating covers their skin, creating an important defense against infection.

Manta rays are the largest rays in the Mobulidae family and until recently, the genus was thought to consist of just a single species, *Manta birostris*. Recent evidence based on morphology and meristic (quantitative features of fish) data has confirmed at least a second species in the genus, *Manta alfredi* (for review see AD Marshall, Compagno, & Bennett, 2009). *M. birostris* herein referred to as "oceanic manta rays" due to their pelagic habitat range, can be differentiated from *M. alfredi* visually in the field by their much larger size, their coloration, and the presence of a caudal spine. At the base of the tail just below the dorsal fin, oceanic manta rays have retained a calcified mass that contains a small, embedded spine, essentially a vestige of their ancestry. *M. alfredi*, herein referred to as "resident manta rays" due to their site fidelity to coastal habitats, do not possess this calcified mass. When differentiating dead specimens, differences in the appearance of the skin and denticle morphology, as well as the number of teeth present on the lower jaw can also be used to identify species. On the bottom jaw exists 12-16 rows of small cusped teeth in oceanic manta rays, and 6-8 rows in resident manta rays, with no teeth existing in the upper jaw for either species. These very small teeth barely penetrate the skin covering and are another vestige of an evolutionary era when their ancestors used their teeth to feed.

1.3.1 Coloration

Each manta ray has a ventral spot pattern that is unique. These patterns are visible at birth (Andrea D. Marshall, Pierce, & Bennett, 2008), and appear to remain unchanged over the life of the individual (T. B. Clark, 2001; Homma, Maruyama, Itoh, Ishihara, & Uchida,

1999; Kitchen-Wheeler, 2010). These unique patterns allow researchers to discriminate and track individuals over time.

The most common resident manta morph has a black dorsal cape with white shoulder patches and a white chevron that stretches anteriorly from the insertion point of the dorsal fin (for review see AD Marshall et al., 2009). The ventral side is typically cream to white in color with variable dark markings that can occupy the entire ventral surface but most are concentrated towards the center of the disc (Figure 1).

Figure 1. *M. alfredi* showing: (a) unique ventral markings, and (b) dorsal shoulder patches with white chevron.

A melanistic morph exists that is almost completely black on both the dorsal and ventral surfaces except for a white blaze along the mid-line that is variable in size. The much less common leucistic "white manta" morph has an almost entirely white dorsal surface and a ventral surface that is much lighter in overall coloration. Less than 20 of these have been observed worldwide (AD Marshall et al., 2009).

The tremendously large body size of manta rays has likely been beneficial in reducing predation pressure. With less predation pressure, the benefits of counter-shading become less important, possibly allowing morph variants to proliferate successfully (G. Notarbartolo-di-Sciara & Hillyer, 1989).

1.3.2 Sensory Systems

Although most elasmobranchs have relatively small brains, both devil rays and galeomorph sharks independently evolved large telencephalons and cerebellums, characterized by high brain:body ratios (Northcutt, 1977). What function the enlarged telencephalon and cerebellum areas play or why they evolved together remains unclear (Hofmann, 1999).

As manta rays move throughout their aquatic environment, they process information through many different sensory channels allowing for a complex repertoire of signals and behaviors to be used for finding food, mates, escaping predators, and to facilitate social interactions with conspecifics. These sophisticated senses incorporate: 1) an olfactory and gustatory system, 2) a visual system, 3) a mechanosensory system, which includes hearing and touch, and 4) an electrosensory system (Bleckmann & Hofmann, 1999).

Elasmobranchs have a very good sense of olfaction and taste (Hodgson & Mathewson, 1978; Kleerekoper, 1978; GH Parker, 1914) and can use these senses to detect biochemical products released by other organisms that may be prey, mates, or conspecifics.

Although often thought of as secondary to olfaction, elasmobranchs have a well-developed visual system (Gruber & Cohen, 1978; Hart, Lisney, & Collin, 2006). The acute vision of elasmobranchs not only assists with detecting prey and avoiding predators, but is also used for inter- and intraspecific communication (Hart et al., 2006). A Manta ray's eyes are located laterally just behind the cephalic fins, giving them the ability to see forward and downward very easily. Binocular vision is likely when looking

downward but the ability to see upward and behind their body appears to be impaired (M. Deakos, pers. observ., 2005-2010).

The movement of animals through the water when traveling, turning, or struggling, creates water displacement and water pressure waves (sound) that can provide a source of biological information for those capable of detecting these signals. Three mechanisms exist in elasmobranchs to detect acoustic (hydrodynamic) events: the inner ear, the lateral line, and the sense of touch.

The hearing sensitivity of manta rays is unknown but that of most elasmobranchs is very acute. Conditioning studies have shown sharks most sensitive to low frequency sounds in the vicinity of 100 Hz (D. Nelson, 1967), the frequency often produced by struggling prey (e.g., D. Nelson & Gruber, 1963).

All elasmobranchs have a lateral line system much like that present in bony fish (D. Nelson, 1967) and aquatic amphibians (Lannoo, 1987). In dorsoventrally flattened batoids, they occur primarily around the expanded pectoral fins (Bleckmann & Hofmann, 1999). Short-tailed Stingrays (*Dasyatis brevicaudata*) were capable of detecting weak vertical jets of water created by buried bivalves, their primary food source, through the elaborate network of lateral line canals on its ventral surface (Montgomery & Skipworth, 1997). Nothing is known about the lateral line system of manta rays.

All elasmobranchs are electroreceptive, detecting electric stimuli passively (Collin and Whitehead, 2004), but some skates and rays are electrogenic (Bratton & Ayers, 1987), capable of using electricity actively to communicate or to stun and capture their prey. Electrosense is used in round stingrays (*Urolophus halleri*) by reproductively active males to locate mates, and by females to locate buried consexuals (Tricas, Michael, & Sisneros, 1995). The electrosensitivity of manta rays is unknown.

Despite their sensitivity to low frequency sound, few elasmobranchs can produce their own sounds beyond the usual hydrodynamic noises (Myrberg, 1981). Coles (1916)

observed and killed 11 oceanic manta rays off North Carolina, USA, and noted a harsh bear-like cough emitted during the kill. Although reported as manta vocalizations, the sounds were more likely the product of air being released from various air-filled cavities. Based on their physiology, it seems unlikely that any audible sounds could be produced by submerged mobulids (G. Notarbartolo-di-Sciara, 1988). Sound production in elasmobranchs has been reported only for the cownose ray (*Rhinoptera bonasus*), which produces sounds by scraping together its dental plates (Myrberg, 1981).

The first and only cognitive experimental research study performed on a captive oceanic manta ray was carried out at the Lisboa Oceanarium in Portugal (Ari & Correia, 2008). The young male manta used visual cues (the feed bucket) and olfactory cues (scent of shrimp) to condition to a feed session that was given at the same time and place each day. The manta's sense of smell was acute, showing detection of 0.3 L of shrimp extract in 4700 m^3 of water, but visual cues were equally likely to generate a food searching response. The characteristics of the bucket that were being used as the visual cue (size, shape, color) were not determined. During later feed sessions that were not cued, the young male was able to remember the time and location of the feed session. This was attributed to use of an internal biological clock and long-term spatial memory.

1.3.3 Morphology

The sheer size of manta rays makes it difficult to obtain morphological measurements unless dead specimens become available (G. Notarbartolo-di-Sciara, 1987). The few morphological reports that do exist on maximum disc width, size at maturity, coloration, tooth counts, and presence of a tail spine have been variable and confusing (AD Marshall et al., 2009), and the use of non-standard metrics has made it difficult to compare between reports.

Although the standard method of reporting size in manta rays is disc width (measured from wing tip to wing tip), proportional measurements using disc length (DL; measured from the tip of the snout to the posterior edge of the pectoral fins), are sometimes used for comparison, especially when a specimen's wings have been severed or are have

deteriorated to the point that an accurate disc width (DW) measurement cannot be made (Andrea D. Marshall et al., 2008). DWs have been reported to be 2.2 – 2.3 times larger than their DLs (Bigelow & Schroeder, 1953; AD Marshall et al., 2009). Determining growth occurs proportionally through all stages of the animal's life is important when making these morphology conversions. For manta rays, other than differences seen in the anterior head region, eye diameter, interspiracle length, and cephalic fin length, which may develop disproportionately, all other morphometric measurements appear to develop proportionately from the fetus to adulthood (Andrea D. Marshall et al., 2008).

A summary of existing documentation for DW measurements on resident manta rays is shown in Table 1.

1.3.4 Reproduction

Manta rays are ovoviparous, giving birth to a live young that hatches from an egg, carried inside the mother. After hatching from the egg case that is attached to the female's oviduct, the pup continues to feed on the mother's uterine milk until fully developed. The pup is born live as a miniature version of the adult.

In free-ranging manta rays some speculate that they may give birth in relatively shallow water where the pups remain for several years before expanding their range (Ginis, 2002). The only reported birth was for a resident manta female in captivity at the Okinawa Expo Aquarium following what was determined to be a 12 -13 month gestation period (Senzo Uchida, Toda, & Matsumoto, 2008). The newborn was abandoned immediately after birth and the mother was seen mating again within hours.

Pregancy rates for resident manta rays have been estimated using long term sighting records of females observed pregnant every 2-3 years on average, with some females becoming pregnant in consecutive years (Homma et al., 1999; AD Marshall & Bennett, 2010). Although giving birth to a single pup at a time appears to be the norm for both species based on disections of pregnant females (Bigelow & Schroeder, 1953; Coles,

1916; Lamont, 1824; Lesueur, 1824), two pups may be conceived on occasion (AD Marshall & Bennett, 2010).

The first detailed description of a manta ray mating sequence was for oceanic manta rays observed off Ogasawara Island, Japan (Yano, Sato, & Takahashi, 1999). The sequence of events involved: (1) a male following directly behind a female for 20-30 minutes making several attempts to bite her pectoral fin as she traveled at a speed of 10 km/hr; (2) the male succeeds in biting the female's pectoral fin positions his ventral side against hers; (3) the male inserts his clasper into the female's cloaca for 60 – 90 seconds; (4) the male remove's his clasper and continues to hold her pectoral fin in his mouth for several minutes; and (5) the male releases the female's pectoral fin and both individuals swim apart. This mating sequence was observed in 10-20 m water depth over rocky reefs and 100-200 m from the beach.

Similar mating train behavior has been described for resident manta rays off Yaeyama Island, Japan (Homma et al., 1999), and at North Male Atoll in the Republic of the Maldives (Stevens & Rubin, 2008). In the Maldives, mating trains are reported to consist of 1 – 21 males chasing a single, fast swimming female. The number of males in the train increases then decreases before copulation takes place.

Marshall & Bennett (2010) reported strong lateralization of mating scars in females with 99% of the scars visible on the left pectoral fin. Yano et al. (1999) reported mating scars on both wings of the female but only fresh scars were observed on the left wing. Mating events appear to be restricted to certain times of the year but the time of year will vary by region (Homma et al., 1999; AD Marshall & Bennett, 2010; Stevens & Rubin, 2008). Off Ogasawara Island, Japan, most mating trains were observed between March and October with occasional chases observed outside the summer season (Yano et al., 1999). Mating behavior in Yaeyama Island was reported during the spring and autumn and lasting for about a one month duration (Homma et al., 1999). The wet season in this area begins in May and lasts through June, and the typhoon season (May – November), typically peaks in September. In the Maldives, the majority of mating trains were

Table 1. Disc Width (DW) in meters for *M. alfredi* reported at pre-birth, birth, maturity, and maximum size, by gender, and for different regions around the world.

Age	Gender	Location	DW Size (m)	Method of Measurement	Reference
Pre-birth	Male	S. Africa	1.33	dead specimen	(Andrea D. Marshall et al., 2008)
Smallest Free-Swimming	Male	S. Mozambique	1.50	field estimate	(AD Marshall & Bennett, 2010)
Maturity	Female	S. Africa	3.90	field estimate	(AD Marshall & Bennett, 2010)
	Male	S. Mozambique	3.00	field estimate	(AD Marshall & Bennett, 2010)
Max	Unknown	S. Mozambique	5.50	field estimate	(AD Marshall & Bennett, 2010)
	Female	Ogasawara, Japan	5.00	field estimate	(Yano et al., 1999)
	Female	Wakayama, Japan	5.00	field estimate, using boat as comparison	(Yanagisawa, 1994)
	Female	Okinawa, Japan	4.65	unknown	(S Uchida, 1994)
	Male	Ogaswara, Japan	4.00	field estimate	(Yano et al., 1999)

between October and November, towards the end of their wet season (April to October), with occasional trains observed outside this season (Stevens & Rubin, 2008). In Mozambique, fresh mating scars were observed primarily during the summer months (AD Marshall & Bennett, 2010).

Although mating trains can be reliably seen, acts of copulation in free ranging manta rays are rare (AD Marshall & Bennett, 2010; Yano et al., 1999).

1.3.5 Range and Habitat

Resident manta rays are more likely to be observed in shallow coastal areas around rocky and coral reef habitats where productive upwellings exist, commonly sighted inshore, within a few kilometers of land. They have been observed as far north as the Yaeyama Islands, Japan, Hawai‘i, the Canary Islands, the Red Sea, Sri Lanka, and Thailand, and as far south as the Solitary Islands, Australia, French Plynesia, Senegal, Durban, South Africa, the Maldives, and Perth, Australia. No sightings exist for the eastern Pacific and except for two sightings off the coast of Senegal from northwest Africa, sightings for the eastern Atlantic are extremely rare (Cadenat, 1958). In general, they can be found in tropical and subtropical regions of the Pacific, Atlantic, and Indian oceans within 30 degrees of latitude to the north and south of the equator (Marshall et al. 2009).

Congregations occur around rich food sources or at specific locations on the reef known as cleaning stations (Losey Jr, 1972) where individuals solicit host cleaner fish to remove parasitic copepods from their body’s surface. Strong site fidelity occurring at specific feeding and cleaning stations (e.g., Homma et al., 1999) has created popular tourist attractions where visitors pay to swim or scuba dive with the manta rays (T. B. Clark, 2001; Dewar et al., 2008).

Some well known resident manta ray aggregation areas worldwide that have become popular tourist destination areas include Komodo Marine Park, Indonesia (Dewar et al., 2008), Yap, Micronesia (Homma et al., 1999), Palau, Yaeyama, Okinawa, Japan

(Kashiwagi, Ito, Ovenden, & Bennett, 2008), Kona , Hawai‘i (T. B. Clark, 2001), Bora Bora, French Polynesia (de Rosemont, 2008), Mozambique (A. D. Marshall, 2009), the Republic of Maldives (C. R. Anderson, Shiham, Joaquim, Kitchen-Wheeler, & Stevens, 2008; Stevens & Rubin, 2008), and Ningaloo Western Australia (Daw & McGregor, 2008; McGregor, Van Keulen, Waite, & Meekan, 2008). In the Republic of Maldives, divers are taken to watch manta rays at the same diving spot each time demonstrating daily site fidelity is strong.

Long term sighting records at established aggregation sites suggest that *M. alfredi* may be philopatric to these areas and may exhibit smaller home ranges (Dewar et al., 2008; Homma et al., 1999; A. Kitchen-Wheeler, 2008; A. D. Marshall, 2009). Areas of high productivity could eliminate the need for manta rays to migrate to other areas (T. B. Clark, 2001) by providing sufficient food resources year-round. Thus far, no evidence of inter-island migration exists from a known manta ray population in Kona, Hawai‘i (T. B. Clark, 2001).

1.3.5.1 Seasonality

Although most resident manta populations appear to have year-round residents (e.g. Bora-Bora, Hawai‘i, Mozambique) some populations (e.g. the Republic Maldive Islands, Yap Island,) occur seasonally (Dewar et al., 2008). In the Republic Maldive Islands, the manta rays are known to migrate from one side of the island to the other coinciding with the seasonally alternating monsoon currents that are responsible for the plankton blooms (C. R. Anderson et al., 2008; Homma et al., 1999). In Yaeyama Island, some individuals are year-round residents but others have been reported to migrate annually to Kerama Island, about 350 km to the east (Homma et al., 1999). In Yap, manta rays use different channels in the summer and winter (T. B. Clark, 2001). Fishers targeting manta rays will set gill nets at the same channel each year for guaranteed catches (Homma et al., 1999). Some migrations are simply diurnal, where manta rays migrate between feeding and cleaning stations (Homma et al., 1999).

1.3.6 Food and Foraging

Manta rays appear to feed on small planktonic organisms such as euphasids, copepods, mysids, decapod larvae, and possibly shrimp, (Bigelow & Schroeder, 1953; T. B. Clark, 2001; Last & Stevens, 1994). Stomach contents from an oceanic manta specimen from South Carolina contained fragments resembling the shells of shrimps as well as remnants of a small crab (Bigelow & Schroeder, 1953).

When feeding, manta rays unfurl their cephalic fins to help direct the plankton rich water into their mouths and over the five pairs of gills. Finger-like projections on the gill arches, known as gill-rakers, strain and capture the food. This type of feeding is termed ram-jet feeding. The manta rays sometimes swim in repeated summersaults through a dense patch of plankton.

Coles (1916) reported mobulids using their cephalic fins to herd small minnows up against the beach and funnel them into their mouth. And although manta rays have been reportedly seen gulping schools of small mullet (Bigelow & Schroeder, 1953), it is more likely that the mullet were simply feeding on the same patches of plankton targeted by the manta rays.

Very little is known about the feeding behavior of resident manta rays. They have been seen feeding during the daytime and at night, as single individuals, and in large aggregations. In some regions, artificial lights above and below the water are used to attract large amounts of zooplankton, which in turn attracts resident manta rays looking to take advantage of an easy meal. Dive operators in Kona, Hawai‘i, have conditioned manta rays to aggregate each night around a group of scuba divers whose underwater lights serve to attract plankton into the area.

The abundance of plankton is much less in the open ocean and tends to be more dense around upwellings and around island groups (Lalli & Parsons, 1993). Nutrient rich upwellings induced by trade winds can also create conditions suitable for high primary

production and thus may create important feeding areas for manta rays (G. Notarbartolo-di-Sciara & Hillyer, 1989).

Temperature is also implicated in the pattern of zooplankton diversity which is correlated closely with sea-surface temperature and decreases rapidly with depth (Rutherford, D'Hondt, & Prell, 1999). Plankton are known to take regular and even daily vertical migrations (Pillar, Armstrong, & Hutchings, 1989) and manta rays may adjust their feeding regime in order to take advantage of these diel cycles. In Bateman Bay, Western Australia, resident manta rays are observed feeding about 40% of the time, throughout the year, on small calanoid copepods (McGregor et al., 2008).

1.3.7 Population Size

Based on photo-identifying the unique spot patterns on the underside of manta rays, population sizes have been estimated for various aggregation areas around the world. These reported population sizes are listed in Table 2.

1.3.8 Behavior

1.3.8.1 Cleaning Stations

Although manta ray congregations do occur around rich food sources (C. R. Anderson et al., 2008), manta rays are also known to congregate at specific locations along the reef referred to as "cleaning stations." A cleaning station is where individuals solicit host cleaner fish to remove parasites and clean wounds from their body's surface (Losey Jr, 1972). When more than one host cleaner fish species shares in the cleaning behavior, each may focus on a different region of the manta's body in order to reduce interspecific competition (A. D. Marshall, 2009).

Although *Mobula* species are reported to be free from parasites, *Mantas* can have parts of their bodies thickly covered with several species of parasitic crustaceans (Coles, 1916). In Japan, the parasitic copepods are thought to belong to the *Pandaridae* family (Homma et al., 1999). For clients, the main benefits to being cleaned are likely a reduction in

ectoparasite load but this has been difficult to measure due to the methodological problems in quantifying ectoparasite density (Coté, 2000).

Host cleaner fish vary by region. In Yaeyama Island, Japan, the cleaner wrasse (*Labroides dimidiatus*) and other small shrimps commonly do most of the cleaning (Homma et al., 1999). In Hawai‘i, predominantly Hawaiian cleaner wrasses (*Labroides phthirophagus*) and saddle wrasses (*Thalassoma duperrey*) are observed removing parasitic copepods from the surface of the manta ray's body (M. Deakos, pers. Obs.). *T. duperrey* will concentrate mostly on the external body surface, whereas *L. phtyirogphagus* will concentrate mainly inside the mouth and around the gill slits. In Mozambique, various butterfly fish (family *Chaetodontidae*) specialize in bite wounds while Seargant Major fish (*Abudefduf saxatilis*) concentrate more around the mouth region (Andrea Marshall & Bennett, 2008; A. D. Marshall, 2009).

Aggregations around cleaning stations are commonly used as reliable areas for guided swim-with manta tours. In some locations, these cleaning stations are active year-round, while in others the presence of manta rays at inshore reefs is seasonal or erratic (Dewar et al., 2008).

1.3.8.2 Breaching

Manta rays are occasionally observed leaping partially or completely out of the water; sometimes one after the other. The purpose of this behavior is unclear (Homma et al., 1999) but some have suggested that it may be related to mating displays, giving birth, or an attempt to get rid of parasites or remoras (*Remorina albescens*) attached to the surface of the manta ray (E. Clark, 1969). One oceanic manta was observed with seven large remoras attached to it's body (Coles, 1916). Manta rays have been observed trying to remove remoras by rubbing against rocks or the sandy bottom (Homma et al., 1999). The splash created when reentering the water during a breach can be audible for some distance away if the seas are calm (Bigelow & Schroeder, 1953) and therefore may be used as an acoustic form of communication.

Table 2. The total number of different individuals photo-identified and the estimated abundance, where available, for manta ray (*Manta alfredi*) populations around the globe.

Location	Total Different Individuals	Estimated Population Size	Time period	Source
Yonara Channel, Japan	185	n/a	1977-1997	Takashi Itoh (Homma et al., 1999)
Yaeyama, Okinawa, Japan	303	n/a	1987 - 2006	(Kashiwagi et al., 2008)
Yap Island, Micronesia	54	n/a	1990-1997	Bill Acker (Homma et al., 1999)
Kona, Hawai‘i, USA	170	n/a	1979 - 2010	*www.mantapacific.org*
French Frigate Shoals, Hawai‘i	40	n/a	1998-1999	(T. B. Clark, 2001)
Ningaloo Reef, Western Australia	300+	n/a	n/a	(McGregor et al., 2008)
Bora Bora, French Polynesia	85	n/a	2002-2005	(de Rosemont, 2008)
Southern Mozambique	449	890	2003-2008	(A. D. Marshall, 2009)
Republic of the Maldives	n/a	2000+	n/a	(C. R. Anderson et al., 2008)

1.3.9 Lifespan

The average lifespan of a manta ray is unknown. The longest reported period between the first and last sighting of the same individual resident manta is 27 years (1980-2006) off Yaeyama Island, Japan (Kashiwagi et al., 2008).

1.3.10 Threats

1.3.10.1 Natural Predators

Large sharks (Homma et al., 1999) and killer whales (*Orcinus orca*) (Visser & Bonoccorso, 2003) have been reported to prey on manta rays. It is unknown how many attacks result in fatalities and whether or not the shark will consume the entire manta ray. Cookie cutter shark bites have also been observed on manta rays, most likely occurring at night during deeper dives (Homma et al., 1999).

1.3.10.2 Anthropogenic Threats

The status of most manta ray populations worldwide is poorly understood. They are classified by the IUCN Red List for Threatened Animals as “near-threatened” (A. D. Marshall et al., 2006), meaning that manta rays could be threatened with extinction in the near future if conservation efforts are not implemented.

The number of manta rays that exist worldwide is unknown, and little is known about their population ecology. The recent reclassification of the genus has new implications for the conservation assessment of both species (AD Marshall et al., 2009). Each population and population stock faces its own regional specific ecological pressures. Understanding how a particular population or population stock is affected by anthropogenic impacts in its region is critical to understanding its conservation status and for directing effective management.

1.3.10.2.1 Directed Fisheries

Traditional hunting for manta rays in places like eastern Australia and the Sea of Cortez (Gulf of California), involved fishers on small boats harpooning slow swimming animals traveling just below the surface. They were hunted for their skin, the oil from their large livers, and simply for shark bait, but never in enough quantity to be of commercial importance (Bigelow & Schroeder, 1953). More recently, large international markets have emerged for shark fins, meat, and hides, resulting in the rapid rise in value for shark products. A new market has also emerged in Asia, creating a demand for dried manta gill rakers to be used in traditional Chinese medicines and in the treatment of cancer (Shen, Jia, & Zhou, 2001). These demands, combined with the existing demand for manta ray cartilage to be used as filler in shark fin soup (Alava, Dolumbaló, Yaptinchay, & Trono, 2002), has led to an exponential increase in the Indonesian fishery in just a few years, threatening to drive manta ray populations into commercial extinction (Dewar, 2002; W. T. White, Giles, Dharmadi, & Potter, 2006). In addition, as a result of over-fishing, fishermen have turned to hunting manta rays as an alternative source of income, leading to a ten-fold increase in manta ray harvesting.

These directed fisheries targeting manta rays have caused populations to decline and even disappear in areas such as Mexico (Homma et al., 1999; G Notarbartolo-di-Sciara, 1995; W. T. White et al., 2006), the Philippines (Alava et al., 2002), Indonesia (Dewar, 2002; W. T. White et al., 2006), India, Sri Lanka, and other parts of Southeast Asia (A. D. Marshall et al., 2006). An estimated 1,500 manta rays were taken over a period of six months in Lamakera, Indonesia (Anon, 1997). Drift gill nets 700 – 1000 m long and 35 m high are set 7 m below the water surface during the migratory passage of mobulids and are reported to entrap as many as 50 manta rays in a single net (Homma et al., 1999). Divers from Palawan Island, Philippines, reported that the local manta ray population had been reduced to a third of its original population over a seven-year period (Homma et al., 1999). In Yaeyama Island, Japan, common aggregations of 50 manta rays in 1982 were reduced to just 30 in 1992, and further reduced to no more than 15 by 1999 (Homma et al., 1999).

1.1.1.1.1 Ecotourism

In light of diminishing populations of manta rays from directed fisheries, new interest has sparked in the area of ecotourism as an alternative and more sustainable use of the resource. Homma et al. (1999) estimated a dead manta ray sells for about $400 US in the Philippines and ecotourism could provide the nation with a total annual revenue of $4,800,000 US. If tourists paid about $400 US each to view manta rays in the wild, 12,000 tourists annually would be needed to generate the same type of revenue gained from direct hunts and the practice becomes sustainable as the resource becomes renewable.

The development of manta ray ecotours is becoming a popular recreational activity and a booming industry in many parts of the world where manta rays are known to aggregate (Dewar et al., 2008; Yano et al., 1999). These programs can generate tens of thousands and even tens of millions of dollars of tourist revenue to local communities annually. However, there are serious concerns over the impact that poorly managed ecotours can have on the behavior of the animals and the habitats they rely on. Some governments are requiring licenses to operate these ecotours to help ensure that they do not damage the resource upon which it relies.

1.3.10.2.1.1 Boat Traffic

Manta rays can be frequently observed traveling just below the surface and will often approach or show little fear towards man or vessel (Coles, 1916), which can also make them extremely vulnerable to boat strikes by vessels traveling at high speed and unaware of an animal in their path. Several manta rays aggregating in Ningaloo Reef, Western Australia, possess deep scars where they were most likely struck by a boat propeller (F. McGregor pers. comm., 2007). Another incident was reported in Hawai‘i in which a manta ray died due to injuries sustained to the head from a boat propeller (T. Clark, pers. comm., 2006).

In the Florida Keys, a cooperative effort between the National Park Service and NOAA’s Florida Key Marine Sanctuary restricted boat traffic through a nurse shark mating and nursery ground by

using navigation control buoys during the peak of the mating season (Carrier & Pratt 1998). They found that the presence of boats during mating activities of nurse sharks was disruptive and often resulted in unsuccessful mating attempts.

1.1.1.1.2 Mooring and Anchor Lines

Although rare, manta rays on occasion entangle themselves in anchor and mooring lines. It is believed that when a line makes contact with the front of the head between the cephalic fins, the reflexive response by the manta is to immediately close the cephalic fins, thereby trapping the rope and entangling the manta ray when they begin to roll in an attempt to get free. This hypothesis was recently supported by video footage of a manta ray colliding with a cameraman and swimming off with the camera after locking its cephalic fins around the camera. Bigelow & Shroeder (1953) documented several records of a manta ray entangling in an anchor line in the same way, sometimes towing the boat along for some distance. On at least two occasions, a manta ray in Hawai‘i was reported to perish after entangling in a mooring line (A. Cummins, pers. comm., 2007, K. Osada, pers. comm., 2009).

1.3.11 Aquariums

The Okinawa Ocean Expo in Motobu, Okinawa Island, Japan, is the only aquarium in the world that has successfully housed and bred resident manta rays (Senzo Uchida et al., 2008). The Atlantis Resort in the Bahamas (Russell, 2008) and more recently the Georgia Aquarium are the only two facilities in the Western Hemisphere to keep manta rays in captivity. The manta rays on display at the Atlantis Resort are removed from the wild and housed temporarily before being returned back to the wild.

The minimum number of aquariums worldwide housing manta rays is a product of the difficulty in keeping manta rays alive during captivity. There is concern about the extraction of even a small number of individuals from the wild, especially when the mortality rate is so high.

1.3.12 Manta Ray Protection

In many parts of the world, measures have been taken to reduce anthropogenic threats on local manta ray populations. For example, codes of conduct for manta ray dive operators have been implemented in Kona, Hawai‘i (unpublished), Western Australia (Daw & McGregor 2008), Mozambique (A. Marshall, pers. comm., 2007), Bora Bora (M. deRosemont, pers. comm.), and in the Maldives (G. Stevens, pers. comm., 2007). Elements of the code include minimizing the number of divers around the manta rays, keeping divers in tight controlled groups, restricting the touching of the animals, and using approach methods that minimize stress on the manta rays. In Mozambique, mooring balls are banned in areas where the manta rays are known to aggregate, and boats are required to minimize their speed. Marine protected areas (MPA) have been established in the Maldives, Mexico, Mozambique, and Yap, to help eliminate fishing pressure and provide a safe refuge for the manta rays.

In Hawai‘i, a state law was passed that prevents the intentional killing or extraction of manta rays from all Hawaiian State waters with an exception for persons granted a special take permit. A special take permit requires the applicant to demonstrate that removal will not harm the population. The proposed removal application must evaluate the potential biological removal (PBR) of the targeted population. PBR is the maximum number of animals, not including natural mortalities, which may be removed from a stock while allowing that stock to reach or maintain its optimum sustainable population (Wade, 1998).

1.3.13 Research

In contrast to sharks, which have been the subject of numerous research studies, little research has been done on manta rays. This difference is primarily due to the difficulty in studying these large animals in the field. Landings from fisheries provide a source of information on morphology, including the size demographics of a fished population, descriptions on the size at sexual and physical maturity, litter sizes, and other important reproductive information. Satellite, acoustic, and pop-up archival tags are becoming more common for tracking animals that travel

large distances and can provide valuable information on home ranges, dive profiles, and diel patterns of behavior. Mark recapture studies are useful for estimating a population size and range, and for developing individual life histories.

1.4 Dissertation Overview

Data on the life history and ecology of resident manta rays is limited, despite the species being circumglobally distributed and aggregation areas being popular attractions for marine tourism (A. D. Marshall, 2009). Like most sharks, manta rays can be considered equilibrium strategists (Winemiller & Rose, 1992), having late maturation, long gestation periods, and producing only a few, large offspring in their lifetime. Their life history characteristics of slow growth and low fecundity make them a greater risk for population decline from human exploitation (Frisk, Miller, & Fogarty, 2001; Holden, 1974).

The aim of this dissertation was to improve our understanding of the ecology and behavior of a manta ray (*M. alfredi*) population in Hawai‘i that frequents an aggregation site located off Maui’s northwestern coastline. An improved understanding of the general ecology of this population would improve management strategies to protect this population locally and other populations globally. Three studies are described:

1) **Using paired-laser photogrammetry as a simple and accurate system to measure the body size of free-ranging manta rays (*Manta alfredi*)**

This study examines the use of paired-laser photogrammetry as a tool for measuring free-ranging manta rays remotely. The accuracy and precision of the technique is quantified and size demographics of the population are described. Minimum and maximum sizes of males and females at physical and sexual maturity are examined.

2) **Characteristics of a manta ray (*Manta alfredi*) population off Maui, Hawai'i, and implications for management**

This study applies mark-recapture methodology to estimate the population size of resident manta rays observed in the study area. Life history information is used to quantify site fidelity. Data from two acoustically tagged individuals and photo-identification comparisons with other aggregation areas are used to determine home ranges and the occurrence of interisland-movements. Basic demographics are described and temporal uses of the study area are examined. Natural and anthropomorphic threats are discussed and incorporated into suggested strategies for local management of this population.

3) **The reproductive ecology of role manta rays (*Manta alfredi*) off Maui, Hawai'i, with an emphasis on body size**

Understanding reproductive strategies and mating behavior is critical for the proper management of marine fish species (S. Rowe & Hutchings, 2003). In this study, the reproductive ecology of this population is examined by looking for seasonal trends in mating, pregnancy rates, and mate choice with an emphasis on how body size plays a role in male and female reproductive success.

1.5General Methods

The majority of surveys were conducted at a single manta aggregation area off the west coast of the island of Maui, Hawai'i, between 2005 and 2010. An area approximately 30,000 m^2 (200 m X 150 m) in size, 450 m offshore, with a depth range of 5 - 30 m was used as the monitoring boundary for the study site. This location was chosen because of the high reliability of encountering manta rays and thereby maximizing encounter rates.

The habitat in this area consisted primarily of fringing coral reef that extends away from the shoreline for approximately 550 m. The substrate cover at depths of 5 m to 15 m was composed

of lobe (*Porites lobata*), rice (*Acroporidae spp.*), cauliflower (*Pocillopora meandrina*), and finger coral (*Porites compressa),* with intermittent patches of sand and rubble. The substrate cover at depths between 15 m and 30 m was composed of sandy bottom and sea grass (*Halimeda spp.*), interspersed with patches of cauliflower and lobe coral.

The main cleaning stations were situated near the starting point of the survey where manta rays have been seen soliciting predominantly Hawaiian cleaner wrasses (*Labroides phthirophagus*) and saddle wrasses (*Thalassoma duperrey*) to remove parasitic copepods from the surface of their bodies. Mating trains have also been observed in this area.

Surveys were conducted with open-circuit SCUBA, over a six-year period between 2005 and 2010. A combination of photo-identification, paired-laser photogrammetry, and occasional active tracking were used as primary methods for collecting data. Detailed accounts of the data collection and analysis procedures used in each study are provided in Chapters 3, 4, and 5.

2 USING PAIRED-LASER PHOTOGRAMMETRY AS A SIMPLE AND ACCURATE SYSTEM TO MEASURE THE BODY SIZE OF FREE-RANGING MANTA RAYS (*MANTA ALFREDI*)[1]

2.1 ABSTRACT

Morphometrics are useful for describing and managing animal populations but measurements can be difficult to obtain, especially on large, free-ranging, aquatic animals. The accuracy and precision of paired-laser photogrammetry was tested as a simple, accurate, precise, and non-invasive remote sensing system for measuring the body size of free-ranging, resident manta rays (*Manta alfredi*), a newly described species that is poorly understood. Based on repeated measurements of a pipe of known size, the paired-laser system proved accurate (mean error of 0.39%) and precise (CV = 0.54%). Repeated measurements on 154 different manta rays visiting a cleaning station off Maui, Hawai‘i, produced a mean CV of 1.46%. Disc length (DL) measurements were more precise than disc width (DW) measurements and an empirically derived disc ratio (DR) function was applied to convert DL to DW measurements for standard comparison with other studies. Sexual dimorphism was present with the largest female (3.64 m DW) 18% larger than the largest male (3.03 m DW). Sexual maturity in females, based on evidence of pregnancy and mating scars, was conservatively determined to be 3.37 m DW. The DW at which 50% of the males were likely to be mature (based on clasper length) was between 2.70 and 2.80 m DW. The absence of individuals smaller than 2.50 m DW suggests age class segregation may be occurring in this population. Paired-laser photogrammetry proved to be a simple, non-invasive, accurate, and precise method for sizing free-ranging manta rays. Repeated measurements on known individuals over time could provide population growth parameters needed for adequate management of this poorly understood species.

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[1] These data have been published in Aquatic Biology (Deakos, 2010) and included here with authorization from the journal

2.2INTRODUCTION

Body size measurements or morphometrics are important components in understanding the life history of an organism. Morphometrics have been used to study individual growth (e.g., S. Clark, Odell, & Lacinak, 2000), physical and sexual maturity (e.g., Waters & Whitehead, 1990), phenotypic differences in closely related species (e.g., Perryman & Lynn, 1993), and size class segregation in a population (e.g., Cubbage & Calambokidis, 1987). Identifying the existence of sexual dimorphism in a population through morphometrics can help to understand reproductive strategies, intrasexual competition, and mate choice (e.g., Breuer, Robbins, & Boesch, 2007).

Obtaining morphometrics on free ranging animals can be challenging, especially if the animals are large in size, and particularly if they live in aquatic environments. Most methods are intrusive, disruptive, and usually involve the capturing or killing of the animal, subjecting both researcher and animal to the risk of injury. The ability to obtain size measurements remotely eliminates many of these risks.

Photogrammetry is a non-invasive, remote sensing technique that uses photography or digital imagery to measure objects, or in the case of animals, morphometrics. The technique has been used successfully to measure the body length of large marine animals such as whales (Best & Ruther, 1992; Cubbage & Calambokidis, 1987; S. S. Spitz, Herman, & Pack, 2000), dolphins (Perryman & Lynn, 1993), seals (Bell, Hindell, & Burton, 1997), and sharks (Klimley & Brown, 1983). With photogrammetry, measurements can be collected quickly, with minimal disturbance to the animal or their associates. However, photogrammetric techniques can be expensive and cumbersome, requiring an aircraft (e.g., Cosens & Blouw, 2003), a boat with a tall mast (e.g., Dawson, Chessum, Hunt, & Slooten, 1995), or multiple cameras operating simultaneously (e.g., Klimley & Brown, 1983), limiting their applicability. Paired-laser photogrammetry uses two parallel laser pointers mounted onto a single camera to project two points of light onto a target,

showing a scale of known size from which the size of the target can be inferred. The technique is relatively simple, compact, and can be implemented by a single photographer. It has been used successfully to measure morphometrics on large free-ranging animals such as the horn length in Alpine ibex (*Capra ibex*) (Bergeron, 2007), and the dorsal fins of killer whales (*Orcinus Orca*) (Durban & Parsons, 2006) and bottlenose dolphins (*Tursiops truncates*) (L. Rowe & Dawson, 2008). It has also been used underwater, with moderate success, to measure small fish at close range (Mueller, Brown, Hop, & Moulton, 2006; Yoshihara, 1997). This study investigates the usefulness of underwater paired-laser photogrammetry for measuring one of the largest fish in the oceans, the manta ray.

Manta rays are the largest ray in the Mobulidae family and still poorly understood. They feed on small planktonic organisms such as euphasids and copepods (T. B. Clark, 2001; Homma et al., 1999; Last & Stevens, 1994; G. Notarbartolo-di-Sciara, 1987) and possibly on small shrimp, crabs, and fish (Bigelow & Schroeder, 1953). They are ovoviviparous and are believed to give birth to a single live young every 2-3 years (Homma et al., 1999) following a gestation period of 12-14 months (A. D. Marshall et al., 2006). Following parturition, the pup is weaned immediately with no further parental care (Senzo Uchida et al., 2008). Natural predators include large sharks (Homma et al., 1999) and killer whales (*Orcinus orca*) (Visser & Bonoccorso, 2003) depending on the region.

The genus *Manta* was thought to consist of just a single species, *Manta birostris*, but recent evidence from morphology has confirmed a second species in the genus, *Manta alfredi* (AD Marshall et al., 2009). *M. birostris*, sometimes referred to as "oceanic manta rays," can grow to a disc width (DW) (measured from wing tip to wing tip) of 6.70 m (Bigelow & Schroeder, 1953), and possibly as large as 9.10 m (L. Compagno, 1999). These manta rays occur in temperate, sub-tropical, and tropical waters globally, spending the majority of their time in deep water, paying occasional visits to coastal areas with productive upwellings, oceanic islands, and offshore pinnacles and seamounts (Bigelow & Schroeder, 1953; L. J. V. Compagno, 1999; AD Marshall et al., 2009).

M. *alfredi*, referred to in the present study as "resident manta rays", have been observed in the Pacific, Atlantic, and Indian ocean between latitudes 30^{o} N and 30 ^{o} S (AD Marshall et al., 2009). Smaller and more tropical than their oceanic relatives, they are more likely to be observed in shallow coastal areas (G. Notarbartolo-di-Sciara & Hillyer, 1989) with rocky and coral reef habitats near productive upwellings, as well as around tropical islands, atolls, and bays (AD Marshall et al., 2009). In some parts of the world they can be reliably seen congregating around rich food sources and cleaning stations (T. B. Clark, 2001; Dewar et al., 2008; Homma et al., 1999). Cleaning stations consist of specific locations along the reef where individuals solicit host cleaner fish that feed on parasites and other unwanted materials on their skin (Losey Jr, 1972).

Worldwide, only a handfull of resident manta rays have been successfully measured for morphology. In waters off southern Mozambique, the smallest free swimming individuals were estimated at 1.5 m DW and the largest estimated at 5.5 m DW (AD Marshall et al., 2009). Males in this region appear to mature around 3.0 m DW while females in coastal waters off South Africa appear to mature at approximately 3.9 m DW (AD Marshall et al., 2009).

The aim of the present study was to examine the practicality, accuracy, and precision of paired-laser photogrammetry as a simple, non-invasive, remote sensing system for measuring free-ranging, resident manta rays from a population off Maui, Hawai'i. The maximum size of male and females at physical maturity, and their minimum size at sexual maturity were quantified, providing useful biological parameters from which to infer new information about the biology and ecology of this species.

2.3 METHODS

2.3.1 Study Area and Population

All manta ray surveys were conducted opportunistically over a three-year period between 2007 and 2009 at a single manta ray cleaning station off the west coast of the island of Maui, Hawai'i.

Approximately 450 m offshore, a region 8000 m^2 (200 m X 400 m) in size, with a depth range of 5-30 m, was chosen as the monitoring boundary for the study area because of the high reliability of observing manta rays and thereby maximizing encounter rates. The habitat consists primarily of fringing coral reef that extends away from the shoreline approximately 550 m. The main cleaning stations were situated near the starting point of the survey where the Hawaiian cleaner wrasse (*Labroides phthirophagus*) and saddle wrasse (*Thalassoma duperrey*) remove small copepod parasites from soliciting manta rays. Manta ray mating trains were also observed in this area consisting of a single female pursued by one or more males (Yano et al., 1999). The exact location of the site is being withheld to avoid the potential commercial exploitation of this unique area.

2.3.2 Equipment

Two underwater, green laser pointers (Lasermate Professional: output power < 5 mw, wavelength = 532 nm, 180 mm in length, 25 mm in diameter) were mounted in parallel with their centers 0.60 m apart onto a 160 X 680 X 5 mm aluminum plate. A 0.60 m separation provided a reference scale small enough to measure the smallest manta ray in the population but large enough to minimize error while maintaining portability. The platform was mounted to the bottom of a Sea & Sea VX-HC1 underwater housing (Figure 2). The housing enclosed a Sony HDR-HC1, high definition video camera with a lens focal zoom length of 5.1 – 51 mm (equivalent to 48 – 480 mm on a 35 mm still camera), lens aperture F/1.8-2.1, and 2.76 megapixels effective still resolution. The camera was fitted with a wide-angle lens attachment (68 mm diameter, 41 mm length, 0.7 magnification). Allen screws, threaded around each laser-mounting bracket, allowed for fine-scale adjustments of each laser pointer to ensure they were exactly parallel.

2.3.3 Accuracy and Precision

Potential sources of measurement error include: (1) image distortion caused by light refraction and the wide-angle lens; (2) non-parallel alignment of the lasers; and (3) parallax error.

Figure 2. A pair of green, underwater laser pointers mounted in parallel to an underwater video housing.

2.3.3.1 Image distortion

Image distortions can occur when light refracts as it passes at an angle from water (refractive index ~1.00) to air (refractive index 1.33) inside the underwater camera housing. Further distortion occurs when the light passes through the wide-angle lens. Wide-angle lenses are designed to severely bend rays of light around the periphery of the field of view (Swaminathan & Nayar, 1999) with pixels toward the center of the image being the least distorted and pixels toward the edges of the image being the most distorted. Some of the distortion, in particular around the edges of the picture, readjusts slightly due to the refraction occurring in air trapped in the camera housing (between water and lens) before the light reaches the lens. By approximating the amount of distortion occurring in the image, a correction factor can be applied to compensate for the distortion.

To quantify the amount of distortion that was occurring, a piece of graph paper was photographed underwater and the image examined in Adobe Photoshop ®. The image was

composed of 16 columns and 10 rows of squares making up a total of 160 squares. Since the squares at the center of the image had the least distortion, the dimensions of these squares were used to represent the expected dimensions of a non-distorted square (as if the image had been photographed with a flat lens). The diagonal length across two of these center squares was measured (in pixels) using the Adobe Photoshop ® line tool. Additional lengths were taken diagonally across 4, 6, and 8 squares with the center of the diagonal passing over the center of the image, essentially expanding the diagonal measurement by 2 square increments. The expected, undistorted lengths for these dimensions were calculated by multiplying the undistorted diagonal length of the two center squares by 2, 3, and 4 respectively.

The expected, undistorted lengths were plotted along the *y*-axis. The actual measured lengths were plotted on the *x*-axis. The data were fitted with a regression curve and a Pearson Correlation Coefficient was calculated to determine its fit. The function of the curve was applied to all measurements to correct for the distortion caused by the light refraction and wide-angle lens.

2.3.3.2 Parallel alignment of lasers

Non-parallel alignment of the laser pointers can cause the spacing between the points of light to change depending on the distance from the target, creating inaccurate measurements. To ensure the lasers were parallel, a plastic pipe with two clear marks on the center of the pipe spaced 0.60 m apart, was placed on the ocean bottom at a depth of approximately 10 m. With the paired-laser system in hand, a scuba diver positioned over the center of the pipe adjusted the Allen screws so that the points of light projected exactly onto the markings (Figure 3A). The laser pointers were confirmed to be parallel when the spacing between the points of light remained 0.60 m, even as the diver moved towards and away from the pipe. On 4 occasions (12 Aug 2008, 21 Sep 2008, 9 Oct 2008, and 9 Jan 2009) the laser pointers were removed from the holding brackets, remounted, and the spacing adjusted to ensure they were parallel.

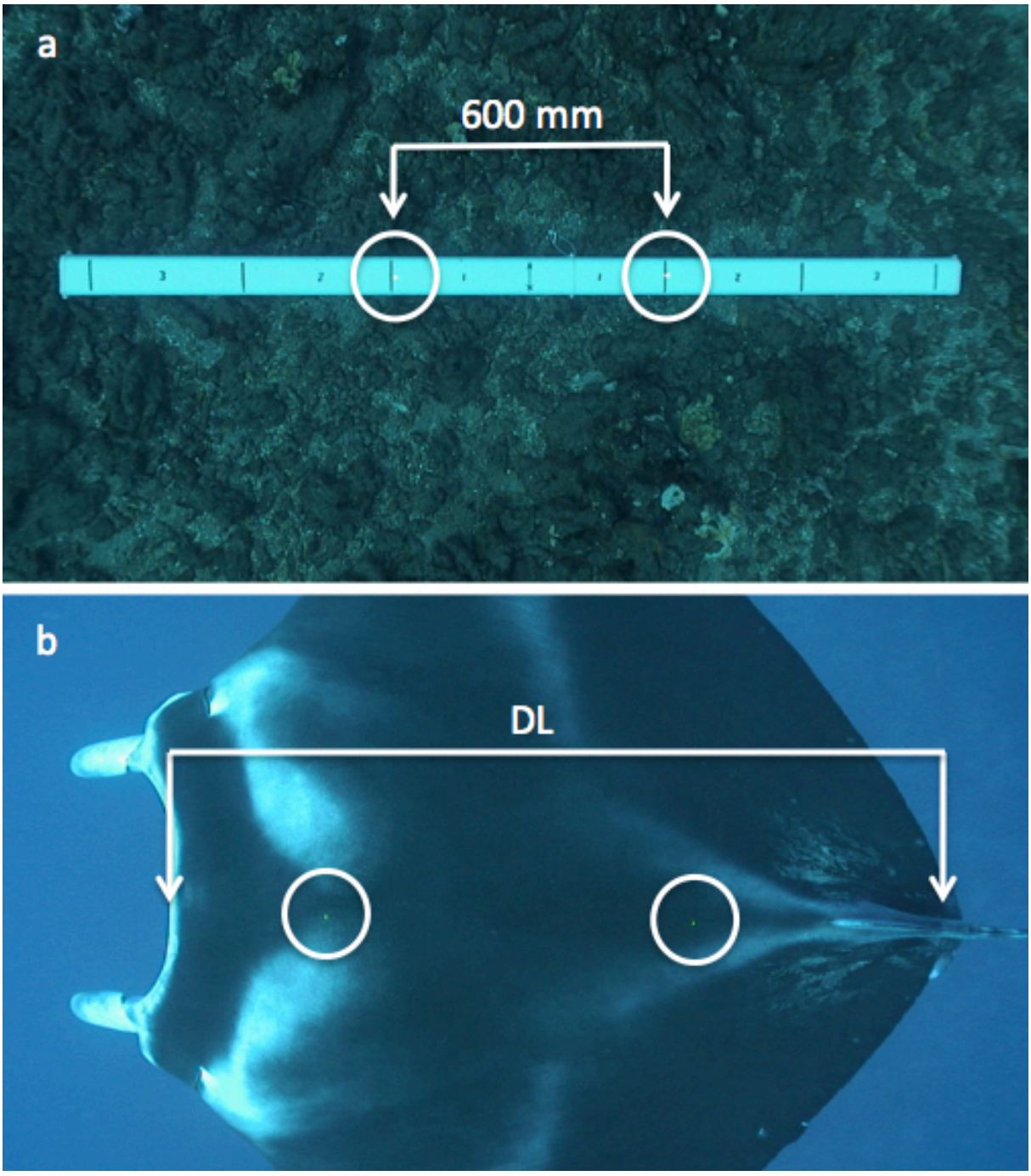

Figure 3. (a) Pipe of known length being measured on the ocean floor showing the projected points of light 60 cm apart; (b) a photograph of a manta ray (*M. alfredi*) from above showing the projected points of light along the spinal axis of the disc from which a DL measurement can be obtained.

2.3.3.3 Parallax error

Parallax error can be a problematic source of error with paired-laser photogrammetry (e.g., Durban & Parsons, 2006). This occurs when the laser projections are not perpendicular to the surface being measured. To investigate how the measurement of an object of known length varies with the horizontal angle of the target to the axis of the lasers (parallax), measurements of a 1.94 m pipe were taken with the diver positioned above the end of the pipe rather than over the center. The distance of the diver above the end of the pipe was calculated by multiplying the tangent of the desired parallax angle (in this case 80, 70, 60, 50, and 40˚) by half the length of the pipe (0.97 m) to produce parallax angles of 10, 20, 30, 40, and 50˚ off the perpendicular axis. The pipe was measured 5 times at each angle. The lens correction function was applied to each measurement and a mean percent error was calculated for all measurements at each angle.

2.3.3.4 Pipe measurements

Accuracy of a measurement is the degree to which the 'measured' matches the 'actual'. Precision of a measurement is the degree to which repeated measurements of the same target show the same results. Both accuracy and precision of the paired-laser system was determined by measuring a 1.94 m pipe on 4 separate days over a 5 mo period. The diver positioned himself over the center of the pipe at a distance that would allow the full length of the pipe to be captured within the camera's field of view along the horizontal plane. With the pipe perpendicular to the direction of the laser projections, the 2 points of light were projected onto the center of the pipe and a photograph was taken using the minimum focal length of 5.1 mm (Figure 3A). After a measurement was taken, the diver moved a short distance away before repositioning to take a second independent measurement. After a minimum of 4 independent measurements, the pipe was moved to a new location and a new series of measurements was taken. Accuracy was determined by measuring the percent error of the estimated length against the known length of the pipe. Precision was measured by calculating the percent coefficient of variation (CV) from repeated measurements of the pipe.

2.3.4 Manta Ray Measurements

2.3.4.1 Surveys

A survey consisted of a 55 to 75 minute SCUBA dive (single tank, open-circuit) with a beach entry to the study site. Each survey began from the exact same location. From this start point, a rectangular search pattern was initiated, enclosing an area approximately 8000 m^2 (200 m X 400 m). The water depth ranged from 6 m to 30 m. When manta rays were encountered, disc length (DL) and DW measurements were attempted.

DL is defined here as the length from the tip of the snout to the posterior edge of the pectoral fins (Francis, 2006). To measure the DL, the diver positioned above the manta ray such that its dorsal plane was perpendicular to the direction of the laser projections, and the DL was captured along the horizontal axis of the field of view. A photograph was taken with the points of light projected onto the center of the anteroposterior axis of the disc (Figure 3B). The diver then turned the camera 180˚ horizontally and repositioned above the manta ray before each repeated measurement. When repositioning, care was taken to stay out of the manta ray's field of view so as not to surprise the animal and cause it to flee. At least 4 independent measurements were attempted on each manta ray when possible.

The same method applied when measuring the DW, except that the wing tips of the manta ray were aligned with the horizontal axis of the field of view. A photograph was taken with the points of light projected onto the center of the mediolateral axis of the disc. It was important that the photograph be taken when both wings were completely open so as not to underestimate the DW.

Sizes were taken from above the manta ray to: (1) minimize disturbance to the manta ray since they do not appear to see directly above their dorsal plane; (2) eliminate any chance of projecting a point of light into the manta ray's eye since the eyes are not visible from above; and (3)

minimize parallax error since the diver, from above, is better able to align the laser projections perpendicular to the axis of the manta ray.

Precision of the paired-laser system on free-ranging manta rays was assessed by calculating the CV from repeated measurements taken on the same animal during the same survey and across different surveys.

2.3.4.2 Disc ratio

The relationship between DW and DL was examined by dividing the DW by its corresponding DL to obtain a disc ratio (DR). The mean DR was compared between females and males to determine if this proportional relationship was the same across sexes, and the same comparison was made between adult and juvenile males to determine if the proportional relationship was constant across age classes. The measured DW for each manta ray was plotted against its corresponding DL and fitted with a linear regression curve. The function of the regression curve was used to convert measurements of DL to an estimate of DW so that direct comparisons could be made with other studies.

2.3.4.3 Photo-processing

Each photograph of a manta ray size was examined in Adobe Photoshop ®. If the dorsal plane of the manta ray in the image did not appear perpendicular to the axis of the laser projections, the image was discarded to eliminate parallax error. For all other images, the number of pixels between the 2 points of light and between each end of the target was measured using the line tool. The length of the target (in pixels) was divided by the distance between the points of light (in pixels) and multiplied by the known distance between the points of light (0.60 m) to obtain the length of the target in m.

2.3.4.4 Photo-identification

Photo-identification involves taking photographs of distinctive characteristics from an animal in order to identify and track individuals of a wild population over time. This technique has been used extensively with large and long-lived vertebrates (for review see Würsig & Jefferson, 1990) for population estimates (e.g., Graham & Roberts, 2007), life history information (e.g., Brault & Caswell, 1993), lifespan information (e.g., Langtimm et al., 2004), migration patterns (e.g., Calambokidis et al., 2006), and social relationships (e.g., Bejder, Fletcher, & Bräger, 1998) of recognized individuals. Since each manta ray possesses a distinct pattern of spots on the ventral surface that are present from birth (Andrea D. Marshall et al., 2008), and the pattern appears to remain unchanged over time (T. B. Clark, 2001; Homma et al., 1999; Yano et al., 1999), this species is well suited for photo-identification studies.

During each manta ray encounter, attempts were made to photograph the ventral spot pattern of each individual sighted. Manta rays frequently make close passes near a diver allowing the diver to be positioned such that a ventral identification photograph can be taken. When possible, the genital area was also captured in the photograph for sex identification. Immediately after each manta ray was photo-identified, a hand signal was also photographed to indicate the sex and age class of that individual. Photo-identifications were taken prior to moving above the animal for size measurements.

Photographs were downloaded to a MacBook Pro computer and the best photo-identification for each individual from a survey was imported into Finbase, a publicly available photo-identification program created in Microsoft Access (Adams, Speakman, Zolman, & Schwacke, 2006). The photo was matched against photos of all previously identified individuals from the study site and determined to be a match or a new individual. A detailed catalog was kept of each individual in the population and its sighting history. The very distinct markings on the underside of each manta ray make the likelihood of missing a match, or falsely identifying a match, unlikely.

2.3.4.5 Sex and age class

Since claspers are present and visible in males from birth (Andrea D. Marshall et al., 2008), the sex of the manta ray was determined by the presence or absence of claspers. Females were documented as sexually mature if they were obviously pregnant, or showed visible mating scars (spot scarring and abrasions usually visible on the dorsal side of the end of the left wing; AD Marshall & Bennett, 2010). A pregnant female close to term was exceptionally rotund in girth and could be identified quite easily. A female that appeared to be pregnant but was questionable was not given an age class. A female being pursued by multiple males in a mating train was documented as a nuclear female.

Among males, calcification of the claspers occurs rapidly over a relatively narrow range of growth (W. T. White et al., 2006), with the majority of calcification occurring once the claspers have extended beyond the length of the pelvic fins (A. Marshall, pers. comm.). Since the onset of clasper calcification in many shark species coincides with a rapid rate of clasper growth and gonadal maturation (e.g., Jones, Hall, & Potter, 2008), claspers extending beyond the pelvic fins were used as a reliable indicator of sexual maturity in male manta rays.

2.3.5 Statistics

Pearson Correlation Coefficients were calculated for both linear regressions describing the relationship between undistorted and actual measured lengths, and the relationship between the DW and DL of individual manta rays. The precision of a single measurement was tested using a Wilcoxon signed-rank test for matched pairs by comparing the first measurement of a manta ray with the mean of repeated measurements on the same manta ray. The variability of repeated DL and DW measurements was compared using a Mann-Whitney *U*-Test. This test was also used to compare the mean DR between females and males, and between adult and juvenile males. Significance for all statistical tests was set at $p < 0.05$. Confidence intervals are reported at 95%.

2.4RESULTS

2.4.1 Accuracy and Precision

The diagonal distance across the 2 center squares in the underwater photograph of the graph paper was 245 pixels. Therefore, expected diagonal distance across 4, 6, and 8 of the center squares were estimated as 2 X 245 = 490, 3 X 245 = 735, and 4 X 245 = 980 pixels respectively. Actual measured diagonal values were 491, 741, and 992 pixels respectively. The expected, undistorted lengths were plotted against the actual, measured lengths (Figure 4). The linear regression of best fit produced a Pearson correlation coefficient of 1.0 ($df = 3$, $p < 0.001$).

Five pipe measurements were made from angles of 10, 20, 30, 40, and 50˚ away from the perpendicular axis to the center of the pipe. This resulted in mean errors of -4.92, -6.13, -8.79, -22.25, and -39.29% respectively. A pipe 1.94 m in length was measured on 4 separate occasions over a 6 mo period for a total of 92 independent measurements. Without a wide-angle lens correction function applied, the overall mean estimated pipe length was 1.97 m (95% CI = ± 0.02, CV = 0.61%). With the lens correction applied the estimated mean length was 1.94 m (95% CI = ± 0.02, CV = 0.54%). The lens correction function reduced the mean error from 1.39% to 0.39%, and reduced the maximum error from 2.76% to 1.43%.

2.4.2 Manta Ray Measurements

2.4.2.1Surveys

A total of 87 surveys were conducted during which the DL of 274 manta rays was measured. The DW of 82 of these manta rays was also measured. Photo-identification matching revealed 154 of these manta rays were distinct individuals.

The variance of repeated DW measurements on the same individual (mean CV = 3.05%) was significantly greater than the variance of repeated DL measurements on the same individual

(mean CV = 1.46%) (Mann-Whitney *U*-Test: $Z_{(0.05)} = -2.692$, n = 264, 51, p = 0.007) indicating that DL is a more precise measurement than DW.

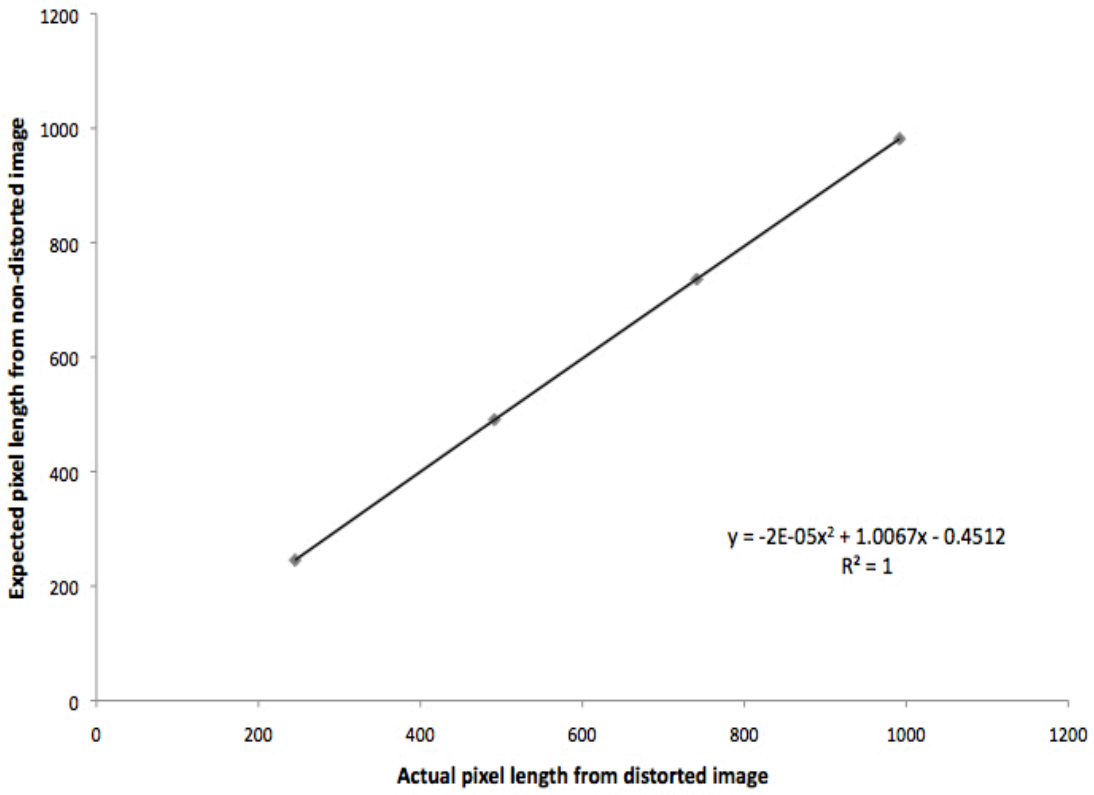

Figure 4. A plot of the expected, undistorted length of an object (in pixels) if measured with a flat lens against the actual measured length of the same object (in pixels) distorted by the wide-angle lens. The data are fitted with a linear regression equation.

2.4.2.2 Disc ratio

The mean DR for all 82 individuals measured was 2.33 (95% CI = ± 0.02). No significant differences were found between the DR of females and males, or between adult and juvenile males (Table 3). For each individual manta ray, the measured DW was plotted against its corresponding DL (Figure 5). The linear regression of best fit produced a Pearson Correlation

Coefficient of 0.923 (df = *63*, $p < 0.001$). The relationship between DW and DL was best described by the following linear regression:

$$DW = 1.958DL + 0.469 \quad (r^2 = 0.923)$$

Results of a Wilcoxon signed rank test found no differences between the first measured DL of an individual manta ray and the mean of repeated independent DL measurements of the same individual ($Z = -0.632$, $n = 274$, $p = 0.527$).

Table 3. Comparison of the mean disc ratio (DR) between male and female, and between adult male and juvenile male manta rays (*M. alfredi*).

	Mean Disc Ratio (m)	n	% CV
Males	2.34[a]	37	3.13
Females	2.33[a]	27	3.50
Adult Males	2.33[b]	23	3.24
Juvenile Males	2.35[b]	12	3.46
All Individuals	2.33	64	3.27

Mann-Whitney *U*-Test

[a] $Z = -0.768$, $df = 63$, $p = 0.442$

[b] $Z = -0.452$, $df = 34$, $p = 0.668$

2.4.2.3 Sex and age class

Of the 154 individual manta rays measured in this population, 71 (46%) were females, and 83 (54%) were males (Figure 6). Females were on average significantly larger than males (Mann-Whitney *U*-Test: $Z = -0.0867$, $n = 71, 83$, $p < 0.001$). The largest female (3.64 m DW) was 18% larger than the largest male (3.00 m DW). The smallest female (2.50 m DW) was only slightly smaller than the smallest male (2.51 m DW).

The smallest pregnant female at 3.37 m DW (n = 16) was also the smallest female with visible mating scars (n = 19). Using the size of this female as a conservative minimum size for sexual maturity in females, at least 48% of the females measured in this population were likely to be of mature size. The smallest female observed in a mating train was 3.24 m DW (n = 12). One third of all nuclear females were never observed pregnant or to have mating scars.

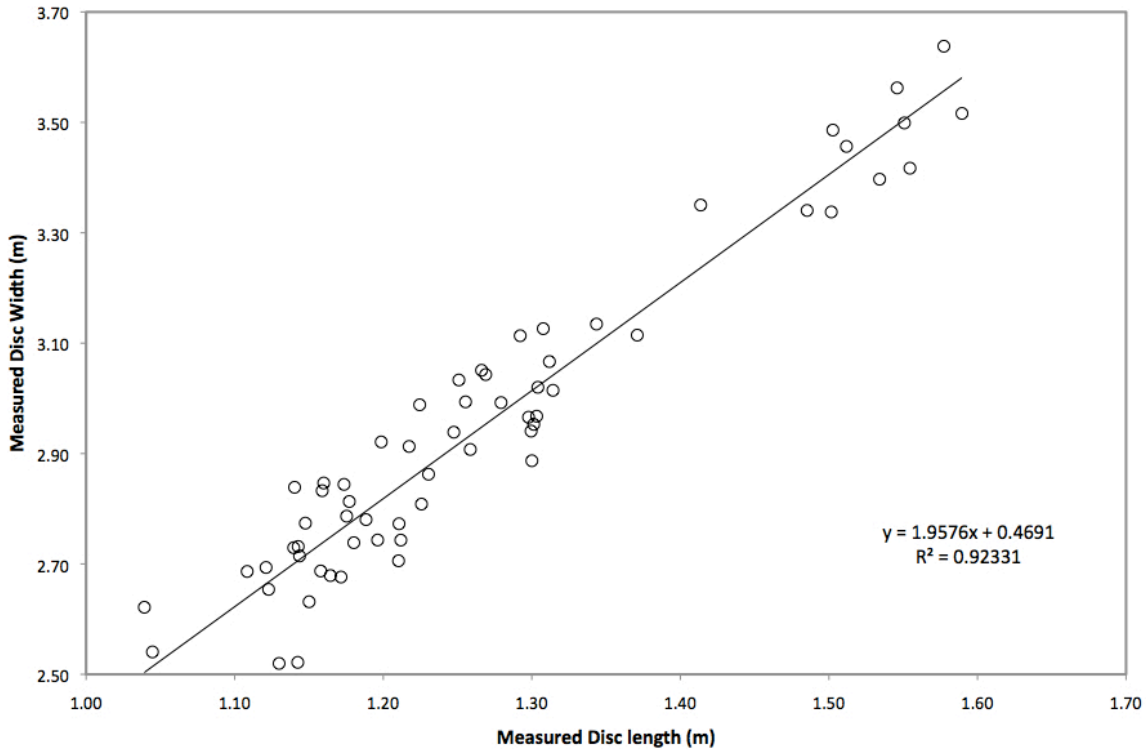

Figure 5. Sixty-four manta ray DW measurements plotted against its corresponding DL measurement and fitted with a linear regression. Measurements are in meters.

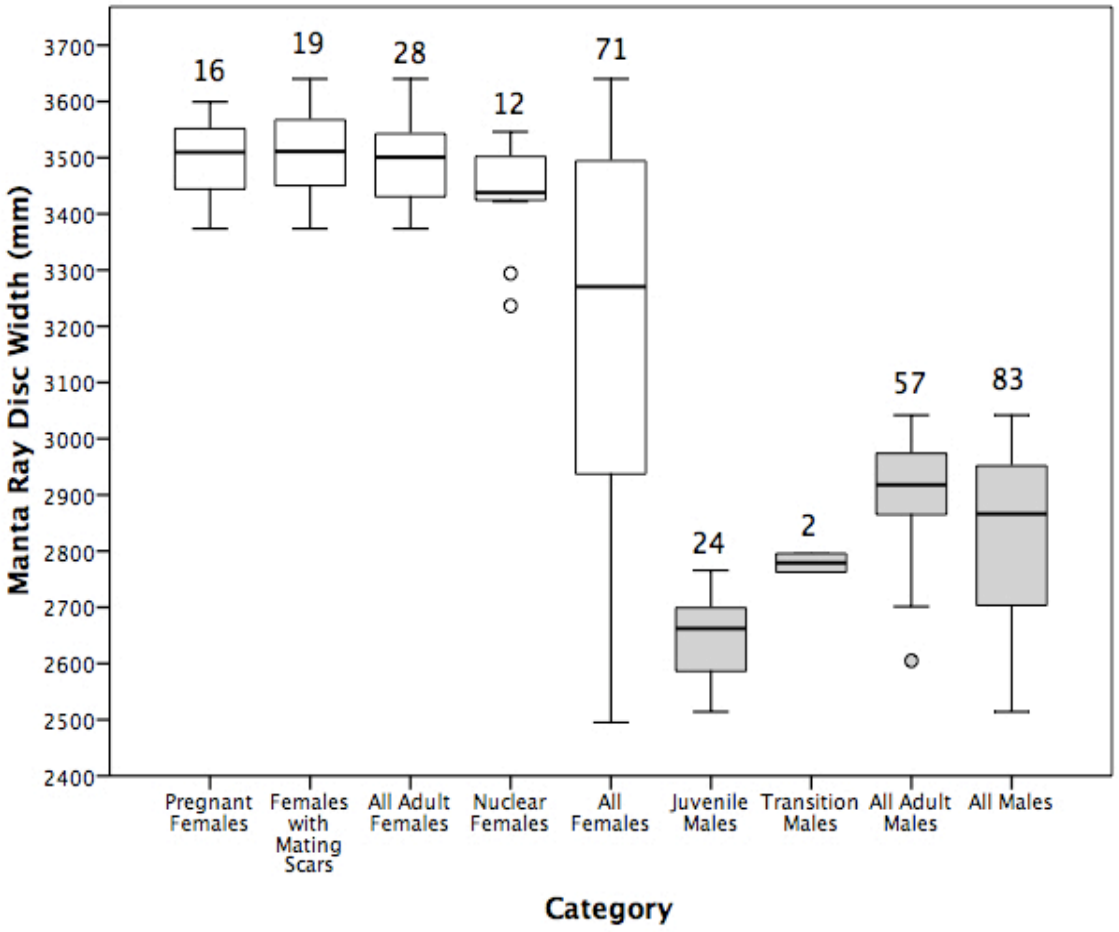

Figure 6. The distribution of manta ray (*M. alfredi*) disc widths by gender and age class. Females were considered adults if obviously pregnant or showed visible mating scars. Not all nuclear females were pregnant or had mating scars. Males with clasper lengths extending beyond the pelvic fins were classified as adult, even with the pelvic fins were classified as transition, and shorter than the pelvic fins were classified as juvenile. White boxes represent female categories, gray boxes represent male categories, numbers represent sample sizes, and circles represent outliers. All measurements are in millimeters.

The smallest adult male was estimated at 2.60 m DW (n = 57) and the largest juvenile male was estimated at 2.77 m DW (n = 24). Transition males, whose claspers were exactly even with the edge of the pelvic fins, were rare. Only two were measured, each with a DW of 2.76 m and 2.80 m. When DW among males was separated into 0.1 m incremental categories, the DW category at which approximately 50% of the males were considered mature (DW_{50}) was 2.7 to 2.8 m (Figure 7).

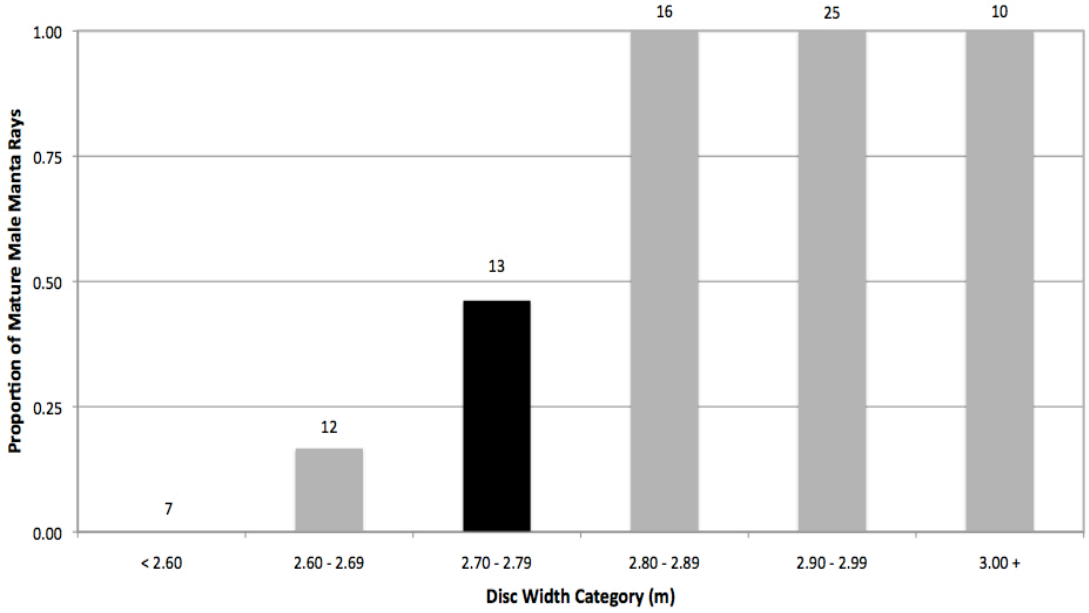

Figure 7. The proportion of males which are sexually mature for each disc width size category. The black column indicates the size category for which nearly 50% of the males can be considered mature (DW_{50}). Numbers above the column indicate the sample size.

2.5DISCUSSION

2.5.1 Accuracy and Precision

When measuring a target of known size, the paired-laser photogrammetry system was accurate to a mean error of 0.39% (0 – 1.43%) and precise to a mean CV of 0.54% (0.02 – 4.01%). This is comparable or better than reports for other photogrammetric systems with accuracy ranging from 0.47 - 6.6% (Bergeron, 2007; Cosens & Blouw, 2003; Cubbage & Calambokidis, 1987; Perryman & Lynn, 1993; S. S. Spitz et al., 2000) and precision (CVs) ranging from 0.84 to 9.03% (Best & Ruther, 1992; Cosens & Blouw, 2003; Cubbage & Calambokidis, 1987; Dawson et al., 1995; J. Gordon, 1990; Klimley & Brown, 1983; Perryman & Lynn, 1993; S. S. Spitz et al., 2000).

Potential sources of error with the paired-laser system were easily controlled for by (1) using study mount to ensure the laser pointers remained parallel, (2) discarding images showing evidence of parallax, and (3) applying a simple, empirically determined correction function to control for image distortion caused by light refraction and the wide-angle lens.

For measuring manta ray sizes, the paired-laser photogrammetric system proved to be simple to use. A single diver was able to take multiple measurements with little or no change to the manta ray's behavior. Occasional reactions by a manta ray during measurements usually occurred when the manta ray performed an abrupt change in direction (e.g., when being bitten by a cleaner wrasse) thus bringing the diver into view and causing the manta ray to move rapidly away.

Although multiple measurements should be taken for insurance, in some situations, such as when multiple animals in a mating train pass through the area rapidly, time may only allow for a single measurement to be taken per individual. The first measurement proved to be just as precise as the mean of repeated measurements on a manta ray.

Measurements of DW were less precise than measurements of DL. This was most likely due to the difficulty in photographing the manta ray with its wings completely open. Even with dead specimens where the fin tips have become curled or the texture has become loose, DW measurements can be unreliable (Andrea D. Marshall et al., 2008; G. Notarbartolo-di-Sciara, 1987), and caution should be taken when using this metric. DL proved to be a more accurate metric for measuring the body size of free-ranging manta rays.

2.5.2 Manta Measurements

For direct comparisons with other studies, DL can be converted to a more conventional DW estimate by applying a DR function. The relationship between DW and DL was constant regardless of sex or age class. Morphometric proportions, including the DW and DL of a measured male manta ray fetus from southern Mozambique were the same as those measured for three juvenile manta rays from South Africa (Andrea D. Marshall et al., 2008), adding further support for isometric growth of this species.

The mean DR was 2.33 (95% CI = ± 0.02), similar to those reported for specimens in South Africa ranging between 2.21 and 2.37, and a fetus from southern Mozambique with a DR of 2.43 (Andrea D. Marshall et al., 2008). DRs for oceanic manta rays include 2.2 reported for an individual from the eastern North Atlantic (Bigelow & Schroeder, 1953) and a range of 2.16 – 2.29 from 4 specimens examined in Indonesia (Andrea D. Marshall et al., 2008).

The largest measured manta ray was a female estimated at 3.64 m DW, substantially smaller than the 5.50 m DW maximum estimate observed in southern Mozambique (AD Marshall et al., 2009) and the 4.30 m DW maximum estimate observed in Japan (Kashiwagi et al., 2008). Geographic variability in size is common for oceanic manta rays ranging in size from 4.94 m DW in Indonesia (W. T. White et al., 2006) to a 6.45 m DW from the eastern North Atlantic (Bigelow & Schroeder, 1953). Additional size measurements of geographically independent populations of *M. alfredi* should be investigated for comparison.

The largest measured female was 18% larger then the largest measured male. This supports the existence of sexual dimorphism in this resident population. In comparison, the largest female reported by Kashiwagi et al. (2008) from a resident population of manta rays off the coast of Japan had a DW of 4.30 m, while their largest male had a DW of 3.60 m, a 19% difference in size. It should be noted that measurements in this study were taken by extending a piece of rope between two divers positioned above the manta ray as it swam (Kashiwagi, pers. comm.).

Sexual dimorphism can occur when natural selection for high female fecundity in a species is stronger than sexual selection for males (Wiklund & Karlsson, 1988). In most vertebrates, natural selection for larger males is well understood, with larger males having an advantage in male-male competition for mating access to females (Thornhill & Alcock, 1983). Natural selection can also favor larger females, with larger females having greater fecundity (Fairbairn, 1997). Female manta rays give birth to a single, large, well-developed pup every 2 to 3 yr (Homma et al., 1999). Since the pup receives no parental care immediately after parturition, larger pups should have greater survivorship (e.g., McMahon, Burton, & Bester, 2000). This immediate independence favors large pups and larger mothers are more able to produce larger offspring (e.g., Pack et al., 2009).

Newborn manta rays have been reported with a DW of 1.1 m–1.5 m (Homma et al., 1999; AD Marshall et al., 2009). The absence of manta rays < 2.5 m DW from the study area suggest that young manta rays may be geographically segregating and may not visit the study area until later in their development. Segregation by body size has been noted for other mobulid species: *Mobula thurstoni*, *Mobula japanica*, *Mobula munkiana*, and *Mobula tarapacana* (G. Notarbartolo-di-Sciara, 1988).

In several species of sharks, females are known to move into specific nursery areas to give birth (e.g., Simpfendorfer & Milward, 1993). The pups remain in the protected area for a length of time before dispersing, presumably for protection against predation. Similarly, female manta rays may retreat to more protected habitats to give birth, where the pup will reside locally until it

reaches a certain age or size. Since no female manta ray has been observed giving birth in the wild, it is not yet known where they go to have their young.

Small manta rays have been observed and photographed in shallow waters along Maui's southeastern shores (B. Blinski, pers. comm.), but not one has been systematically measured. This southeast Maui area is approximately 20 km from the study site and may constitute an area where females give birth and young animals reside until they are more mature and begin to expand their range. Future efforts should focus on obtaining body size measurements from manta rays frequenting this area.

Using pregnancy and mating scars as an indicator of sexual maturity in females, a DW of 3.37 m constitutes a conservative estimate of the size at sexual maturity achieved by females in this Maui population. Although females measuring 3.24 m DW were observed as nuclear females in mating trains, the lack of observed mating scars and pregnancy suggest immature females may also be pursued by males in mating trains. Otherwise, 3.24 m DW may represent a lower limit on sexual maturity in females. Males appear to reach sexual maturity between 2.75 and 2.80 m DW, at the time when their claspers grow rapidly and begin to extend beyond their pelvic fins.

2.5.3 Future Research

Paired-laser photogrammetry is a practical tool for collecting and comparing morphometric data on resident manta rays throughout their range. By visiting areas where manta rays are known to aggregate, it is relatively easy to obtain length measurements from a large part of the population in a relatively short period of time. The ability to relate individual identities with morphometrics can be applied to longitudinal studies looking at growth rates, and allows for the incorporation of life history information about those individuals. By measuring the body size of captive and free-ranging animals of known ages, future applications include identifying age specific survival rates, age at first pregnancy, and other important variables for modeling population growth. Morphometrics on free-ranging manta rays can also help to identify stock depletion, evident from fewer older and larger animals in the population (Cubbage & Calambokidis, 1987),

primarily in regions where they are overfished (A. D. Marshall et al., 2006; W. T. White et al., 2006). This is particularly important with large, slower-growing species such as manta rays that are at greater risk of population decline from exploitation (Frisk et al., 2001).

2.5.4 Summary

The equipment needed to carry out paired-laser photogrammetry is simple, allowing a single diver to collect a large number of manta ray sizes quickly, with high accuracy and precision. Information about the individual's identity, sex, and age class can be obtained simultaneously. These types of information from known-aged animals can be applied to population growth models and used for population management. By adjusting the distance between the lasers, the projected points of light can be customized for measurements of other species. Limitations to the use of this system include the ability to fit the target being measured within the field of view of the camera, the distance from the target at which the light points are still visible (largely dependent on the clarity of the water), and the ability to get into position such that the target is perpendicular to the axis of the laser projections.

3 CHARACTERISTICS OF A MANTA RAY (*MANTA ALFREDI*) POPULATION OFF MAUI, HAWAIʻI, AND IMPLICATIONS FOR MANAGEMENT

3.1 ABSTRACT

Late maturity, few offspring, and a residential nature, typical of *Manta alfredi*, make this species particularly vulnerable to localized anthropogenic threats. A total of 229 surveys were conducted between 2005 and 2009 at a manta ray aggregation site off Maui, Hawai'i, to describe this population's abundance, characteristics, and temporal use of the area. Photo-identifications revealed 290 different individuals. A discovery curve showed no asymptotic trend, indicating the number of individuals using the area was much larger than the total identified. Resights and manta follows revealed that manta rays used Maui County waters but did not appear to mix with a neighboring island population off the Big Island suggesting the possibility of independent island associated stocks. High resight rates within and across years provided strong evidence of site-fidelity. Findings were consistent with a population of manta rays moving into and out of the Maui aggregation area, with a varying portion of the total population temporarily resident at any given time. Males accounted for 53% of all individuals. Manta rays were usually absent at first light with numbers increasing throughout the day. More frequent mating trains were observed during the winter months. Mating appears to occur primarily during the winter. Shark predation was evident in 24% of individuals, and 10% had an amputated or non-functional cephalic fin. This small, demographically independent population appears vulnerable to the impacts from non-target fisheries, primarily from entanglement in fishing line, and could suffer from exploitation by "unregulated swim-with manta ray" programs. Management on an island-area basis is recommended.

3.2INTRODUCTION

Understanding the basic biology and ecology of a species is necessary for the proper conservation and management of that species. For elasmobranchs, which are slow-growing, slow to mature, and have low fecundity (Holden, 1974), understanding how populations are affected by anthropogenic impacts comes with added urgency as they are less likely to recover from population depletion (Hoenig & Gruber, 1990; Pratt & Casey, 1990). Furthermore, populations that are isolated geographically are subject to regional ecological pressures and may require a management strategy that is tailored to that specific population. Batoids (rays) are among the most susceptible marine taxa to fisheries exploitation (Dulvy et al., 2008; Dulvy & Reynolds, 2002) since their large body size is associated with later maturation, thereby putting them at greater risk of overexploitation, extirpation, and in some cases extinction. Manta rays (*Manta sp*), the largest of the batoids are especially vulnerable.

The status of most manta ray populations worldwide is poorly understood. They are classified by the IUCN Red List for Threatened Animals as "near-threatened" (A. D. Marshall et al., 2006). Fisheries targeting manta rays in many parts of the world (L. Compagno, 1999; Dewar, 2002; A. D. Marshall et al., 2006; G. Notarbartolo-di-Sciara, 1987) are fueled by an increasing demand for branchial filter plates and cartilage. The branchial filter plates are used in traditional Chinese medicines, and the cartilage for filler in shark-fin soup (Alava et al., 2002; W. T. White et al., 2006). These directed fisheries have caused significant population declines in areas such as Mexico (Homma et al., 1999), the Philippines (Alava et al., 2002), Indonesia (Dewar, 2002; W. T. White et al., 2006), India, Sri Lanka, and other parts of Southeast Asia (A. D. Marshall et al., 2006).

Manta rays are ovoviviparous, giving birth to a single pup every 2-3 years (Homma et al., 1999; AD Marshall & Bennett, 2010). The only manta ray birth ever witnessed was captured on video at the Okinawa Churaumi Aquarium in Japan (Senzo Uchida et al., 2008). The mother gave birth to a single pup following a twelve month gestation period. Parturition was immediate and the

mother was observed mating within a few hours after giving birth. No information exists on the development and growth of free-ranging manta ray pups.

Mating behavior in manta rays has been described as a mating train, where multiple males pursue and attempt to mate with a single female (Yano et al., 1999). Although these mating trains can be observed at all times of the year, seasonal peaks have been reported for the summer months (July – August) in Ogasawara, Japan (Yano et al., 1999), and the austral summer (October – November) in Mozambique (AD Marshall & Bennett, 2010).

The number of species within the *Manta* genus has long been debated among scientists (Beebe & Tee-Van, 1941; Bigelow & Schroeder, 1953; T. B. Clark, 2001; L. J. V. Compagno, 1984; Fowler, 1941; Last & Stevens, 1994; J. Nelson, 1984; Nishida, 1990; Whitley, 1936) but recent evidence supports at least two species: *Manta birostris* and *Manta alfredi* (AD Marshall et al., 2009). *M. birostris* are the larger of the two species, found in tropical, sub-tropical and temperate waters. Although occasionally seen visiting shallow, coastal areas, they spend the majority of their time in pelagic waters, migrating over thousands of kilometers (Marshall, pers. comm., 2009). Their disc width (DW: measured from wing tip to wing tip) can span 6.7 m (Bigelow & Schroeder, 1953) with one specimen reportedly as large as 9.1 m (Last & Stevens, 1994). *Manta alfredi* are more likely to be observed in shallow coastal areas around rocky and coral reef habitats where productive upwellings exist. They can be found in tropical and subtropical regions of the Pacific, Atlantic, and Indian Oceans within 30 degrees of latitude to the north and south of the Equator (AD Marshall et al., 2009). Congregations can occur around rich food sources or at specific locations on the reef known as cleaning stations (Losey Jr, 1972) where individuals solicit host cleaner fish to remove parasitic copepods from their body's surface. Strong site fidelity occurring at specific feeding and cleaning stations (e.g., Homma et al., 1999), has created popular tourist attractions where visitors pay to swim or scuba dive with the manta rays (T. B. Clark, 2001; Dewar et al., 2008). *M. alfredi* are much smaller than their oceanic cousins with females reaching a maximum DW between 3.6 m (Deakos, 2010) and 5.5 m (AD Marshall et al., 2009) depending on the region. The maximum lifespan is unknown but the longest reported time

period between first and last sightings of a *M. alfredi* is 27 years (1980-2006) off Yaeyama Island, Japan (Kashiwagi et al., 2008).

For management purposes, differentiating between *M. birostris* and *M. alfredi* is extremely important since each could be exposed to a very different set of anthropogenic impacts. While *M. birostris* may be targeted by large-scale directed fisheries, or succumb to bycatch in longline and tuna purse seine operations (Paulin, Habib, Carey, Swanson, & Voss, 1982; Romanov, 2002), *M. aflredi* populations may be more vulnerable to nearshore anthropogenic impacts such as coastal development, storm water runoff, pollutant loadings, boat strikes, entanglement in fishing and mooring lines, and increased pressure from "swim-with manta" programs. A basic understanding of the abundance, home range, and use of popular aggregation areas by *M. alfredi* is needed for effective management.

The aim of this study was to use photo-identification and active tracking to describe for the first time, the abundance, minimum geographic range, and population structure of *M. alredi* frequenting a known aggregation area in waters off West Maui, Hawai'i. Temporal patterns and reproductive use of the area were investigated. Both natural and anthropogenic threats were quantified and their implications for management of this population discussed.

3.3 METHODS

3.3.1 Main study area

All surveys were conducted at a single manta aggregation area off the west coast of the island of Maui, Hawai'i. The exact location of the site is being withheld to avoid the potential commercial exploitation of this unique site. An area approximately 30,000 m^2 (200 m X 150 m) in size, 450 m offshore, with a depth range of 5 - 30 m was the monitoring boundary for the study site (Figure 8). This area was chosen because of the high reliability of encountering manta rays and thereby maximizing encounter rates. Habitat consisted primarily of fringing coral reef that extended away from the shoreline for approximately 550 m. The main cleaning stations were

situated near the starting point of the survey where manta rays are seen soliciting predominantly Hawaiian cleaner wrasses (*Labroides phthirophagus*) and saddle wrasses (*Thalassoma duperrey*) to remove parasitic copepods from the surface of their bodies. Mating trains were also observed in this area.

3.3.2 Surveys

Surveys, carried out with open-circuit SCUBA, were conducted opportunistically over a five-year period between 2005 and 2009. Surveys were done at different times of the day and attempts were made to conduct at least one survey during each month of each year. Due to an apparent diurnal trend on manta ray sighting rates, 10 days in which a pair of surveys were conducted in a single day were compared using a Wilcoxon Signed-Rank Test to determine if sighting rates later in the day were significantly different than sighting rates earlier in the day. The majority of surveys were conducted during late afternoon since more manta rays were likely to be encountered during that time.

A survey involved a 55 to 75 minute SCUBA dive. Divers entered the water from the beach and transited at the surface 450 m to the survey start point before descending (Figure 8). A dive flag was attached to the ocean floor in ten meters of water. Midway down the tether, a fluorescent green target (30 cm x 20 cm) was attached and used as a visual cue to determine visibility. The distance at which point the target was no longer visible was recorded from the north and south of the flag and the mean was used as the visibility rating for that survey. A rectangular search pattern was initiated from the start point (see Figure 8). When manta rays were encountered, the search was interrupted in order to collect information on that individual. Once the desired information was collected, the search pattern was resumed.

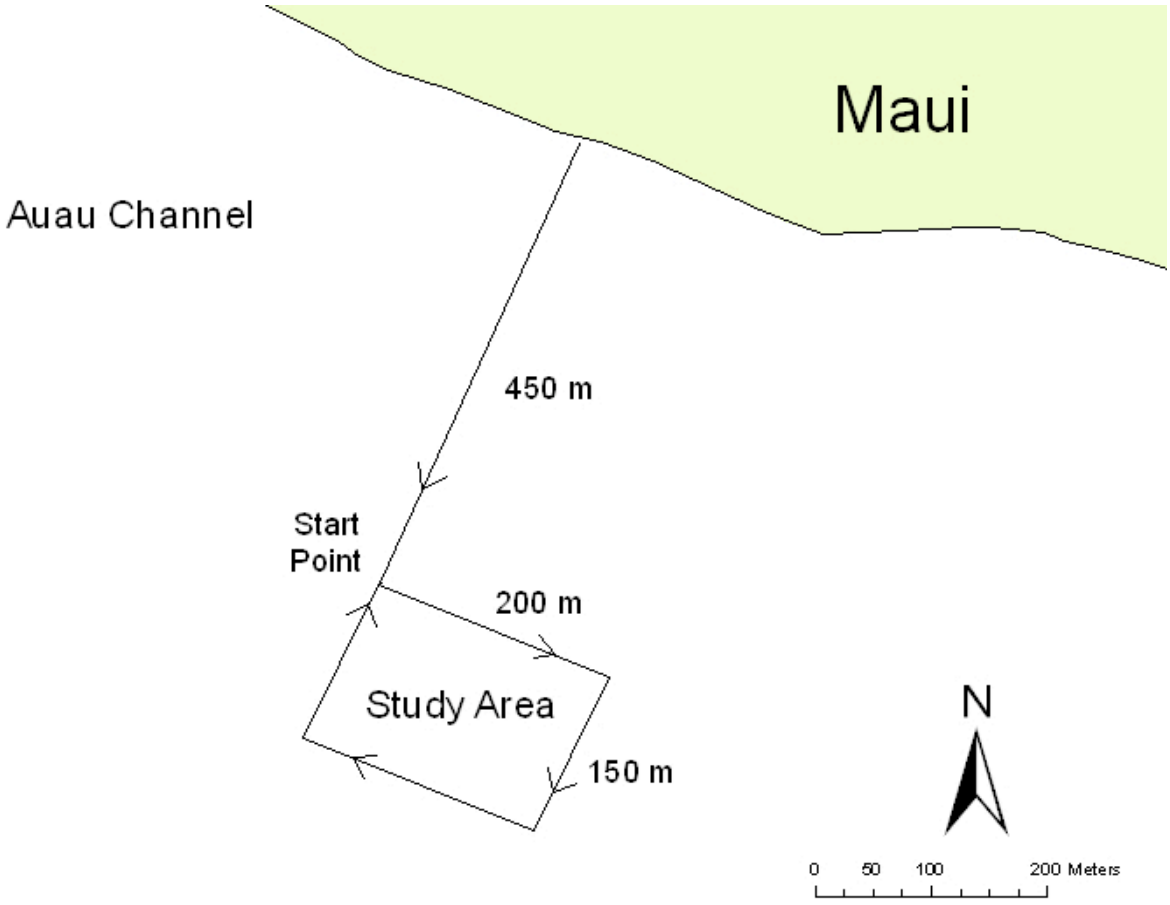

Figure 8. Map showing the study area 450 m from the shoreline. The start point of each survey and the clockwise survey route are shown.

3.3.3 Photo-identification

Photo-identification involves taking photographs of distinctive characteristics of an animal to identify and track individuals of a wild population over time. This technique has been used extensively with large and long-lived vertebrates (for review see Würsig & Jefferson, 1990), for population estimates (e.g.: Graham & Roberts, 2007), life history information (e.g.: Brault & Caswell, 1993), lifespan information (e.g.: Langtimm et al., 2004), migration patterns (e.g.: Calambokidis et al., 1996), and social relationships (e.g.: Bejder et al., 1998) of an individual.

Each manta ray is born with a unique pattern of spots on its ventral side (Andrea D. Marshall et al., 2008), which appears to remain unchanged for the duration of the animal's life (T. B. Clark, 2001; AD Marshall & Bennett, 2010; Yano et al., 1999), even after 20 years (Homma et al., 1999). This makes manta rays highly suitable for photo-identification studies.

During each manta ray encounter, a diver equipped with either a Canon Powershot S70 in an underwater housing, or a Sony HDR-HC1 video camera in a Sea & Sea VX-HC1 underwater housing, attempted to photograph the ventral pattern of each individual. Images were downloaded to a MacBook Pro computer and the best identification for each individual was imported into Finbase, a publicly available photo-identification program created in Microsoft Access (Adams et al., 2006). The photo was matched against photos of all previously identified individuals from the study site and recorded as either a match or as a new individual. The very distinct markings on the underside of each manta ray make the likelihood of missing a match, or falsely identifying a match very unlikely.

3.3.4 Abundance and Survivorship

3.3.4.1 Discovery Curve

To illustrate the rate at which new individuals were encountered, a discovery curve showing the cumulative number of individual manta rays identified was plotted against the cumulative number of identifications made. Winter and summer season identifications were differentiated on the curve to visually demonstrate if new individuals were entering the population more often during a particular season.

3.3.5 Population Range

3.3.5.1 Active Tracking

Two manta rays were tagged on separate occasions with Vemco V16 continuous acoustic pingers. Each pinger was programmed to emit a unique pulse frequency (52 and 56 kHz respectively). The signal was received through a VH110 directional hydrophone (frequency range 50 – 84 kHz) and decoded by a Vemco VR100 receiver/decoder that was kept onboard a 28 ft Glass Pro vessel. A crew of 3 rotated every 4 hours tracking the manta ray in real time from the vessel. Tracking was continuous throughout the day and night until weather conditions made it unsafe to continue. The acoustic detection range of the pingers was approximately 1 km. A continuous track of the boat was recorded onto a Garmin GPSMAP 276C. The acoustic tags were attached to the dorsal side of the right pectoral fin by a snorkeler swimming above the manta ray. The tags were deployed using a modified Hawaiian sling and anchored to the manta ray by embedding a small stainless steel barb under the skin. The barb was tethered to the acoustic tag with 15 cm of stainless steel wire and crimps.

3.3.5.2 Regional comparisons

Photo-identifications from our study area were compared to opportunistic photo-identifications taken of manta rays off the southwestern coast of Maui (n=18), Molokini Crater (n=11), and the

southeastern coast of Molokai (n=11). Comparisons were also made to a catalog of 146 individual manta rays from a well-monitored population off Kona on the Island of Hawai‘i (Big Island) (*www.mantapacific.org*), to look for potential movements between Maui and the Big Island.

3.3.6 Population Structure

Gender was determined by the presence of claspers in males and their absence in females. Females were only classified as sexually mature if they had visible mating scars (spot scarring and abrasions usually on the dorsal or ventral side of the left wing tip) or were obviously pregnant (AD Marshall & Bennett, 2010). A pregnant female close to term was exceptionally rotund and unmistakable. A female that appeared to be pregnant but was questionable was not given an age class.

Among males, calcification of the claspers occurs rapidly over a relatively narrow range of growth (W. T. White et al., 2006) and the majority of calcification occurs once the claspers have extended beyond the length of the pelvic fins (AD Marshall & Bennett, 2010). Since the onset of clasper calcification in many shark species coincides with a rapid rate of clasper growth and gonadal maturation (e.g., Jones et al., 2008) claspers extended beyond the pelvic fins were used as a reliable indicator of sexual maturity in male manta rays. Since juvenile females could not be determined, comparisons between adults and juveniles were done only with males.

3.3.7 Use of the Aggregation Area

3.3.7.1 Temporal Trends

Sighting rates were computed as the total number of manta rays photo-identified divided by the number of hours surveyed and were compared by time of day, by month, by season, and by year.

The start time of each survey was categorized as “AM” (6:00 – 10:00), “MIDDAY” (10:00 – 14:00) and “PM” (14:00 – 18:00) surveys. Surveys from November through April were

categorized as “winter season” surveys, and May through October were categorized as “summer season” surveys. The effect of diver visibility and tidal state on sighting rate was also examined. Linear regression was used to determine the correlation between sighting rates and diver visibility. A Kruskal-Wallis Test was used to assess the significance of year, season, month, and tidal states in explaining variance of manta ray sightings. Tide tables were used to determine the tidal state, which was categorized as “incoming” (flood tide), “incoming/outgoing” (high tide), “outgoing” (ebb tide), or “outgoing/incoming” (low tide) for each survey.

3.3.7.2 Reproduction and New Individuals

The presence or absence of mating trains and pregnant females were recorded for each survey as well as the proportion of males to females. Chi-square statistics were used to compare the proportion of mating trains between winter and summer seasons. The mean number of new individual sighting rates (total number of newly identified individuals divided by the total amount of time surveyed) was computed for each survey, by month, by season, and by year. A Kruskal-Wallis Test was used to test the significant difference in the rate of new individuals occurring by month and by season.

3.3.8 Threats

Physical characteristics of an individual were also recorded and included: a missing or damaged cephalic fin, and the presence of a large wound, large scar, or large section of the body missing (i.e. disc or tail) indicative of having been attacked by a large predator. Chi-square statistics were used to compare the proportions of natural and anthropogenic injuries between gender and age class. The probability level at which significance was determined was 0.05. Statistical analyses were performed using SPSS version 17.0 (SPSS Inc., 2007)

3.4 RESULTS

3.4.1 Surveys

A total of 229 surveys were conducted between 2005 and 2009 (Table 4). Surveys carried out later in the day were more likely to have a higher sighting rate (Wilcoxon Signed-Rank: $Z = -2.912$, $n = 20$, $p = 0.004$). Due to this diurnal trend, the majority of dives were conducted in the PM (82%) to increase encounter rates. The remaining surveys were conducted in the AM (8%) and at MIDDAY (10%). Fifty-seven percent of surveys were conducted in the summer months and 43% during winter months. A total of 1494 manta rays were encountered and photo-identified, revealing 290 different individuals. Manta rays were observed on 201 (88%) surveys. The number of manta rays encountered during each survey ranged from 0 to 31.

3.4.2 Abundance and Survivorship

3.4.2.1 Discovery Curve

The discovery curve (Figure 9) illustrates a decreasing trend of new individuals entering the population with increasing identifications. The curve has a steep slope during early surveys and begins to decrease with additional surveys but never reaches asymptote.

Table 4. The number of surveys conducted and mean sighting rates for years 2005 through 2009, broken down into time of day (600-1000, 1000-1400, 1400-1600) and season (Nov-Apr, May-Oct). Sighting rates are calculated as the mean number of manta rays observed per hour of survey effort.

	No. of Surveys					
Year	AM	MIDDAY	PM	Winter	Summer	Total
2005	0	0	33	10	23	33
2006	1	1	22	6	18	24
2007	16	14	29	22	37	59
2008	1	8	86	42	53	95
2009	0	1	17	18	0	18
Overall	18	24	187	98	131	229
Sighting Rate	1.40	4.17	6.90	8.14	4.77	6.21

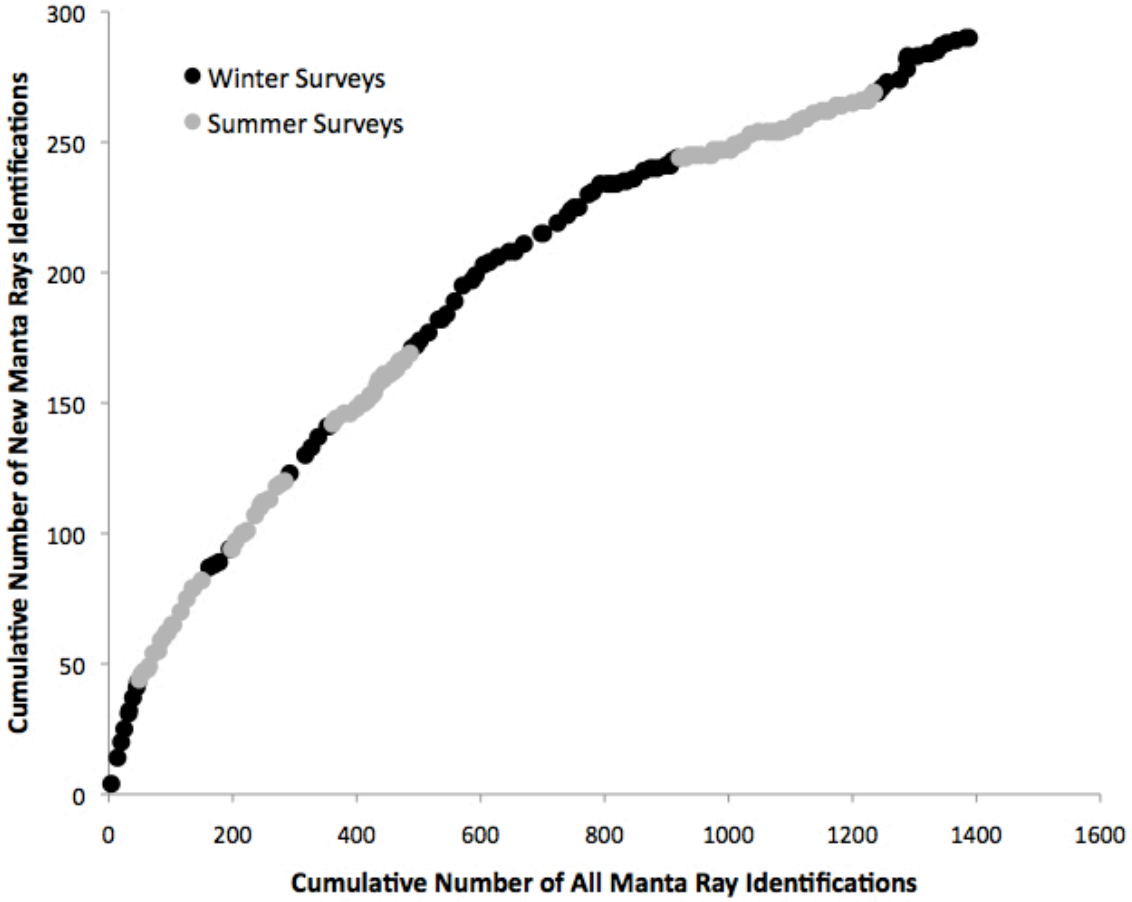

Figure 9. Discovery curve illustrating the cumulative number of new manta ray identifications against the cumulative number of all identifications. Dark circles represent surveys conducted in the winter (November – April) and light circles represent surveys conducted in the summer (May - October).

3.4.3 Population Range

3.4.3.1 Active tracking

On separate occasions in December of 2008, an adult male and an adult female manta ray were tagged with an acoustic transmitter in the study area. Both animals were in a mating train at the time of tagging. The adult male was tracked for 28 hours and traveled across the Auau channel to the north coast of the island of Lanai, a linear distance of 40 km from the study area where he was tagged (Figure 10). The maximum depth traversed was 93 m. The adult female was tracked for 51 hours and traveled to the northwest side of the island of Kahoolawe, a linear distance of 32 km from the study area where she was tagged (Figure 10). The maximum depth traversed was 324 m.

3.4.3.2 Regional comparisons

Of the 290 individuals identified from the study area, 2 matches were made to south Molokai (based on 11 photo-ids), 3 matches to Molokini Crater (based on 11 photo-ids), and one match to a southwest Maui sighting (based on 18 photo-ids; Figure 10). No matches were found between the 290 individual manta rays from the Maui study area with the 146 individuals photo-identified in waters off Kona, Big Island, a transit distance of approximately 150 km from the study area.

3.4.4 Population Structure

The 290 photo-identified individuals were composed of 128 (44%) females, and 153 (53%) males. Nine of these individuals were of unknown sex. At least 44% of the females were considered to be sexually mature based on the appearance of being pregnant or with mating scars. Among the males, 72% were considered sexually mature based on claspers extending beyond the pelvic fins, 26% were recorded as immature, and 2% were never confirmed.

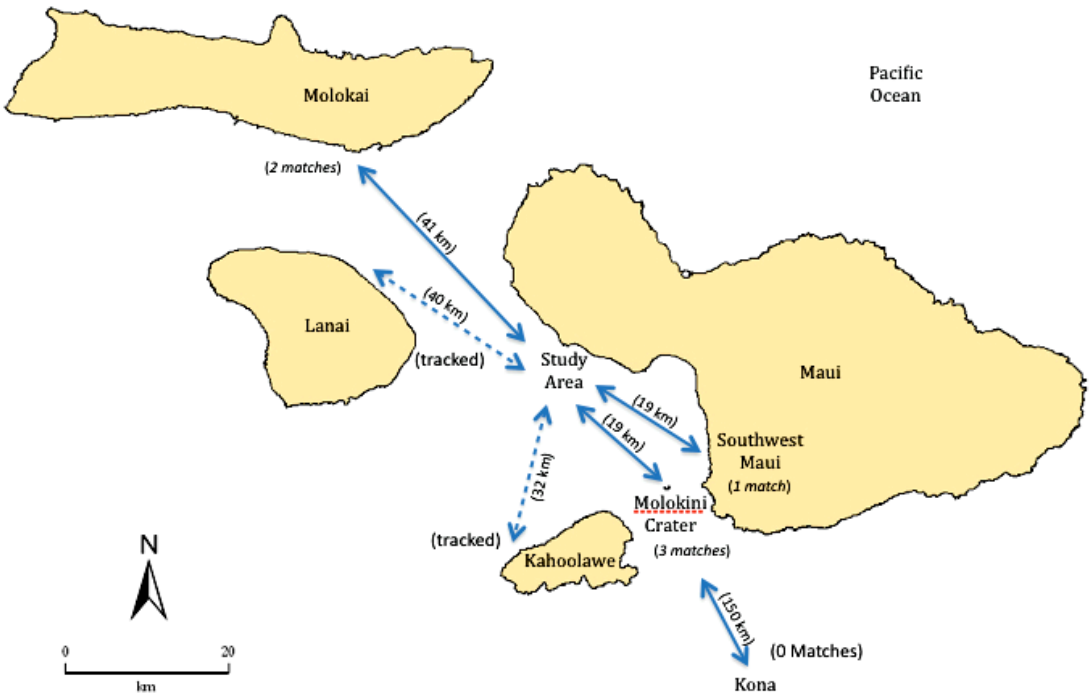

Figure 10. Map showing the range of individual manta rays (*M. alfredi*) either matched with photo-identifications (solid arrows) or tracked with an acoustic tag (dashed arrows).

3.4.5 Use of the Aggregation Area

3.4.5.1 Temporal Trends

It was rare to see manta rays during early morning surveys. The sighting rate during AM surveys was 1.40 manta rays per hour (SD = 3.24), 4.17 (SD = 4.25) for MIDDAY surveys, and 6.94 (SD = 5.16) for PM surveys (Table 4). The number of surveys conducted and mean sighting rates for years 2005 through 2009, broken down into time of day (600-1000, 1000-1400, 1400-1600) and season (Nov-Apr, May-Oct). Sighting rates are calculated as the mean number of manta rays observed per hour of survey effort. To eliminate the diurnal effect on sighting rates described previously, analyses of sighting rates incorporated only the 187 PM surveys.

3.4.5.2 Other variables affecting sighting rate

The survey month was a significant predictor of the mean sighting rate (Table 5; Kruskal-Wallis Test: $\chi^2 = 26.14$, df = 11, $p = 0.006$), with significantly greater sighting rates during the winter months (Kruskal-Wallis Test: $\chi^2 = 19.35$, df = 1, $p < 0.001$). For years 2006, 2007, and 2008, during which surveys were conducted in both summer and winter months, mean sighting rates did not differ significantly across years (Kruskal-Wallis Test: $\chi^2 = 0.91$, df = 2, $p = 0.634$). Sighting rates were not significantly affected by visibility ($r^2 = 0.031$, $p = 0.075$) or tidal state (Kruskal Wallis Test: $\chi^2 = 5.616$, df = 3, $p = 0.132$).

Table 5. Surveys, mean sighting rates, mean rate of new individuals, proportion of males, and proportion of mating trains with standard deviations (SD) listed by month and season.

	Month	No. Surveys	Mean Sighting Rate	SD	% with Mating Trains	% Males	SD	Mean Rate of New Individuals	SD
Winter	NOV	14	7.73	5.24	0	61	0.16	0.25	0.18
	DEC	10	10.31	6.47	10	57	0.07	0.14	0.13
	JAN	13	11.13	7.29	31	64	0.23	1.40	1.49
	FEB	14	7.38	4.79	43	54	0.24	2.02	2.52
	MAR	10	9.37	7.80	20	67	0.23	1.40	1.96
	APR	22	7.90	4.28	32	58	0.23	1.85	1.79
Overall		83	8.76[a]	5.80	24[b]	60[c]	0.21	1.79[d]	1.84
Summer	MAY	15	4.45	3.08	7	63	0.26	0.45	0.87
	JUN	16	6.67	4.36	13	49	0.30	0.66	0.97
	JUL	24	5.26	4.14	17	48	0.29	1.35	1.55
	AUG	17	3.40	2.88	0	54	0.28	0.60	0.85
	SEP	19	6.78	3.95	11	50	0.29	1.68	1.79
	OCT	13	6.49	5.04	8	56	0.25	0.67	0.64
Overall		104	5.49[a]	4.05	10[b]	53[c]	0.28	0.97[d]	1.31
Grand Total		187	6.94	5.16	16	56	0.25	1.33	1.62

[a, b, c] *significantly different ($p < 0.05$)*

[d] *not significantly different ($p > 0.05$)*

3.4.5.3 Residency

Of the 290 different individuals, 78 (27%) were observed only once, 212 (73%) were observed more than once, 198 (68%) were resighted within a one year period, and 95 (33%) were resighted across multiple years (Figure 11). Resights were made on 76% of the females, 74% of the males, 78% of the adult males, and 59% of the juvenile males. Of the top ten most resighted individuals, 6 were male and 4 were female. The most resighted individual was an adult male, seen 41 times between April 2005 and April 2009. The most resighted female was sexually mature and seen 30 times between April 2005 and December 2008.

The mean period between resights for all individuals was 181 days (SD = 195), ranging from a single day to as long as 3.6 yrs. For the highest resighted individual, 31 (78%) resights had a lag period of less than 2 months, but on two occasions his lag periods lasted 7 and 10 months in duration.

3.4.5.4 Reproduction

Mating trains were observed during 10 months of the year with most surveys containing mating trains between December and April. Significantly more mating trains were observed during the winter season (24%) compared with the summer season (10%) (Chi-square Test: $\chi^2 = 195.2$, df = 1, $p < 0.001$; Table 5). The proportion of males to females during winter months (0.60) was not significantly different than the proportion during the summer months (0.53; Kruskal-Wallis Test: $\chi^2 = 3.65$, df = 1, $p = 0.056$; Table 5).

3.4.5.5 New Individuals

The overall mean rate of newly encountered manta rays was 1.33 per hour of observation. This rate decreased each year from 2.41 in 2005 to 0.77 in 2008 but increased again in 2009 to 1.02 (Table 5). The month played a significant role in the rate of new individuals

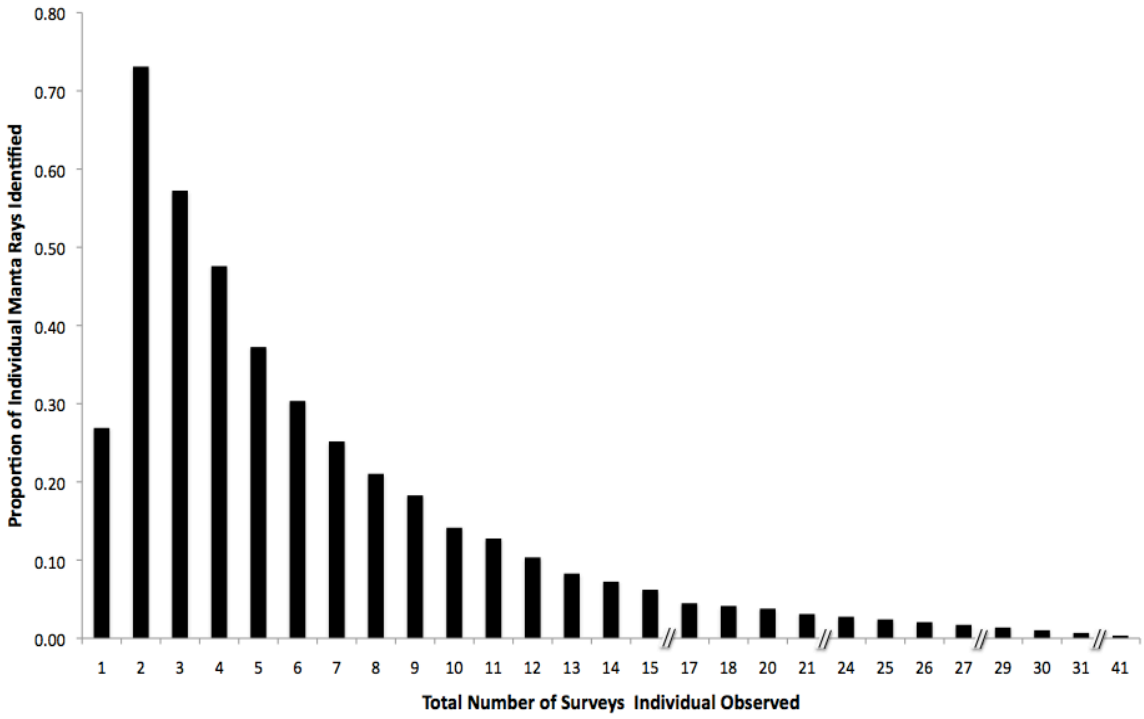

Figure 11. The proportion of individual manta rays identified plotted against the number of surveys in which they were observed.

observed (Kruskal-Wallis Test: χ^2 = 23.596, df = 11, p = 0.015) with a higher rate of new individuals observed during the winter months (Chi-square Test: χ^2 = 10.355, df = 1, p = 0.001; Table 5).

3.4.6 Threats

3.4.6.1 Natural

A total of 70 individuals (24%) had an injury that appeared to have been caused by a shark attack based on wound characteristics described for shark predation on marine mammals and turtles (Corkeron, Morris, & Bryden, 1987; MR Heithaus, 2001; M Heithaus, Frid, & Dill, 2002). Males and females were both equally likely to have these injuries (Chi-Square Test: (χ^2 = 1.389, df = 1, p = 0.239), but juvenile males were significantly less likely to possess these injuries when compared with adult males (Chi-Square Test: (χ^2 = 7.509, df = 1, p = 0.023). Only a single juvenile (3%) had shark related injuries compared with 31 (30%) adult males. Since juvenile females could not be determined, the proportion of injuries in adult and juvenile females could not be compared.

3.4.6.2 Anthropogenic

Twenty-eight individuals (10%) had an amputated or disfigured, non-functioning cephalic fin. The proportion of males and females with cephalic fin injuries were not significantly different (Chi-Square Test: χ^2 = 1.567, df = 1, p = 0.211). The proportion of adult males and juvenile males with cephalic fin injuries were also not significantly different (Chi-Square Test: χ^2 = 1.676, df = 1, p = 0.433).

Eight individuals had physical evidence of entanglement in fishing line. These included two with fish hooks embedded in the cephalic fin, two with monofilament line wrapped around the cephalic fin, two with clear injuries where line had begun to cut part-way

through the cephalic fin, and two with visible scars from line that had been wrapped around the cephalic or pectoral fin.

3.5DISCUSSION

3.5.1 Abundance and Survivorship

The population of manta rays utilizing the Maui aggregation site consisted of at least 290 individuals, and the rate of new individuals shows no sign of leveling off, suggesting that the overall population is much larger than all individuals identified. Other reported population sizes include 185 different individuals identified over a twenty-year period from an aggregation site off the Yaeyama Islands in Japan, and 54 different individuals identified over a seven-year period off the Island of Yap in the Western Pacific (Homma et al., 1999). An estimated 890 individuals, of which 449 individuals were identified over a five-year period were reported for a resident population along the west coast of Mozambique, Africa (A. D. Marshall, 2009). In areas where anthropogenic impacts are not impeding population growth, the size of the local population may be a reflection of local food availability and the carrying capacity this resource can sustain. For example, the presence of manta rays around the atolls of the Republic of the Maldives coincide with the seasonally alternating monsoon currents, supplying rich zooplankton blooms that support a manta ray population numbering into the thousands (C. R. Anderson et al., 2008).

3.5.2 Population Range

Photo-identification matches combined with tracks from acoustically tagged animals provide evidence that individuals from the study area are moving between the 4-islands that represent the Maui County area (Maui, Molokai, Lanai, and Kahoolawe). with distances between these neighboring islands ranging from 11 to 15 km. The closest distance between the Big Island and the island of Maui is 49 km, which would seem be within the range of attainment for individuals in this Maui population. *M. alfredi* in Japan were reported to travel distances of 350 km (Homma et al., 1999), and individuals from a

population in the Maldives reportedly travelled 160 km (A.-M. Kitchen-Wheeler, 2008). However, the absence of photo-identification matches between the Maui population and the Kona population, for which individual identities have been well documented by commercial dive operators for the past 10 years, brings to question if movement between these islands is occurring. The deepest area transited by one of the acoustically tracked individuals was 324 m. The 2000 m depth in the middle of the Alenuihaha channel could present a barrier preventing individuals from crossing Maui to the Big Island. A more likely explanation is that sufficient resources exist within the 4-island region to sustain the Maui population, making the transit unnecessary. Species such as Cuvier's (*Ziphius cavirostris*) and Blainville's (*Mesoplodon densirostris*) beaked whales, spinner dolphins (*Stenella longirostris*), and bottlenose dolphins (*Tursiops truncatus*) have been reported to have independent island-associated stocks among the main Hawaiian Islands (Andrews et al., 2006; Baird et al., 2009; McSweeney, Baird, & Mahaffy, 2007).

The deepest area transited by one of the acoustically tracked individuals was 360 m. The 2000 m depth in the middle of the Alenuihaha channel could present a barrier preventing individuals from crossing Maui to the Big Island. A more likely explanation is that sufficient resources exist within the 4-island region to sustain the Maui population, making the transit unnecessary. Species such as Cuvier's (*Ziphius cavirostris*) and Blainville's (*Mesoplodon densirostris*) beaked whales, spinner dolphins (*Stenella longirostris*), and bottlenose dolphins (*Tursiops truncatus*) have been reported to have independent island-associated stocks among the main Hawaiian Islands (Andrews et al., 2006; Baird et al., 2009; McSweeney et al., 2007).

The greatest depth needed to transit from Molokai to Oahu is 600 m. If depth does not represent an inter-island barrier, future research should compare individuals photo-identified on Oahu with those from the Maui study area. Additional acoustic tracking of individuals and genetic sampling could help to confirm whether or not these individuals are crossing the deep channels to neighboring islands outside the Maui County area.

3.5.3 Population Structure

The male to female ratio was near parity with a slight bias towards males (1:0.83). Marshall & Bennett (2010) reported a strong female biased sex ratio (1:3.5) within a population off the eastern coast of Mozambique. The attractiveness of an aggregation area may vary according to the sex or age class of an individual. Aggregation areas in close proximity to suitable pupping grounds may be more favorable to pregnant females (AD Marshall & Bennett, 2010). The lack of female bias in this aggregation area may reflect the absence of a nearby birthing area.

The disc width (DW) of 154 different individuals from this population were measured using paired-laser photogrammetry (Deakos, 2010), and were all larger than 2.5 m. The smallest free-swimming individuals for both *M. birostris* and *M. alfredi* have been reported between 1.2 – 1.5 m DW (Bigelow & Schroeder, 1953; L. J. V. Compagno, Marshall, Kashiwagi, & Bennett, 2008). This suggests that very young animals in the Maui population may be segregated geographically and staying out of the study area, making them unavailable for sighting. In some coastal shark species, females seek out discrete, inshore habitats where they give birth and the young spend their first weeks, months, or years of life protected from predation by larger sharks (e.g., Castro, 1993). Very young individuals in this population may exhibit similar behavior. This age class may represent a significant portion of the population that is not accounted for in the population estimate.

3.5.4 Use of the Aggregation Area

Frequent resights of individuals within and across years support long-term site fidelity to the Maui study area. Although sight-fielity was highly variable between individuals, males and females, or adult males and juvenile males were equally likely to revisit the study site. The times between resights ranged from a single day to over three years with an average of about 6 months between sightings. Even individuals with the strongest fidelity to the study site, on occasion, were not resighted for periods of 6 months or more.

This is consistent with animals residing in the area for a period of time before dispersing to a new area. The absence from the study area for long durations may be a product of decreasing food resources or potential mates in the area during that period. Caution should be taken when interpreting these results since effort was not continuous and individuals could have been present in the study area when surveys were not conducted.

Time of day and the time of year were the best predictors of manta ray sighting rates. They were typically absent in the early morning with sighting rates increasing as the day progressed. The two individual manta rays equipped with acoustic tags both moved offshore and out of the study area after sunset. Since both individuals were part of a mating train when tagged, it is unclear if these offshore movements were representative of all individuals or specific to individuals in mating trains. Both individuals remained in the study area for several hours before moving offshore making it unlikely that their movements were a response to being tagged.

This diurnal trend may be due to manta rays moving out of the study area at night to feed, since they were never seen feeding while in the study area. Whether or not these animals were feeding during the night was not confirmed. Although zooplankton distribution and abundance can be highly variable across space and time (Greene, Wiebe, Pelkie, Benfield, & Popp, 1998), certain changes in the vertical abundance of zooplankton, termed deep vertical migration (DVM) can be predictable (for review see Hays, 2003). Planktivorous elasmobranchs such as basking sharks (*Cetorhinus maximus*) and whale sharks (*Rhincodon typus*) can take advantage of these predictable diel trends by resting more during the day and foraging more at night when the plankton moves closer to the surface (Rowat, Meekan, Engelhardt, Pardigon, & Vely, 2007; Sims, Southall, Tarling, & Metcalfe, 2005). By feeding at night, manta rays could be taking advantage of more easily accessible euphasid and copepod concentrations. Further research is needed to better understand when and where this population is feeding.

Although mating trains were observed during 10 months of the year, most occurrences were concentrated during the winter months, primarily January through April. This was

also the time when the proportions of new individuals sighted during a survey were highest. It is possible that during the reproductive season more individuals visit the aggregation area in search of mates rather than for use of the cleaning stations. A mating system is based on the potential of one sex to monopolize key resources or mates of the limiting sex (Emlen & Oring, 1977). The limiting sex is usually more heavily invested in parental care, and the greater the imbalance, the more intrasexual competition exists between members of the other sex (Darwin, 1871). Female manta rays are likely the limiting sex since they provide the only parental investment in the form of a 12-month gestation period, and multiple males appear to compete for access to a single female in a mating train (Yano et al., 1999). The dispersion of females, or resources essential to females, limits the ability for a male to monopolize multiple females.

A male dominance polygyny mating system could explain shorter residency times calculated for males, who may move more frequently between aggregation areas in search of reproductively available females. The shorter residency time for males would create a greater turnover of males in the study area, making more males available for sighting. This could explain why the estimates of abundance using mark-recapture were much larger for males utilizing the study area compared to females, even though the proportion of photo-identified males and females was nearly equal. Adult females may benefit from residing longer in a popular aggregation area where she may have a greater selection of potential mates, provided food resources are also available nearby.

3.5.5 Threats

Both natural threats and anthropogenic threats were documented in this population. Large sharks (Homma et al., 1999) and killer whales (*Orcinus orca*) (Visser & Bonoccorso, 2003) have been reported to prey on manta rays. Since killer whales are extremely rare in Hawaiian waters (Mobley, Mazzuca, Craig, Newcomer, & Spitz, 2001), the most likely predator would be large sharks such as the common tiger shark (*Galeocerdo cuvier)*. About one in four individuals showed injuries likely caused from a shark attack. Although males and females were equally likely to possess these injuries,

adult males were 10 times more likely to have these injuries compared with juvenile males. This may suggest that juveniles are less susceptible to attacks by sharks, possibly because they may be geographically segregated in more protective areas during their early years of development, whereas adults foraging in deeper waters are more susceptible. The proportional difference could also be an artifact of adults having more exposure to sharks during their lifetime, or if they are more likely to survive a shark attack due to their larger body size.

If young manta rays are spending the early years of development in shallower, more protective geographically segregated to areas that make them less prone to shark predation, this might explain the low proportion of shark attack injuries on juveniles. However, if shark attacks on juveniles are fatal, these would go undetected whereas adults may be more likely to survive an attack.

One out of ten manta rays in the population had an amputated or non-functioning cephalic fin, most likely due to entanglement in monofilament line. Considering the function of the cephalic fins to guide food into the mouth during feeding, an animal reduced to a single cephalic fin would likely suffer a reduction in feeding efficiency. Individuals in this population with only a single functioning cephalic fin appeared healthy but further research should investigate how the absence of a cephalic fin affects the size, growth rate, and reproductive success of these individuals.

All amputated cephalic fins had straight edge cuts, consistent with being severed with line. Some deformed cephalic fins had straight cuts half way through the fin, most likely having shed the line before the fin was completely severed. Shark predation as the cause of cephalic fin damage seems unlikely, as the 70 individuals with shark attack scars, 65 had scars either on the posterior part of their body or on the wing tip. Only five individuals possessed attack scars anterior to the midline of the body. This suggests that most attacks are occurring from behind or from the side where the shark is less likely to be detected. Additionally, eight individuals were observed with either fish hooks embedded into their cephalic fins, fishing line wrapped around a cephalic fin, or fishing

line scars around a cephalic fin and the pectoral fin, providing further support that entanglement in fishing lines is a significant threat. Further research is needed to determine the impaired fitness of a manta ray reduced to only a single cephalic fin. This could be achieved by monitoring their growth and reproductive success over time.

Manta rays have also been known to die from entanglement in boat anchor lines (Bigelow & Schroeder, 1953), and mooring lines. Two manta ray entanglements in mooring lines were documented on video in Hawai‘i. The first was reported inside Molokini Crater, Maui, on 12 Jun 2007 (A. Cummins, pers. comm., 2007), and the second off Kona, Hawai‘i, on 19 Jun 2009 (K. Osada, pers. comm., 2009). Both manta rays perished and were consumed by sharks immediately thereafter.

Additional acoustic tracking could assist in determining areas frequented by manta rays that may be heavily fished and pose a higher risk of entanglement. Managing fishing practices in these areas or simply educating fishers who utilize these areas could help to reduce the frequency of manta ray entanglements.

Several manta ray aggregation sites worldwide are being utilized commercially to put paying clients in the water to swim with the manta rays. Unregulated, these operations can impose undue stress on the local manta ray population, potentially causing the animals to abandon the area. Sustained pressure from divers, snorkelers, boaters, and jet skiers visiting a manta ray aggregation site in Bora Bora, French Polynesia, reportedly caused the manta rays to completely abandon this area (de Rosemont, 2008). The biological significance of displacing manta rays from these aggregation sites is unknown and worthy of investigation. A study conducted by Semeniuk (2009) in which tourists interacted with a wild population of southern stingrays (*Dasyatus americana*) resulted in higher parasite loads, higher injury rates, and suppression of the immune system in these animals, putting their long-term survival at serious risk.

Recent success in Japan’s manta ray captivity program (Senzo Uchida et al., 2008) has sparked global interest from aquariums looking to add manta rays to their exhibits. In

certain aggregation areas where manta rays are easily accessible, and where no regulatory protection exists, populations may be exposed to indiscriminant non-sustainable extraction of individuals for profit, especially those that are small and geographically isolated.

3.5.6 Population Management

In many parts of the world, measures have been taken to reduce anthropogenic threats on local manta ray populations. For example, codes of conduct for manta ray dive operators have been implemented in Kona, Hawai‘i, Western Australia (Daw & McGregor, 2008), Mozambique, Bora Bora, French Polynesia (de Rosemont, 2008), and in the Maldives (R. Anderson, Adam, Kitchen-Wheeler, & Stevens, 2011). Elements of the code include minimizing the number of divers around the manta rays, keeping divers in tight controlled groups, restricting the touching of animals, and using approach methods that minimize stress on the manta rays. In Mozambique, mooring balls are banned in areas where the manta rays are known to aggregate, and boats are required to minimize their speed. Marine protected areas (MPA) have been established in the Maldives, Mexico, Mozambique, and Yap, to help eliminate fishing pressure and provide a safe refuge for the manta rays.

In 2009, the State of Hawai‘i passed a law making it illegal to intentionally kill or extract manta rays within state waters with an exception given to persons granted a special take permit. Obtaining such a permit requires the applicant to demonstrate the potential biological removal (PBR) of the targeted population. PBR is the maximum number of animals, not including natural mortalities, which may be removed from a stock while allowing that stock to reach or maintain its optimum sustainable population (Taylor, Wade, De Master, & Barlow, 2000). This approach was originally designed and implemented in 1994 as an amendment to the Marine Mammal Protection Act to ensure more sustainable levels of incidental takes of marine mammals, especially in data poor situations. PBR is the product of a minimum population estimate of the stock (N_{MIN}), one-half of the maximum population growth rate (R_{MAX}), and a recovery factor F_R.

$$PBR = N_{MIN} * 0.5R_{MAX} * F_R$$

The model has been made simplistic for management purposes and utilizes parameters that are readily available, and more importantly, that are conservative when biological parameters are uncertain.

3.5.7 Conclusions

The findings of this study are consistent with a population of more than 290 manta rays moving into and out of the Maui study area with a varying portion of the total population temporarily resident in the study area at any given time. Although strong site-fidelity exists to the study area, individuals range throughout the Maui County area. The Maui County population appears to be geographically distinct from its neighboring island populations but further research through active and passive tracking and genetics is needed to confirm the existence of independent Hawaiian island stocks of *M. alfredi*.

The biological significance of the study area is not well understood but appears to be an important staging area where individuals from the population make routine, year-round visits to either rid themselves of parasites or to find available mates. The absence of very young individuals (<2.5 m DW) and a biased sex ratio towards adult males indicates that not all individuals in the population make use of the area equally and segregation is occurring based on age class and sex. The predominance of adult males and the high frequency of mating trains observed indicate the study area may also be a significant mating area, primarily between the months of December through April.

If island-associated *M. alfredi* populations are indeed geographically independent from neighboring stocks, with little or no transfer occuring between individuals, regional management of these population stocks is needed to deal with specific threats that are unique to each region. Small, isolated populations can be at serious risk of rapid and unrecoverable decline (Musick, 1999), and the frequent occurrence of large aggregations

of manta rays to a small area makes them even more vulnerable to localized anthropogenic impacts.

The greatest immediate threat to this population appears to be entanglement in monofilament fishing line, which appears to result in disabling or dismembering the cephalic fin, likely impacting an individual's feeding efficiency. Anticipated threats in the near future include: unregulated swim with manta ray programs adding increased pressure on animals utilizing this natural aggregation area; and entanglement in proposed mooring lines for this area.

The recent differentiation of the genus *Manta* into two separate species raises new concerns about anthropomorphic impacts placed on highly resident populations. Due to the slow population growth and low fecundity typical of elasmobranchs (Holden, 1974), monitoring of changes in population size, population growth, and impact on these parameters from anthropogenic impacts are recommended. An understanding of population characteristics and basic ecological information is needed on a regional basis.

4 THE REPRODUCTIVE ECOLOGY OF MANTA RAYS (*MANTA ALFREDI*) OFF MAUI, HAWAIʻI, WITH AN EMPHASIS ON BODY SIZE

4.1 ABSTRACT

A combination of photo-identification and photogrammetry was used to study the reproductive ecology of a resident population of manta rays (*Manta alfredi*) in what appears to be an important staging area off Maui, Hawai‘i. Reproductive cycles including mating, birthing, and estrus were investigated. Although reproductive activities occur year-round, mating trains and late-term pregnant females were significantly more likely to be observed during the winter months, and mature females seem capable of ovulating multiple times during a year if their initial mating attempts are unsuccessful. Sexual maturity appears delayed until growth exceeds 90% of their maximum size, an indicator that large body size provides a reproductive advantage in both sexes. Larger females had higher pregnancy rates, and were more likely to reproduce in successive years, but did not have more escorting males in their mating train. The mean pregnancy rate for all females was close to biennial and the operational sex ratio was male biased with 2.68 adult males per reproductively available female. Males do not appear to compete physically for access to females and body size was not a predictor of a male's position in the mating train. Further research is needed to determine how size plays a role in male mating success.

4.2 INTRODUCTION

In many species, larger body size provides a reproductive advantage for males and females (Ralls & Mesnick, 2002). For females, large size generally equates to greater physiological resources for reproduction, and often results in the production of larger,

healthier, or more frequent offspring. For males that compete physically with one another for access to mates, larger males generally win out over smaller males.

The proportion of maximum growth reached at sexual maturity can be an indicator of the importance of large body size for reproductive success and can also be an indicator of the availability and predictability of food resources (Shine, 1988). This study examines the reproductive ecology of a resident manta ray (*Manta alfredi*) population off Maui, Hawai‘i, and the relationship of body size and reproductive activities.

Two species of manta ray are currently recognized (AD Marshall et al., 2009): *Manta birostris,* herein referred to as oceanic manta rays, and *Manta alfredi,* herein referred to as resident manta rays (Deakos, 2010). The oceanic manta is the larger of the two species with a maximum disc width (DW; measured from wing tip to wing tip) reported at 6.7 m (Bigelow & Schroeder 1953) and is widely distributed, occurring in tropical, sub-tropical and temperate waters around the globe. Members of this species spend the majority of their time in deep waters but are commonly sighted along productive coastlines with regular upwellings, oceanic island groups, and near offshore pinnacles, seamounts, and submarine ridge systems (LJV Compagno & Last, 1999; AD Marshall et al., 2009; Rubin, 2002).

Resident manta rays average smaller than oceanic manta rays with a maximum recorded DW of 5.5 m (AD Marshall et al., 2009). This species is found in tropical and subtropical regions of the Pacific, Atlantic, and Indian oceans within 30 degrees of latitude to the north and south of the equator where they occur primarily along shallow coastal areas that are often associated with coral reef habitats within a few kilometers of land (Homma et al., 1999; A. D. Marshall, 2009).

Most female resident manta rays become pregnant on average every 2-3 yrs, though some are capable of becoming pregnant in consecutive years (Homma et al., 1999; AD Marshall & Bennett, 2010). Birthing has never been reported in free-ranging manta rays and birthing areas are unknown. The only documented birthing event occurred at the

Okinawa Churaumi Aquarium in Japan, where a resident females produced a single, precocial pup, following what was determined to be a twelve-month gestation period (S Uchida, Toda, & Kamei, 1990). Parturition was immediate and the mother was seen mating again within hours. The smallest free-swimming manta rays of either species have been reported to be between 1-1.5 m DW (Bigelow & Schroeder, 1953; Homma et al., 1999; AD Marshall et al., 2009). Parental care is absent beyond gestation and no information exists about pup development or survival.

Pre-copulatory behavior of manta rays involves multiple, escorting males, pursuing a single, fast-swimming female in what is known as a "mating train" (Yano et al., 1999). Rapid swimming is interrupted by periods of quick turns and somersaults initiated by the female and often mimicked by the pursuing males (AD Marshall & Bennett, 2010). When copulation occurs, a male directly behind the female moves over her while biting her pectoral fin, almost always on the left side, and twists his body so that his ventral side is against hers and a clasper is inserted into her cloaca for insemination. Although mating trains are commonly observed, reports of actual copulation in free-ranging manta rays are rare (AD Marshall & Bennett, 2010; Yano et al., 1999).

Resident manta rays are sexually dimorphic with females as much as 16% larger than males (Deakos, 2010; AD Marshall & Bennett, 2010). This size difference make it unlikely that males are able to force an unwilling female to mate. Because females carry all the burden of parental investment in a 12 month gestation, they are likely the choosier sex (Trivers, 1972), and should select males that are the most fit (Fisher, 1930). Mating trains are likely a way for reproductively available females to evaluate potential mates. By moving rapidly through an area while advertising her willingness to mate, a female may recruit male escorts in an attempt to find the best suitor. These "female recruitment runs" have been observed in other species. For example, female bison (*Bison bison*) seek to replace a lower-ranking, tending male by running away from him and towards higher-ranking males, usually resulting in her tending male being replaced by one of higher rank (Wolff, 1998). Humpback whales (*Megaptera novaeangliae*) also engage in similar behavior, in which two or more males, (sometimes as many as 20 or more males),

compete physically for the primary escort position (N1E) closest to the female (Baker & Herman, 1984; Herman et al., 2007; Tyack & Whitehead, 1982). In humpback whales, the N1E is typically the largest male in the group (S. Spitz, Herman, Pack, & Deakos, 2002).

Our limited understanding of elasmobranch reproduction is in large part due to the difficulty in studying them in the wild. Their wide distribution in an aquatic environment poses many challenges. However, two characteristics of resident manta rays facilitate our ability to study their life history. First, each individual manta is born with a unique pattern of spots on their ventral surface (Andrea D. Marshall et al., 2008), that appear to remain unchanged over its lifetime and can be reliably identified (T. B. Clark, 2001; Homma et al., 1999; Kitchen-Wheeler, 2010; A. D. Marshall, 2009; Yano et al., 1999). Second, though the home range of individuals in this population is broad and extends throughout Maui County waters, an area comprising over 3,210 km^2 (Deakos, Baker, & Bejder, submitted), resident manta rays are known to congregate at specific locations on the reef known as cleaning stations. Cleaning stations are where individuals solicit host cleaner fish to remove parasitic copepods from their body's surface (Coté, 2000; Losey Jr, 1972). Strong site fidelity to these cleaning stations allows for reliable encounters (e.g., Homma et al., 1999), making these aggregation areas ideal for studying resident manta rays (T. B. Clark, 2001; Deakos, 2010; Dewar et al., 2008; Homma et al., 1999; A. D. Marshall, 2009).

This paper focuses on the reproductive cycles and role of body size in the reproductive ecology of a resident manta ray population off Maui, Hawai'i. Several aspects of their reproductive ecology were investigated.

4.2.1 Reproductive Cycles

Most viviparous elasmobranchs follow annual reproductive cycles with somewhat synchronous mating, gestation, and parturition (for review see Hamlett & Koob, 1999). Seasonal reproduction generally occurs if it maximizes a female's chance to successfully

produce offspring. This is often influenced by the seasonal availability of food so that young are developing during a time of year when food is more plentiful. A reduction in predation and improved weather conditions could also influence the occurrence of seasonal breeding.

Reproductive seasonality in the Maui population of resident manta rays was investigated. Females observed in mating trains were assumed to be ovulating. The hypothesis tested was that a reproductive advantage to seasonal breeding should reveal mating trains and late-term pregnancies (based on a 12 month gestation period) to occur more often during certain times of the year.

4.2.2 Role of Body Size

The role of body size in the Maui population of resident manta rays was investigated by the observations of the relationship between body size and reproductive activity. Based on data collected by Deakos (2010), the estimated size of the largest female and male in this population using paired-laser photogrammetry was 3.62 m DW and 3.05 m DW respectively. The minimum size at sexual maturity was estimated at 3.37 m DW for females, and 2.80 m DW for males. Assuming these maximum sizes are representative of maximum growth, sexual maturity in both sexes is delayed until growth reaches greater than 90% of maximum size. This suggests that the reproductive advantage of larger size must strongly outweigh the cost of a reduced reproductive time period. Given that larger body size should provide a reproductive advantage to both males and females in this population, several hypotheses were tested:

1) Larger females should have higher pregnancy rates, and more consecutive year pregnancies than smaller females. Larger females, being more fecund, should attract a greater number of escorts to her mating train than do smaller females (cf. Pack et al., 2009).

2) Larger males should be more likely to hold the position closest to the female (N1E), in a mating train (cf. S. Spitz et al., 2002). These males should be larger, on average, than all other males in the train, primarily those that have never been observed in the N1E position. Larger males should also be more likely to choose larger females and therefore should be associated with larger females (cf. Pack et al., 2009).

These hypotheses are based on the assumptions: a) males physically compete with other males for access to limited females, and b) that an operational sex ratio (OSR), defined as the average ratio of fertilizable females to sexually active males at any given time (Emlen, 1976; Emlen & Oring, 1977), skewed towards males, should favor male competition over limiting females.

The OSR for the Maui resident manta population was approximately two adult males for every adult female (Deakos et al., submitted). Generally the sex with the lower parental investment will be the sex towards which OSR is biased (Trivers, 1972). The level of bias in the OSR will determine how intense the sex that is in excess will compete for access to the other. The sex in shortage may afford to be selective if there are many potential mates to choose among (e.g., Berglund, 1994) or they may be unselective and simply mate with fitter mates as an outcome of contest competition (Cox & Le Boeuf, 1977).

4.3 METHODS

4.3.1 Data Collection

Surveys of the resident manta rays were conducted with SCUBA at an aggregation area off the west coast of the island of Maui over 6-year period from 2005 through 2010. A description of the study area and detailed methodology is presented in Chapter 3. A survey consisted of a rectangular search pattern originating from the same location covering an area approximately 200 m X 150 m. When manta rays were encountered, attempts were made to collect the following information on each individual: (1) photo-

identification, (2) sex, (3) age class, (4) female pregnancy status, (5) DW measurements, and (6) behavioral role when in a mating train.

Ventral markings of each individual were photographed for identification using either a Canon Powershot S70 still camera in an underwater housing, or a Sony HDR-HC1 high definition camcorder housed in a Sea & Sea VX-HC1 underwater housing. Sex was determined by the presence (males) or absence (females) of claspers. Calcification of claspers occurs rapidly over a relatively narrow range of growth with the majority of calcification occurring once the claspers have extended beyond the length of the pelvic fins (AD Marshall & Bennett, 2010; W. T. White et al., 2006). Since the onset of clasper calcification in elasmobranchs coincides with a rapid rate of clasper growth and gonadal maturation (e.g., L. Marshall, White, & Potter, 2007), claspers extending well beyond the margins of the pelvic fins were used as a reliable indicator of sexual maturity. Females were considered mature if they possessed mating scars (abrasions on the wing tip), or were obviously pregnant based on the extreme distention of her abdomen (AD Marshall & Bennett, 2010). The distention of the abdomen does not become apparent until at least 6.5 months into a female's pregnancy (AD Marshall & Bennett, 2010). A female without mating scars, or that was never observed pregnant was given an age class status of "unknown." DW measurements (measured from wing to tip to wing tip) were obtained using paired-laser photogrammetry as described in Deakos (2010). A mating train consisted of a single female being pursued by at least two adult males. Rarely a female leading a mating train was seen to follow a second female for a brief period; this second female was not considered part of the mating train. The female considered part of the mating train was given the behavioral role of "nuclear female" (NF). The male closest to the NF, usually directly behind, was called the "primary escort" (N1E). The male directly behind the N1E was called the "secondary escort" (N2E). All additional males in the train were call "escorts" (NE). Since the position of males often changed while observing a mating train, males could receive multiple behavioral roles as part of any given train. Mating train events were recorded using the high-definition, underwater, video camera.

4.3.2 Data Analysis

Photo-identification images of each individual from a survey were matched against a catalog of all identified individuals from the study area to determine if that individual had been previously seen (a resight) or was a first sighting. Size measurements were processed as described in Chapter 2.

For a given time period, the number of surveys in which trains were observed was divided by the total number of surveys to obtain a proportion of mating trains observed. Proportions were calculated for each month and for each season. Seasons were categorized as "winter" (November through April) and "summer" (May through October) encompassing the coldest and wettest months and the warmest and driest months of the year, respectively. The proportion of sightings an individual was observed in a particular behavioral role was calculated for each individual, and for each behavioral role. If an individual was observed in more than one behavioral role during a sighting, a proportion was calculated for each.

4.3.3 Reproductive Cycles

Chi-square analyses were used to compare the proportion of surveys containing mating trains, and the proportion of surveys containing a pregnant female (PF), by month and by season. Mean train sizes were calculated by month and season and analyzed for significant differences using a Kruskal-Wallis Test, and a Mann-Whitney *U*-Test respectively.

Pregnancy rates were estimated by dividing the number of years in which a female was determined to be visibly pregnant by the total number of years seen. Years with insufficient sightings to determine if a female was pregnant during that year were omitted. The overall mean pregnancy rate for the population was an average of all individual pregnancy rates.

A successful consecutive year pregnancy was scored if a female was observed pregnant during two consecutive years, with a minimum of 7 months occurring between the two pregnancies. This minimum delay between pregnancies was to ensure that the same pregnancy was not counted in both years. A failed consecutive year pregnancy was scored if a female was not observed pregnant the year following a pregnancy. The rate of consecutive year pregnancies for a female was calculated by dividing successful scores by the sum of successful and failed scores. Only years with enough sightings to determine whether or not a female was pregnant during that year, were used in the analysis.

OSR was determined by calculating the total number of males prepared to mate (adult males) divided by the total number of males and females prepared to mate (adult females and reproductively available females) (Kvarnemo & Ahnesjo, 1996).

4.3.4 Role of Body Size

Minimum, maximum, and mean DWs, and standard deviations (SD) were quantified for NFs, PFs, N1Es, N2Es, and NEs. A behavioral proportion was calculated for each individual in each behavioral role as the number of sightings that individual was observed in the behavioral role by the total number of sightings. The mean behavioral proportion was calculated for all individuals observed in that role as a measure of the proportion an individual may occupy a particular behavioral role. Thus, if the same individual were observed in multiple behavioral roles during a single survey, a proportion was calculated for each behavioral role.

The mean DW of NFs was compared to the mean DW of PFs using a Mann-Whitney *U*-Test. Linear regression was used to determine if larger PFs were correlated with higher pregnancy rates, and more consecutive year pregnancies. Linear regression was also used to determine if larger NFs were positively correlated with more males in her train.

A Kruskal-Wallis Test was used to compare mean sizes between all adult males, NEs, N1Es, N2Es, and NEs that have never been observed as a N1E. Linear regression was used to determine if larger N1Es were positively correlated with larger NFs. A Mann-Whitney *U*-Test was used to compare the mean DW of all N1Es to the mean DW of all NEs that had never been observed as an N1E.

All linear regression tests were directional (one-tailed) since the hypothesis was that larger predictor variables would correlate positively with larger body size. Significance was determined at a 0.05 probability level. Statistical analyses were performed using SPSS version 17.0 (SPSS & Inc., 2007).

4.4RESULTS

Of the 309 individual manta rays identified, 159 (51%) were males and 150 (49%) were females. Based on clasper length (Deakos, 2010), 112 (70%) males were adults and 57 (30%) were juveniles. Based on size at sexual maturity for this population (Deakos, 2010), of the 163 sized individuals, among males, 51 (60%) were of adult size with 34 (40%) of juvenile size, and among females, 37 (47%) were of adult size with 41 (53%) of juvenile size. Applying these proportions to the total number of males and females in the population, 95 adult males and 71 adult females were estimated to be available for mating, producing an OSR of 1.34 adult males per adult female.

A total of 286 surveys were conducted between February 4, 2005 and July 14, 2010 (Table 6). Even though it was somewhat common to see a single male in pursuit of a female, the interaction was generally brief lasting only a few seconds before the male abandoned the female and therefore was not considered a mating train. Mating trains were observed on 32 (11%) surveys and ranged in size from 3 to 18 individuals. Generally, only one mating train was seen on a survey. Mating trains were observed each year and in all months of the year except May and August.

Table 6. Summary of surveys conducted by month and season showing the total number and proportion of surveys observed with mating trains and pregnant females. The proportions of surveys with trains and pregnant females were significantly higher during the winter season.

	Month	No. Surveys	No. Surveys with Trains	Proportion with Trains	Mean Train Size	Surveys with PFs	Proportion with PFs
Winter	NOV	22	1	0.05	6.00	5	0.23
	DEC	24	7	0.29	8.29	6	0.25
	JAN	16	3	0.19	6.33	1	0.06
	FEB	15	5	0.33	6.20	4	0.27
	MAR	16	3	0.19	4.67	5	0.31
	APR	31	4	0.13	8.25	5	0.16
	Winter Subtotal	124	23	0.19[a]	6.62	26	0.21[b]
Summer	MAY	25	0	0.00	-	1	0.04
	JUN	19	2	0.11	9.00	3	0.16
	JUL	30	4	0.13	5.00	0	0.00
	AUG	23	0	0.00	-	1	0.04
	SEP	34	2	0.06	14.50	4	0.12
	OCT	31	1	0.03	4.00	6	0.19
	Summer Subtotal	162	9	0.06[a]	8.13	15	0.09[b]
	Grand Total	286	32	0.11	7.22	41	0.14

[a, b] Significantly different, $p < 0.05$

4.4.1 Reproductive Cycles

The proportion of surveys containing a mating train varied significantly by month (Chi-Square Test: $\chi^2 = 27.255$, df = 11, n = 286, p = 0.004) and by season (Chi-Square Test: $\chi^2 = 11.932$, df = 1, n = 286, p = 0.001). Mating trains were three times more likely to be observed during the winter months (Table 6). Mean mating train size was 7.22 animals (SD = 4.10) with the smallest containing a female and two males (by definition) and the largest containing a female and 17 males. The most common train size was 2 males (25%); 9 trains (28%) had 10 or more males. Train size did not vary significantly by month (Kruskal-Wallis Test: $\chi^2 = 9.220$, df =9, n = 32, p = 0.417) or by season (Mann-Whitney *U*-Test: $Z = -0.407$, n = 32, p = 0.705). Of the 28 trains observed, 21 different NFs were identified (Table 7). On average, these females were observed as NF's 21% of the time (4% - 50%). Most were seen only once as a NF (n = 16) and one was seen four times. Five NFs (24%) were also pregnant while leading the mating train.

The mean proportion of sightings containing a pregnant female varied significantly by month (Chi-Square Test: $\chi^2 = 19.917$, df = 11, n = 286, p = 0.046) and by season (Chi-Square Test: $\chi^2 = 7.841$, df = 1, n = 286, p=0.006). Pregnant females were more than twice as likely (21%) to be observed during the winter compared to the summer (9%). During 51 (18%) surveys, at least one pregnant female was observed (Table 6). A total of 20 individual females were observed pregnant (Table 8). On average these females were observed pregnant on 25% of the surveys (3% - 100%).

Table 7. The resight history of 21 nuclear females (NF) observed between years 2005 and 2010. Numbers indicate the month in which they were observed as a NF during that year with train size indicated in brackets. Bolded IDs indicate females observed in a train during a summer month.

Nuclear Female ID	DW (m)	2005	2006	2007	2008	2009	2010	Total Sightings
1021	n/a		4(18)					2
1029	n/a				2(9)			5
1062	n/a					1(10)		4
2002	n/a				12(7)		1(3)	5
3011	n/a	9(16)						4
3041	n/a					3(8)		4
3056	n/a					2(4)		2
5023	n/a				12(8)			5
5029	n/a					4(5)		3
13005	3.30	9(13)			12(10)	7(5)		15
7006	3.37			7(11)				25
3003	3.39				10(4)		3(3)	14
3019	3.39					1(6)		19
12010	3.41				2(9)			9
5001	3.42					1(10)		9
5003	3.44	7(3)			4(6), 6(7), 12(10)			21
7002	3.45				12(3)			13
8008	3.48				2(3)			15
6011	3.48				12(10)			7
3060	3.52					2(4)		4
3030	3.62						3(3)	10
mean	3.44							

Table 8. The resight history and disc width (DW), if available, of 20 pregnant females during the years 2005 through 2010. P indicates she was observed pregnant and her pregnancy was new for that year; N indicates she was observed enough times during that year to determine she was unlikely pregnant; U indicates the she was observed during that year but not sufficiently often to determine if she was visibly pregnant; and a dash (-) indicates she was not sighted during that entire year. Bolded IDs indicate females observed pregnant during a summer month. Estimated Pregnancy Rates (EPR) based on a minimum of 3 yrs with sufficient data, and Estimate Consecutive Pregnancy Rates (ECPR) based on a minimum of 2 consecutive yrs with sufficient data are shown.

Pregnant Female ID	DW (m)	2005	2006	2007	2008	2009	2010	EPR	ECPR	Total Sightings
2036	n/a	-	-	P	-	-	-	-	-	1
3041	n/a	-	-	U	N	P	P	0.67	1.00	4
3056	n/a	-	-	-	U	P	-	-	-	2
12009	3.35	-	-	-	P	-	-	-	-	6
12011	3.39	-	-	-	P	-	-	-	-	4
3003	3.39	P	-	N	P	N	U	0.50	0.00	14
3019	3.39	-	N	P	N	N	-	0.25	0.00	19
5013	3.41	-	-	-	P	N	-	-	0.00	21
5008	3.44	-	U	-	P	-	-	-	-	6
5003	3.44	N	N	U	N	P	-	0.25	-	21
10002	3.45	N	N	P	N	-	N	0.20	0.00	31
8008	3.48	N	U	U	P	-	-	0.50	-	15
3001	3.51	U	P	U	N	N	-	0.33	-	6
10000	3.52	U	-	-	P	-	-	-	-	7
3060	3.52	-	-	-	U	P	-	-	-	4
7000	3.53	P	P	-	P	N	-	0.75	0.50	6
3008	3.55	P	U	U	U	P	P	1.00	1.00	17
12005	3.58	-	-	P	U	N	-	-	-	5
1007	3.59	U	U	U	P	P	P	1.00	1.00	15
3030	3.62	-	P	-	U	N	P	0.66	-	10
mean	3.48							0.56		

One notable female (ID# 5003) was observed as a NF on 4 occasions over an eight-month period (Figure 12). On 22 Apr 2008, she was an NF with 6 males, periodically following a larger pregnant female (ID#5013), who was not considered part of the mating train. On 23 Apr 2008 she was being pursued briefly by a single male (ID#8002) that had not been present the day before, and again periodically following a larger, pregnant female (ID#5008), one different from the day before. Fresh mating scars were visible on her left pectoral fin indicating that mating had at least been recently attempted. On 24 Apr 2008, she was observed on her own. The same male (ID#8002) was pursuing her two days later on 26 Apr 2008. On 15 May 2008, she was observed on her own with other mating trains in the vicinity. On 12 Jun 2008 she was leading a train of 7 males. On 24 Jun 2008 she was on her own and was not seen again until 10 Dec 2008, when she was leading a train of 9 males. On 8 Feb 2009 she was observed on her own, and on 21 Mar 2009 she was observed on her own while a separate eight animal train passed through the area. During both of these latter sightings, she did not appear pregnant, but on 24 Aug 2009, she was visually confirmed to be pregnant.

4.4.2 Role of Body Size

The mean, minimum, and maximum body size of all females, NFs, PFs, all males, juvenile males, transition males, adult males, all escorting males, N1Es, N2Es, and NEs that have never been seen as an N1E are shown in Figure 13. The average female DW was 3.18 m (SD = 0.31). A total of 21 NFs and 20 pregnant females were observed. NFs averaged 16% larger than all males measured in mating trains, but NF DWs did not significantly differ from PF DWs (Mann-Whitney U Test: $Z = -1.196$, $n = 29$, $p = 0.232$). Larger PFs had significantly higher pregnancy rates than smaller PFs (Linear Regression: $R^2 = 0.520$, $df = 8$, $F = 8.684$, $p = 0.009$), and were significantly more likely to be observed pregnant in consecutive years (Linear Regression: $R^2 = 0.882$, $df = 5$, $F = 37.470$, $p = 0.001$). Mating trains with larger females did not contain significantly more males (Linear Regression: $R^2 = 0.277$, $df = 11$, $F = 3.841$, $p = 0.078$).

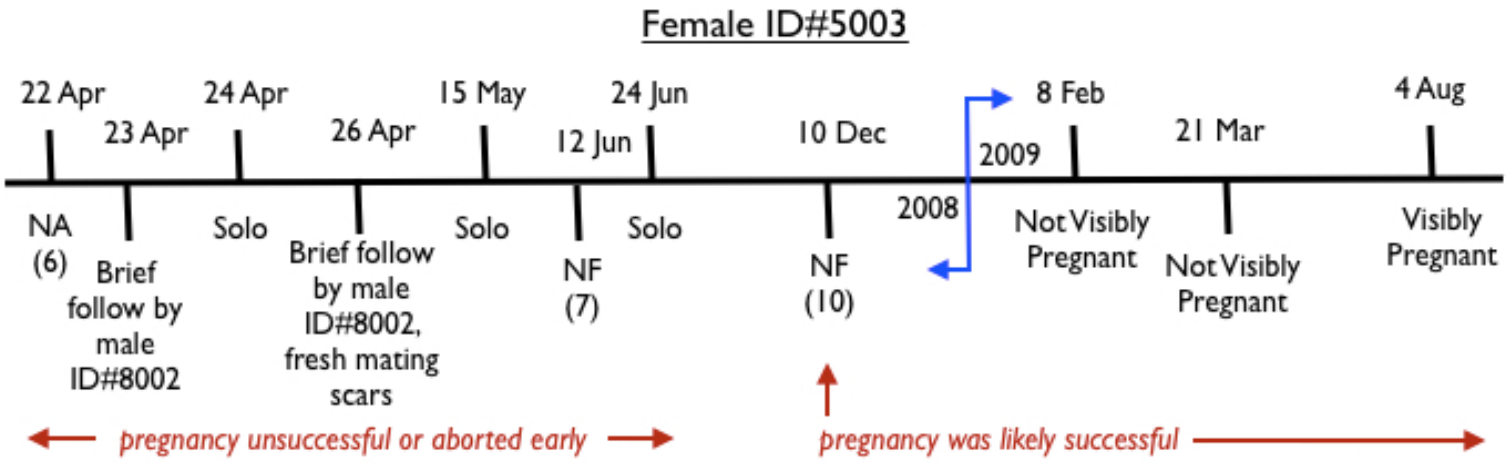

Figure 12. The sighting history of a notable female (ID#5003) observed multiple times as a NF. Numbers in brackets indicate the number of animals in the mating train.

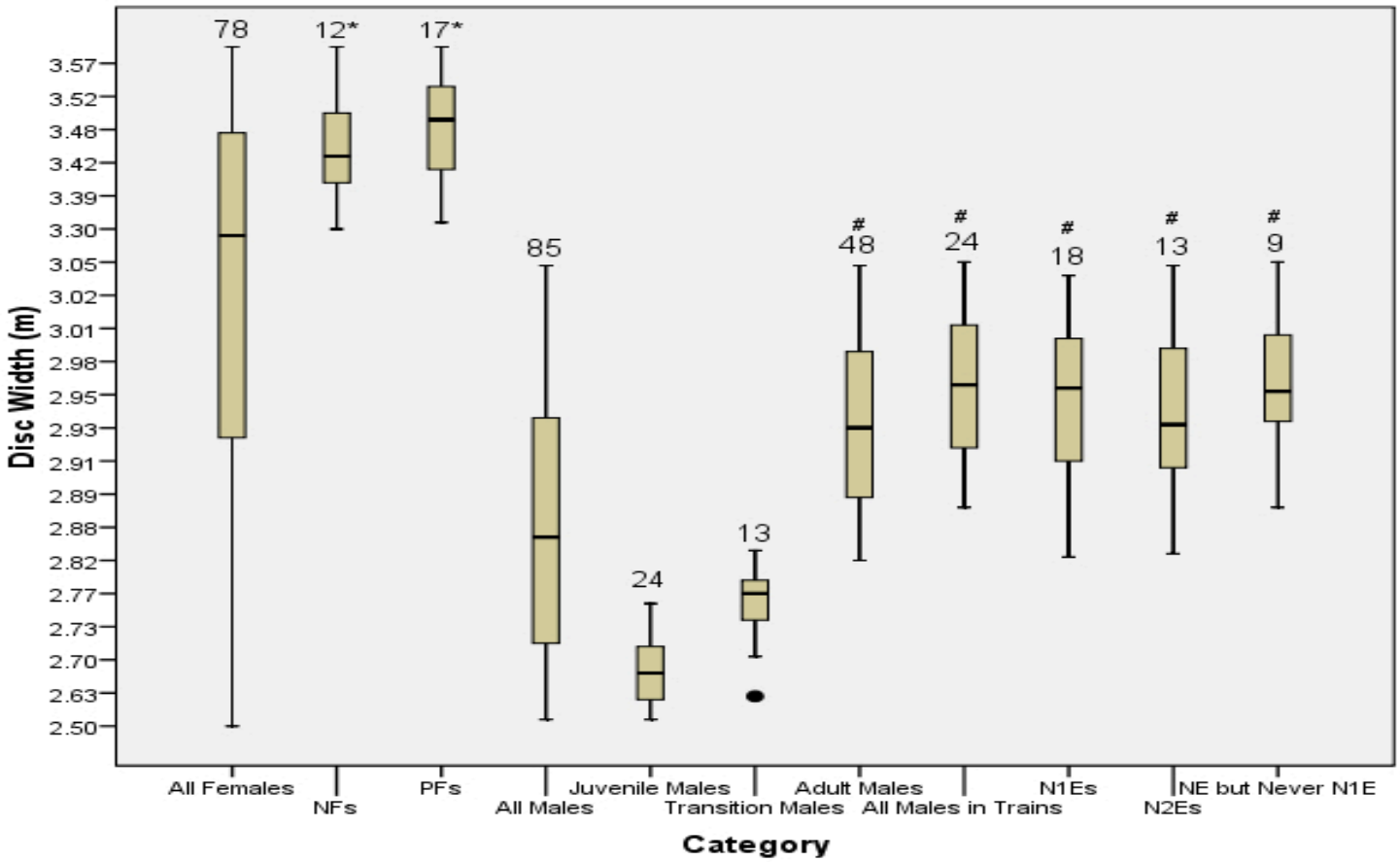

Figure 13. Distribution of manta ray disc widths. Heavy black lines = means, box boundaries = 25th and 75th percentiles, whiskers = smallest and largest observed values that are not statistical outliers, circles = statistical outliers, numbers = sample sizes. *not significantly different, p = 0.232, #not significantly different, p = 0.363.

Most pregnant females were observed to be pregnant only once during the 6 years of the study (n = 15). Two were observed pregnant in two separate years, and three were observed pregnant in three separate years. The latter three were among the nine largest females of the 77 measured. Three females were confirmed pregnant in two consecutive years. One female, confirmed pregnant in three consecutive years, was the second largest female measured in the population.

Based on 11 females observed pregnant at least once, with sufficient sightings to determine pregnancy status for at least 3 separate years, the estimated mean pregnancy rate was 0.56 pups per year (0.20 – 1.0; Table 8). Based on a biennial pregnancy rate, nearly half of these females may not be available for mating, thereby inflating the OSR to 2.68 adult males per reproductively available female.

The most likely months for giving birth based on the earliest confirmation that a pregnant female was no longer pregnant were November through April. One half of the PFs were confirmed with mating scars, and at least two PFs were confirmed without. Of the 41 females observed with mating scars, all had scars on the dorsal side of their left wing tip, and two (5%) females had visible mating scars on the dorsal surface of both the left and right wing tip.

The average male DW was 2.83 m (SD = 0.14). A total of 22 different males were identified occupying the N1E position in a mating train (Table 9). On average these males were observed as the N1E on 14% of the surveys (2% - 40%), and the majority (73%) were only seen once as the N1E during the 6 years. A total of 19 different N2Es were observed. On average these males were observed as N2E's 17% of the time (7% - 50%). A total of 40 different NEs were observed. On average these males were observed as NEs on 22% of the surveys (4% - 100%). No significant differences were found between the mean sizes of all adult males, NEs, N1Es, N2Es, and NEs never seen as N1Es (Kruskal-Wallis Test: $\chi^2 = 4.328$, df = 4, n = 112, p = 0.363). Larger N1Es were not significantly correlated with larger NFs (Linear Regression: $R^2 = 0.001$, df = 14, F = 0.011, p = 0.918). Adult males were frequently observed following females briefly

Table 9. The resight history and disc width (DW), if available, of 22 individual nuclear primary escorts (N1Es) during the years 2005 through 2010. N1E indicates he was the primary escort in a mating train, and the bracketed numbers represent the month followed by the train size. S indicates the male was sighted but not as a N1E during that year. A dash (-) signifies he was not sighted during any surveys for that entire year. Bolded IDs indicate a male observed escorting in a train during a summer month.

N1E ID#	DW (m)	2005	2006	2007	2008	2009	2010	Total Sightings
1033	n/a	-	-	S	S	N1E(3:8)	-	4
2014	n/a	S	N1E (4:18)	-	N1E (12:10)	-	S	5
3017	n/a	-	S	S	N1E (12:10)	S	-	14
3055	n/a	-	-	-	N1E (6:7)	-	-	5
6023	n/a	-	-	-	S	-	N1E(3:3)	3
8017	2.84	S	S	-	N1E (6:7,6:11)	S	S	32
2005	2.89	N1E (7:3)	S	S	S	S	S	16
1001	2.90	S	S	-	S	S	N1E(1:3)	12
3064	2.90	-	-	-	S	N1E(1:6)		4
2039	2.91	-	-	-	S	S	N1E(3:3)	8
4000	2.92	S	S	S	N1E (2:9)	N1E(1:10,2:4)	-	27
7010	2.92	-	-	-	N1E (12:10)	-	-	5
3007	2.94	S	-	N1E (7:11)	S	S	-	12
13007	2.94	S	S	S	S	N1E(1:10,2:4)	S	45
5019	2.96	-	-	-	N1E(4:6)	S	-	12
8012	2.98	-	S	-	S	N1E(1:10)	-	10
3033	2.98	-	-	S	S	S	N1E(3:3)	6
8002	2.99	N1E (9:16)	S	N1E (7:11)	N1E(10:4)	N1E(7:3)	-	34
2037	3.00	S	-	-	N1E(2:9,12:7)	S	-	8
1003	3.02	S	S	-	S	N1E(2:4)	-	10
3023	3.02	-	S	S	S	N1E(4:5)	S	21
8009	3.03	S	S	S	N1E(12:3)	N1E(1:10)	-	31
mean	2.95							

from behind, or occasionally turning abruptly in order to pass through an area where a female recently defecated. One notable male (ID#13007) was the most frequently sighted individual in the study. He was observed on 41 occasions over six years, and during seven of these sightings he was observed pursuing a female (Figure 14). Except for encounters on 22 Apr 2008 and 24 Apr 2008, in which the female was the same, all other females were different. On 20 Jan 2009, one of the few occasions when more than one mating train was in the area, he was observed switching back and forth between the two NFs. His behavioral role within the train varied frequently as did others but he often moved to the N1E position with what appeared to be little effort and without any conflict from those males already holding that primary position, even if that male was larger in size.

4.5DISCUSSION

4.5.1 Reproductive Cycles

Although mating trains and late-term pregnant manta rays in this study were observed at all times of the year, they were significantly more likely to be observed during the winter season indicating some reproductive advantage. Seasonal breeding may help to concentrate adult males during certain times of the year, thereby increasing a female's access to more or better mate choices. Seasonality may also coincide with improved food resources available to pups or reduced predation. Without knowing the location of birthing areas, or what resources pups use during their initial years of development, it is difficult to identify the benefits that may exist for pups born during the winter.

The preponderance of mating trains observed in winter, combined with 2 of the 5 females in summer trains were also seen in a train during the previous winter, suggest that females prefer to mate in winter. Summer mating trains may function to allow females who were unsuccessful in getting pregnant during winter, or ones that aborted, a chance of mating again during a less favorable time of the year. This was further supported by a female who was not visibly pregnant

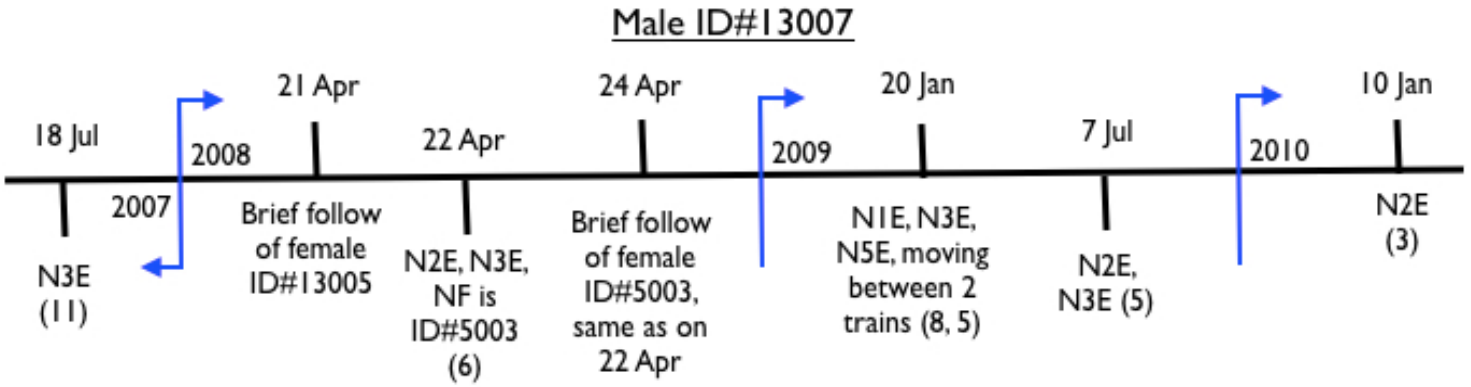

Figure 14. The sighting history of a notable male (ID#13007) with a DW of 2.94 m, observed pursuing a female on seven occasions. The behavioral role(s) observed are indicated for each sighting as well as the train size in brackets.

until the summer of 2009, indicating her mating attempts 14 and 16 months earlier were unsuccessful, or that her pregnancy was aborted.

The rarity of two mating trains at one time suggests that ovulations may be staggered, perhaps reducing competition among females, and providing all reproductively available females an opportunity to mate with higher-ranking males in the area. Staggered ovulation would make it possible for a select subset of higher-ranking males to dominate paternity, at least within a localized area. However, since the study area represents only a small portion of the estimated home range, additional mating trains may have been present in other areas.

Females ovulating outside the primary reproductive season should find less competition for male mates if the population of adult males remains constant throughout the year. If true, larger train sizes would be expected during the summer with fewer available females, but train sizes did not differ significantly between seasons, a possible indication that the OSR remains unchanged with fewer adult males available as well. Females seeking mates during the summer may have access to fewer mate choices and possibly fewer quality males.

The existing OSR of an aggregation area may reflect the habitat choices of different individuals in the population, which could vary by season. A female's lifetime reproductive success is dependent on her ability to raise offspring to the age of independence (Clutton-Brock, Guinness, & Albon, 1982), and the habitat she chooses is often a trade-off between an area rich in food resources and the needs and security of her offspring. Females that are preparing to give birth may choose a habitat that is near sufficient food resources and cleaning stations, but also near a desired birthing area. Therefore, aggregation sites in close proximity to birthing areas may be biased towards pregnant females, while non-pregnant females and others members of the population take advantage of better food resources at locations that may be distant from any birthing area. This could explain a difference in sex ratios between the Maui aggregation site, where

females comprise 47% of individuals, and an aggregation site off Mozambique, in which females comprise 75% of the individuals (AD Marshall & Bennett, 2010). The absence of very small individuals (Deakos, 2010) and a nearly equal number of males and females utilizing the Maui study site could reflect the absence of a nearby birthing area.

Elasmobranchs are known to have a very good sense of olfaction and taste (Hodgson & Mathewson, 1978; Kleerekoper, 1978) and can use these senses to detect biochemical products released by other organisms, including females trying to attract potential mates (I. Gordon, 1993; Johnson & Nelson, 1978). Ari & Correia (2008) reported an acute sense of smell from a captive oceanic manta ray. Brief investigations of females by adult males were likely attempts by males to sense a female's reproductive state through her bodily excretions.

The low pregnancy frequencies for this resident population of manta rays is consistent with reported biennial mating in many elasmobranch females, most likely to allow post-partum recovery to rebuild reproductive reserves before mating again (Pratt & Carrier, 2001). Due to the small sample size in determining the mean pregnancy rate, and the possibility that females classified as non-pregnant in earlier years of the study could have been immature at that time, the mean pregnancy rate should be used with caution. Additionally, if some pregnant females had less distended abdomens during late-term pregnancy, they could have been misdiagnosed as non-pregnant.

4.5.2 Role of Body Size

Larger females had significantly higher pregnancy rates and were significantly more likely to become pregnant in consecutive years, consistent with larger females benefiting from greater reproductive success. Larger females did not have a greater number of male escorts, contrary to what was expected if males were exhibiting mate choice and choosing larger, more fecund females. However, given that mating trains could last several hours and possibly several days, the number of males observed during the survey may consist of only a fraction of the total.

Males holding the N1E position in a mating train were not significantly larger than any other adult male, contrary to what was expected if males were competing physically for access to the female (cf. S. Spitz et al., 2002). There was also no evidence that larger males were choosing larger females to escort.

Occasionally males that were not part of the mating train inserted themselves into the primary position for very brief periods. When this occurred, the N1E at the time simply retreated to the N2E position without any confrontation. It is possible that dominance had already been established among these males and risking injury by fighting a more dominant male was not advantageous. The absence of male combats suggests that this is not a preferred strategy of males or perhaps other mating opportunities are available that reduce the need to engage in fighting for potential mates.

A variable OSR across seasons can strongly influence the success of male tactics and the predominant mating system for that season (Madsen & Shine, 1993). When OSR is low (female bias), body size seems to show little advantage in reproductive success since many females are available, few combats occur, and smaller males receive mating opportunities. Factors that may affect OSR include biased adult sex ratios, differences between the sexes in age at maturity, reproductive longevity, migration schedules, spatial distribution, mortality during the reproductive season (for review see Clutton-Brock & Vincent, 1991), or momentary differences in the distribution of the sexes (e.g., Höglund, Montgomerie, & Widemo, 1993).

Although the overall number of males and females in this population were nearly equal, the sex ratio of adults was estimated at 1.34 males per female. Biennial mating would reduce the number of reproductively available females by half, which will lead to an OSR of 2.68 mating males for every mating female in this population. This male bias should favor more intense competition between males but the absence of male combats in mating trains indicates such competition may not be directly physical.

Some studies have found that in the absence of intense physical competition between males, male reproductive success is influenced more by social factors than by morphological traits associated with size (Bercovitch, 1989). In whitetip reef sharks (*Triaenodon obesus*), group courtship has been observed where multiple males at the same time bite, mount, and attempt to copulate with a single female (Whitney 2004). Cooperation between males has also been suggested in order to achieve successful mating with a female (Carrier, Pratt Jr, & Martin, 1994).

Competition between males may be occurring through sperm competition. Observations of some female sharks copulating with multiple males during a mating bout suggest sperm competition may be occurring in some elasmobranch species (Carrier et al., 1994; Pratt & Carrier, 2001). Sperm competition may be an alternative mating strategy by males, in which the male's sperm compete for fertilization of the eggs during a single fertile period (GA Parker, 1970). Yano (1999) reported two male oceanic manta rays mating in succession with the same female, although this has been the only report of a female manta mating with more than one male in the same day (AD Marshall & Bennett, 2010).

Among mammals, relative testes size is a good indicator of whether or not sperm competition exists (Gomendio , Harcourt , & Roldan 1998). Right whales (*Eubalaena australis*) for example, which have multiple males mating almost in unison with a single female, have testes weighing over one ton each, more than 1% of their total body weight, while those of sperm whales and humpback whales, which are known to fight aggressively for mates, have testes weighing less than 0.5% of their total body weight (Brownell & Balls 1986). The relative weight of manta ray testes in mature males should be further examined to determine the likelihood of sperm competition as a mating strategy. In birds and mammals, where frequency of copulation is high, testes tend to be large, and where it is low, testes tend to be small (e.g., TR Birkhead, Briskie, & Miller, 1993; Harvey & Harcourt, 1984). The rarity of observed copulation acts suggests that sperm competition is not likely a predominant male mating strategy in resident manta rays.

Endurance rivalry, which can be defined as the ability to remain reproductively active during a large part of the mating season (Andersson, 1994), can favor larger males for reasons of energetics (Andersson & Iwasa, 1996). In manta rays, larger males may be more able to endure a long lasting mating-train consisting of rapid swimming, abrupt turns, and somersaulting. This study showed that mating trains could last for more than one day (see Chapter 2). Females selecting males based on endurance would likely select those males capable of remaining with the train over time, and not simply by their proximity to her within the train.

The absence of any observations of copulation make it difficult to know which males are mating more often. Future work with genetic sampling would be beneficial in deciphering paternity and could help to identify which traits may be contributing to male reproductive success.

4.5.3 Conclusion

A winter breeding and birthing season exists in a resident population of manta rays off Maui, Hawai‘i. Late maturation by females suggests that food resources are likely readily available and predictable and that large body size is advantageous. Females, primarily larger females, have the ability to give birth in consecutive years, but the energy requirements are likely so great that most females will rest for one or more years between pregnancies. Late maturation by males for larger body size also suggests a reproductive advantage in males but how larger males are benefiting is unclear. Since direct physical combats with other males do not occur, understanding the benefits of larger size needs further study. The Maui aggregation area appears to be an important breeding area due to the recurrence of the same reproductively active individuals across years. Combining long-term field studies with the use of genetics to identify paternity and reproductive success among males is suggested for future work in helping to improve our understanding of resident manta ray reproductive ecology.

5 SUMMARY AND GENERAL DISCUSSION

Manta rays are one of the most susceptible marine taxa to population depletion from fisheries exploitation (Dulvy et al., 2008; Dulvy & Reynolds, 2002), primarily due to their life history characteristics of slow growth, late maturation, and low fecundity. The size and status of manta populations globally are unknown. Manta rays are currently classified by the IUCN Red List as Near Threatened (A. D. Marshall et al., 2006), but this list does not currently differentiate between the larger, pelagic species (*M. birostris*), and the smaller, coastal species (*M. alfredi*).

For management purposes, differentiating between oceanic and resident manta rays is extremely important. Each species occupies a very different habitat and therefore may be vulnerable to very different anthropogenic impacts. *M. alfredi,* which appears to consist of small, geographically isolated populations, with little or no exchange of individuals between populations, is more vulnerable to nearshore anthropogenic impacts such as coastal development, storm water runoff, pollutant loadings, boat strikes, entanglement in fishing and mooring lines, and increased pressure from "swim-with manta" programs.

Existing information on resident manta ray life history and ecology is severely limited and much needed for proper management decisions. Data presented in this study contribute new information on: (1) an effective method for measuring sizes of free-ranging manta rays, (2) size demographic for a resident manta population, (3) an estimate of population size and home range, (4) temporal use of an aggregation area, (5) existing natural and anthropomorphic threats, (6) reproductive seasonality, and (7) the role of body size in the reproductive success of females and males. Findings presented in this paper are based on 6 years of research collected from 2005 through 2010 and provide a broader understanding of resident manta ecology and behavior to better assist with management of this species.

5.1 Using paired-laser photogrammetry as a simple and accurate system to measure the body size of free-ranging manta rays (*Manta alfredi*)

In Chapter 2, paired-laser photogrammetry was shown to be a simple, accurate, and precise remote measuring tool, providing a single diver with the ability to obtain a large number of manta ray sizes quickly, while concurrently gathering information about the individual's identification, sex, age class, and behavioral role. Paired-laser photogrammetry proved to be as, or more, accurate and precise than other reported photogrammetry systems (Bergeron, 2007; Cosens & Blouw, 2003; Cubbage & Calambokidis, 1987; Perryman & Lynn, 1993; S. S. Spitz et al., 2000).

Disc width (DW), the standard metric for measuring the size of rays, was not always reliable for measuring free-ranging manta rays. Disc length measurements were much more reliable and could be converted to DW using an empirically derived disc ratio (DR) function for standardized comparisons with other studies.

Measurements on 154 different individual manta rays provided information about maximum size and size at sexual maturity. Data collected indicate that resident manta rays are sexually dimorphic in size, with females larger on average (mean = 3.18 m DW, SD = 0.31) than males (mean = 2.83 m DW, SD = 0.14), and that size varies geographically. The largest female in this Maui population was estimated at 3.64 m DW, much smaller than 5.5 m DW for the largest female reported in southern Mozambique (AD Marshall et al., 2009), or 4.3 m DW reported in Japan (Kashiwagi et al., 2008). The largest male in this Maui population was estimated at 3.03 m DW, much smaller than the largest male reported in Japan at 3.6 m DW (Kashiwagi et al., 2008).

Using pregnancy as an indicator of sexual maturity in females, a DW of 3.37 m was a conservative estimate of the size at sexual maturity. Males appeared to achieve sexual maturity between 2.75 and 2.80 m DW, at the time when their claspers grow rapidly and extend beyond the margins of their pelvic fins.

Very small manta rays were not observed in the Maui aggregation area providing the first evidence of age class segregation in resident manta rays. Newborn manta rays have been reported between 1.1 – 1.5 m DW (Homma et al., 1999; AD Marshall et al., 2009). At the Maui study site, no manta rays less than 2.5 m DW were observed. Manta rays have never been observed giving birth in the wild, and birthing areas are unknown. Female manta rays may retreat to more protected habitats to give birth, where pups may reside, without parental care, until they reach a certain age or size. Age class segregation is commonly reported in many shark species (Klimley, 1985; Pratt & Carrier, 2001) and some mobulids (G. Notarbartolo-di-Sciara, 1988), and female sharks of several species are known to move into protected, nursery areas to give birth (Bass, 1978; Springer, 1967). Pups remain in these areas during early development, presumably for protection against predation.

By visiting areas where manta rays are known to aggregate, length measurements using paired-laser photogrammetry can be obtained from a large part of the population in a relatively short period of time. The ability to integrate individual identities and life histories with morphometrics can be beneficial in longitudinal studies of growth. The presence of fewer older and larger animals in the population can help to identify stock depletion (Cubbage & Calambokidis, 1987). Population parameters such as growth and survival rates, and age at first and last pregnancy can be obtained by measuring captive and free-ranging manta rays of known age over time.

5.2 Characteristics of a manta ray (*Manta alfredi*) population off Maui, Hawai‘i, and implications for management

Chapter 3 describes how photo-identification and active tracking were used to determine population age and sex structure, abundance, home range, and temporal use of the Maui aggregation area.

Findings indicated that more than 290 manta rays, consisting of nearly equal numbers of males and females, occurred within the study area but that individuals moved in and out

of the area such that only a portion of the population was resident at any given time. Sizes of resident manta ray populations in other parts of the world appear to vary widely with reports ranging from 54 individuals in Yap Island, Micronesia, to 890 in Southern Mozambique, to more than 2,000 reported in the Republic of the Maldives (C. R. Anderson et al., 2008; T. B. Clark, 2001; Homma et al., 1999; A. D. Marshall, 2009). Population sizes may reflect food availability of the various regions.

The study area appears to be an important staging area where individuals make routine, year-round visits to rid themselves of parasites or find available mates. The predominance of adult males compared to juvenile males and the high frequency of mating trains observed indicate the study area is likely a significant mating area, primarily between the months of December through April.

High resight rates within and across years provided strong evidence of site fidelity to the study area. Evidence from two actively tracked individuals and photo-identifications revealed that individuals in this population range throughout waters of the Maui County region (Maui, Molokai, Lanai, and Kahoolawe), and appear to be geographically distinct from a neighboring island population off Kona, Hawai‘i. Additional active tracking of individuals combined with genetic sampling is needed to determine the extent of overlap in these populations.

If island-associated resident manta ray populations are geographically independent, with little genetic transfer occuring between populations, local management is needed to address potential threats that may be unique to each region. Small, isolated populations can be at serious risk of rapid and unrecoverable decline (Musick, 1999), and the frequent occurrence of large aggregations of manta rays in a small area makes them more vulnerable to localized anthropogenic impacts.

One of the greatest immediate concerns to this population is entanglement in monofilament fishing line, which can result in disabling or dismembering the manta’s cephalic fin. Alarmingly, 10% of individuals in this population have lost the use of one of

their cephalic fins. Such an injury is likely to impact an individual's feeding efficiency but to what extent requires further investigation. Additional potential threats facing this population include: (1) unregulated swim with manta ray programs adding increased pressure on animals utilizing this natural aggregation area, (2) increased boat strikes on manta rays that frequently travel just below the surface, and (3) entanglement in proposed mooring lines.

5.3 The reproductive ecology of manta rays (*Manta alfredi*) off Maui, Hawai'i, with an emphasis on body size

In Chapter 4, photo-identification and paired-laser photogrammetry were used to investigate reproductive seasonality, and the role of body size in the reproductive success of male and female resident manta rays off Maui, Hawai'i.

This study revealed a reproductive season with mating trains and late-term pregnant females observed more often during winter. The time of year when mating is observed in other populations globally seems to vary according to region (C. R. Anderson et al., 2008; T. B. Clark, 2001; Homma et al., 1999), and may reflect differences in temporal food sources, and weather systems. Breeding seasons generally occur if it improves a female's chance for successful offspring, and is usually guided by a greater availability of food resources, a reduction in predation, improved weather conditions, or a combination of these. Most viviparous elasmobranchs follow annual reproductive cycles with somewhat synchronous mating, gestation, and parturition (for review see Hamlett & Koob, 1999).

Though mating activities were mostly observed during winter, females in this population appear capable of ovulating any time of year and multiple times within a year. This was supported by observations of the same females seen in mating trains in winter and summer during the same year. If initial mating attempts were unsuccessful or aborted, females may mate again outside the primary reproductive season. Although fewer

competing females may occur during the summer, the number of male mate choices may also be reduced.

Males seem capable of detecting a female's reproductive state through her bodily excretions. This was supported by observations of males positioning themselves directly behind females for brief periods, or turning abruptly to pass through her bodily excretions. Elasmobranchs are known to have a very good sense of olfaction and taste (Hodgson & Mathewson, 1978; Kleerekoper, 1978) and can use these senses to detect biochemical products released by other organisms, including females trying to attract potential mates (I. Gordon, 1993; Johnson & Nelson, 1978).

A nearly biennial pregnancy frequency estimated for female manta rays in this population is consistent with information for many elasmobranchs, most likely to allow for post-partum recovery and the rebuilding of reproductive reserves before mating again (Pratt & Carrier, 2001). Based on a biennial mating cycle, the operational sex ratio (OSR) in this population, appears skewed towards males with an estimated 2.7 adult males per available female.

The demographics of a population affect the OSR, which can ultimately impact the predominant mating system and the success of male tactics (Madsen & Shine, 1993). For example when the OSR is skewed toward females, larger male body size seems to show little advantage in reproductive success. Since many females are available, few male-to-male combats occur, and smaller males receive mating opportunities. The males-biased OSR found in the Maui population combined with females providing the only parental care in the form of a 12-month gestation, would predict that males should compete for access to limited numbers of reproductively available females as was observed within the mating trains consisting of a single female followed by multiple males.

Delayed sexual maturity can indicate that the benefits of large body size outweigh the cost of a reduced reproductive lifespan (Shine, 1988). Larger females tend to be more fecund and produce larger, healthier offspring, while larger males, who compete

physically for access to mates, generally outcompete smaller males due to their size and strength (Ralls & Mesnick, 2002). In this manta population, sexual maturity is delayed in both males and females until their body size exceeds 90% of their maximum size (Deakos, 2010), an indicator that large body size provides a reproductive advantage. Larger females had a higher frequency of pregnancies and a greater likelihood of reproducing in successive years. Among males, the reproductive advantage of large body size was not clear, but the absence of observed combats in mating trains suggests that males use strategies other than direct physical competition to gain access to mates. Observations of escorts changing their position frequently in a mating train, sometimes moving into the position directly behind the female, with no apparent conflict from other males, suggest that position in the train may not be an indicator of male fitness. If females select males based on their endurance, demonstrated by their ability to stay with her mating train over long-durations, larger males may benefit from greater energy reserves (Andersson & Iwasa, 1996), especially if males are unable to feed while in a mating train.

Sperm competition has been documented in some species of sharks (Carrier et al., 1994; Pratt & Carrier, 2001), but does not seem to be an important mating strategy in this population of manta rays since sperm competition is generally correlated with increasing bouts of copulation (e.g., T Birkhead, 2000), and no copulations were observed during the six years of this study.

5.4SUMMARY

The information presented in this study broadens our understanding of the social behavior and ecology of *M. alfredi*. These data describe a resident population of ranging throughout Maui County waters, with most individuals showing residency to the main study area off Maui, Hawai‘i, for cleaning and reproductive behaviors. Size data provided evidence of sexual dimorphism, geographical variation in size, and reproductive benefits of late maturation in *M. alfredi*. Reproductive data provided evidence of a mating season, and a nearly biennial female reproductive cycle. Larger females were more fecund and larger males may benefit more from endurance rivalry rather than direct physical

competition between males for limited reproductively available females. The location where females are birthing is unknown but the absence of very small individuals at the study area suggests newborns are segregated, possibly in shallow, protected areas where they reside during early development. Tracking and photo-identification results suggest that resident manta rays in Hawai‘i may consist of independent, island-associated stocks.

In many parts of the world, measures have been taken to reduce anthropogenic threats on local manta ray populations. Some of these measures include: laws making it illegal to kill or capture manta rays, the establishment of marine protected areas in certain parts of the world where manta rays are known to aggregate, and the establishment of codes of conduct for interacting with manta rays underwater. In areas subject to direct removal of manta rays, limiting take numbers to the Potential Biological Removal (PBR) is highly recommended in order to allow small, geographically isolated stocks to reach or maintain their optimum sustainable population (Taylor et al., 2000). This requires knowledge of the minimum population size and maximum population growth rate.

Future research should include active and passive tracking to better describe individual home ranges, to locate birthing areas, to identify areas frequented that pose a high risk of entanglement in monofilament line, and to determine the extent of overlap between neighboring island populations. Genetic sampling of resident populations throughout the Hawaiian Islands could help to identify which males have the greatest reproductive success, and could further determine the extent of mixing occurring between populations.

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