Laser photogrammetry improves size and demographic estimates for whale sharks

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Abstract Whale sharks *Rhincodon typus* are globally threatened, but a lack of biological and demographic information hampers an accurate assessment of their vulnerability to further decline or capacity to recover. We used laser photogrammetry at two aggregation sites to obtain more accurate size estimates of free-swimming whale sharks compared with visual estimates of size. Laser photogrammetry revealed individual whale sharks ranged from 432–917 cm total length (TL) (mean ± SD = 673 ± 118.8 cm, N = 122) in southern Mozambique and from 420–990 cm TL (mean ± SD = 641 ± 133 cm, N = 46) in Tanzania. By including direct measurements of stranded individuals with photogrammetry measurements, we calculated length at 50% maturity for males in Mozambique at 916 cm TL. Repeat measurements on individual whale sharks measured over periods from 347–1068 days yielded inconclusive results about growth rates. The amount of growth over this period of time does not appear to be sufficient to be detected using laser photogrammetry. The sex ratio of both populations was biased towards males (74% in Mozambique, 89% in Tanzania), the majority of which were immature. The population structure for these two aggregations was similar to most other documented whale shark aggregations around the world. Information on small (<400 cm), mature, and female whale sharks in this region is lacking, but necessary to inform conservation initiatives for this globally threatened species.

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Introduction

The whale shark *Rhincodon typus* (Smith 1828) is the world's largest fish species, measuring up to 2000 cm total length (TL) and 34 t in mass (Chen, Liu & Joung, 1997). Their large size, tendency to spend much of their time in surface waters (Wilson et al., 2006; Brunnschweiler et al., 2009; Motta et al., 2010) and predictable aggregative behaviour in certain coastal areas, make them susceptible to human threats such as directed fisheries (Pravin, 2000), boat strikes and net entanglement (Speed et al., 2008). Similar to most large sharks (Cortés, 2002), whale sharks are likely to grow and reach maturity slowly, leaving them vulnerable to depletion caused by human pressures (Wintner, 2000; Cheung, Pitcher & Pauly, 2005).

Whale sharks were listed as Vulnerable on the IUCN Red List of Threatened Species following rapid and substantial declines caused by targeted fisheries in the 1990s and early 2000s in the Indo-Pacific (Norman, 2005). Although a decrease in whale shark sightings may not necessarily indicate a decrease in actual whale shark numbers due to the highly mobile nature of these animals and variability in sighting conditions, studies that controlled for environmental factors in southern Mozambique (2005–2011; Rohner et al., 2013) and at Ningaloo Reef, Western Australia (1995–2004; Bradshaw et al., 2008) revealed substantial declines in sightings. This suggests that some aggregations in the Indian Ocean have suffered population declines. Additional studies at Ningaloo Reef proposed that an apparent decline in mean length of whale sharks (Bradshaw et al., 2008) may have resulted from increased recruitment of smaller sharks to this location (Holmberg, Norman & Arzoumanian, 2008), rather than a decrease in survivorship of larger individuals (Bradshaw, Mollet & Meekan, 2007; Bradshaw et al., 2008). Such an interpretation would suggest that this regional population is recovering. These apparently conflicting results may be due partly to methodological differences among studies. Holmberg et al. (2008; 2009) used mark-recapture population models and excluded transient sharks, whereas Bradshaw et al. (2007) used demographic models, which are highly sensitive to variation in key biological parameters such as age or size at maturity. These parameters are poorly-known for whale sharks, and this high uncertainty decreases the predictive capability of demographic models (Simpfendorfer, 1999; Bradshaw et al., 2007).

Generally, vertebral ageing studies are the source of most demographic data for elasmobranchs (Cailliet et al., 2006; Pierce & Bennett, 2010), but whale shark studies have been hampered by limited sample sizes and the difficulty in validating results (Wintner, 2000). An alternative approach has been the use of growth rates on free-ranging sharks through the marking and

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recapture of individuals (Pierce & Bennett, 2009). In whale sharks, the common use of imprecise visual size estimation (Rohner et al., 2011) has precluded routine collection of growth data, and consequently long-term trends in length-frequencies should be interpreted cautiously.

Whale sharks show some degree of site fidelity (Holmberg et al., 2009; Rowat et al., 2011) that has allowed for basic biological parameters to be estimated through visual assessment, despite most aggregations being dominated by juvenile males. The length at which 50% of males reach maturity (TL_{50}) was estimated to be ~810 cm at Ningaloo Reef (Norman & Stevens, 2007), while growth rates were estimated to be 3–70 cm $year^{-1}$ in Belize (Graham & Roberts, 2007) and 45 cm $year^{-1}$ in the Maldives (Riley et al., 2010). However, visual size estimates can lack accuracy and repeatability, particularly where multiple observers are involved (Holmberg et al., 2009). By contrast, laser photogrammetry (photogrammetry henceforth) is likely to be more accurate and precise (Rohner et al., 2011).

Here, we use photogrammetry to measure whale sharks at two coastal aggregation sites in the southwestern Indian Ocean offshore of Praia do Tofo (Tofo Beach) in southern Mozambique and offshore of Kilindoni on Mafia Island, Tanzania. First, we aimed to describe the size ranges and sex ratios of sharks at these sites. Second, we aimed to assess TL_{50} of males with photogrammetry (southern Mozambique) and direct measurements (northern South Africa) of clasper lengths. Third, we aimed to test whether photogrammetry can detect growth rates estimated between a 1-3 year time period.

Methods

Study locations and whale shark searches

Photogrammetry data were collected from whale sharks off Praia do Tofo (23.85° S, 35.56° E) in southern Mozambique between January 2010 and October 2013 and off Mafia Island, Tanzania (7.90° S, 39.66° E) between October 2012 and December 2013 (Fig. 1). Whale sharks were spotted during boat-based searches (see Pierce et al., 2010), and all data were collected while snorkeling alongside the sharks. Direct measurements of stranded sharks were obtained from Pomene, southern Mozambique (22.92° S, 35.56° E) and from the northern South African coast (~29.10° S, 31.64° E, Fig. 1). Unpublished photographic identification data (WildMe, 2014) and satellite tagging results (Rohner, 2013) have demonstrated regular movements between northern South Africa and southern Mozambique, hence we treat them as

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a single population. Data collection in Mozambique was cleared by The University of Queensland's animal ethics committee (GPEM/184/12/MMF/SF) and research in Tanzania was approved by the Tanzania Commission for Science and Technology (COSTECH).

Photographic identification

A laser photogrammetry system mounted on a housed digital camera, as described in Rohner et al. (2011), was used to project two spots of green laser light onto the flank of each shark while a photograph suitable for individual identification was taken (Arzoumanian, Holmberg & Norman, 2005). Identification photographs were submitted to the Wildbook for Whale Sharks library (www.whaleshark.org) and processed to assign a unique identity to each shark. Sightings were compared with images in the archived database of sharks to identify broader connectivity with other sites.

Photogrammetry analysis

All photographs were taken with Canon G11/G12 compact digital cameras. The zoom function was not used. Only sharks with a suitable photogrammetry image were included in all analyses. Four observers took photogrammetry images, with the majority taken by CAR and SJP (~90%). Total length was extrapolated from a measurement of the flank between the 5th gill slit and the origin of the 1st dorsal fin (B_{P1} in Rohner et al., 2011). Where possible, multiple laser photogrammetric images were taken of the shark in each encounter to measure TL and improve the morphometric relationship between TL and the distance from the 5th gill slit to the origin of the 1st dorsal fin. All shark lengths are reported as total length unless otherwise specified.

Assessment of the laser photogrammetry set-up

Image distortion: The airspace between the camera lens and the underwater housing refracts the incoming light and the shape of the lens itself can lead to image distortion. We thus assessed image distortion of the photogrammetry setup empirically underwater. A grid of 10 x 10 squares was photographed and the number of pixels (length L) that formed the diagonal of the middle two squares was determined. If the measurement was extended across another square then a linear multiple of this count would occur if image distortion was absent. Deviation from linearity can be quantified from a plot of observed versus expected pixel counts. A linear regression was fitted to obtain the image distortion function:

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Comment [17]: Maybe a sentence describing why this ratio is used, for example was shown to be constant at any growth stage of the shark

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As the zoom on the camera was never used, this distortion function was constant (Harvey & Shortis, 1998) and was applied to all photogrammetry image measurements.

Parallel alignment of lasers: Lasers must be parallel to provide accurate data from varying distances to the target. The photogrammetry set-up was therefore regularly calibrated on land by measuring points 50 cm apart from 8 m to the target. Photogrammetry images of whale sharks for size analysis were consistently taken at ~4 m from the shark, so that the maximum tested distance (8 m) was about twice that used for size estimation, and errors would have been, on average, half as large.

Parallax error: Parallax error would lead to an underestimate of shark length if a photogrammetry image was not taken perpendicular to the target. The parallax error for our setup was assessed by measuring a 50 cm long object 5 times each from an angle of 10°, 20°, 30°, 40° and 50°. The percentage error was 2.9%, 8.3%, 16.6%, 27.5% and 39.1%, respectively. In the field, we had no means of estimating this angle for each photograph and thus correcting for potential parallax error. Instead, we visually assessed the photos and compared them with the photos from this test to exclude all images at >10° angle.

Finally, the accuracy and precision of the photogrammetry setup were assessed by measuring a 258.6 cm pole 30 times.

Male maturity assessment

The sex of each whale shark was determined visually by examining the pelvic fins for the presence of claspers, the external, paired reproductive structures of male sharks. Maturity in male sharks was assessed by examining the size and thickness of the claspers (Norman & Stevens, 2007). Immature sharks have relatively small claspers, and mature sharks have thick claspers that extend past the pelvic fins. Claspers of 46 male sharks from Mozambique and 22 sharks from Tanzania were measured using photogrammetry, while claspers from 11 males that were stranded along the northeastern coast of South Africa were measured directly. Clasper length (CL) was defined as the distance from the anterior end of the cloaca to the posterior tip of the clasper, equivalent to clasper inner length in Compagno (2001). The TL and CL at which 50%

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Comment [23]: I don't understand how comparing whale shark photos in the field to the pipe photos would allow you to identify parallax

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of males were mature (TL_{50} and CL_{50}) were each calculated using generalised linear models (GLM), with a binary logit function. We minimised potential differences among measurements of live, free-swimming sharks and dead, stranded specimens by measuring natural TL (Francis, 2006) where possible, or scaling pre-caudal length (PCL) to TL based on a previously-derived morphometric relationship: $TL = 1.2182 * PCL + 33.036 (N = 41)$ in 4 of the 11 stranded sharks (Wintner, 2000; Rohner et al., 2011).

Reproductive status

Three whale sharks were found stranded on 16 August 2009 at Pomene Beach in southern Mozambique (Fig. 1) and dissections were conducted on-site. The maturity status of the two female sharks was based on the condition of the ovary and the uteri, and of the male through examination of claspers, testes and accessory organs, similar to criteria in Pierce et al. (2009).

Age determination

Vertebrae anterior to the first dorsal fin were extracted from two of the stranded whale sharks from Mozambique; a 738 cm male and a 630 cm female. Vertebrae were stored frozen until x-radiography images were taken (Eklin EDR3 Mark III) to visualise band pairs following Wintner (2000) as a method of determining age. Band pairs, consisting of one opaque and one translucent band, were counted on two vertebrae from each shark. Three readers assessed each vertebra three times, independently of one another, after which the median was taken as a consensus count.

Growth rates

We tested whether *in situ* photogrammetry had enough precision to determine growth rates of 13 whale sharks measured and subsequently re-measured over 340 days. These growth rate estimates were compared to those derived from band-pair counts from stranded sharks of known size from South Africa (Wintner 2000) and from two of the sharks we dissected at Pomene, Mozambique, assuming annual band pair formation. A linear regression with 95% confidence intervals (CI) was produced from back-calculated size at age values. The zero value was set at 42 cm PCL following Wintner (2000), as this is the approximate size of newly-born whale sharks (Chang, Leu & Fang, 1997).

Results

Photogrammetry assessment

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Length estimates of the 258.6 cm pole made with our photogrammetry equipment under controlled conditions were accurate, with a mean error of 1.2% or -3.2 cm. Lengths ranged from 254.7–256.6 cm and all measurements underestimated the true length. Precision was high, with a coefficient of variation of 17%.

Morphometric relationship for TL

The morphometric relationship used for estimating TL was updated with the inclusion of additional data from 14 fully-measured live sharks and removal of morphometric data for 4 sharks measured outside southern Mozambican and northeastern South African waters (Rohner et al., 2011). The updated equation was:

$$TL = 4.902 \times P1 + 72.579 \; (r^2 = 0.92, N = 37).$$

Population structure

The 123 measured whale sharks in southern Mozambique ranged from 439–934 cm, with a mean ± SD of 684 ± 118 cm (Table 1). A significant sex bias was observed, with 75.7% male and 24.3% female in the 115 sharks for which sex was determined (Chi-square test, χ^2 = 26.420, P < 0.001). Average male size (range = 445–934 cm, mean ± SD = 692 ± 119 cm, N=87) did not differ significantly from average female size (range = 439–858 cm, mean ± SD = 670 ± 108 cm, N=28) (t test, t = 0.67, df=49.65, p = 0.506), although all 6 sharks >860 cm were male (Fig. 2a).

The 56 whale sharks measured in Tanzania ranged from 420–990 cm, with a mean of 655 ± 129 cm (Table 1). A significant sex bias was present, with 87.5% male and 12.5% female in the 56 measured sharks for which sex was determined (Chi-square test, χ^2 = 56.3, P < 0.001). The mean length of males (660 ± 131 cm, N=49) and females (620 ± 117 cm, N=7) were not significantly different (t = 0.84, df = 8.32, p = 0.425) (Fig. 2b).

Size at maturity

Inner clasper lengths (CL) were measured for 46 sharks from Mozambique, 11 from South Africa and 22 from Tanzania. Eight sharks ranging from 823–1032 cm were mature and had clasper lengths ranging from 75–106 cm. The largest immature male was 928 cm and clasper length of immature males ranged from 26–84 cm (Fig. 3).

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Based on the established connectivity between Mozambique and South Africa, we combined maturity data from stranded whale sharks in northeastern South Africa and Pomene (Mozambique) with data from whale sharks measured with photogrammetry in Mozambique. Maturity (TL_{50}) was attained at 916 cm (Residual Deviance = 19.9; p=0.012; AIC=23.9), and CL_{50} was 81.0 cm (Residual Deviance = 6.81; p=0.02; AIC=10.81; Fig. 4). One 876 cm mature male from Tanzania had a CL of 89 cm, slightly smaller than the largest immature shark (cf. 903 cm), but with longer claspers (cf. 74 cm). The 3 stranded sharks examined at Pomene measuring 738 cm (male), 630 cm (female) and 820 cm (female), were immature. The larger female had thin, strap-like uteri and a lattice-like ovary structure. No ovarian follicles were observed.

Ageing and natural growth rates

Vertebrae of the 738 cm male and the 630 cm female had 26 and 22 band pairs, respectively. These data were added to the 15 band pair counts from Wintner (2000) to create an updated regression for band pair counts and length: $PCL = 22.44 * \text{band pairs} + 29.46$ ($r^2 = 0.99$, $N = 17$).

Over the study period, we resighted 72% and 96% of measured individuals from Mozambique and Tanzania, respectively. Seven sharks from Mozambique and 24 sharks from Tanzania were measured multiple times over the study period, with a time gap of 3–1068 days. Of these, 13 individuals were re-measured after more than 340 days had elapsed since the time of the initial size estimate. Mean growth rate was 5.6 cm $year^{-1}$ (± 47.3), with 6 sharks having decreased in length when re-measured (Fig. 5).

Discussion

Photogrammetry improved the accuracy of whale shark size estimates by almost an order of magnitude. While the estimated error in visually-determined lengths of whale sharks was ~10% (Rohner et al., 2011), our controlled tests showed that photogrammetry reduced this to 1.2%. Precision was also high, with a CV of 17%, so length estimates were consistent across photographs. Jeffreys et al. (2012) also found high accuracy and precision in experimental tests of a similar photogrammetry set-up. The major challenge with photogrammetry of whale sharks remains taking an image from the correct horizontal and vertical angle while the

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Comment [34]: This represents the error of your photogrammetry setup measuring a stationary pipe, not sure how the error was calculated using visually determined lengths and not sure comparing the two is appropriate. Compare free-ranging animals with free-ranging animals, see next comment.

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Comment [35]: A stationary pipe is very different from a moving animal, would be good to get precision on multiple independent measurements of the same whale shark either by different people or the same whale shark a few days later, this would seem to be a more accurate measure of precision for whales sharks, which incorporates parallax.

shark is in a straight, flexed position. Measuring only a portion of the body, such as PCL or B_{P1}, enhances precision as it excludes the caudal fin or the whole posterior part of the body which can be flexed when the shark is swimming and result in out of plane (foreshortened) images. We used B_{P1} to scale TL in preference over the distance from the spiracle to the 5^{th} gill slit (A1 in Jeffreys et al., 2012). This was because sharks in our study were mostly surface feeding, which resulted in a dorso-ventral flexion of the head that precluded an assessment of the A1 metric. Although the TL data used in our study are derived from a morphometric relationship between B_{P1} and TL, our measurements are considered to be more accurate than those derived from visual estimates.

Sex- and size-based segregation

Whale sharks measured in Mozambique and Tanzania exhibited pronounced sex- and size-based segregation. Most sharks were juvenile males of 550–850 cm, which is similar to other known whale shark aggregation sites in the Indian Ocean and elsewhere (Fig. 6 with references in the caption). Given that whale sharks can reach 2000 cm (Chen, Liu & Joung, 1997), the size structure observed in these aggregations show that only a proportion of a whale shark population is seen at these coastal sites. Mean sizes of 684 cm in Mozambique and 640 cm in Tanzania were larger than that recorded from Djibouti (370 cm), Saudi Arabia (400 cm), Taiwan (460 cm), inshore sites in the Gulf of Mexico (490 cm), the Seychelles (580 cm) and the Maldives (598 cm), but considerably smaller than at offshore sites in the Gulf of Mexico (1085 cm), in South Africa (804 cm), India (740 cm) and Ningaloo Reef (720 cm) (Fig. 6 with references in the caption). Size ranges of 439–934 cm observed in Mozambique and 415–971 cm in Tanzania were smaller than reported for most other locations, although this may be a consequence of the improved precision of size estimates from photogrammetry in comparison to visual estimates and the comparatively short time-frame of this study.

A male sex bias is common at monitored whale shark aggregation sites. The percentage of male sharks in Mozambique (76%) was similar to northeastern South Africa (73%) and inshore sites in the Gulf of California (75%), but lower than that in Tanzania (88%), the Maldives (95%), Djibouti (83%), Ningaloo Reef (85%) or the Seychelles (82%) (Fig. 6). By contrast, the coastal aggregation in Saudi Arabia had about equal numbers of juvenile males and females, whereas offshore sites in the Gulf of California and the Galapagos Islands mainly had large females (Ketchum, Galván-Magaña & Klimley, 2012; Ramírez-Macías, Vázquez-Haikin & Vázquez-Juárez, 2012b; Hearn et al., 2013; Berumen et al., 2014). The

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Comment [36]: Would be good to provide some type of variability measure around this relationship, since it is not likely a perfect correlation and this variability gets incorporated into the variability of your measurements.

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Comment [37]: Much of this is results regurgitated, don't think you need the actual numbers here but rather discuss why you see these differences in sex and size across regions

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Comment [38]: Pretty big assumption here, Figure 6 does show some areas with smaller size ranges of animals (Saudi Arabia, Djibouti), need to explain that away. Would be good to see sample sizes as this can influence your range as well.

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Comment [39]: How come not showing in Figure 6?

apparent sex bias and the narrow size range of whale sharks across the Indian Ocean aggregation sites raises intriguing questions concerning the location of newborn, female, and larger mature sharks. Whale sharks are born at ~45–60 cm (Joung et al., 1996; Aca & Schmidt, 2011), but <250 cm individuals are rarely seen anywhere in the world and there are only 19 reports of sharks <150 cm (Rowat & Brooks, 2012). The sex ratio of whale shark embryos was almost equal among males and female (1: 0.98 females to males, N = 297) in the only pregnant shark investigated to date (Chang et al., 1997). Chang et al. (1997) found no inter-sex difference in the length or mass of embryos and hence female neonates are assumed to have similar survival rates to males. The pronounced segregation in most coastal whale shark aggregations suggests that whale sharks occupy different habitats, or use the same habitats differently, depending on their sex and size.

While the sex bias and the predominance of immature whale sharks at coastal sites could conceivably be an artifact of the previous targeted fisheries activities in the Indian Ocean and Western Pacific, there are several arguments against this being the case in Mozambique and Tanzania. First, the largest whale shark aggregations in the Indian Ocean appear to have little or no connectivity (Wilson et al., 2006; Brooks et al., 2010; Sleeman et al., 2010). This suggests that fisheries in the Maldives, India, or further away in Taiwan and the Philippines should not have affected the population structure in the Western Indian Ocean, although they may have led to declines in the east at Ningaloo Reef (Bradshaw et al., 2007, 2008) and off Thailand (Theberge & Dearden, 2006). Second, evidence suggests that the majority of sharks caught in fisheries were males or juveniles. Most sharks landed in Taiwan were juvenile males (Hsu, Joung & Liu, 2012). A large proportion of the catch from India contained immature sharks, though the sex of the sharks was not reported (Pravin, 2000). Interviews with fishers and catch records from the Philippines also indicate that landed sharks are largely immature, again with no information on the sex ratio (Alava & Dolumbalo, 2002). Last, coastal whale shark aggregations in and around the Caribbean Sea, where there is no history of fishing for whale sharks, are also dominated by immature male sharks (Graham & Roberts, 2007; Ramírez-Macías et al., 2012a, Fox et al., 2013). Therefore, data suggest that juvenile and male dominated whale shark aggregations are not necessarily an artifact of selective fishing pressures.

Segregation is common in many shark species, with populations usually divided socially and/or geographically into units of sub-adults, mature males and mature females (Springer,

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Comment [40]: Different references used in methods section

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Comment [41]: Where were these small sharks observed and can you use that information here to bring context to the size ranges you are describing?

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Comment [42]: To what?

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1967; Klimley, 1987; Richardson et al., 2000; Bansemer & Bennett, 2011). This is thought to be due to differences in diet or swimming capabilities or to reduce intra-specific competition, aggression and predation (Springer, 1967; Wearmouth & Sims, 2008). The reason for the prevalence of juvenile male whale sharks at known aggregation sites is unclear, and although different diet preferences for juveniles and adults has been suggested (Ketchum et al., 2012), this does not explain the sex bias. The segregation observed in Mozambique, Tanzania and elsewhere indicates that larger individuals and neonates use different habitats than juveniles. Similarly, mature sharks of both sexes are not often seen at coastal sites and may be completely oceanic. Although few data are available from the Indian Ocean, large mature sharks are regularly seen in the open ocean in other areas (Hazin et al., 2008; Hearn et al., 2013; Afonso, McGinty & Machete, 2014). Their larger size and superior swimming efficiency may enable them to move further horizontally and vertically and thus forage more successfully in a patchy offshore prey landscape (Sims et al., 2006).

Size at maturity

Our TL$_{50}$ of male whale sharks was 916 cm, ~100 cm larger than that visually estimated for Ningaloo Reef sharks (Norman & Stevens, 2007), and ~200 cm larger than those off the Yucatan coast of Mexico (Ramírez-Macías et al., 2012a). These large differences are potentially significant, and suggest genuine biological differences among sharks using these sites. Regional differences among life-history traits of elasmobranch species are not uncommon, and have been documented in bonnethead sharks *Sphyrna tiburo* (Lombardi-Carlson et al., 2003), greeneye spurdog shark *Squalus mitsukurri* and porbeagle sharks *Lamna nasus* (Francis & Duffy, 2005), and cownose rays *Rhinoptera bonasus* (Neer & Thompson, 2005), among others. Evidence from mitochondrial and microsatellite DNA showed that Atlantic and Indo-Pacific whale sharks never or rarely mix, while no evidence of stock structure was found within the Indian Ocean (Vignaud et al., 2014). The marked difference in TL$_{50}$ between Mozambique and the Yucatan coast of Mexico thus is consistent with these genetic results. It is unclear whether our photogrammetric results are directly comparable with the visual size estimates from Ningaloo Reef (Norman & Stevens, 2007) due to the differing methods employed. While genetic results do not support genetically distinct stocks of whale sharks within the Indian Ocean (Vignaud et al., 2014), photo-matching (Brooks et al., 2010) and tracking studies (Wilson et al., 2006; Sleeman et al., 2010) have not demonstrated any interchange between the eastern and western Indian Ocean populations. There may thus be population differentiation among Indian Ocean whale shark

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Comment [43]: May want to discuss why only adult females observed in offshore Mexico

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Comment [44]: I wouldn't mind seeing a percentage difference here to get a better grasp of how different the comparison is

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Comment [45]: What traits are you referring to here, we were discussing size

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Comment [46]: Maybe provide a suggestion for the selection pressures that would select for larger size in one region versus another.

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aggregations on a shorter time-scale than detected in genetic studies. A significant size-at-maturity difference between the eastern and western Indian Ocean, if it does exist, would suggest population-level separation within this ocean basin.

Mature female whale sharks are rarely observed and all sharks >900 cm observed in our study were male. While it is impossible to assess maturity in females externally, in the absence of visible pregnancy, females of 820 cm (this study), 870 cm (Beckley et al., 1997) and 880 cm (Pai, Nandakumar & Telang, 1983) examined in the Indian Ocean were immature. The only directly-measured mature female to date was 1060 cm (Joung et al., 1996), and mature females in the Gulf of California were visually-estimated at 900–1300 cm (Ramírez-Macías et al., 2012b). Potential stock differences notwithstanding, this suggests that none of the females in our study were mature.

Growth rates and age

We found a slow mean growth rate (5.6 cm year^{-1} ± 47.3 cm) and 6 sharks showed negative growth. While capture stress may either retard or potentially reverse growth in tag-and-release studies on sharks (Davenport & Stevens, 1988), the minimally-invasive nature of photogrammetry makes this implausible for whale sharks. Based on the assumption of annual band-pair formation in the vertebral centra, the back-calculated mean growth rate from whale sharks in northeastern South Africa was 21.45 cm yr^{-1} PCL or 27.0 cm yr^{-1} TL, assuming linear growth (PCL to TL regressions used from Wintner, 2000 and this study) or 28.8 cm yr^{-1} TL for directly-measured sharks (Wintner, 2000). Based on our results, laser photogrammetry would have been able to measure a growth rate of this magnitude over the time period of this study. One study has suggested that whale sharks have biannual band-pair formation (Hsu et al., in press), which would suggest the growth rate maybe twice as fast.

Our results do not support these growth estimates, although our resighting sample size was small (n = 13), and whale sharks are likely to have variable growth rates. Substantial variation in TL was evident in individuals with same band pair counts included in Wintner (2000) and this study, with three female sharks of 577 cm, 630 cm and 778 cm and a male of 866 cm all having 22 band pairs. Aside from counting errors (Cailliet et al., 2006), growth is probably associated with the condition of the individuals (Natanson et al., 2008), which in turn can be influenced by environmental variables, such as temperature, or by food availability (Stevenson & Woods, 2006; Hussey et al., 2009). The maximum longevity of whale sharks

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Comment [47]: Explain why this would not be detected with genetics? What would select for size that may not be captured with genetics? Food?

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Comment [48]: Judging by your confidence interval, I don't think you found any growth. Would be good to put into context the change in growth measured based on time between measurements. Was there a lot of variation with measurements taken very close together, which would be an indicator of lack of precision, or did you find that as measurements had greater time lapses, the growth rate estimates seemed to increase?

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Comment [49]: Is that a fair assumption, seems that sharks would grow rapidly at first and begin to slow with age, so possibly the growth rate of your animals had slowed way down.

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Comment [50]:

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has been estimated to be 80 years (Hsu et al. in press), so the relatively short timeframe of this study may have been insufficient for growth rate estimation. It is important to note that the basking shark *Cetorhinus maximus*, which is ecologically similar to the whale shark, and other orectolobiform sharks have asynchronous growth band deposition (Chidlow, Simpfendorfer & Russ, 2007; Natanson et al., 2008, Huveneers et al., 2013). Age estimation through vertebral counts has been further complicated in some long-lived shark species because bands either cease to be deposited or become unresolvable with age (Francis, Campana & Jones, 2007). The uncertainties that relate to growth and ageing based on vertebral band counts suggest that photogrammetry, applied to free-swimming whale sharks over long time frames, may provide the best means of determining age at maturity, growth rates and longevity in this species.

Laser photogrammetry, as implemented in this study, may also be too inaccurate to obtain valid growth measurements over short time frames. Although we determined the accuracy of measurement on a static target of known length it was not possible to know how accurate the technique was when applied to a free-swimming whale shark, as there was no way of knowing the true length of the subject shark. A complementary technique for size estimation, such as stereo-videography, is required to further validate the applicability of photogrammetry. It is also important to keep in mind that we only attempted to measure growth in a single dimension, length. It is entirely plausible that individual sharks may have increased significantly in mass, while showing little or no increase in length.

In conclusion, laser photogrammetry estimates are more accurate and precise than visual estimates of length and size at maturity, but we suggest that they are not used for growth rate estimates over short time periods. Accurate measurement of life-history parameters can improve demographic models for the whale shark and thus facilitate better assessment of its vulnerability to fishing pressures or recovery from population declines. We also show that the size range and sex ratio of whale sharks from Mozambique and Tanzania are similar to those at most other aggregation sites globally, in that the population consisted largely of ~450-950 cm juvenile sharks, most of which were males. The observed population segregation by size and sex reinforces the need to determine the whereabouts of young-of-the-year and small juvenile sharks, immature female sharks, and mature sharks of both sexes to improve conservation and management for this globally threatened species.

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Comment [51]: You still need to know the birth date of the shark or some type of age-related growth curve, measuring size alone won't help determine age

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Comment [52]: Also the shark has to be dead to get the age using vertebral bands, that's a negative

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Comment [53]: You may not be able to measure accuracy but you can measure precision (how variable are your repeated measurements) and then use accuracy on your static object as an estimate but highlight the parallax problem. You did calculate parallax error based on your static measurements, you could use that to infer parallax error, how likely were you to be at 5, 10, 20, 50 degree off axis when taking measurements? How difficult is it to line up perpendicular to your target?

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Comment [54]: I would remove this statement as it isn't really applicable to the growth rates.

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Comment [55]: To make this statement you would need to have people visually estimate the size of the pipe and compare those results to the laser photogrammetry results.

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Comment [56]: If the measurements are accurate and the sharks are growing extremely slow, you can still measure growth rate but it may be too small based on precision of the technique.

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Table 1. The size of whale sharks measured with laser-photogrammetry in Mozambique and Tanzania by sex, with male clasper measurements from South Africa included under Mozambique.

Mark Deakos 10/30/2014 12:03 AM
Deleted: and sex structure

	Total length (cm)			Clasper length (cm)		
	N (%)	Mean (±SD)	Range	N	Mean (±SD)	Range
MOZAMBIQUE						
Males	87 (75.7%)	692 (±119)	445 - 934	57	54 (20)	27 - 106
Females	28 (24.3%)	670 (±108)	439 - 858			
Total	123	684 (±118)	439 - 934			
TANZANIA						
Males	49 (87.5%)	660 (±131)	420 - 990	22	51 (15)	31 - 89
Females	7 (12.5%)	620 (±117)	541 - 871			
Total	56	655 (±129)	420 - 990			

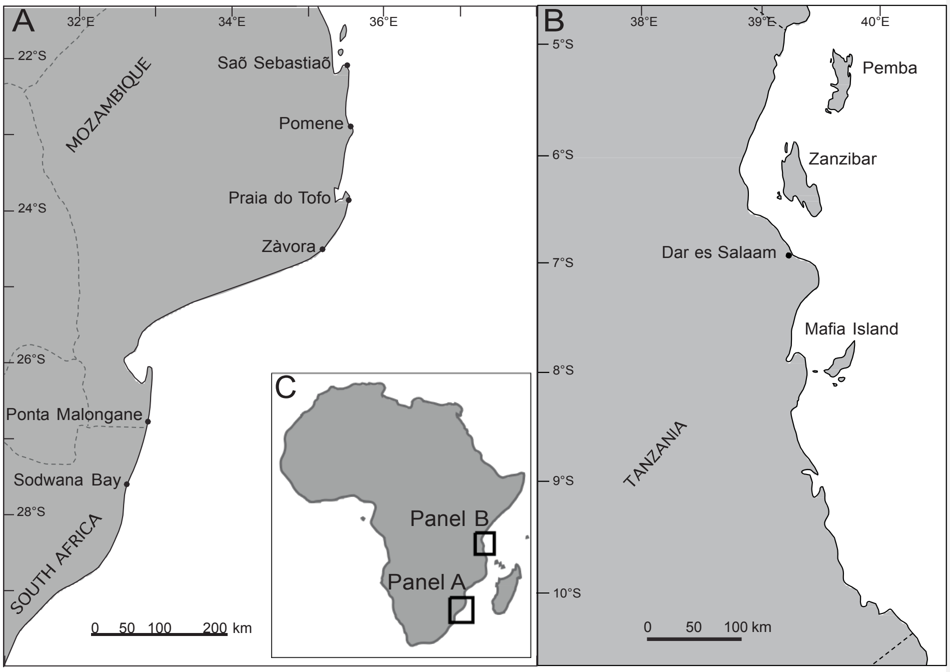

Fig. 1 The study locations off (A) Praia do Tofo in southern Mozambique and (B) Mafia Island in Tanzania, with (C) an inset of Africa for overview.

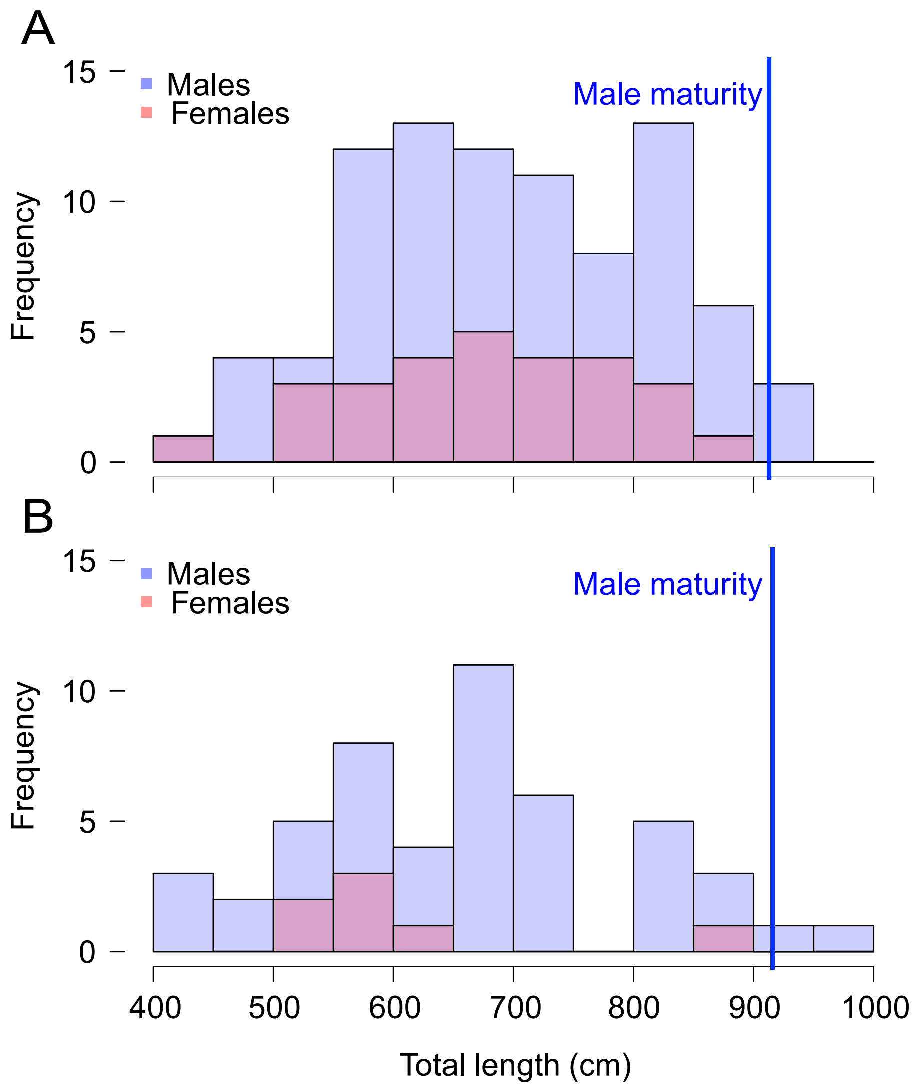

Fig. 2 The length-frequency of whale sharks (red = females, blue = males) measured with photogrammetry in (A) Mozambique and (B) Tanzania.

Mark Deakos 10/29/2014 10:07 PM
Comment [57]: Frequency seems more of a temporal measurement (count per unit of time), perhaps label as No. of sharks?

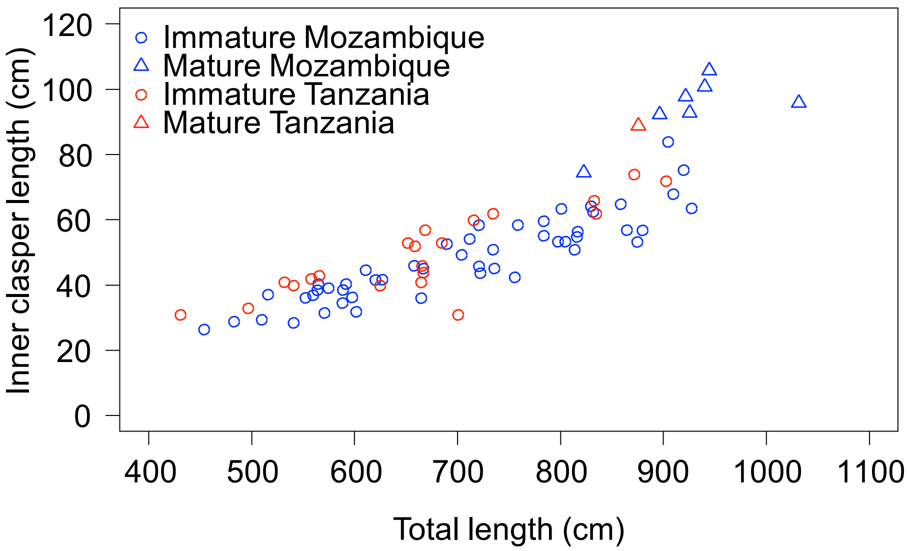

Fig. 3 Total length and inner clasper length of male whale sharks (¢=immature; **Δ**= mature) in Mozambique and South Africa (blue) and Tanzania (red).

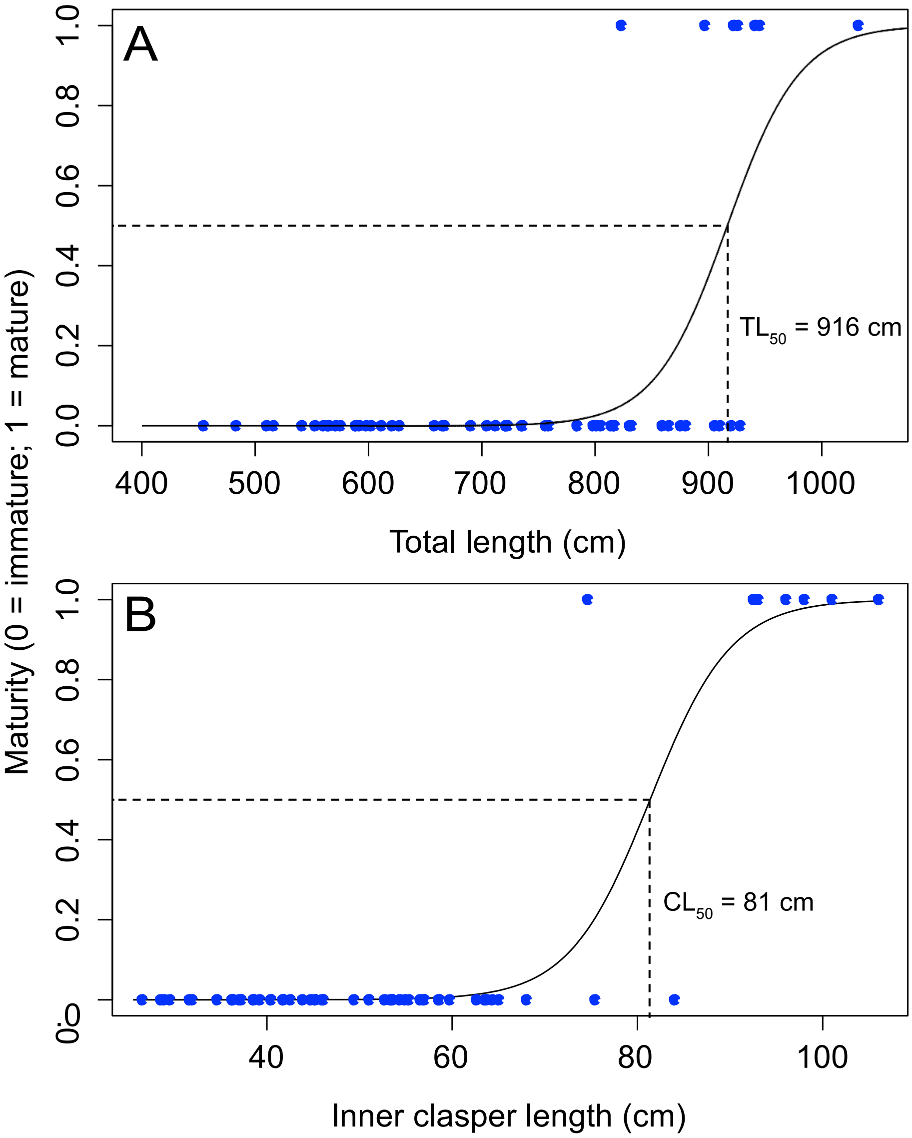

Fig. 4 Binary logistic plot of maturity in male whale sharks against (A) total length, with TL_{50} = 916 cm; and (B) inner clasper length, with CL_{50} = 81.0 cm.

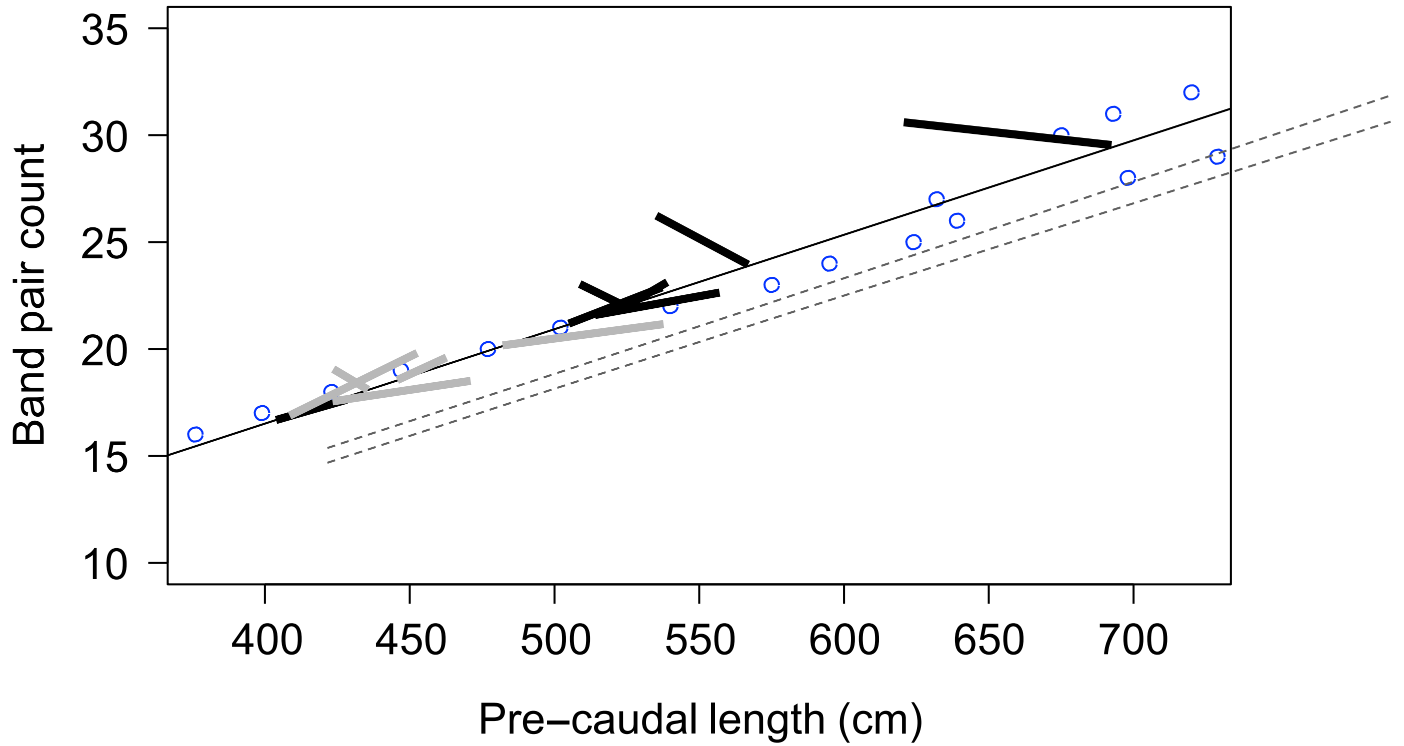

Fig. 5 Observed growth increments of male (black) and female (grey) whale sharks plotted as size at age based on back-calculated lengths from vertebral band pair counts (Wintner, 2000) with 95% CI indicated. The initial size measurement was placed on the PCL/band pair count regression line.

Mark Deakos 10/29/2014 10:19 PM
Comment [58]: By what, dashed lines?

Mark Deakos 10/29/2014 10:19 PM
Comment [59]: Dark line?

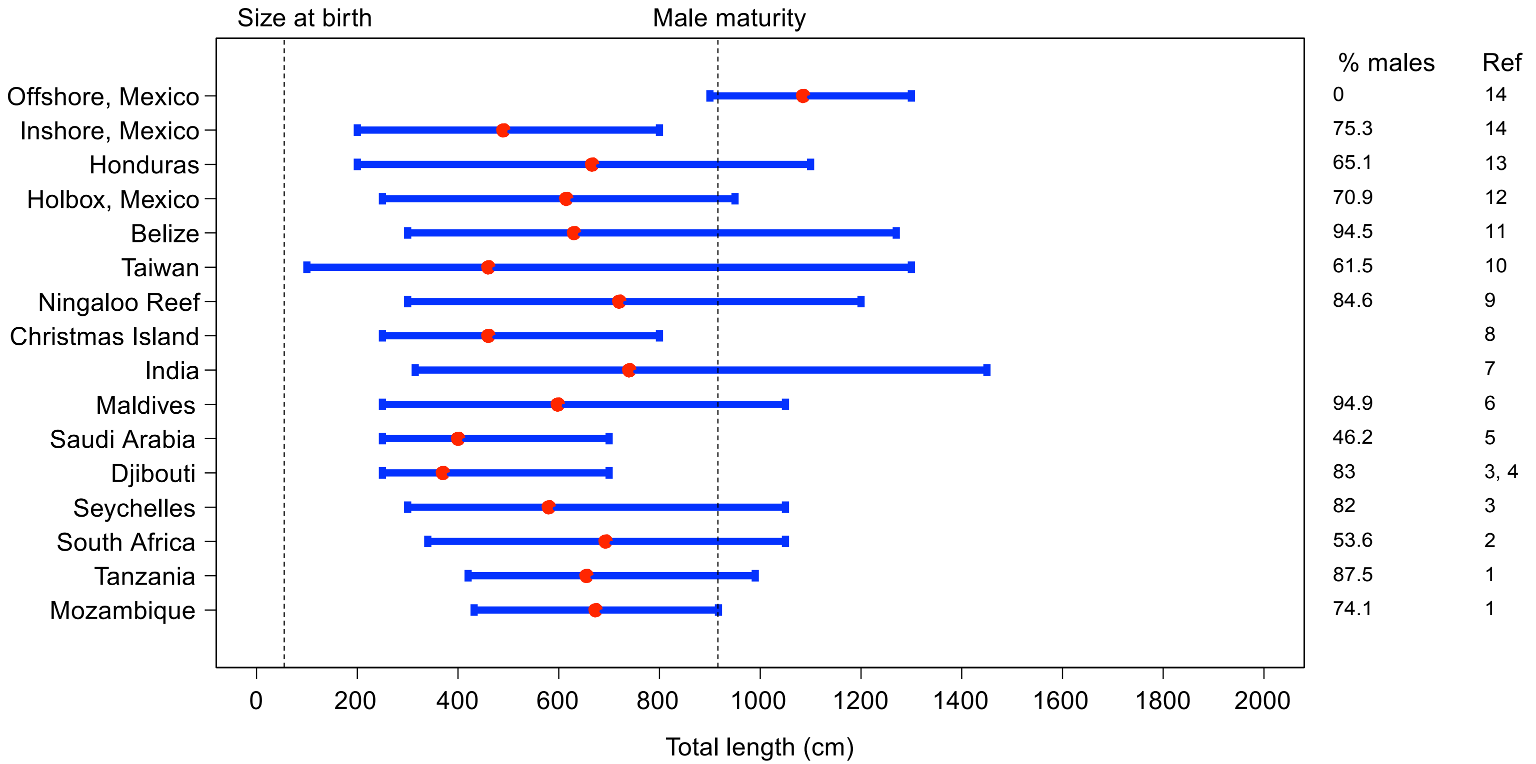

Fig. 6 The population structure of whale shark aggregations around the world, with mean total length in red and length range in blue, plotted on the total length range for the species. References: 1 (this study), 2 (Beckley et al., 1997), 3 (Rowat et al., 2011), 4 (Brooks et al., 2010), 5 (Berumen et al., 2014), 6 (Riley et al., 2010), 7 (Pravin, 2000), 8 (Hobbs et al., 2009), 9 (Norman & Stevens, 2007), 10 (Hsu et al., 2012), 11 (Graham & Roberts, 2007), 12 (Ramírez-Macías et al., 2012a), 13 (Fox et al., 2013), 14 (Ramírez-Macías et al., 2012b).

Mark Deakos 10/29/2014 10:54 PM
Comment [60]: Would be useful to see sample sizes for each of the ranges