

An integrated mark-recapture and genetic approach to estimate the population size of white sharks in South Africa

Sara Andreotti¹, Michael Rutzen², Stéfan van der Walt³, Sophie Von der Heyden¹, Romina Henriques¹, Michael Meÿer⁴, Herman Oosthuizen⁴, Conrad A. Mathee^{1,*}

¹Evolutionary Genomics Group, Department of Botany and Zoology, Private Bag X1, Stellenbosch University, Stellenbosch 7600, South Africa

²Shark Diving Unlimited, Kleinbaai, Gansbaai 7220, South Africa

³Applied Mathematics, Stellenbosch University, Matieland 7602, South Africa

⁴Department of Environmental Affairs, Branch Oceans and Coasts, Private Bag X2, Roggebaai 8012, South Africa

ABSTRACT: The loss of apex marine predators has been reported to have a cascade of detrimental effects on marine ecosystems; however, the general lack of empirical data can severely limit our understanding of the ecological interactions among marine species. In this study we propose an integrated approach using mark-recapture and genetic techniques to assess population estimates of white sharks *Carcharodon carcharias*. Between 2009 and 2011, 4389 dorsal fin photographic identifications were collected in Gansbaai, South Africa, from 426 white sharks and used in mark-recapture analyses. Saturation of new sightings occurred once 400 individuals were catalogued and the open population model POPAN suggested ranges between 353 and 522 individuals (95% confidence) and a point estimate of $N = 438$. Between 2010 and 2013, 302 biopsy samples were collected from 233 white sharks and used for a comparative genetic population estimate. Analyses of 14 microsatellite markers revealed a contemporary effective population size (CN_e) of 333 individuals (95% CI = 247–487, $p_{crit} = 0.02$). These values were at least 52% less than those estimated in previous mark-recapture studies. Using this combination of techniques, we propose a $N_e:N$ ratio of 0.76 for white sharks, which advances our ability to accurately make inferences on elasmobranch population numbers in general. Given the low population numbers of white sharks along the South African coastline, we predict a negative effect on the ecological stability of the marine environment in this region.

KEY WORDS: *Carcharodon carcharias* · Effective population size · Microsatellites · Photographic identification · Population abundance · Shark conservation

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

A delicate and complex balance exists between the ocean and its inhabitants. Sharks as top predators are particularly important since they directly influence the population size of prey communities, as well as prey behaviour and space utilization (see also 'risk effect' in Heithaus et al. 2008). The problems stemming from the loss of apex predators are complex,

but quantifiable negative implications for the marine ecosystem have been reported (Baum et al. 2003, Baum & Myers 2004, Myers et al. 2007, Ferretti et al. 2010, Heithaus et al. 2012). Generally, the population reduction of a top predator causes a collapse in resource species (consumers at lower trophic levels, like small teleosts or primary producers), by increasing the population of meso-consumers (predatory or herbivorous species at risk of predation from top

predators; Heithaus et al. 2008). The potential for meso-consumer dispersal in previously high predatory-risk areas can result in a cascade alteration of the local ecological structure and general functioning of marine communities (Heithaus et al. 2008, Ferretti et al. 2010).

Models used in ecology predict that sharks have a strong impact on marine ecosystems (Stevens et al. 2000, Kitchell et al. 2002, Okey et al. 2004), but without empirical data on population numbers (and reliable historical data), it is difficult to draw strong ecological conclusions (Ferretti et al. 2010). It is thus not surprising that elasmobranch population estimates are regarded as particularly important (Dudgeon & Ovenden 2015). The white shark *Carcharodon carcharias* has a circumglobal distribution (Compagno et al. 2001, Boustany et al. 2002, Bonfil et al. 2005, Domeier & Nasby-Lucas 2008, Jorgensen et al. 2010), is an apex marine predator and is currently listed as 'Vulnerable' (IUCN Red List, Category VU A1cd+2cd). The elusive nature of the species limits the collection of baseline information critically needed for its management and long-term survival. A recent phylogeographic study on South African white sharks, including samples collected at 5 aggregation sites (False Bay, Gansbaai, Struisbaai and De Hoop, Mossel Bay and Algoa Bay), revealed the presence of a panmictic population with frequent movement of individuals between sites (mtDNA: $\phi_{st} = 0.018$, $p = 0.71$; nDNA: $F_{st} = 0.0014$, $p = 0.38$; Andreotti et al. 2016, see also Johnson & Kock 2006). At the local scale, the South African population also shows very low genetic diversity, and despite the evidence for potential migrations across oceanic basins (e.g. between South Africa and Australia; Bonfil et al. 2005), they represent elements of a unique evolutionary lineage (Andreotti et al. 2016).

Previous studies suggest that the South African white shark population has a census size in the lower thousands: for example Cliff et al. (1996) based on 6 sharks recaptured from 73 tagged individuals estimated a mean of $N = 1279$. More recently, by making use of the unique trailing edge of the dorsal fin of white sharks (similarly used by Anderson et al. 2011, Chapple et al. 2011, Andreotti et al. 2014, Burgess et al. 2014) and the software DARWIN (Stanley 1995), Towner et al. (2013) suggested that in the Gansbaai region alone, the estimated total number of sharks ranges between 808 and 1008 individuals. Given the low numbers of opportunistic recaptures in the first study ($m_i = 6$; Cliff et al. 1996), and since the software DARWIN has previously been considered unreliable for matching white shark dorsal fins (Chapple et al. 2011), these estimates may not accurately reflect the

population status of the species in the region. In addition, these estimates were exclusively based on capture-mark-recapture (CMR) models, which can be problematic when dealing with elusive species (Schwartz et al. 2007).

Modern model-based CMR methods rely on a series of assumptions that are often difficult to achieve in the field, especially when the study species have large geographic distributions. The model assumptions include (1) that the tagging method will not affect subsequent catchability, (2) homogenous sampling and re-sampling of all individuals, (3) zero loss of tags, (4) that sampling is random and covers a representative area related to the distribution of the population, and (5) accurate designation of whether the population is closed or open (Sutherland 2006). Although non-invasive methods such as photo-identification techniques (Wursig et al. 1990, Whitehouse & Hall-Martin 2000) can assist in meeting some of these assumptions (Carbone et al. 2001, Jackson et al. 2006, Kelly et al. 2008, Schofield et al. 2008, Chapple et al. 2011, Marshall & Pierce 2012, Andreotti et al. 2014), it is virtually impossible to meet all the required criteria (also see Burgess et al. 2014). Thus, the integration of CMR with other techniques is particularly appealing when aiming to obtain a more robust assessment of the size of a population.

Genetic estimates of population size can provide a powerful alternative or supplement to the CMR model (Schwartz et al. 2007, Luikart et al. 2010). For example, studies on large vertebrates have used molecular fingerprinting of hair and/or faecal DNA for estimating population census size (Taberlet et al. 1999, Woods et al. 1999, Mowat & Strobeck 2000). In this instance 're-captures' are inferred from a multi-locus genotype (i.e. individual) captured 2 or more times in a single sampling section (Luikart et al. 2010). However, despite the promise of the method, these studies also suffer from several pitfalls including un-quantified genotyping error rates or biases related to mixed samples (Taberlet et al. 1999, Mills et al. 2000, Waits et al. 2001, McKelvey & Schwartz 2004, Roon et al. 2005). Many of these problems are largely due to human error and can be minimised by removing duplicate samples, re-genotyping and blind rescoring of genotypes.

The most common genetic technique to determine population estimates is based on measuring contemporary effective population size (CN_e). It approximates the mean number of breeding individuals contributing offspring per generation (Huson & Bryant 2006, Portnoy et al. 2009, Dudgeon et al. 2012) and is calculated based on the levels of pairwise linkage

disequilibrium (LD) between microsatellite loci (measured as the deviation between observed and predicted 2-locus genotype frequencies; Luikart et al. 2010, Waples et al. 2014). The CN_e has direct conservation relevance since a low number of breeders can increase the extinction risk of populations (Luikart et al. 2010). The only study to date documenting the effective population size of a white shark population was carried out in Australia but was based on only 6 microsatellite loci ($CN_e = 1512$; p_{crit} of 0.06, Blower et al. 2012).

Matching genetic data with phenotypic information (i.e. photographic identification and estimated length of the individuals sampled) should increase the precision of population size estimates. To gain more insight into the size of the South African white shark population, we assessed both population abundance (census size: N) and effective population size (N_e) in a combined framework. This study aims to (1) use photographic CMR and a genetic technique to evaluate the population size of white sharks occurring in the largest known aggregation area in South Africa (Gansbaai), (2) provide the first population estimate for white sharks along the entire coastline and (3) calculate the ratio between CMR estimates and genetic N_e estimates which can provide the baseline for future assessments of white shark population sizes. The results of this study provide powerful insights into the status of this top predator along the South African coastline, information essential for future management decisions when dealing with white sharks.

MATERIALS AND METHODS

Data collection

Between April 2009 and April 2014 photographic data and biopsy samples were collected by attracting white sharks close to a vessel with natural fish chum (Department of Environmental Affairs of South Africa Permit Numbers: RES2009/18, RES2010/71, RES2011/55, RES2012/38, RES2013/41, RES2014/39, RES2015/50). In CMR studies the frequency at which different individuals can be trapped may vary: some may become 'trap shy' (e.g. they avoid coming to the vessel after the first attraction) and others may become 'trap happy' (e.g. they tend to visit the vessel more because it is baited, thereby underestimating population size). The baited condition of this study exposed all individuals attracted (whether 'trapped' by the camera or not) to the same treatment, which is

comparable to 'setting the trap open and baited', a practice used to reduce 'trap happy' bias (Sutherland 2006). Dorsal fin photographs and tissue biopsies were collected from all free ranging individuals at 5 known white shark aggregation sites around the coastline: Gansbaai (34° 40' 614" S, 19° 23' 934" E), False Bay (34° 08' 036" S, 18° 34' 930" E), Struisbaai (34° 35' 403" S, 20° 24' 786" E), Mossel Bay (34° 08' 985" S, 22° 07' 220" E) and Algoa Bay (33° 44' 753" S, 26° 13' 523" E). Only photographs collected in Gansbaai were used for the mark-recapture analyses; these were collected over a 28 mo period (298 sampling days).

Capture-mark-recapture data

Dorsal fin pictures were taken in RAW format with high-resolution cameras following Andreotti et al. (2014). The sex and total length of each individual was documented by visually recording the presence or absence of claspers at the posterior portion of the pelvic fins and by comparing the size of the sharks (estimated from the rostrum to the higher part of the tail in natural position) with a fixed size length (see also Leurs et al. 2015). Photo-identification data collected during the study period were stored in a separate folder (referred to as 'the full database' in Andreotti et al. 2014) per individual shark, named after the date of first capture (i.e. for all the sharks captured on 5 March the 2 first numbers of their folders would be '0305...'). To separate each shark, the third number indicates the sequence in which the animal approached the vessel: the folder for Shark 4 for that day would then be '030504'). All subsequent photographs of the same shark obtained a re-sighting code (RS) based on the 'month/day' of recapture (i.e. the photograph of Shark 030504, re-captured on 11 April, was labelled: 030504_RS 0411). The use of a storage system together with the creation of a document summarizing all the additional morphological information (length and sex) allowed for confirmation of the match of the recaptured shark, since new visual matches could be visually compared to the suite of all the previous photographs of the same shark.

The final photo-identification dataset was double-checked manually for duplicates (e.g. false negative matches: same shark then catalogued as 2 different individuals) prior to CMR analyses. To minimise human error in the data analyses, an ad hoc algorithm was created in Python to extrapolate the recapture data directly from the storage folders of each

shark (van der Walt unpubl. data). The algorithm generated a binomial recapture history matrix (1: captured; 0: non-captured) of all the sharks (y -axis) captured on each sampling occasion (x -axis). The recapture history matrix generated by the ad hoc algorithm was manually double-checked by 2 of the authors.

The relatively small number of captures per capture occasion (averaging 4–5 sharks daily), for a prolonged period of time (298 d) can potentially inflate estimates of population numbers (Holmberg et al. 2008); therefore, the time intervals of the capture-recapture history matrix were collapsed from daily to monthly time intervals. Two history matrices for recaptures were generated: (1) per month (27 capture occasions) and (2) per winter season (3 capture occasions between May and July each year). Population sizes (N) were estimated based on these matrices using CMR models.

Since population size is a broad term that can vary across studies (Sutherland 2006, Luikart et al. 2010) and white sharks have long life spans (Calliet et al. 1985), low fecundity and low estimated natural mortality (Smith et al. 1998), in the present study population census (or abundance) was conservatively defined as the maximum number of individuals identified, after reaching saturation (Sutherland 2006). The population estimate N , based on CMR analyses of photographic identifications, is defined as the estimated number of all individuals in the study area. When model assumptions are met and a large number of individuals are captured, N should not differ significantly from the true population census (Sutherland 2006).

Due to the lack of phylogeographic structure around the South African coastline (regardless of the genetic marker used), Andreotti et al. (2016) suggested that the population sampled in Gansbaai can be considered an open system. However, to further assess which model was most appropriate for estimating population abundance, the history matrix was also subjected to a Close Test. The latter uses Otis et al. (1978) and Stanley & Burnham (1999) closure tests to detect closure violation in CMR datasets (Stanley & Richards 2005). The Otis et al. (1978) model allows for heterogeneity in capture probabilities under closure, and the Stanley & Burnham (1999) allows for time-specific variation in capture probabilities under closure (Stanley & Richards 2005). The results of both tests confirmed that the Gansbaai population is open, with both losses and additions to the area. The open population model POPAN provided in MARK, Version 6.1 (White & Burnham 1999), was thus chosen. The following assumptions were made:

(1) the marking (notches on the fin) remained constant during the sampling period (Anderson et al. 2011), (2) the photographic process did not affect the catchability of individuals (Chapple et al. 2011) and (3) the population numbers did not change sufficiently to influence the estimates during the 3 yr study period, due to the life-history features of white sharks (e.g. long life span coupled with low fecundity and low estimated natural mortality; Calliet et al. 1985, Smith et al. 1998).

The POPAN model estimates population size (N) based on apparent survival rate (Φ), probability of entry into the population (β) and capture probability (ρ). Different variations of the model were applied to both capture-recapture matrices (e.g. history matrix) of 27 monthly encounter occasions and the 3 winter season encounters, by setting Φ , β and ρ either to constant (.) or time dependent (t). Link functions were specified as the Logit link for Φ and ρ and the multinomial Logit (MLogit) link for β (GC White, Program MARK Help files). Selection of the most parsimonious model was based on Akaike's information criterion with correction for finite sampling sizes (AICc). Goodness of fit (GoF) of the data under the open population model assumptions was tested using Tests 2 and 3 in RELEASE GOF (available in MARK).

Genetic data

During 115 successful sampling occasions (field trips), a total of 302 tissue biopsies were collected from 233 unique white sharks (duplicates were confirmed using both photo-identification and genetic fingerprint techniques). Biopsy samples were taken from a small region at the base of the dorsal fin by making use of a 2.53 m long pole equipped with a sterilised biopsy sample; dermis tissue was then stored in 80% alcohol. In the laboratory, DNA was extracted using the DNeasy Blood and Tissue Extraction Kit (Qiagen) following the manufacturer's protocol. Fourteen microsatellite markers: Ccar1, Ccar13, Ccar6.27x, Ccar9, Iox10, Cca1419, Cca83, Cca1536, Cca1273, Cca711, Cca1072, Cca1466, Cca1276 and Cca1226 (Pardini et al. 2000, Gubili et al. 2011, O'Leary et al. 2013) were amplified in 3 multiplex and 1 simplex reaction (see Andreotti et al. 2016 for details; see Table S2 in the Supplement at www.int-res.com/articles/suppl/m552p241_supp.pdf).

Genotype scoring was performed in Geneious Version 5.6.5 (©2005–2012 Biomatters Ltd.). Assessment of amplification errors, such as large allele drop out, stuttering and null alleles was conducted in Micro-

checker Version 2.2.3 (Van Oosterhout et al. 2004). Deviations from the expectation of outcrossing (Hardy-Weinberg equilibrium) and linkage disequilibrium were determined in FSTAT Version 2.9.3.2 (Goudet 1995) and in Genepop Version 4.2.1 (Rousset 2008). Additional properties of the markers utilised are reported in Andreotti et al. (2016). The software SHAZA Version 1.0 (Macbeth et al. 2011) was used to assess duplicated samples based on genetics, and these were eliminated from subsequent analyses. The program COLONY (Jones & Wang 2010) was used to assess the relatedness among individuals.

Our dataset exceeded the minimum sample size (20% of the population) and number of markers (at least 10 loci) as set out by Dudgeon & Ovenden (2015). To maximise the power of the estimates (Dudgeon & Ovenden 2015), we analysed all uniquely identified sharks together (233 genotypes and 14 markers). Following Blower et al. (2012), the LD estimate of CN_e was determined using the program N_e Estimator Version 2.0 (Do et al. 2014) and represents the size of an ideal population that is experiencing a known amount of genetic drift. Random mating over monogamy was assumed, as this is the most likely system for white sharks (also see Blower et al. 2012). The software provides a range of CN_e point estimates (with 95% accuracy), and these values vary depending on the optimal allele frequency exclusion criterion p_{crit} (Waples 2006, Waples & Do 2010, Do et al. 2014). The large sample size (>100) and the 14 nuclear markers utilised in this study suggest that acceptable estimates of CN_e can be obtained with optimal allele frequency exclusion criteria $p_{crit} = 0.01$ and $p_{crit} = 0.02$ (Waples & Do 2010). However, for comprehensiveness we varied p_{crit} from 0.05 to 0+; CN_e estimates are listed in 'Results of the different CN_e values obtained varying p_{crit} ' in the Supplement. For large, long-lived animals with low fecundity such as elasmobranchs, estimates of N_e are expected to be similar to the census size N (Portnoy et al. 2009, Dudgeon & Ovenden 2015).

N_e was initially calculated for the entire dataset and then re-calculated for sub-portions of the dataset, based on the length of the sampled shark. The latter was performed to avoid biasing the estimate due to overlapping generations that can be found in iteroparous species (Waples & Do 2010, Waples et al. 2013, 2014). Thus, to give a broader perspective on the estimate of CN_e for all white sharks, we separately analysed the genotypes of sharks from different size groups. The 2 groups selected were: (1) adults (males ≥ 3.8 m and females ≥ 4.5 m; see Mollet et al. 1996) and (2) a single juvenile group (length ≥ 2.5 and ≤ 3.0 m). The sample sizes of adults ($n = 33$) and juve-

niles ($n = 66$) were still large enough to select the optimal estimate of CN_e at a p_{crit} of 0.02 (Waples & Do 2010). Although the sharks grouped as adults are likely to represent overlapping generations (due to the species' long lifespan), the juvenile group most likely includes individuals born approximately at the same time (Tanaka et al. 2011). In theory, CN_e calculated on a single age group reflects the effective number of breeders in 1 reproductive cycle (N_b).

The effective population size per generation (N_e) can also be determined by sampling a single age group (or cohort) when the ratio between N_e and N_b (effective number of breeders in 1 reproductive cycle) is known (Waples et al. 2013, 2014). The ratio between N_e and N_b varies among taxa, but it reflects the life traits (longevity and reproduction strategy) of the species investigated (Waples et al. 2013). This ratio is of particular value for conservation, especially when data are available for a single cohort of a long-lived species (Waples et al. 2013, 2014). Therefore, the $N_b:N_e$ ratios available in Waples et al. (2014) for bison (*Bison bison*, ratio = 0.966) and dolphin (*Tursiops truncatus*, ratio = 0.964), which have comparable life traits to white sharks, were used to calculate the true N_e , using the N_b estimated from the juvenile group. However, due to a 2-locus Wahlund effect (Nei & Li 1973, Sinnock 1975), even individuals sampled from a single age group can be a source of bias as they can derive from parents of different ages (i.e. different cohorts). Thus, we assessed the true N_e based on the raw N_b , and subsequently recalculated it, adjusting the N_b of the juvenile group by applying Eq. (8) from Waples et al. (2014). In the present study we used CN_e to replace N_e in the formula. Finally, we provided the $N_b:N_e$ ratio for white sharks calculated from our data, with the raw and adjusted value of N_b of the juvenile group and the N_e estimated by using all the samples together. The $N_e:N$ ratio was estimated with the N_e for all the samples and the N value given by the CMR analyses.

RESULTS

Capture mark-recapture population estimate

A total of 426 individual white sharks were recorded in Gansbaai from 4398 photographic identifications. The sex ratio (male:female) was 1:1.09, with a Gaussian distribution across the size ranges from 1.6 to 5 m (see also Fig. S1 in the Supplement. The number of 'informative photographs' obtained for CMR analyses was 1034, after excluding multiple

photographs of the same sharks collected on the same day. Three false negatives were found and excluded after manual checking (Andreotti et al. 2014).

A total of 58% of the sharks in the database were re-captured on 2–18 subsequent occasions (877 re-sighting / 1304 total capture events, see details in Table S1 and Figs. S2 & S3 in the Supplement. Forty-seven sharks identified in 2009 were recaptured in 2010, and 128 sharks from previous years were recaptured in 2011. The latter exceeded the number of newly identified sharks for the same year (n = 100). Saturation of new sightings occurred once 400 individuals were sampled (Fig. 1), indicating sufficient sampling effort (Sutherland 2006). RELEASE GOF Tests 2 and 3 suggested that the monthly capture history matrix was consistent with the open population model assumptions ($\chi^2 = 251.9$; $p < 0.005$). Irrespective of the history matrix used (per month or per winter season) the most parsimonious model for the POPAN variants, provided in MARK Version 6.1 (White & Burnham 1999), was when the capture probability (ρ) was time dependent, and the survival rate (Φ) and the probability of entry into the population (β) were kept constant (Table 1). As expected, the population estimate derived from the larger monthly capture history matrix (n = 426) showed a narrow interval ranging between N = 429 and N = 431 ($\chi^2 = 0.66 \times 10^6$), while the same estimate from the smaller dataset comprising the winter season (n = 255), ranged between N = 353 and N = 522 ($\chi^2 = 0.34 \times 10^3$) (Table 1).

Genetic population estimate

Likelihood-based genotype matching identified 60 duplicated samples among all the tissue biopsies taken. These duplicate samples were indicated blindly

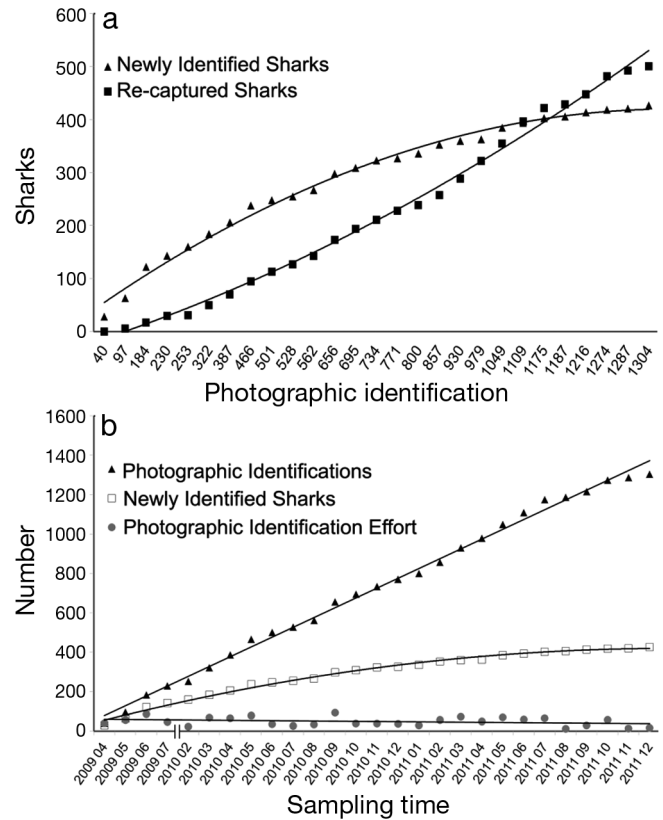


Fig. 1. *Carcharodon carcharias*. (a) Inverse relationship between newly sighted and re-captured individuals. (b) Cumulative curves of photographic identifications, newly identified sharks and photographic identification effort measured as the effective number of sampling days

and subsequently re-confirmed by photo-identification (Fig. 2). The duplicated samples were removed from all further analyses, leaving 233 sharks in the genotype database. The adult group (males ≥ 3.8 m and females ≥ 4.5 m) included 33 individuals, and the juvenile group (length between 2.5 and 3 m) included 66 sharks.

Table 1. *Carcharodon carcharias*. Selected parameter estimates and model selection criteria for 4 POPAN model variants for the 2 capture history matrices. The 2 sampling intervals (SI) include 27 capture occasions in the monthly dataset and 3 capture occasions in the seasonal dataset. Survival rate (Φ), probability of entry into the population (β) and capture probability (ρ) are assumed either constant (.) or time dependent (t). The population estimate (\hat{N}) and interval (N-i) are calculated with CI = 95%. The models are sorted by model likelihood (ML). Bold print indicates the most parsimonious model for the POPAN variants based on ML. AIC_c: Akaike's corrected information criterion

SI	Model	AIC _c	Δ AIC _c	AIC _c weight	ML	Deviance	\hat{N}	N-i
Monthly	{$\Phi(\cdot)\rho(t)\beta(\cdot)$}	3963.86	0	1	1	0	430 ± 1	429–431
	{ $\Phi(t)\rho(t)\beta(t)$ }	39961.08	35997.21	0	0	35710.3	3161 ± 150	2867–3455
	{ $\Phi(t)\rho(\cdot)\beta(t)$ }	40177.58	36213.71	0	0	35987.16	2715 ± 131	2457–2972
Seasonal	{$\Phi(\cdot)\rho(t)\beta(\cdot)$}	347.55	0	0.51	1	0	438 ± 42	353–522
	{ $\Phi(t)\rho(\cdot)\beta(t)$ }	347.75	0.2	0.28	0.9	0	471 ± 45	382–531
	{ $\Phi(\cdot)\rho(t)\beta(t)$ }	349.31	1.73	0.21	0.4	0	408 ± 36	336–479

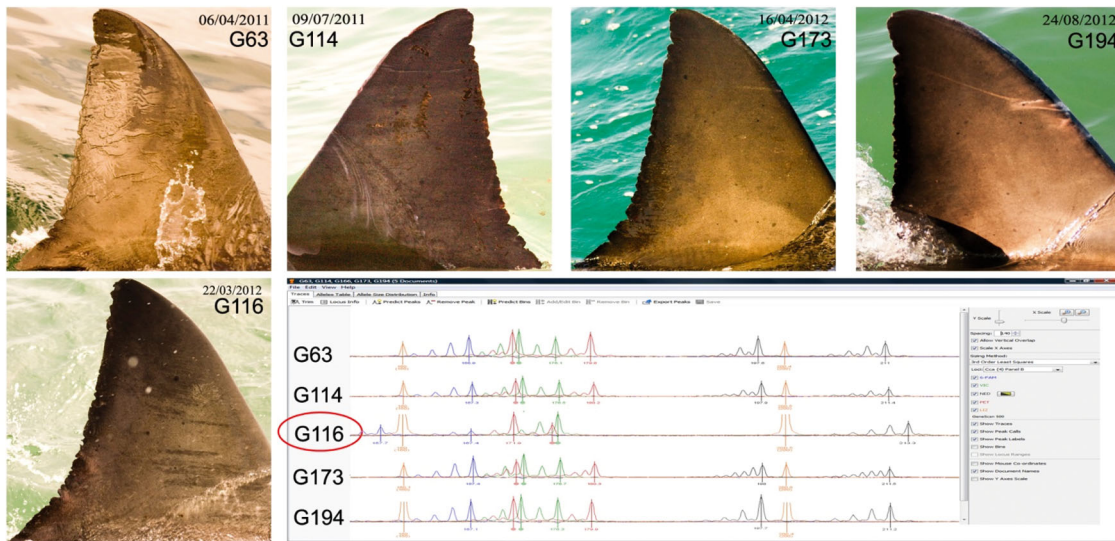


Fig. 2. *Carcharodon carcharias*. Correspondence between photo-identification and genetic fingerprint of a shark (C_050606B) sampled on 4 different occasions across 2 yr. Each genetic sample has been uniquely coded (G63, G114, G173 and G194) to allow for blind scoring of duplicates using a genetic fingerprint. The sample G116 belongs to a different individual (C_040705), as confirmed by the genetic profile and the different notch pattern on the dorsal fin

Overall, microsatellite genotypes did not exhibit evidence of amplification errors, or linkage disequilibrium. Four loci (Ccar1419, Ccar1072, Ccar1466 and Ccar9) consistently exhibited deviations from Hardy-Weinberg equilibrium in both tests for some of the populations analysed ($p < 0.05$ —see Tables S3–S6). These results were not statistically significant once we applied a correction for multiple tests ($p \geq 0.001$, as implemented in FSTAT—see Table S3 in the Supplement). The loci Ccar1419 and Ccar1466 exhibited evidence of a heterozygote excess, which may be linked to a small effective population size (Waples 2015), while loci Ccar1072 and Ccar9 exhibited signs of heterozygote deficit. When the overall sample was analysed, only loci Ccar1072 and Ccar9 still exhibited deviations from Hardy-Weinberg equilibrium (see Tables S5 & S6 in the Supplement), due to a deficit of heterozygotes linked to the presence of

null alleles (Andreotti et al. 2016). However, since there were no signs of inconsistencies in scoring, the frequency of null alleles was found to be $< 5\%$, and no significant deviations were observed when all samples and loci were analysed together (exact tests: heterozygote excess $p = 0.280$ and heterozygote deficit $p = 0.720$, as implemented in GENEPOP), the 2 markers were retained in subsequent analyses (Andreotti et al. 2016).

Estimates of CN_e for Gansbaai alone ($n = 167$) obtained a point estimate of 351 (95% CI = 247–642, $p_{crit} = 0.02$). The estimate for the entire coastline ($n = 233$) provided a point estimate of 331 (95% CI = 252–468) at $p_{crit} = 0.01$ and 333 (95% CI = 247–487) at $p_{crit} = 0.02$. The estimate based on the adult group ($n = 33$) provided a point estimate of 200 (95% CI = 68 – ∞ , $p_{crit} = 0.02$), while the estimate based on the juvenile group ($n = 66$) was 303 (95% CI = 152–3048,

Table 2. *Carcharodon carcharias*. Estimate of the contemporary effective population size (CN_e) for South African white sharks based on 14 microsatellite loci. The CN_e estimate for a single age group (Juv. group) represents the number of breeders that contributed to the selected group (N_b). To adjust for the Wahlund effect, the raw N_b value of the juvenile group was transformed with Eq. (8) from Waples et al. (2014). The $N_b:N_e$ ratio for white sharks was then calculated between N_e calculated with all sharks and the N_b raw and adjusted (*). The $N_e:N$ ratio was calculated between CN_e of all the sharks and the population size estimate based on CMR ($N = 438$)

Samples	Size (m)	N	p_{crit}	CN_e	95% CI	N_b	$N_b:N_e$	$N_e:N$
All sharks	All	233	0.02	333	247–487	–	–	0.76
Gansbaai	All	167	0.02	351	233–642	–	–	–
Adults	(female ≥ 4.5 ; male ≥ 3.8)	33	0.02	200	68 – ∞	–	–	–
Juv. group	(between 2.5 and 3)	66	0.02	303	151–3049	293*	-0.87*, 0.90	–

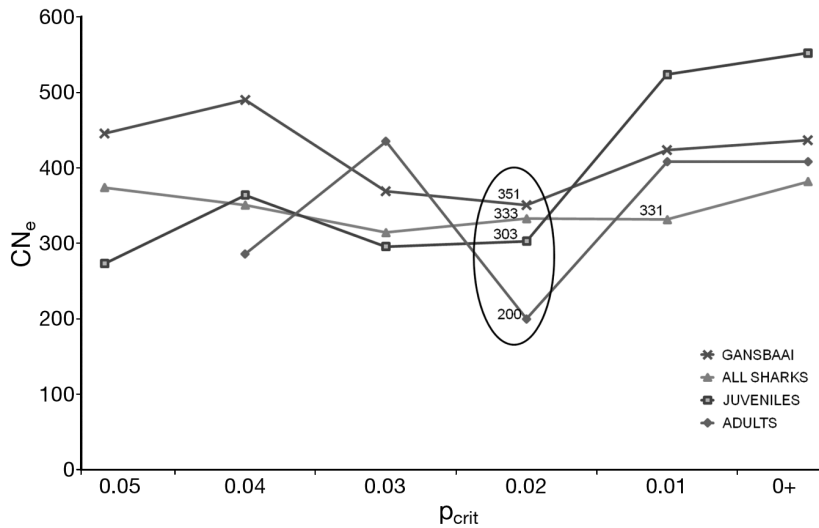


Fig. 3. *Carcharodon carcharias*. Comparison of the contemporary effective population size (CN_e) estimates of white sharks in South Africa, based on the complete dataset (all sharks, $n = 233$), the dataset from Gansbaai alone ($n = 167$), the size group of juveniles ($n = 66$) selected between 2.5 and 3 m in length and the adults alone ($n = 33$), when selecting different p_{crit} values ranging from 0.05 to 0+

$p_{crit} = 0.02$). Results obtained using the conversion formula based on the ratio for bison (0.966) or dolphin (0.964) and the raw N_b of the juvenile group were $N_e = 314$ and $N_e = 303$, for the adjusted N_b . The raw and adjusted values for N_b of the juvenile group, the $N_b:N_e$ ratio and the $N_e:N$ ratios are provided in Table 2 (variations of p_{crit} from 0.05 to 0+ are provided in Fig. 3 and Table S7). The relatedness among individuals could not be accurately determined due to limited samples and limited variability in the data.

DISCUSSION

One of the key components for the management and conservation of rare and endangered species is the use of reliable information based on established techniques and comprehensive datasets (Keith et al. 2011). The ability to estimate population size parameters has vastly improved over the past decade (Luikart et al. 2010); however, empirical and simulated results need to be integrated in order to improve their overall reliability. The results of this study provide novel population estimates relevant to the conservation of white sharks, with a striking overlap in population size estimates derived from genetic ($CN_e = 333$; 95% CI = 247–487) and mark recapture ($N = 438$; 95% CI = 353–522) methods. These values are also in line with the census number of individuals (426 sharks) reported in Andreotti et al.

(2014). The congruence in estimates is, however, in contrast with the reported difference between N and CN_e for other marine species, where N can be 2–6 orders of magnitude higher than CN_e (Hill 1981, Hoarau et al. 2005, Waples 2006, Hauser & Carvalho 2008, Waples & Do 2010). This similarity in estimates may have resulted from an inflated value of N_e due to gene flow from another population not yet sampled (Hare et al. 2011). However, we believe that it is most likely influenced by the different life-history traits of elasmobranchs when compared to other marine fishes, in particular the slow mode of reproduction (Hoenig & Gruber 1990). Indeed, congruence between effective population size and census size has also been reported for sandbar sharks (*Carcharhinus plumbeus*; Portnoy et al. 2009), grey nurse sharks (*C. taurus*; Ahonen et al. 2009), zebra sharks (*Stegostoma fasciatum*; Dudgeon & Ovenden 2015) and reef manta rays (*Manta alfredi*; Ovenden 2014). Hence our findings support the notion that agreement between these 2 measures is to be expected in marine species with high or constant lifetime survivorship and low fecundity rates (Luikart et al. 2010). Direct comparison of the $N_e:N$ ratio across studies must be approached with caution due to possible differences in the interpretation of 'N' as the number of adults only (Waples et al. 2013), the census size (Ahonen et al. 2009, Portnoy et al. 2009, Dudgeon & Ovenden 2015), or the population estimate (present paper). The $N_e:N$ ratio of 0.76 for white sharks presented in this study should thus be seen in context and can only provide a baseline for future population assessments where the same values are employed. Despite this, it is interesting to note that the $N_e:N$ ratio of white sharks estimated in this study falls between 0.5 and 1, as also observed for sandbar sharks (Portnoy et al. 2009) and zebra sharks (Dudgeon & Ovenden 2015). We attribute this to the long life span and late maturation of white sharks rather than the mode of reproduction (Dudgeon & Ovenden 2015). When sampling a single age group, however, the genetic signal shows that the number of successful breeders which contributed to this group was $N_{b(Adj)} = 293$, in a population conservatively estimated to be between 353 and 522 individuals (all size classes). If this estimate is correct, it would suggest that nearly all individuals contributed

equally to the next generation. Since the number of adults observed in the CMR study is much lower than this value, it is instead possible that the adult population experienced a drastic decline in numbers during the last few generations (Antao et al. 2011). N_e is known to lag behind contemporary changes in the population size (Antao et al. 2011, Waples et al. 2013, 2014), which would explain the observed overlap between the CMR and genetic estimates. Another informative value for future population assessments, particularly useful for species in which only 1 age group can be sampled, is the ratio between N_e and effective number of breeders in 1 reproductive cycle (N_b). This has been examined for several taxa and can be calculated if there is available knowledge of life-history features (e.g. age at maturity, life span; Waples et al. 2013, 2014). Unfortunately white shark longevity and age of maturity are currently unknown, but we could calculate the true N_e by applying the conversion formula of Waples et al. (2013) based on the ratios of bison and dolphin. Using N_b estimated from 1 age group we obtained a true $N_e = 314$ which does not differ significantly from $N_e = 333$ when all individuals were included. The latter finding suggests that the sampled non-mature individuals were likely to only contain a number of cohorts approximately equal to the generation length (Waples et al. 2014) and that the 6% of adults included in the dataset were probably not enough to cause an underestimation of N_e (Waples et al. 2014).

Furthermore the estimated $N_b:N_e$ ratio, either when using the adjusted $N_{b(Adj)}$ ($N_b:N_e = 0.87$), or when using the raw N_b ($N_b:N_e = 0.90$), is similar to the ratio observed for other elasmobranchs (with long life span, low fecundity, late maturity and high survivorship to adult stage; Dudgeon & Ovenden 2015) and for long-lived marine mammals such as dolphins (ratio = 0.90; Waples et al. 2013, 2014). The estimates based on adults only ($CN_e = 200$; 95% CI = 68– ∞) were lower than the other estimates, but in line with the study of Waples et al. (2014), who found that in every modelled species (in which the number of cohorts within the adult group does not correspond closely to the generation length), N_e based on randomly sampled adults is 25–30% lower than the true N_e ; the upper value of ∞ in the confidence interval is a reflection of the small sample size of the subsample (Do et al. 2014, Dudgeon & Ovenden 2015).

The comparison between datasets not only provides corroborating evidence for population estimates, but also a mean to optimise each method and to test the validity of some of the assumptions made. CMR studies in general have the disadvantage of possibly

missing some individuals due to differences in behavioural patterns among individuals (Sperone et al. 2010, Delaney et al. 2012, Jacoby et al. 2014) or even weather conditions (Marshall & Pierce 2012, Andreotti et al. 2014). The advantage of employing molecular markers when using the linkage disequilibrium method is that estimates are not based on recapture, but information contained in the genetic diversity of the sampled individuals. It is thus very unlikely to mistake a population with a moderately small CN_e with one with a large CN_e (Waples & Do 2010). In our study, the information derived from the photographic dataset highlights that most of the assumptions for estimating CN_e from microsatellite analyses were met. Specifically, equal sex ratio could be confirmed for our population (1:1.09), non-overlapping age groups (cohorts) could be selected with reasonable certainty by knowing the size of the sharks sampled and duplicate sampling (and genotype errors) could be avoided by cross checking the 2 datasets. Thus, some of the difficulties associated when dealing with elusive and difficult to sample species could be minimised by combining the 2 techniques.

Our CMR result, supported by genetic evidence, is in sharp contrast to the recent study by Towner et al. (2013), who estimated 808–1008 sharks in the Gansbaai region. We employed exactly the same models in the CMR analyses, the time period of sampling overlapped, and many of our sharks were sampled in the same geographic area. The only clear difference between the 2 studies is the usage of the software DARWIN (Stanley 1995) by Towner et al. (2013) and the manual fin matching technique (Andreotti et al. 2014) used in the present study. For Towner et al. (2013), no mention is made regarding the accuracy of the DARWIN software (no mention of the rankings given), and we propose that the discrepancy between results is more than likely due to the inclusion of false negatives in the previous dataset (Sutherland 2006). To test this prediction, we validated the accuracy of DARWIN by making use of our own dataset. A total of 426 individual high-quality photographs were used to test-match 122 additional images from 53 unique sharks. A paired *t*-test was used to validate the null hypothesis that the software DARWIN could score the correct matching image from the database within the top 20 ranked positions (see details of the test in Fig. S5). 'Fin trace' failed to automatically draw an accurate edge around the dorsal fin, and manual optimization was needed in each instance (Fig. S4 in the Supplement). The paired *t*-test rejected the null hypothesis that DARWIN can successfully match a white shark dorsal fin image in the

upper range of up to 20 ranked images (t -stat = 5.73, $p = 3.72 \times 10^{-8}$, see details in the Supplement), providing empirical support for the notion that the software is not suitable for automated white shark identification (Chapple et al. 2011). Circumstantial evidence for the inclusion of false negatives (resulting in an inflated number for N) can also be found in the fact that the curve of newly identified sharks in the Towner et al. (2013) study never reached saturation over a 3 yr period (Fig. 2 in Towner et al. 2013, cf. Fig. 1 in the present study).

In the present study, the CN_e point estimates and the confidence intervals for including individuals from the entire coastline ($n = 233$) or just from Gansbaai ($n = 167$) were remarkably close (Coastline $CN_e = 333$, 95% CI = 247–487, $p_{crit} = 0.02$; Gansbaai $CN_e = 351$, 95% CI = 234–642, $p_{crit} = 0.02$), with an expected broader CI for Gansbaai alone, since the precision of the linkage-disequilibrium method increases proportionally to the number of samples and markers used (Waples & Do 2010, Dudgeon et al. 2012, Do et al. 2014). If these values are correct, it seems reasonable to suggest that sharks sampled at Gansbaai over a 3 yr period are likely representative of the entire South African population. These findings are also well supported by a mtDNA and microsatellite study showing panmixia among sampling sites (Andreotti et al. 2016), previous acoustic tagging studies (Bonfil et al. 2005) and also photo-identification evidence (Andreotti et al. 2016).

The magnitude of both the population estimate (N) and the effective population size (CN_e) and their ratio can assist management actions directed towards maintaining genetic variation (Cooper et al. 2010). In particular, it has been proposed that N_e values of at least 500 are needed for a species to retain long-term evolutionary potential (Franklin 1980, Frankham et al. 2010). If this holds, the effective population size of white sharks along the south African coastline (irrespective of whether the total data or the age group data are used) never exceeded 350 ($CN_{e(All)} = 333$; $CN_{e(Adults)} = 200$; $CN_{e(Juvenile\ group)} = 303$; true N_e (inferred from the juvenile group) = 314; see also Fig. 3). These low numbers are further supported by the CMR estimate of <500 white sharks. The drivers for such low numbers could have been the depletion of food resources (Stevens et al. 2000, Anticamara et al. 2011), bioaccumulation of pollutants (Cliff et al. 1989, Compagno et al. 1997, Chapman et al. 2003, Worm et al. 2013), and also direct anthropogenic mortality (between 1978 and 2008 approximately 1063 white sharks were killed as part of shark protection measures by the Kwazulu Natal Shark Boards alone; Peschak 2009).

Regardless of the driver of population decline, the low estimates of CN_e show that the white shark population in South Africa could potentially face serious difficulties in the future.

Carcharodon carcharias has been legally protected in South African waters since 1991, but, based on the results of this study, a more concerted effort should be made to protect the environment and resources utilised by white sharks. We predict that failure to do so will result in the complete loss of this vulnerable predator along the South African coastline. Considerable genetic evidence suggests that, despite being highly mobile, white shark populations across the globe do not form a single panmictic population (Pardini et al. 2001, Jorgensen et al. 2010, Blower et al. 2012, Andreotti et al. 2016). Southern African white sharks appear to have closest genetic connectivity with the Northwest Atlantic population and likely represent a recent founder population that is genetically distinct from other regions (Andreotti et al. 2016). In fact, the majority of white shark populations worldwide would qualify as distinct management units and therefore deserve separate management plans (Ryder 1986, Palsbøll et al. 2007, Andreotti et al. 2016). The ecological consequences of the loss of a top predator such as white sharks should not be underestimated, especially when only considering direct predation at the lower levels of the trophic nets. In a South African context, the reduction of white shark numbers will impact the numbers and space utilization of meso-consumers such as the Cape fur seal (*Arctocephalus pusillus pusillus*; also see Heithaus et al. 2008). In addition, long-lived species such as marine mammals are predicted to invest more heavily in anti-predator behaviour than shorter lived ones (Heithaus et al. 2008). Given that Cape fur seals seasonally change the utilization of refugia based on the presence of white sharks around their colonies (Wcisel et al. 2015), the predator loss in this specific case can severely affect the community by increasing the foraging area of Cape fur seals and ultimately eliminating spatial refugia for resource species (see also Heithaus et al. 2008, 2012). An empirical effect of the loss of large sharks in South Africa has already been documented in the 50 yr shark netting program of the Kwazulu-Natal Province: from 1956 to 1976 the number of large sharks caught in nets declined by >99% (Ferretti et al. 2010). The reduction of large predatory sharks in turn benefited the survival of smaller and more resilient shark pups, as indicated by the reporting of catches during inshore fishing competitions in the same areas (van Der Elst 1979). The latter may have also caused a substantial in-

crease of smaller elasmobranchs and a reduction in the catch of bony fishes (van Der Elst 1979).

In conclusion, the results of our study paint a gloomy picture for the future persistence of white sharks in South Africa. The survival of this population and the ecological structure of the coastline will be seriously compromised if management measures aimed at halting population declines are not improved in the short term and maintained into the future.

Acknowledgements. We thank Shark Diving Unlimited for financial support, the vessels, logistical assistance and the help of its staff, particularly L. Rutzen and J. Grodes. We thank F. Rutzen, T. Dose, J. Burger, J. de Bruyn, J. P. Botha, S. McCue, K. Moss and S. Muller for assistance with fieldwork. Dr. Steve Kirkman provided valuable insights in the usage of the software MARK, and Dr. Craig O'Connell provided valuable comments on an earlier draft of this paper. Thank you to Kerry Moss for helping with the validation of the program DARWIN. We thank Ruth-Mary Fisher, South African National Parks and Cape Nature for the permits to sample in protected areas. The authors are also grateful for the comments of 3 anonymous reviewers who greatly helped to improve this manuscript. S.A. was financed by the Department of Botany and Zoology Postgraduate Bursary Scheme, and laboratory work was funded through an NRF incentive to C.A.M.

LITERATURE CITED

- Ahonen H, Harcourt RG, Stow AJ (2009) Nuclear and mitochondrial DNA reveals isolation of imperilled grey nurse shark populations (*Carcharias taurus*). *Mol Ecol* 18: 4409–4421
- Anderson SD, Chapple TK, Jorgensen SJ, Klimley AP, Block BA (2011) Long-term individual identification and site fidelity of white sharks, *Carcharodon carcharias*, off California using dorsal fins. *Mar Biol* 158:1233–1237
- Andreotti S, Rutzen M, Wesche PS, O'Connell CP, Meÿer M, Oosthuizen WH, Matthee CA (2014) A novel categorisation system to organize a large photo identification database for white sharks *Carcharodon carcharias*. *Afr J Mar Sci* 36:59–67
- Andreotti S, von der Heyden S, Henriques R, Rutzen M, Meÿer M, Oosthuizen H, Matthee CA (2016) New insights into the evolutionary history of white sharks, *Carcharodon carcharias*. *J Biogeogr* 43:328–339
- Antao T, Pérez-Figueroa A, Luikart G (2011) Early detection of population declines: high power of genetic monitoring using effective population size estimators: early detection of population declines. *Evol Appl* 4:144–154
- Anticamara JA, Watson R, Gelchu A, Pauly D (2011) Global fishing effort (1950–2010): trends, gaps, and implications. *Fish Res* 107:131–136
- Baum JK, Myers RA (2004) Shifting baselines and the decline of pelagic sharks in the Gulf of Mexico: pelagic shark declines. *Ecol Lett* 7:135–145
- Baum JK, Myers RA, Kehler DG, Worm B, Harley SJ, Doherty PA (2003) Collapse and conservation of shark populations in the Northwest Atlantic. *Science* 299:389–392
- Blower D, Pandolfi J, Bruce B, Gomez-Cabrera M, Ovenden J (2012) Population genetics of Australian white sharks reveals fine-scale spatial structure, transoceanic dispersal events and low effective population sizes. *Mar Ecol Prog Ser* 455:229–244
- Bonfil R, Meÿer M, Scholl MC, Johnson R and others (2005) Transoceanic migration, spatial dynamics, and population linkages of white sharks. *Science* 310:100–103
- Boustany AM, Davis SF, Pyle P, Anderson SD, Le Boeuf BJ, Block BA (2002) Expanded niche for white sharks. *Nature* 415:35–36
- Burgess GH, Bruce BD, Cailliet GM, Goldman KJ and others (2014) A re-evaluation of the size of the white shark (*Carcharodon carcharias*) population off California, USA. *PLoS ONE* 9:e98078
- Cailliet GM, Natanson LJ, Weldon BA, Ebert DA (1985) Preliminary studies on the age and growth of the white shark, *Carcharodon carcharias*, using vertebral bands. *Mem South Calif Acad Sci* 9:49–60
- Carbone C, Christie S, Conforti K, Coulson T and others (2001) The use of photographic rates to estimate densities of tigers and other cryptic mammals. *Anim Conserv* 4:75–79
- Chapman DD, Abercrombie DL, Douady DJ, Pikitch EK, Stanhope MJ, Shiviji MS (2003) A streamlined, bi-organelle, multiplex PCR approach to species identification: application to global conservation and trade monitoring of the great white shark, *Carcharodon carcharias*. *Conserv Genet* 4:415–425
- Chapple TK, Jorgensen SJ, Anderson SD, Kanive PE, Klimley AP, Botsford LW, Block BA (2011) A first estimate of white shark, *Carcharodon carcharias*, abundance off central California. *Biol Lett* 7:581–583
- Cliff G, Der Elst RP Van, Govender A, Witthuhn TK, Bullen EM (1996) First estimates of mortality and population size of white sharks on the South African coast. In: Klimley AP, Ainley DG (eds) *Great white sharks: the biology of Carcharodon carcharias*. Academic Press, San Diego, CA, p 393–400
- Cliff G, Dudley SFJ, Davis B (1989) Sharks caught in the protective gill nets off Natal, South Africa. 2. The great white shark *Carcharodon carcharias* (Linnaeus). *S Afr J Mar Sci* 8:131–144
- Compagno LJ, Marks MA, Fergusson IK (1997) Threatened fishes of the world: *Carcharodon carcharias* (Linnaeus, 1758) (Lamnidae). *Environ Biol Fishes* 50:61–62
- Compagno LJV, Dando M, Fowler S (2001) *Sharks of the world. An annotated and illustrated catalogue of shark species known to date. Bullhead, mackerel and carpet sharks: (heterodontiformes, lamniformes and orectolobiformes)*. Food and Agriculture Organization of the United Nations, Rome
- Cooper AM, Miller LM, Kapuscinski AR (2010) Conservation of population structure and genetic diversity under captive breeding of remnant coaster brook trout (*Salvelinus fontinalis*) populations. *Conserv Genet* 11:1087–1093
- Delaney DG, Johnson R, Bester MN, Gennari E (2012) Accuracy of using visual identification of white sharks to estimate residency patterns. *PLoS ONE* 7:e34753
- Do C, Waples RS, Peel D, Macbeth GM, Tillett BJ, Ovenden JR (2014) NeEstimator v2: re implementation of software for the estimation of contemporary effective population size (N_e) from genetic data. *Mol Ecol Resour* 14:209–214
- Domeier M, Nasby-Lucas N (2008) Migration patterns of white sharks *Carcharodon carcharias* tagged at Guadalupe Island, Mexico, and identification of an east-

- ern Pacific shared offshore foraging area. *Mar Ecol Prog Ser* 370:221–237
- Dudgeon CL, Ovenden JR (2015) The relationship between abundance and genetic effective population size in elasmobranchs: an example from the globally threatened zebra shark *Stegostoma fasciatum* within its protected range. *Conserv Genet* 16:1443–1454
- Dudgeon CL, Blower DC, Broderick D, Giles JL and others (2012) A review of the application of molecular genetics for fisheries management and conservation of sharks and rays. *J Fish Biol* 80:1789–1843
- Ferretti F, Worm B, Britten GL, Heithaus MR, Lotze HK (2010) Patterns and ecosystem consequences of shark declines in the ocean: ecosystem consequences of shark declines. *Ecol Lett* 13:1055–1071
- Frankham R, Ballou JD, Briscoe DA (2010) Introduction to conservation genetics, 2nd edn. Cambridge University Press, Cambridge
- Franklin IR (1980) Evolutionary change in small populations. In: Soulé ME, Wilcox BA (eds) Conservation biology: an evolutionary-ecological perspective. Sinauer, Sunderland, MA, p 135–150
- Goudet J (1995) FSTAT (Version 1.2): a computer program to calculate *F*-statistics. *J Hered* 86:485–486
- Gubili C, Bilgin R, Kalkan E, Karhan SU and others (2011) Antipodean white sharks on a Mediterranean walkabout? Historical dispersal leads to genetic discontinuity and an endangered anomalous population. *Proc R Soc B* 278:1679–1686
- Hare MP, Nunney L, Schwartz MK, Ruzzante DE and others (2011) Understanding and estimating effective population size for practical application in marine species management: applying effective population size estimates to marine species management. *Conserv Biol* 25:438–449
- Hauser L, Carvalho GR (2008) Paradigm shifts in marine fisheries genetics: Ugly hypotheses slain by beautiful facts. *Fish Fish* 9:333–362
- Heithaus MR, Frid A, Wirsing AJ, Worm B (2008) Predicting ecological consequences of marine top predator declines. *Trends Ecol Evol* 23:202–210
- Heithaus MR, Wirsing AJ, Dill LM (2012) The ecological importance of intact top-predator populations: a synthesis of 15 years of research in a seagrass ecosystem. *Mar Freshw Res* 63:1039–1050
- Hill WG (1981) Estimation of effective population size from data on linkage disequilibrium. *Genet Res* 38:209
- Hoarau G, Boon E, Jongma DN, Ferber S and others (2005) Low effective population size and evidence for inbreeding in an overexploited flatfish, plaice (*Pleuronectes platessa* L.). *Proc R Soc B Biol Sci* 272:497–503
- Hoenig JM, Gruber SH (1990) Life-history patterns in the elasmobranchs: implications for fisheries management. NOAA Tech Rep NMFS 90:1–15
- Holmberg J, Norman B, Arzoumanian Z (2008) Robust, comparable population metrics through collaborative photo-monitoring of whale sharks *Rhincodon typus*. *Ecol Appl* 18:222–233
- Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. *Mol Biol Evol* 23:254–267
- Jackson RM, Roe JD, Wangchuk R, Hunter DO (2006) Estimating snow leopard population abundance using photography and capture recapture techniques. *Wildl Soc Bull* 34:772–781
- Jacoby DMP, Fear LN, Sims DW, Croft DP (2014) Shark personalities? Repeatability of social network traits in a widely distributed predatory fish. *Behav Ecol Sociobiol* 68:1995–2003
- Johnson R, Kock A (2006) South Africa's white shark cage-diving industry: Is there cause for concern? Finding a balance: white shark conservation and recreational safety in the inshore waters of Cape Town, South Africa. In: Nel DC, Peschack TP (eds) Proceedings of a specialist workshop. WWF South Africa Report Series – 2006/Marine/001, p 3
- Jones OR, Wang J (2010) COLONY: a program for parentage and sibship inference from multilocus genotype data. *Mol Ecol Resour* 10:551–555
- Jorgensen SJ, Reeb CA, Chapple TK, Anderson S and others (2010) Philopatry and migration of Pacific white sharks. *Proc R Soc B* 277:679–688
- Keith DA, Martin TG, McDonald-Madden E, Walters C (2011) Uncertainty and adaptive management for biodiversity conservation. *Biol Conserv* 144:1175–1178
- Kelly MJ, Noss AJ, Di Bitetti MS, Maffei L and others (2008) Estimating puma densities from camera trapping across three study sites: Bolivia, Argentina, and Belize. *J Mammal* 89:408–418
- Kitchell JF, Essington TE, Boggs CH, Schindler DE, Walters CJ (2002) The role of sharks and longline fisheries in a pelagic ecosystem of the central Pacific. *Ecosystems* 5: 202–216
- Leurs G, O'Connell CP, Andreotti S, Rutzen M, Vonk Noordegraaf H (2015) Risks and advantages of using surface laser photogrammetry on free-ranging marine organisms: a case study on white sharks *Carcharodon carcharias*. *J Fish Biol* 86:1713–1728
- Luikart G, Ryman N, Tallmon DA, Schwartz MK, Allendorf FW (2010) Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches. *Conserv Genet* 11:355–373
- Macbeth GM, Broderick D, Ovenden JR, Buckworth RC (2011) Likelihood-based genetic mark-recapture estimates when genotype samples are incomplete and contain typing errors. *Theor Popul Biol* 80:185–196
- Marshall AD, Pierce SJ (2012) The use and abuse of photographic identification in sharks and rays. *J Fish Biol* 80: 1361–1379
- McKelvey KS, Schwartz MK (2004) Genetic errors associated with population estimation using non-invasive molecular tagging: problems and new solutions. *J Wildl Manag* 68:439–448
- Mills LS, Citta JJ, Lair KP, Schwartz MK, Tallmon DA (2000) Estimating animal abundance using noninvasive DNA sampling: promise and pitfalls. *Ecol Appl* 10:283–294
- Mollet HF, Cailliet GM, Klimley AP, Ebert DA, Testi AD, Compagno LJV (1996) A review of length validation methods and protocols to measure large white sharks. In: Klimley AP, Ainley DG (eds) Great white sharks: the biology of *Carcharodon carcharias*. Academic Press, San Diego, CA, p 91–107
- Mowat G, Strobeck C (2000) Estimating population size of grizzly bears using hair capture, DNA profiling, and mark-recapture analysis. *J Wildl Manag* 64:183–193
- Myers RA, Baum JK, Shepherd TD, Powers SP, Peterson CH (2007) Cascading effects of the loss of apex predatory sharks from a coastal ocean. *Science* 315:1846–1850
- Nei M, Li WH (1973) Linkage disequilibrium in subdivided populations. *Genetics* 75:213–219
- O'Leary SJ, Feldheim KA, Chapman DD (2013) Novel microsatellite loci for white, *Carcharodon carcharias* and

- sandtiger sharks, *Carcharias taurus* (order Lamni-formes). *Conserv Genet Resour* 5:627–629
- Okey TA, Banks S, Born AF, Bustamante RH and others (2004) A trophic model of a Galápagos subtidal rocky reef for evaluating fisheries and conservation strategies. *Ecol Modell* 172:383–401
- Otis DL, Burnham KP, White GC (1978) Statistical inference for capture data on closed animal populations. *Wildl Monogr* 62:3–135
- Ovenden JR (2014) Elasmobranchs are ideal for studying the relationship between abundance and genetic effective population size. Oral presentation for the Shark International Conference, Durban, South Africa. www.sharksinternational.org/Documents/SI%202014%20Oral%20Presentations%20Program%20and%20abstracts.pdf
- Palsbøll PJ, Berube M, Allendorf F (2007) Identification of management units using population genetic data. *Trends Ecol Evol* 22:11–16
- Pardini AT, Jones CS, Scholl MC, Noble LR (2000) Isolation and characterization of dinucleotide microsatellite loci in the great white shark, *Carcharodon carcharias*. *Mol Ecol* 9:1176–1178
- Pardini AT, Jones SC, Noble RL, Kreiser B and others (2001) Sex-biased dispersal of great white sharks. *Nature* 412:139–140
- Peschak T (2009) Shark nets—the real killers of the sea? *Afr Geogr* 17:36–44
- Portnoy DS, McDowell JR, McCandless CT, Musick JA, Graves JE (2009) Effective size closely approximates the census size in the heavily exploited western Atlantic population of the sandbar shark, *Carcharhinus plumbeus*. *Conserv Genet* 10:1697–1705
- Roon DA, Thomas ME, Kendall KC, Waits LP (2005) Evaluating mixed samples as a source of error in non invasive genetic studies using microsatellites. *Mol Ecol* 14:195–201
- Rousset F (2008) Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Mol Ecol Resour* 8:103–106
- Ryder OA (1986) Species conservation and systematics: the dilemma of subspecies. *Trends Ecol Evol* 1:9–10
- Schofield G, Katselidis KA, Dimopoulos P, Pantis JD (2008) Investigating the viability of photo-identification as an objective tool to study endangered sea turtle populations. *J Exp Mar Biol Ecol* 360:103–108
- Schwartz MK, Luikart G, Waples R (2007) Genetic monitoring as a promising tool for conservation and management. *Trends Ecol Evol* 22:25–33
- Sinnock P (1975) The Wahlund effect for the two-locus model. *Am Nat* 109:565–570
- Smith SE, Au DW, Show C (1998) Intrinsic rebound potentials of 26 species of Pacific sharks. *Mar Freshw Res* 49:663–678
- Sperone E, Micarelli P, Andreotti S, Spinetti S and others (2010) Social interactions among bait-attracted white sharks at Dyer Island (South Africa). *Mar Biol Res* 6:408–414
- Stanley R (1995) DARWIN: identifying dolphins from dorsal fin images. Eckerd College, St. Petersburg, FL
- Stanley TR, Burnham KP (1999) A closure test for time-specific capture-recapture data. *Environ Ecol Stat* 6:197–209
- Stanley TR, Richards JD (2005) Software review: a program for testing capture-recapture data for closure. *Wildl Soc Bull* 33:782–785
- Stevens JD, Bonfil R, Dulvy NK, Walker PA (2000) The effects of fishing on sharks, rays, and chimaeras (chondrichthyans), and the implications for marine ecosystems. *ICES J Mar Sci* 57:476–494
- Sutherland WD (2006) *Ecological census techniques: a handbook*, 2nd edn. Cambridge University Press, Cambridge
- Taberlet P, Waits LP, Luikart G (1999) Non-invasive genetic sampling: Look before you leap. *Trends Ecol Evol* 14:323–327
- Tanaka S, Kitamura T, Mochizuki T, Kofuji K (2011) Age, growth and genetic status of the white shark (*Carcharodon carcharias*) from Kashima-nada, Japan. *Mar Freshw Res* 62:548–556
- Towner AV, Wcisel MA, Reisinger RR, Edwards D, Jewell OJD (2013) Gauging the threat: the first population estimate for white sharks in South Africa using photo identification and automated software. *PLoS ONE* 8:e66035
- van Der Elst RP (1979) A proliferation of small sharks in the shore-based Natal sport fishery. *Environ Biol Fishes* 4:349–362
- van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Notes* 4:535–538
- Waits LP, Luikart G, Taberlet P (2001) Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Mol Ecol* 10:249–256
- Waples RS (2006) A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. *Conserv Genet* 7:167–184
- Waples RS (2015) Testing for Hardy-Weinberg proportions: Have we lost the plot? *J Hered* 106:1–19
- Waples RS, Do C (2010) Linkage disequilibrium estimates of contemporary N_e using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. *Evol Appl* 3:244–262
- Waples RS, Luikart G, Faulkner JR, Tallmon DA (2013) Simple life-history traits explain key effective population size ratios across diverse taxa. *Proc R Soc B* 280(20131339):1–9
- Waples RS, Antao T, Luikart G (2014) Effects of overlapping generations on linkage disequilibrium estimates of effective population size. *Genetics* 197:769–780
- Wcisel M, O'Riain MJ, de Vos A, Chivell W (2015) The role of refugia in reducing predation risk for Cape fur seals by white sharks. *Behav Ecol Sociobiol* 69:127–138
- White GC, Burnham KP (1999) Program MARK: survival estimation from populations of marked animals. *Bird Study* 46:S120–S139
- Whitehouse AM, Hall-Martin AJ (2000) Elephants in Addo Elephant National Park, South Africa: reconstruction of the population's history. *Oryx* 34:46–55
- Woods JG, Paetkau D, Lewis D, McLellan BN, Proctor M, Strobeck C (1999) Genetic tagging of free-ranging black and brown bears. *Wildl Soc Bull* 27:616–627
- Worm B, Davis B, Kettner L, Ward-Paige CA and others (2013) Global catches, exploitation rates, and rebuilding options for sharks. *Mar Policy* 40:194–204
- Wursig B, Jefferson TA, Hammond PS, Mizroch SA, Donovan GP (1990) Methods of photo-identification for small cetaceans. In: Donovan GP (ed) *Reports of the International Whaling Commission*, Cambridge, Special 12:42–43