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ABSTRACT

Populations of many large marine species are under threat. Here, we conducted a demographic analysis of a reef manta ray (*Manta alfredi*) population off the southern coast of Mozambique, in order to ascertain the main drivers of its population changes. Using six years (2009–2014) of mark-recapture data collected using a photo-identification method, we estimated the annual apparent survival rate as 0.67 (± 0.155 standard error) per year, with 7% re-sights every year. The female-to-male ratio was 1.7:1. We also constructed a stage-structured matrix population model, and estimated generation time as 7.9 years, the population growth rate as 0.780 per year and lifetime reproductive success as 0.28 offspring per year. The latter two results indicate that this population is declining at the same rate as another population 100 km further north. An elasticity analysis revealed that, as in other long-lived species with comparable life history characteristics, population growth rate is most sensitive to changes in adult survival rate. This suggest that conservation of this population would benefit from focusing on how to best protect adult *M. alfredi*.

INTRODUCTION

Over-fishing, climate change and continuous habitat degradation by human activity pose great threats to the persistence of wild populations; consequently, stringent management policies are required to ensure their viability and persistence. However, information regarding the criteria for accurate management recommendations is often insufficient, or completely absent. When prioritizing management policies, one must assess a population's response to various candidate management policies. Therefore, knowledge of demographic rates and the life stages that most affect the population growth rate is indispensable. Investigating the consequences of changes in a population's demography due to perturbations such as climate change, fishing or changes in management policies may be crucial to a species' survival (Nelson & Peek, 1982; Wisdom & Mills, 1997).

Manta rays are among the largest planktivorous elasmobranchs in the world (Deakos, 2010), can reach a disc width, i.e. the distance between the two pectoral fin tips, of 5–7 m (Marshall et al., 2011), and mainly feed on epipelagic and demersal zooplankton (Couturier et al., 2013). Reef manta rays (*Manta alfredi*) have a slow life-history speed, e.g. late maturity, a long gestation period and a low mean lifetime reproductive success (Heppell, Caswell & Crowder, 2000; Dewar et al., 2008; Marshall & Bennett, 2010; Deakos, Baker & Bejder, 2011; Braun et al., 2014). Therefore, once a manta ray population starts to decrease or reaches critically low numbers it is very difficult for it to recover (Jennings, 2001; Denney, Jennings & Reynolds, 2002; Russ & Alcala, 2004; Cavanagh & Gibson, 2007; Dulvy et al., 2008), so understanding how population growth rates are affected by different demographic rates is particularly important (Hoenig & Gruber, 1990; Reynolds, Dulvy & Roberts, 2002; Dulvy et al., 2008; Deakos, Baker & Bejder, 2011).

Reef manta rays are currently listed as "Vulnerable" by the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, and their populations are decreasing due to targeted and accidental fishing (Marshall et al., 2011; Couturier et al., 2012). They are fished for their gill rakers, which is a key ingredient in Chinese pseudo-medicine, and cartilage, which serves as a filler in shark-fin soup (White et al., 2006; Deakos, Baker & Bejder, 2011; O'Malley, Lee-Brooks & Medd, 2013; Ward-Paige, Davis & Worm, 2013). Recently, *M. alfredi* and the giant manta (*M. birostris)* were listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). The Convention determined that any international trade of manta rays from September 2014 must be controlled. However, in many countries, particularly underdeveloped ones, manta ray populations are still decreasing at an alarming rate, due to a lack of national management and protection. Despite their importance for tourism (Tibiriçá et al., 2011), their large size and frequent inshore occurrence, manta rays have escaped the national attention of Mozambique, even though there has been a 88% decrease in manta sightings (Rohner et al., 2013). Manta rays are not protected by law in Mozambique, their main aggregation areas are not inside marine protected areas and there has been a rapid increase in the use of gill nets, which have significantly increased manta ray by-catch (Marshall, Dudgeon & Bennett, 2011; Pereira et al., 2014).

Although manta rays are difficult to overlook and are often easy to approach, a paucity of data is still a problem that hampers an in-depth understanding of their population dynamics. If conservation management policies are to be effective, knowledge regarding a population's sensitivity to disturbance is essential. Population models have already shown that in some long-lived organisms, investing in the protection of adults yields a larger increase in population growth rate than the protective rearing of newborns (Heppell, Crowder & Crouse, 1996).

Therefore, a very small decrease in the annual survival rate of (sub)adults can have serious repercussions for the persistence of such populations (Brault & Caswell, 1993; Heppell, Crowder & Crouse, 1996; Heppell, Caswell & Crowder, 2000).

The aim of this study was to ascertain ~~what~~ the main drivers of population changes ~~are~~ in a reef manta ray population off the coast of southern Mozambique. We developed and parameterized a stage-structured population projection model (PPM) (Nichols et al., 1992; Caswell, 2001; Fujiwara & Caswell, 2001). Estimates for growth and reproductive rates within the PPM were taken from the literature; survival rates were estimated by applying a Cormack-Jolly-Seber (CJS) model to capture-mark-recapture (CMR) data taken from the customized Manta ID database (MID 3.0 Beta Version) over a six-year period (2009–2014), using photo-identification methods. We applied this population model to obtain, to our knowledge for the first time, a demographic characterization of *M. alfredi*, including its population growth rate, lifetime reproductive success and generation time. Elasticity analysis is an important tool in answering questions in population ecology (Caswell, 2001), and is widely applied by conservation biologists to aid in developing sustainable management strategies (Benton & Grant, 1999; Carslake, Townley & Hodgson, 2009); therefore, we conducted an elasticity analysis to assess how sensitive the *M. alfredi* population growth rate is to perturbations in growth, survival and reproductive rates.

METHODS

Study site

Surveys were conducted on the inshore reefs off Zavora beach, Mozambique (S24.30 E35.12E), along an approximately 13-km stretch of coastline, at depths ranging from 7 to 15 m. Three main manta ray aggregation sites within 800 m (Fig. 1: areas in A) were chosen, because of their high manta ray encounter rates. Opportunistic manta ray surveys were also conducted at two other locations with less frequent manta encounters, about 3 to 5 km north of the main aggregation site (Fig. 1: areas in B). Reef manta rays tend to stay inshore, and exhibit a high affinity for particular cleaning stations (Dewar et al., 2008; Marshall, 2009; Marshall, Compagno & Bennett, 2009; Deakos, Baker & Bejder, 2011), making these locations well suited for photo-identification studies.

Data collection using photo-identification

A suitable method for data gathering with minimal disturbance is photographic identification. Due to its non-invasive nature, photo-identification is particularly suitable for endangered species, such as manta rays (Brault & Caswell, 1993; Bansemer & Bennett, 2008; Marshall & Pierce, 2012). Instead of physically marking an animal, photographs of permanent phenotypic characteristics can serve as points for recognition, turning conventional physically intrusive mark-recapture methods into a sight-re-sight technique. Photo-identification has already successfully been used in marine species, e.g. cetaceans (Hammond, Mizroch & Donovan, 1990; Brault & Caswell, 1993) and whale sharks (*Rhincodon typus*) (Arzoumanian, Holmberg & Norman, 2005; Graham & Roberts, 2007; Speed, Meekan & Bradshaw, 2007; Holmberg, Norman & Arzoumanian, 2009). Manta rays have a distinctive spot and patch pattern on their ventral side that allows for individual recognition. These markings are already present in the foetus (Marshall, Pierce & Bennett, 2008) and remain unchanged over time (Yano, Sato & Takahashi, 1999; Kitchen-Wheeler, 2010; Marshall, Dudgeon & Bennett, 2011). The markings extend across the entire ventral surface, but due to its diversity, a small surface area is sufficient to ensure positive identification results. We used the same rectangle-shaped area as used by Marshall, Dudgeon & Bennett (2011). This area allows sex determination and a clear image of the most important part of the spot pattern, without any deformation as a result of pectoral fin flexing (Marshall, Dudgeon & Bennett, 2011).

Using the photo-identification method, *M. alfredi* presence was surveyed over a six-year period from February 2009 to October 2014, with an average of 135 dives per year. Photographs of *M. alfredi* individuals were taken in an opportunistic manner, and depended on weather, funding and sea conditions. Occasionally, recreational divers also contributed photographs. On average, two dives were conducted per day, three times a week, with most diving activity taking place during December–January and April–June of each year. Each dive lasted 60 min at the most. Upon encountering a reef manta ray, photographs were taken of the pigment patterns on the ventral side, scars, condition of the tail and other markings to aid individual identification. Where possible, sex was determined by the presence or absence of ~~male~~ claspers on the pelvic fins. Photographs were taken using a Canon PowerShot G11 inside an Ikelite underwater casing. A GoPro 360 camera was attached to a custom-made aluminium structure, which supported two parallel green lasers that were fixed 50 cm apart, in order to increase the precision of the size estimates (Deakos, 2010; Marshall, Dudgeon & Bennett, 2011). All of the data collection conducted in this study complied with the laws of Mozambique as no permits are required.

Manta ID database

When at least one quality image was taken of a reef manta ray, the picture was uploaded onto a custom-designed Manta ID database (MID 3.0 Beta Version). This database was specifically designed for the cross-matching of photographs of manta rays, using an algorithm that takes the following criteria into account:

1. Species; *M. birostris* or *M. alfredi*. Both species are recorded in MID, but only observations of *M. alfredi* were used in this study.

2. Sex; manta rays were classified as female, male or unknown.

3. Gill pattern; a "yes or no question" regarding the presence of spots in four different areas between the anterior 1st gill slits and the most posterior 5th gill slits.

4. Areas 1 & 2; the number of spots in Area 1 (consisting of the surface between the 1st and 3rd gill slits) and the number of spots in Area 2 (the surface between the 3rd and 5th gill slits).

5 & 6. Distal right/left side; the presence or absence of black markings on the outer edge of the right/left gill set.

7. Ventral area; the number of spots (0–5, 6–10, 11–15, 16–20, 20–25 or >25) and grey patches on the ventral side, ranging from the 5th gill slits to the end of the pelvic fins.

The first two criteria are easily observable *in situ* for the trained eye, and can be confirmed with a photograph. The spot pattern in the standardized area is the most important characteristic with which to identify individual manta rays (criteria 3 to 7), and the other characteristics provide confirmation. The program MID uses a mathematical algorithm that takes into account the position, presence/absence and number of spots in relation to each gill. After entering new data, MID will show several options of similar manta rays for which a re-sight can be verified or a new manta ray can be added to the database. As different observers may interpret photographs differently, the logarithm does not eliminate any potential options; it allows up to three mathematical differences in order to avoid eliminating possible matches by accident.

Statistical analyses

Firstly, to gain an insight into the structure of the *M. alfredi* population, we conducted a Chi-square test in R v. 3.1.2 software (Crawley, 2007; R Development Core Team, 2013) to assess the overall sex ratio within the study population. Secondly, we used statistical analyses to estimate *M. alfredi* recapture rates and survival rates; estimates of survival rates were required to parameterise our population model. We used a CMR approach to estimate recapture and survival rates from data on individual manta rays, which were photo-identified and later re-encountered. We only estimated these rates for adult females, because (i) the population model was constructed for females (assuming that female demography determines population growth (Caswell, 2001)), and (ii) there were no re-sights of juveniles, so we could only estimate these rates for adults. Of the different CMR approaches that exist, we used open-population CJS models (Cormack, 1964; Jolly, 1965; Lebreton et al., 1992) and assumed that manta rays seen at the different study sites comprise a single population, which was open to immigration/birth and emigration/death. CJS models rely on the following three assumptions (Cooch & White, 2014): (1) every marked animal present in the population at time t has the same probability of survival and recapture at time $t + 1$; (2) marks are not lost or missed; (3) all samples are instantaneous relative to the interval between occasion t and $t + 1$, and each ray is released immediately after sampling. The first assumption was tested using a goodness-of-fit test in MARK v. 7.2 (White & Burnham, 1999), which revealed that our dataset conformed to this assumption ($\chi^2 = 2.5$, *d.f.* = 1, $p = 0.444$). Assumptions 2 and 3 were met, as no manta rays were overlooked during the dives due to their large size (divers reported the same number of manta rays seen per dive), and photographs were taken immediately upon encountering a manta ray.

We used four different open CJS models to investigate whether the recapture rate (p, yr^{-1}) and apparent survival rate (σ, yr^{-1}), defined as the probability that the animal survives and is available for recapture, were constant or varied over time. In model 1, apparent survival rate and recapture rate are constant, denoted as $\sigma(.)\ p(.)$; in model 2, apparent survival rate is constant and recapture rate varies across years, denoted as $\sigma(.)\ p(t)$; in model 3, apparent survival rate varies across years and recapture rate is constant, denoted as $\sigma(t)\ p(.)$; in model 4, both apparent survival rate and recapture rate vary across years, denoted as $\sigma(t)\ p(t)$. To assess which of the four models best fitted our data, we applied model selection using Akaike's Information Criterion ($\mathrm{AIC_c}$) to compare the models, where the subscript c indicates that the AIC is corrected for a finite sample size and a decreased chance of overfitting. The model with the lowest $\mathrm{AIC_c}$ value was considered to be the best-fitting (Akaike, 1987). We then applied a model selection procedure to compare nested models (model 2 is nested within model 1; model 3 is nested within model 1; and model 4 is nested within models 2 and 3). CJS model analysis and selection were conducted using the standard "live recaptures (CJS)" setting in MARK. We used estimates of apparent survival rate from the best-fitting model.

Population model

To construct the population model, we divided the *M. alfredi* life cycle into the following three life stages: yearling (individuals in the first year of life), juvenile (which do not reproduce) and adult (which reproduce) (Fig. 2). The addition of further life stages may have increased model accuracy, but these are the only currently distinguishable stages in *M. alfredi*. The rate at which individuals survive and stay in the same life stage equals P_i, where i indicates Y (yearling), J (juvenile) or A (adult), and was calculated as:

$$P_i = \sigma(1 - \gamma_i) \qquad \text{(Equation 1)}$$

where σ is the apparent survival rate estimated using the CMR analysis (see above). We assumed that the apparent survival rate σ did not differ between life stages, as we could only calculate σ for adults (see above). The parameter γ_i is the transition rate from one life stage to the next (expressed per year); from yearling to juvenile (γ_Y) or from juvenile to adult (γ_J). Each transition rate γ_i was calculated as $\gamma_i = 1/S_i$, where S_i is the duration (in years) of either the yearling (i = Y) or juvenile life stage (i = J) (Table 1). The rate (per year) at which individuals survive and grow into the next life stage is defined as:

$$G_i = \sigma\gamma_i \qquad \text{(Equation 2)}$$

where i indicates Y (yearling) or J (juvenile). The number of female offspring that an adult female produces each year equals F_A. Putting it all together results in the following population projection matrix:

$$\mathbf{A} = \begin{bmatrix} P_Y & 0 & F_A \\ G_Y & P_J & 0 \\ 0 & G_J & P_A \end{bmatrix} \qquad \text{(Equation 3)}$$

The projection interval from time *t* to $t + 1$ is one year. Using the population model, we calculated the population growth rate λ from the dominant eigenvalue of matrix **A** and the stable stage distribution by its right eigenvector. Generation time T was calculated as $T = \log\left(R_0\right)/\log(\lambda)$, where R_0 is mean lifetime reproductive success as calculated by taking the dominant eigenvalue of the matrix, $\mathbf{R} = \mathbf{FN}$. The matrix **F** is a fertility matrix that describes the production of new individuals:

$$\mathbf{F} = \begin{bmatrix} 0 & 0 & F_A \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix} \qquad \text{(Equation 4)}$$

The matrix **N** is calculated as $\mathbf{N} = (\mathbf{I} - \mathbf{U})^{-1}$, where **I** is the identity matrix and **U** the transient matrix that describes the growth and survival rates of the different stages:

$$\mathbf{U} = \begin{bmatrix} P_Y & 0 & 0 \\ G_Y & P_J & 0 \\ 0 & G_J & P_A \end{bmatrix} \qquad \text{(Equation 5)}$$

Using the population projection matrix **A**, we performed an elasticity analysis to investigate how sensitive the population growth rate λ is to perturbation of each of the different growth, survival and reproductive rates. We calculated the elasticity matrix **E**, where each element on row *m* and column *n* of matrix **E**, e_{mn} represents the proportional contribution of this demographic rate corresponding to this element to the population growth rate λ. The elasticities were calculated as follows (Caswell, 2001):

$$e_{mn} = \frac{a_{mn}}{\lambda} \frac{\partial \lambda}{\partial a_{mn}} \qquad \text{(Equation 6)}$$

where a_{mn} are the elements of **A**, and the second part of the equation are the sensitivities of λ to changes in the elements a_{mn} of **A** (Caswell, 2011). The elasticities sum to 1, and give the proportional contributions of the matrix elements to the population growth rate λ. Therefore, the higher an elasticity value is relative to other elasticity values, the greater is the effect of the demographic rate that that elasticity value is associated with on the population growth rate. All of the population model analyses were conducted in MATLAB® R2014b (MathWorks®, MA, USA).

RESULTS

A total of 2281 images of 327 *M. alfredi* individuals were analysed. Of these 327 individuals, 21 were re-sighted at least once over the six-year observation period, 7 of which were male and 14 female. The maximum number of times that an individual was re-sighted was three, with one female that was seen at the same location twice in 2013 and once in 2014. A total of 39 individuals could not be sexed; of the 288 sexed individuals, 63% were female and 37% male, indicating a female-biased sex ratio of 1.7:1, which was significantly different from a 1:1 ratio ($\chi^2 = 20.05$, *d.f.* = 1, $p < 0.0001$). Overall, the total number of males and females seen varied between years (Fig. 3).

In order to construct our population model to run a demographic analysis for *M. alfredi*, we estimated annual apparent survival rates of adult females using a CMR analysis, in which we compared four open CJS models where apparent survival and recapture rates are both constant, both vary over time or one was constant and the other varies over time. Both model selection procedures revealed that the best-fitting model was model 2, with a constant apparent survival rate σ and a time-varying recapture rate p ($\Delta\mathrm{AIC_c} > 2$, $\mathrm{AIC_c}$ weight = 0.748) (Tables 2 and 3). According to model 2, the apparent survival rate was $\sigma = 0.666$ year^{-1} (standard error, 0.155; 95% confidence interval, 0.338–0.887), and the yearly recapture rates $p_{t,t+1}$ from year t to year $t + 1$ were $p_{2009–2010} = 0.000$, $p_{2010–2011} = 0.000$, $p_{2011–2012} = 0.022$, $p_{2012–2013} = 0.209$ and $p_{2013–2014} = 0.094$. The overall recapture rate over the whole study period was 0.065 per year. We calculated the rates at which individuals transitioned from the yearling to the juvenile stages ($\gamma_Y = 1/S_Y = 1/1$) and from the juvenile to the adult stage ($\gamma_J = 1/S_J = 1/8$) from published observations of how many years individuals stay in the yearling ($S_Y = 1$) or juvenile stage ($S_Y = 8$) (Table 1). We then parameterised the population projection matrix **A**. Using a constant apparent survival rate σ value of 0.666 and the life-stage transition rates $\gamma_Y = 1$ and $\gamma_J = 0.125$ (Table 1), we calculated P_i and G_i (Equations 1 and 2). We set adult fertility rate F_A at 0.375, because mature female manta rays produce one offspring every one or two years (Dewar et al., 2008; Marshall & Bennett, 2010; Couturier et al., 2014), resulting in an average of 0.5 × (1 + 0.5) offspring per year = 0.375 female offspring per year. Inputting all of these values resulted in the following population projection matrix:

$$\mathbf{A} = \begin{bmatrix} P_Y & 0 & F_A \\ G_Y & P_J & 0 \\ 0 & G_J & P_A \end{bmatrix} = \begin{bmatrix} 0 & 0 & 0.375 \\ 0.666 & 0.583 & 0 \\ 0 & 0.083 & 0.666 \end{bmatrix} \qquad \text{(Equation 7)}$$

Note that $P_Y = 0$ (top-left element in **A**, Equation 7), because the projection interval of one year is equal to the time that a yearling remains in the yearling life stage. This matrix yields a population growth rate of $\lambda = 0.780$, meaning that each year, the population ‘grows’ by 78%; therefore, the population is projected to decrease each year by 1 – 0.78 = 0.22, or 22%. The stable stage distribution reveals that the population is predicted to be mainly comprised ~~of~~ juveniles (52.4%), followed by 32.1% adults and 15.5% yearlings. Unfortunately, we had no observations with which to compare the predicted population structure to. Mean female lifetime reproductive success is estimated to be 0.28 offspring per year, and generation time is estimated to be 7.9 years.

We then calculated the elasticity matrix **E**, which summarises how influential each demographic rate is to the population growth rate λ, relative to other demographic rates:

$$E = \begin{bmatrix} 0 & 0 & 0.093 \\ 0.093 & 0.276 & 0 \\ 0 & 0.093 & 0.445 \end{bmatrix}. \qquad \text{(Equation 8)}$$

The highest value is the bottom-right element, $e_{3,3} = 0.445$. This matrix element corresponds to the adult survival rate P_A (Equation 3), indicating that the population growth rate is most sensitive to changes in adult survival rate. The next-highest elasticity value is $e_{2,2} = 0.276$, which corresponds with juvenile survival rate P_J (Equation 3), so the population growth rate λ is also sensitive to changes in juvenile survival rate. The other elasticity values are all much lower (Equation 8), which indicates that the population growth rate is relatively insensitive to changes in adult fecundity rate (F_A), the rate of transitioning from the yearling to juvenile stage (G_J) and the rate of transitioning from the juvenile to adult stags (G_A).

DISCUSSION

Demographic analysis

Here, to our knowledge, we present the first demographic analysis of a reef manta ray population. Manta ray populations have globally declined according to the IUCN Red List for Threatened Species. Our results confirm this pattern, as both the predicted population growth rate and mean lifetime reproductive success were lower than unity (population growth rate λ = 0.78 per year; lifetime reproductive success R_0 = 0.28 offspring per year), and both are indicative of population decline. Before drawing any firm conclusions on the population dynamics of this *M. alfredi* population, a couple of factors that could have compromised our results should be discussed. Firstly, the CMR analysis revealed an overall 7% yearly re-sight rate of *M. alfredi* individuals, which is considerably lower than in other manta ray studies that have applied CMR analyses. For example, Marshall, Dudgeon & Bennett (2011) re-sighted over 40% of their photo-identified individual manta rays within a four-year period; at Lady Elliot Island, Australia, over 66% were re-sighted in a five-year study (Couturier et al., 2014), and over 36% of the manta rays identified revisited the same aggregation site in the central Maldives within a nine-year period (Kitchen-Wheeler, Ari & Edwards, 2012). The low re-sight rate that we obtained may have been due to temporal emigration. Once photographed, a manta ray might disperse to other regions and only occasionally return to the study site, thereby decreasing the number of re-sights. However, a more probable cause for the low number of re-sights in this study could have been the irregular sampling effort. Regular daily and/or weekly data sampling was not feasible, because of unfavourable weather conditions or for financial reasons. As a result, revisiting manta rays may have been missed, thereby lowering the number of re-sights to below the actual number of re-visits by the same individuals. Secondly, the low percentage of re-sights in our population probably affected the confidence interval of our survival rate estimate, although our estimate of a mean annual survival rate of σ = 0.666 was not significantly different from what has previously been estimated using *M. alfredi* sightings further north along the coast of Mozambique (0.683 ± 0.147 (mean ± standard error); Marshall, Dudgeon & Bennett, 2011). Whether a higher percentage of re-sights would have affected our estimate of mean survival rate is unclear, but the survival rates within our population model were probably an overestimation of actual survival rates. Because we had so few re-sights, we assumed that juveniles and adults had the same survival rate; however, this was probably not the case. Typically, juvenile survival rates are lower than those of adults, because juveniles are more vulnerable to predation (Begon, Townsend & Harper, 2005). However, decreasing yearling and/or juvenile survival rates in our population model would have resulted in an even lower population growth rate and lifetime reproductive success; therefore, our predictions probably underestimated the actual population growth rate and lifetime reproductive success. On the basis of our demographic analysis, we can conclude that our study population is decreasing in size. Our estimated population growth rate of λ = 0.780 per year indicates that, for example, a population of 500 individuals would, over a period of 10 years, decrease to only $500 \cdot \lambda^{10} = 42$ individuals. Such a projected decline in population size of over 90% in 10 years is not unrealistic, given that the number of *M. alfredi* sightings further north along the coast of Mozambique (Praia do Tofo) has decreased by 88% over six years between 2005 and 2011 (Rohner et al., 2013). It is important to consider that both of these populations might be part of the same meta-population. The cause of the latter's decline is mainly attributed to increased fishing pressure (Rohner et al., 2013), indicating the direct need for marine protected areas around manta ray aggregation sites. Harvesting has probably also contributed to the decline of our study population.

Sex ratio

In the study population, 63% of the individuals identified were female. The observed 1.7:1 female-to-male ratio was significantly different from a 1:1 ratio, and was therefore female-biased. Off the coast of Praia do Tofo (Mozambique), approximately 100 km north of our study site, the percentage of females in the population is 78% (3.5:1 female-male ratio) (Marshall, Dudgeon & Bennett, 2011). Female-biased sex ratios comparable to our observations have been observed around the Lady Elliot Islands, eastern Australia (1.3:1) (Couturier et al., 2014) and the Maldives (1.8:1) (Kitchen-Wheeler, Ari & Edwards, 2012). In contrast, Deakos (2010) found a male-biased sex ratio of 0.9:1 females to males at Maui, Hawaii. Skewed sex ratios have also been reported in other populations of elasmobranch species. ~~Great~~ white sharks (*Carcharodon carcharias*) and whale sharks (*R. typus*), for example, exhibit male-biased sex ratios (Graham & Roberts, 2007; Robbins, 2007), whereas nurse sharks (*Ginglymostoma cirratum*) have a higher number of females than males (Castro & Rosa, 2005). Other elasmobranch species, e.g. scalloped hammerhead sharks (*Sphyrna lewini*), even exhibit almost complete sexual segregation outside the mating season (Klimley, 1987). The female-biased sex ratio in our reef manta ray population might indicate that our study sites are important mating and birthing sites, where females have higher return rates and stronger site affinity than males.

Conservation

Our elasticity analysis revealed that the population growth rate is most sensitive to perturbation of adult survival rate. The demographic rates that comprise our population matrix are determined by the underlying parameters σ (the apparent survival rate) and γ_i (the stage-specific transition rate). However, because the adult survival rate P_A equals σ and is independent of γ_i, population growth rate is indeed most sensitive to perturbation in adult survival, which is typical for long-lived animals. For example, there is a minimal impact of so-called “headstarting” of turtle hatchlings on the population growth rate. Elasticity analyses have revealed that targeting sub-adult and adult turtle survival would yield a higher rate of population growth, and thus form a more effective management strategy than the protective rearing of newborns (Crouse, Crowder & Caswell, 1987; Heppell, Crowder & Crouse, 1996). The importance of adult survivorship is also evident in northern fur seals *Callorhinus ursinus* (Trites & Larkin, 1989), marbled murrelets *Brachyramphus marmoratus* (Beissinger, 1995) and cheetahs *Acinonyx jubatus* (Crooks, Sanjayan & Doak, 1998). In the case of *M. alfredi*, conservation efforts and population management policies should be aimed at protecting both adults and juveniles, in order to increase their survival and hence population growth.

Our results suggest that the *M. alfredi* population studied is decreasing in size, and effective management and legislation is urgently needed to avoid its local extinction. Two main approaches should be taken: (1) the species should be protected at the national level against fishing, including accidental catch; (2) aggregation areas should be protected. The behaviour of reef manta rays at cleaning stations makes targeted fishing a potential threat, but also creates an opportunity for site-specific protection. By protecting aggregation sites, both juveniles and adults could profit from increased survival, resulting in a higher population growth rate. The importance of adult survival makes manta rays an unsustainable fishing resource. Their socio-economic value has yet to be realised to its full potential, but one thing is clear: manta rays are worth more alive than dead (O’Malley, Lee-Brooks & Medd, 2013; Ward-Paige, Davis & Worm, 2013).

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Table 1 Model parameters used to calculate survival rate $P_i = \sigma(1 - \gamma_i)$ of yearlings (i = Y), juveniles (i = J) and adults (i = A) and growth rate $G_i = \sigma\gamma_i$ of yearlings (i = Y) and juveniles (i = J). Apparent survival rate, σ (year^{-1}) was estimated using capture-mark-recapture analyses (see Methods); estimates for life stage duration S_i of yearlings (i = Y), juveniles (i = J) and adults (i = A) were based on International Union for Conservation of Nature estimates (Marshall et al., 2011).

	Life stage		
Parameter	Yearling	Juvenile	Adult
Apparent survival rate, σ (year^{-1})	0.666	0.666	0.666
Duration of stage i, S_i (years)	1	8	31
$\gamma_i = 1/S_i$	1	0.125	0.032

Table 2 Model selection using Akaike's Information Criterion (AIC) for four open-population Cormack-Jolly-Seber models. In model 1, apparent survival rate (σ, year^{-1}) and recapture rate (p, year^{-1}) are constant, denoted as $\sigma(.)\ p(.)$; in model 2, apparent survival rate is constant and recapture rate varies across years, denoted as $\sigma(.)\ p(t)$; in model 3, apparent survival rate varies across years and recapture rate is constant, denoted as $\sigma(t)\ p(.)$; in model 4, both apparent survival rate and recapture rate vary across years, an denoted as $\sigma(t)\ p(t)$.

Model		AIC$_c$	ΔAIC$_c$	AIC$_c$ weight	Model likelihood	No. parameters
1	$\sigma(.)\ p(.)$	126.299	6.874	0.024	0.032	2
2	$\boldsymbol{\sigma(.)\ p(\mathrm{t})}$	**119.426**	**-**	**0.748**	**1.000**	**6**
3	$\sigma(t)\ p(.)$	127.163	7.737	0.016	0.021	6
4	$\sigma(t)\ p(\mathrm{t})$	121.945	2.520	0.212	0.284	8

Notes.

AIC$_c$, corrected AIC; ΔAIC$_c$, difference in AIC$_c$ when compared to the lowest AIC$_c$ model; AIC$_c$ weight, Akaike weight. The best-fitting model is in bold.

Table 3 Step-by-step model selection procedure using log-likelihood tests to identify the best-fitting, open-population Cormack-Jolly-Seber model. At each step, a log-likelihood test was conducted to assess whether a more complex, alternative model provided a better description of the data than the less complex, null model (within which the alternative model is nested). The procedure ended when the alternative model did not provide a significantly better fit than the null model (step 3). In each model, the apparent survival rate (yr^{-1}), σ, and recapture rate (yr^{-1}), p, were either set as constant (.) or time-varying (t) (see also Table 2). The best-fitting model is in bold.

Step	Null model	Alternative model	Log-likelihood	*d.f.*	*p*
1	$\sigma(.)\ p(.)$	$\sigma(t)\ p(.)$	7.669	4	0.105
2	$\sigma(.)\ p(.)$	$\sigma(.)\ p(t)$	15.406	4	0.004
3	**$\sigma(.)\ p(t)$**	**$\sigma(t)\ p(t)$**	**1.937**	**2**	**0.380**

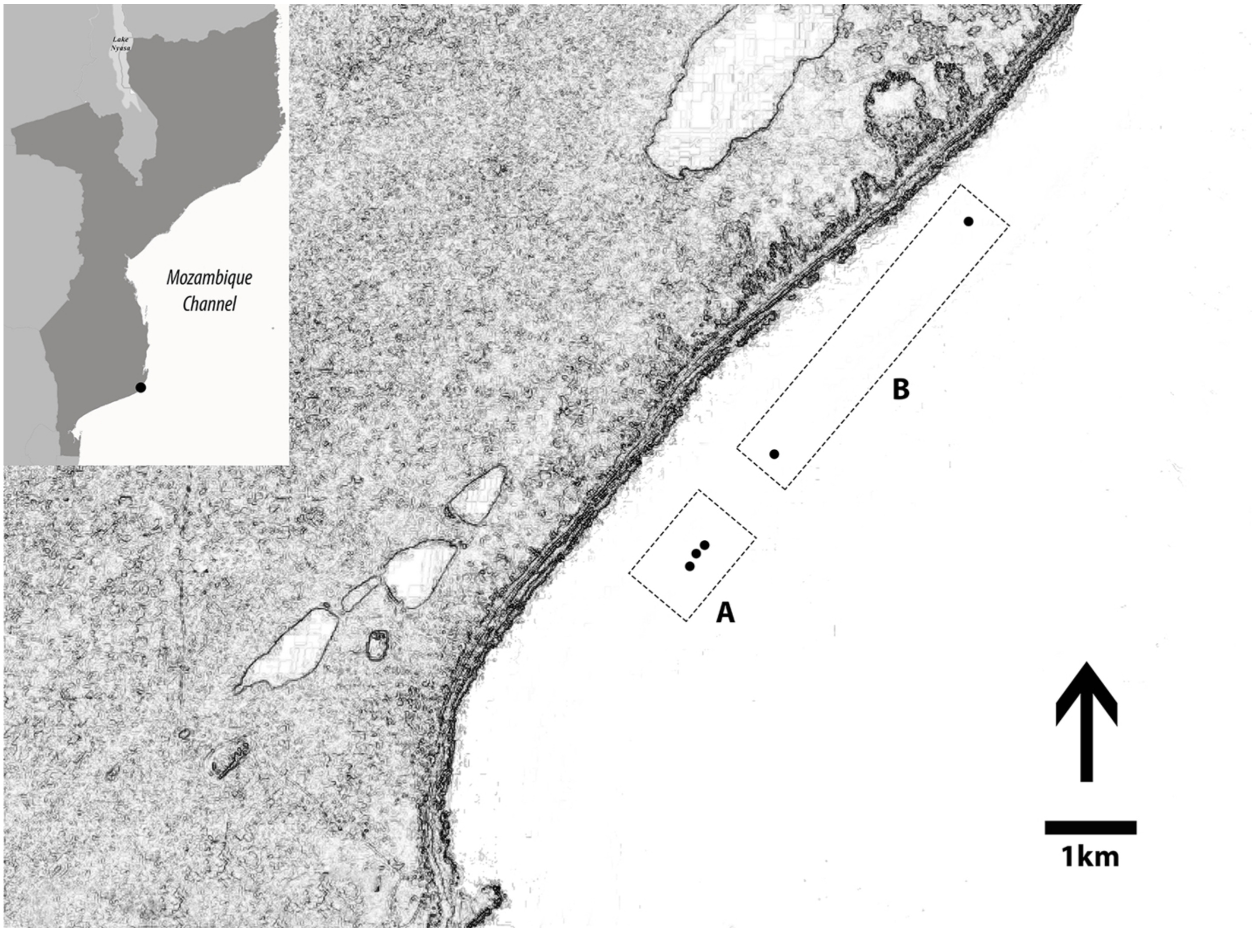

Figure 1 Locations of study sites off the coast of southern Mozambique. Main surveys were conducted at three main manta ray aggregation sites (areas in A) and opportunistic surveys were conducted at two other locations with less frequent manta encounters (areas in B).

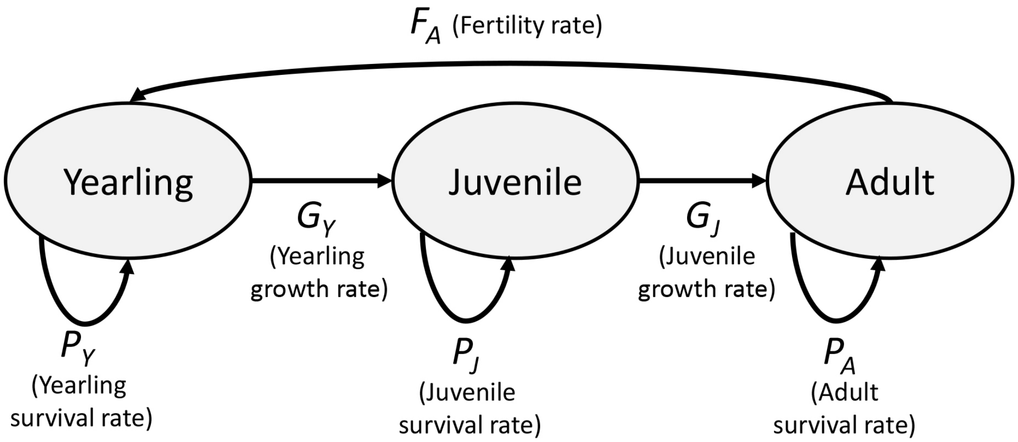

Figure 2 Life cycle of *Manta alfredi*. We distinguished three life stages: yearlings (Y), juveniles (J) and adults (A). The rate at which individuals survive and stay in the same life stage equals P_i, where i indicates Y (yearling), J (juvenile) or A (adult); the rate at which individuals survive and grow to the next life stage equals G_i, where i indicates Y (yearling) or J (juvenile); the rate at which adults produce yearlings equals F_A. See also Equations 1–3.

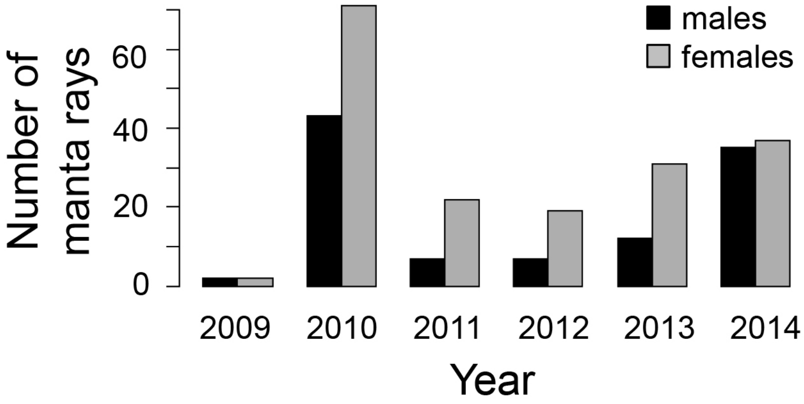

Figure 3 Number of adult male (black bars) and adult female (grey bars) manta rays summed per year over a period of six years. The associated female-male sex ratios for each year within the study period (2009–2014) were 1:1 (2009), 1.7:1 (2010), 3.1:1 (2011), 2.7:1 (2012), 2.6:1 (2013) and 1:1 (2014).