Title: Effective number of white shark (*Carcharodon carcharias,* Linneaus) breeders is stable over four successive years in the population adjacent to eastern Australia and New Zealand

Article type: Original research

Running Head: *C. carcharias* number of breeders

Keywords: effective population size; effective number of breeder, population genetics; conservation; monitoring

Authors:
Danielle Davenport[a], Paul Butcher[b], Sara Andreotti[c], Conrad Matthee[c], Andrew Jones[a], Jennifer Ovenden[a]

Corresponding Author:
Danielle Davenport, Molecular Fisheries Laboratory, School of Biomedical Sciences, University of Queensland, St. Lucia, Australia.
Email: danielle.davenport@uq.edu.au; Voice: +61 4322030509

Paul Butcher <paul.butcher@dpi.nsw.gov.au>
Sara Andreotti <andreottisara@gmail.com>
Conrad Matthee <cam@sun.ac.za>
Andrew Jones <andrewthomasjones@gmail.com>
Jennifer Ovenden < j.ovenden@uq.edu.au>

[a] Molecular Fisheries Laboratory and Schools of Biomedical Sciences, University of Queensland, St. Lucia 4072, Australia
[b] New South Wales Department of Primary Industries, PO Box 4321, Coffs Harbour, NSW 2450, Australia
[c] Evolutionary Genomics Group, Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Stellenbosch 7600, South Africa

Animal Ethics Permit Information

NSW DPI provided scientific (Ref. P01/0059(A)), Marine Parks (Ref. P16/0145-1.1) and Animal Care and Ethics (ACEC Ref. 07/08) permits

Conflicts of Interest

This project was funded by the New South Wales Department of Primary Industries, Australia.
The authors have no conflicts of interest to declare.

Contributions

D.D performed sample preparation, data analysis, simulations and wrote the manuscript. AJ calculated combined estimates. J.O. & P.B coordinated and led the project. P.B collected NSW samples. S.A and C.M collected SA samples and performed microsatellite genotyping. All authors contributed to and approved the final manuscript.

Abstract

Population size is a central parameter for conservation, however monitoring abundance is often problematic for threatened marine species. Despite substantial investment in research, many marine species remain data-poor resulting in uncertain population forecasts and restricting the evaluation of past and present conservation actions. Such is the case for the white shark (*Carcharodon carcharias*), a highly mobile apex predator for whom population monitoring is a conservation priority following substantial declines recorded through the 20th century. Here, we estimate the effective number of breeders that successfully contribute offspring in one reproductive cycle (Nb), providing a snapshot of recent reproductive effort in an east-Australian New Zealand population of white shark. Nb was estimated over four consecutive age cohorts (2010, 2011, 2012, 2013) using two genetic estimators (linkage-disequilibrium; LD and sibship assignment; SA) based on genetic data derived from two types of genetic markers (single-nucleotide-polymorphisms; SNPs and microsatellite loci). While estimates of Nb using different marker types produced comparable estimates, microsatellite loci were the least precise. The LD and SA estimates of Nb within cohorts using SNPs were comparable, for example the 2013 age-cohort $Nb(SA)$ was 289 (95%CI 200-461) and $Nb(LD)$ was 208.5 (95%CI 116.4-712.7). We show that over the time period studied Nb was stable and ranged between 206.1(±45.9) and 252.0(±46.7) per year using a combined estimate of $Nb(LD + SA)$ from SNP loci, and a simulation approach showed that in this population effective population size (Ne) per generation can be expected to be larger than Nb per reproductive cycle. This study demonstrates how breeding population size can be monitored over time to provide insight into the effectiveness of

69 recovery and conservation measures for the white shark, where the methods described here may
70 be applicable to other data-poor species of conservation concern.
71

Introduction

Assessing the size of natural populations is a key objective of monitoring programs which are vital for understanding the conservation status of species, the regulating effects of biotic and abiotic factors, and for the assessment of management efforts (Lindenmayer et al., 2020). However, for many marine populations, there is a lack of consistent monitoring programs at appropriate spatial and temporal scales for conservation and policy needs (Papa, Oosting, Valenza-Troubat, Wellenreuther, & Ritchie, 2020). This presents a significant problem for chondrichthyans (sharks, skates, rays and chimaeras), where more than half of known species are characterised by insufficient data and one-quarter are estimated to be at risk of extinction (Dulvy et al., 2014). Within the elasmobranchs (sharks, skates and rays), each contributes significantly to connect ecosystems and regulate marine food webs (Heupel, Knip, Simpfendorfer, & Dulvy, 2014). However, habitat loss and continued pressures on mortality though bycatch and targeted-fishing have resulted in many populations of elasmobranchs being depleted at a rate that exceeds their natural recovery potential (Worm et al., 2013). Given the significant challenges facing elasmobranchs and the importance of their role in regulating marine ecosystems, improvements for monitoring changes in natural populations is critical.

Monitoring threatened elasmobranch species is particularly challenging for many reasons. In the case of the white shark, *Carcharodon carcharias* (Linnaeus, 1758), where monitoring is a both a social and conservation priority, efforts to establish long term population trends have been hampered by issues including detectability [misidentification in photo-ID surveys (Burgess et al., 2014), lack of re-sightings in mark-recapture studies (Gore, Frey, Ormond, Allan, & Gilkes, 2016),

effects of environment on heterogeneity of behaviour (Jacoby, Croft, & Sims, 2012) and a lack of catch statistics (i.e. illegal, unregulated and unreported fishing). The need for alternate methods to index shark populations has therefore led to the increasing use of molecular markers to evaluate change and inform management (Blower, Pandolfi, Bruce, Gomez-Cabrera, & Ovenden, 2012; Bruce et al., 2018; Hillary et al., 2018). In this study, we focus on the concept of genetic effective population size (herein effective population size − *Ne*), which can be used to evaluate change in abundance from allele frequencies (Schwartz, Luikart, & Waples, 2007). When populations are small, genetic models predict that the evolutionary force of genetic drift (stochastic changes in allele frequencies) will predominate over other evolutionary forces such as natural selection, to reduce genetic diversity, population viability and evolutionary potential (Frankham, 1996; Franklin, 1980). The extent to which a population is vulnerable to such effects is inversely related to the magnitude of *Ne*, where the effects of drift will occur more slowly in populations with larger effective sizes than those with smaller effective sizes (Wang, 2005). When a genetic sample contains only individuals from a single age-cohort (a group of individuals having the same age-class), then the estimate of *Ne* corresponds to the effective number of breeders (*Nb*) which contributed offspring to that cohort (Wang, Santiago, & Caballero, 2016; Waples, Luikart, Faulkner, & Tallmon, 2013). For long-lived, iteroparous species, estimates of *Nb* are generally considered more useful for monitoring as they apply to a single breeding season and represent an accessible parameter for monitoring population trends at ecological timescales most relevant to conservation and management needs (Ovenden et al., 2016; Schwartz et al., 2007; Waples & Do, 2008). Past research has confirmed the power and usefulness of *Nb* as a tool to

monitor population trends (Antao, Pérez-Figueroa, & Luikart, 2011; Nunziata & Weisrock, 2018). For instance, quantifying changes in *Nb* over time has helped to identify factors relevant to shaping populations (i.e. management interventions, demographic parameters) with successful outcomes reported for populations of commercially important bony fishes. Examples include salmon (Bacles et al., 2018; Perrier, April, Cote, Bernatchez, & Dionne, 2016), trout (Ruzzante et al., 2019; Whiteley et al., 2013; Wood, Belmar-Lucero, Hutchings, & Fraser, 2014), snapper (Jones et al., 2019) and tuna (Waples, Grewe, Bravington, Hillary, & Feutry, 2018). In these examples, both *Nb* and *Ne* were used to investigate demographic (i.e. variance in reproductive success under commercial harvest conditions) and environmental (i.e. stream productivity, competition, habitat quality, year-of-the-young development) effects on long-term population viability, with significant implications for management and conservation.

In this study, we trialled a sampling and genotyping protocol aimed at estimating *Nb* over four breeding seasons (2010-2013) in a population of *C. carcharias* of conservation concern. We focus on the east Australia New-Zealand population (EAP) of *C. carcharias* which, due to patterns of coastal residency and site fidelity (Bruce, Harasti, Lee, Gallen, & Bradford, 2019; Spaet, Patterson, Bradford, & Butcher, 2020) is genetically distinct from other identified populations in the North-Pacific, South-West Australia, Atlantic, South Africa, and Mediterranean (Andreotti et al., 2016; Blower et al., 2012; Gubili et al., 2010; O'Leary et al., 2015; Tanaka, Kitamura, Mochizuki, & Kofuji, 2011). The EAP has experienced a large (greater than 90%) decline during the 20th century due to targeted fishing and mortalities associated with bather protection programs (Reid, Robbins, & Peddemors, 2011; Roff, Brown, Priest, & Mumby, 2018), however recovery is now

anticipated due to protection through international conventions and jurisdictional legislature [i.e. International Plan of Action for the Conservation and Management of Sharks (FAO, 2000) and the Environment Protection and Biodiversity Conservation (EPBC) Act of 1999 (EPBC, 1999)]. Previous efforts to detect population recovery using historical catch data (Roff et al., 2018) and genetic close-kin-mark-recapture (Bruce et al., 2018; Hillary et al., 2018) found no significant evidence of population growth or recovery in the EAP. Updated bather-protection programs along parts of east coast Australia (i.e. SMART drumlines, see Tate et al., 2019) aimed at minimising unfavourable interactions with marine environment users offer an opportunity for non-lethal tissue sampling and to determine the usefulness of this genetic monitoring method in the EAP. Our specific objectives were to: (i) use two genetic methodologies to estimate Nb over time in the EAP [sibship assignment (SA) (Wang, 2009) and linkage-disequilibrium (LD) (Hill, 1974, p. 197; Waples, 2006)]; (ii) validate these results using two types of nuclear genetic markers (single-nucleotide polymorphisms and microsatellites); (iii) investigate $\frac{Nb}{N}$ ratios using published estimates of the adult population size (*Na*) and (iv) develop expectations for generational Ne in the EAP using life-history information and simulations. Our results for the EAP of *C. carcahrias* suggest that Nb has not changed significantly year-to-year and provides insight into the effectiveness of recovery and conservation measures following historical declines.

153 ## *Methods*

154 ### *Tissue Sampling*

155 To obtain genetic data to estimate Nb in the east coast Australia-New Zealand population
156 of *C. carcharias* (EAP) tissue samples (*n* = 247) were non-lethally collected during 2015 to 2018
157 from juvenile and sub-adult *C. carcharias* between Buckley Beach, Narrawalle (-35.29873,
158 150.48331) and Seven Mile Beach, Lennox Head (-28.76130, 153.62020). Individuals were
159 captured, restrained, tagged and released as part of the New South Wales (Australia) Shark
160 Management Strategy. Fin-clips were collected for genetic purposes and fork length (FL) and total
161 length (TL) measurements were taken from each individual. Since migration between populations
162 can bias genetic estimates of both Ne and Nb (Macbeth, Broderick, Ovenden, & Buckworth,
163 2011), the population of origin of individuals was resolved through the inclusion of tissue samples
164 of white sharks collected from other locations (Western Australia *n* = 3; South Australia *n* = 9;
165 South Africa *n* = 20; total *n* = 279, see Table S1). All samples were used in the SNP discovery and
166 genotyping pipeline.

167 ### *Cohort Assignment*

168 To group individuals into age-cohorts, a year-of-birth was assigned to each sample using
169 the year the individual was sampled minus the age of the individual in that given year. To estimate
170 the age of individuals, we used the von Bertalanffy growth function (VBGF) (Supplementary
171 Appendix 2) to transform the relationship of TL to relationships at age using growth parameters
172 specific to the EAP (O'Connor, 2011). We considered fork length (FL, defined here as the

measurement from the tip of the rostrum to the fork in the tail over the body) the more accurate measurement at the time of sampling. Conversion of FL to TL was achieved by linear regression based on measurements of study samples using the *lm* function in R (O'Connor, 2011). Assumptions of linearity, normality, and heteroscedasticity were checked by means of residual and quantile plots and the following conversion was used to transform measurements:

$$TL\ (cm) = 6.80 + FL\ (cm) * 1.07$$

Equation 1

SNP and microsatellite loci datasets

DNA was extracted from all samples (*n* = 279) using a standard salt precipitation procedure. The samples were genotyped by DArT P/L laboratory using DArTseq™ technology (Kilian et al., 2012). Sequencing steps followed Kilian et al., (2012) and were completed using an Illumina Hiseq2500. Resulting sequences were processed using the proprietary DArT analytical software, DArTsoft14. DArTsoft14 uses technical sample replicates to optimize its algorithm parameters and ensure scoring consistency (see Georges et al., 2018). Post-processing of SNPs was completed in R (R Core Team, 2018) using the R-Package *radiator 0.0.5* (Gosselin, 2017) and custom R-scripts following current best practice (O'Leary, Puritz, Willis, Hollenbeck, & Portnoy, 2018; Shafer et al., 2017). A two-stage post-processing approach was employed to the SNP dataset to identify and remove 1) migrants and 2) outlier (non-selectively neutral) loci. SNP data representing all samples (East Australia, South Africa, Western Australia, South Australia) were filtered following the steps outlined in Table S3.1 (Supplementary Materials 3), and was

subsequently used for sample population assignment and initial outlier loci discovery. Strongly divergent individuals create strong mixture LD which downwardly bias estimates of $Ne(LD)$ (Waples & England, 2011) and may contribute to upward bias in estimates using the SA method (Ackerman et al., 2017). To identify divergent individuals, we performed a Discriminant analysis of principal components (DAPC) (Jombart, Devillard, & Balloux, 2010) implemented in the R-package *adegenet* (Jombart et al., 2010). The optimal number of discriminant functions to retain was calculated using the function *xvalDAPC* using 80% of the data in the training set, and the number of PCs retained in the final DAPC were associated with the lowest Mean Squared Error. As indicated in Figure S3.1 (Supplementary Materials 3), two samples collected from east Australia appeared distinct from other EAP samples (subsequently confirmed using tracking data from acoustic tagging, Spaet et al., 2020). These samples were removed from subsequent analysis. We also performed tests for outlier loci which deviate from the assumptions necessary for estimating Ne (Waples & England, 2011). We used *pcadapt* (Luu, Bazin, & Blum, 2017) which identifies outlier loci in a multidimensional space (we used k = 3 principal components). We removed loci when the q-value (test statistic) was smaller than the false-discovery rate ($\alpha = 0.05$). In the second stage of SNP post-processing, we used a dataset (herein Dataset-2) containing all SNP loci except those identified as outliers and including samples representing genotypes of EAP origin only. We then filtered Dataset-2 using reproducibility greater than 98%, a minor-allele-count greater than three, coverage (minimum 5, maximum 25), retained only one SNP per locus and removed individuals missing greater than 20% of SNP loci. Loci were further removed where Hardy-Weinberg disequilibrium mid-p $\alpha < 0.1$ and if F_{IS} was less than or equal to

214 + 0.5 and greater than - 0.5 (see Table S3.2, Supplementary Materials 3). Dataset-2 was then used
215 to make estimates of *Nb*.

216 Extracted DNA from 192 EAP samples were further genotyped in another laboratory
217 (Stellenbosch University) with nineteen species-specific microsatellite loci to provide alternate
218 estimates of Nb. Fourteen of the loci were derived from previous studies: Ccar1, Ccar13,
219 Ccar6.27x, Ccar9, Iox10, Cca1419, Cca83, Cca1536, Cca1273, Cca711, Cca1072, Cca1466, Cca1276,
220 Cca1226 (Gubili et al., 2010; O'Leary et al., 2015; Pardini et al., 2001). Five loci (CcSA1, CcSA2,
221 CcSA3, CcSA4 and CcSA5) were developed using the methods described in Andreotti et al. (2016).
222 Wet lab genotyping was performed as described by Andreotti et al. (2016) and genotype scoring
223 was performed in *Geneious* v.5.6.5 (©2005 - 2012 Biomatters Ltd). Assessment of amplification
224 errors, such as large allele drop-out, stuttering and null alleles was conducted in *Microchecker*
225 v.2.2.3 (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004). The program SHAZA (Macbeth et
226 al., 2011) was used to detect duplicates in the dataset. Descriptive statistics, including observed
227 heterozygosity (H_o) and expected heterozygosity (H_e) were calculated using the R-package
228 *hierfstat* (Goudet, Jombart, & Goudet, 2015). Hardy-Weinberg equilibrium (HWE) was evaluated
229 using an exact test based on 10,000 Monte Carlo permutations of alleles and implemented in
230 *Genepop* (Rousset, 2008).

231 ### *Estimation of Nb*
232
233 Two methods were used to estimate Nb from data derived from SNP and microsatellite
234 loci: (1) the linkage-disequilibrium method (LD) (Hill, 1974; Waples, 2006) and (2) the sibship
235 assignment method (SA) (Wang, 2009). Estimates are referred to as $Nb(LD)$ and $Nb(SA)$.

Broadly, the LD method determines the size of the parental generation using a measure of the genetic association (or LD) in a given age-cohort. In finite populations, random genetic drift leads to associations of alleles at different loci. The LD method uses the extent of non-random association between alleles at different loci to estimate genetic *Ne* and reflects the inbreeding *Ne* when loci are unlinked (Hill, 1981; Waples & Do, 2010). The formulation of the LD method uses the observed average disequilibrium between pairs of independent (ie. non-linked), neutral loci in a sample of individuals taken from a single, isolated, randomly mating population. Estimates of *Nb(LD)* are based on the theoretical relationship between $\hat{r}^2$ and Ne as described in Hill (1981);

$$\widehat{N_e} = \frac{1}{3(\hat{r}^2 - \frac{1}{S})}$$

Equation 2a from (Waples & Do, 2010)

where r^2 is the mean squared correlation of allele frequencies at different gene loci adjusted for sampling error (i.e. the observed average disequilibrium) and *S* is the number of individuals sampled. We implemented this method using the program *NeEstimator* v2.1 (Do et al., 2014). In contrast, the SA method uses the direct relationship between genetic relatedness and inbreeding *Ne*, such that any two individuals sampled randomly from a population with a small *Ne* will have a higher probability of sharing the same parent or parents (Wang, 2009). The SA method (Wang, 2009) determines the size of the parental generation by estimating the probability that dyad relationships are either full or half siblings in a sample from the same cohort, sharing two, one or zero parents, respectively;

$$\frac{1}{Ne} = 1 + \frac{3\alpha}{4}(Q_1 + Q_2 + 2Q_3) - \frac{\alpha}{2}(\frac{1}{N_1} + \frac{1}{N_2})$$

Equation 10 from Wang (2009)

where Q_1, Q_2, Q_3 are the paternal, maternal half-sibs and full-sibs respectively, N_1 and N_2 are the number of male and female parents, and α is a measurement of the deviation from Hardy-Weinberg proportions in genotype frequencies (Wang, 2009). The SA method was implemented in the program COLONY (Wang, 2009).

Both $Nb(LD)$ and $Nb(SA)$ were estimated for the EAP across four year-of-birth-cohorts (2010, 2011, 2012, 2013) where sample size per cohort was greater than 25 individuals. Estimates of $Nb(LD)$ and $Nb(SA)$ were made using either SNP or microsatellite marker data sets. To estimate $Nb(LD)$ with *NeEstimator v2.1* (Do et al., 2014) a random mating model was specified, rare alleles which upwardly bias estimates were excluded using the criterion $Pcrit$ = 0.05 as recommended in Waples and Do (2010), and jack-knife confidence intervals that accounts for pseudo-replication due to physical linkage and overlapping loci pairs were used (Jones, Ovenden, & Wang, 2016; Waples & Do, 2010). To estimate $Nb(SA)$, relatedness coefficients were estimated for individuals within each year of birth cohort using COLONY v2.0.5.6 (Jones & Wang, 2010). COLONY estimates the likelihoods of full, maternal-half and paternal-half siblings depending on the mating system chosen in the programs settings, which may impact the final estimate of $Nb(SA)$. We tested different COLONY parameters to determine any effects on the final estimates of $Nb(SA)$ (Supplementary Appendix 4). Results are presented for the maximum-likelihood with random mating model, with male polygamy/female monogamy, no update of allele frequencies, medium sibship prior (sibship size per parent $k = 10$, run for 5 replicate runs, error rate 0.001.

Inference of Nb/Na ratios

To calculate Nb/Na ratios we used Na as described in Bruce et al., (2018), where *Na* is the number of adults in the population. As the species has a low intrinsic capacity for population increase, low fecundity and low life-time variance in reproductive success (Bruce, 2008), the *Na* estimates from Bruce et al. (2018) apply to the time period corresponding to our study; Na=750 with an uncertainty range 470 to 1,030 (Bruce et al., 2018). Our estimates of $Nb(LD)$ and $Nb(SA)$ were combined (herein $Nb(LD + SA)$ to provide a single-value of Nb with which to infer Nb/Na ratios. $Nb(LD)$ and $Nb(SA)$ were combined by taking the harmonic mean of the two values, weighted by the inverse of their variances as suggested in previous studies (see Waples & Do, 2010); see Appendix A for a worked example. In our study, the differences between the estimates from the LD and SA methods were not overly large, so using a combined estimate of Nb to determine the Nb/Na ratio would not change the conclusions described herein.

Expectations for Ne

To develop expectations for generational *Ne* in the EAP of *C. carcharias*, we use a simulation-based approach. This route was taken as the assumptions of single-sample genetic estimators of Ne, including LD and SA methods used herein, dictate that data used to make estimates represents a random sample of a population across an entire generation (Hare et al., 2011). Since the white shark is long-lived and samples in this study were mostly juvenile or sub-adults, we instead characterise the expected $\frac{Nb}{Ne}$ ratio using simulations based on published methods and parametrised using the life-history of white sharks. This indirectly allowed the inference of an expected Ne/Nb ratio, which may permit a better understanding of inbreeding and implied

298 fitness of the population. We use both deterministic and forward-time population simulations
299 following methods described in Waples & Antao, (2014), to determine Ne and Nb. First, we
300 implemented the discrete-time, deterministic hybrid Felsenstein–Hill method for calculating Ne
301 in iteroparous species (Waples, Do, & Chopelet, 2011). The model was implemented in the
302 software *AgeNe* (Waples et al., 2011), herein $Nb(ageNe)$, and parametrised using life-history
303 information from white sharks in the EAP (Supplementary Appendix 7). Furthermore, since the
304 Felsenstein-Hill method assumes the probability of reproduction is not affected by events in
305 previous time periods, we also use forward-time population simulations implemented in simuPOP
306 (Peng & Kimmel, 2005), to create a single, isolated, randomly mating population to further
307 characterise the $\frac{Nb}{Ne}$ ratio under two intermittent-breeding scenarios as in Waples & Antao (2014).
308 Each simulation was parametrised using outputs from AgeNe, including total population size and
309 stable age distribution in the population, given the specified vital rates and a specified number of
310 offspring produced per cycle that survived to age 1 (N1), here N1 = 1000. Each individual was
311 represented by 100 microsatellite-like loci, each having 10 possible allelic states, no mutation,
312 and data was tracked for 50 reproductive cycles after a burn-in period of 50 cycles. We forced a
313 number of females to skip either zero, one or two cycles of breeding (a proportion of females)
314 hypothesised in this species (Domeier & Nasby-Lucas, 2013; Mollet & Cailliet, 2002). Intermittent
315 or skipped breeding occurs when sexually mature adults skip breeding opportunities (Last &
316 Stevens, 2009; Shaw & Levin, 2011), in this case likely due to the costs of reproduction or
317 prolonged gestation period in females (Bruce, 2008). We directly calculated mean (k) and
318 variance (Vk) lifetime reproductive success, and Ne and Nb directly from simulation

demographic data for each reproductive cycle using Equation 1 and Equation 2 from Waples et al., (2014), where presented values represent the arithmetic mean of k, Vk and the harmonic means of Ne and Nb calculated across 10 population replicates, herein $Ne(demo), Nb(demo)$.

Data Availability

Data for this study are available at UQ espace: https://doi.org/10.14264/uql.2020.634. All plots in this study were made using the *ggplot2* package in R (Wickham, 2016).

Results

Cohort Assignment

Using the relationship between TL and age we found that one individual was born in 2005 with various years represented by the following number of individuals; 2007 (n = 3), 2008 (n = 6), 2009 (n = 10), 2010 (n = 30), 2011 (n = 43), 2012 (n = 53), 2013 (n = 67), 2014 (n = 23), 2015 (n = 9) and 2016 (n = 2). The physical size of individuals within age-cohorts increased with age (Figure S2.1). The range of FL between age-cohorts overlapped principally driven by heterogeneous year-of-capture sampling; 2010 (*n* = 30, 224 cm and 296 cm FL), 2011 (*n* = 43, 207 cm and 276 cm FL), 2012 (*n* = 52, 187 cm and 255 cm FL) and 2013 (*n* = 67, 174 cm and 268 cm FL) (Table S5). As low sample sizes can bias estimates of Nb using the methods of this study, only age-cohorts containing greater than 25 samples were used (2010, 2011, 2012 and 2013).

SNP and microsatellite loci data

The DArTsoft14 pipeline delivered 9841 SNPs across 9180 loci. The final SNP dataset after filtering consisted of 3668 diallelic SNPs consisting of 236 EAP individuals with high quality SNP

genotypes (Dataset-2). Nineteen microsatellite loci were successfully genotyped across 181 EAP individuals. No evidence of null alleles or scoring errors were detected. The genotypic distribution of microsatellite genotypes per locus showed three loci did not conform to the expectations of Hardy-Weinberg equilibrium using $\alpha = 0.05$ (loci Cca1419, Cca1072, CcSA2). These markers were removed from further analysis (LD method only). One locus (CcSA5) was not polymorphic (see Table S6.1) and was also excluded. Per individual, 97% had no missing loci while the remaining 3% of samples had three or less missing loci.

Empirical estimation of Nb

Using SNP data, Nb estimates per year-of-birth cohort were similar between the LD and SA methods and had overlapping 95% confidence intervals (Table 1). Estimates of $Nb(SA)$ were not sensitive to changes in model parameters such as the sibship prior, inbreeding settings, error rate and polygamy settings (Table S4.1). This was consistent with the expectations of the SA estimator which becomes increasingly independent of the prior with increasing marker information and sample size. Although confidence intervals overlapped, estimates of $Nb(SA)$ were generally higher than those determined from $Nb(LD)$ across all cohorts. The 2011 cohort showed the largest difference between estimates; $Nb(SA_{2011})$ = 344 (95%CI 204-923), $Nb(LD_{2011})$ = 195.1 (95%CI 104-952.9).

Comparing between the SA and LD method using data from microsatellite loci, estimates of $Nb(LD_{MSAT})$ were higher than the equivalent estimate of $Nb(SA_{MSAT})$. The number of estimated full and half sib-ships in each cohort sample was high and pairwise-probabilities were

low (data not shown) compared to those sib-ships estimated using SNPs. This resulted in $Nb(SA_{MSAT})$ estimates being substantially lower than the equivalent SNP estimate, with the exception of 2011 $Nb(SA_{MSAT})$(Table 1). The $Nb(LD_{MSAT})$ were the least precise estimates, where all but one cohort (2013) did not return an upper (95% CI) estimate.

The ratio Nb/Na was estimated using combined estimates of $Nb(LD + SA)$. The SNP-based Nb estimate for the 2010 cohort contained at least one infinite upper estimate of Nb, so in this case we did not calculate a combined estimate. For cohorts 2011 to 2013, $Nb(LD + SA)$ ranged from the smallest estimated value in 2012, $Nb(LD + SA_{2012}) = 206(45.9\,SD)$, to the largest in 2013, $Nb(LD + SA_{2013}) = 252(46.7\,SD)$ (Table 1). The inferred ratio of Nb/Na ranged from 0.27 to 0.34; Nb/Na_{2012} = 0.27 (0.44-0.2) to Nb/Na_{2013} = 0.34 (0.54-0.24). The intervals (in parentheses) were calculated using the lower and upper uncertainty estimates of Na from Bruce et al., (2018).

The ratio of Nb/Ne was evaluated using simulations. Using a standard model implemented in *AgeNe* yielded estimates of $Nb(demo)$, $Ne\,(demo)$ of 372.7 and 857.2 respectively, and an $Nb(demo)/Ne(demo)$ ratio of 0.43. To account for variations in breeding biology, further forward-time population simulations in SimuPop showed the equivalent no-skip breeding model closely reflected AgeNe results (Nb_{demo} = 365.46, Ne_{demo} = 860.67), validating the model, while alternate breeding models decreased the Nb/Ne ratio (see Table S7.2).

Discussion

Using data from SNP and microsatellite loci and two single sample genetic estimators, our results show the effective breeding population (*Nb*) of the EAP remained unchanged across four successive years (2010 - 2013). We caution that these results may not be indicative of a broader temporal trend, as monitoring (using either genetic or census methods) would require more than five generations to pass between sampling events to correctly identify a population trend (Tallmon et al., 2010). Nonetheless, our study supports existing evidence (Hillary et al., 2018; Roff et al., 2018) that the white shark population has not changed significantly in size over the years studied herein, despite measures implemented to rebuild the population. The white shark recorded substantial declines through the 20th century in Australia and New Zealand and has since been the subject of legislated protection and management interventions targeted toward population recovery (i.e. National Plans of Action for the Conservation and Management of Sharks Shark Advisory Group, 2004; EPBC, 1999; Commonwealth of Australia, 2013). Regrettably, the long monitoring period required to detect a population trend as recommended in Tallmon *et al*., (2010) contrasts with the needs of management and conservation which requires prompt and regular information to offer insights into the effects of current management actions and co-occurrences such as environmental changes. To this end, we recommend using *Nb* to track year-to-year changes in the effective number of breeders as a timely assessment of population status over time. As in this study, future tissue samples for *Nb* monitoring could be obtained as part of existing bather protection programs (i.e. SMART drumlines, see Tate et al., 2019).

In this study, we used two genetic marker types (SNPs and microsatellites) and two estimators (LD and SA) to estimate *Nb*. Both estimators showed more precision and power when

399 SNPs were used to estimate *Nb* compared to microsatellites, and we therefore recommend the
400 use of SNPs for the future monitoring of the EAP. Although $Nb(LD)$ estimated from both SNP and
401 microsatellite were comparable, and results reflected those reported in previous studies (eg.
402 Beebee, 2009), here microsatellite loci were unable to estimate upper CIs for age-cohorts without
403 significant sampling effort (> 50 samples). Of note, estimates of $Nb(LD_{MSAT})$ were consistently
404 higher than the equivalent estimates of $Nb(SA_{MSAT})$. This may be attributed to the
405 overestimation of sibship dyads, which can be expected to decrease estimates of Nb (Table 1).
406 This has been noted in previous studies (Ackerman et al., 2017; Wang, 2009) which have
407 demonstrated that false sibships (type I errors) occur with a higher frequency compared to false
408 non-sibships (type II errors) when either genetic information or true sibship within a sample is
409 insufficient (i.e. few loci, low polymorphism, small sample size relative to total population size,
410 low inclusion of siblings). Nb estimated using SNPs differed between methods such that $Nb(LD)$
411 was lower compared to $Nb(SA)$, although differences were not significant having overlapping
412 CIs. $Nb(LD)$ estimated using SNPs showed those cohorts with larger numbers of samples (i.e.
413 2013) provided more precise estimates, a result expected given genetic methods for estimating
414 contemporary effective size depend on signals that are proportional to $1/Ne$ (Waples, Antao, &
415 Luikart, 2014; Waples et al., 2018).

416 Monitoring studies are often focused on the number of individuals in a population,
417 however the relationship between effective size and population size (i.e. Ne/Na , Nb/Na) can
418 also be useful for examining how different ecological factors influence genetic variation (Nunney,
419 1996). In this study, the ratio of *Nb/Na* was approximately 1/3 for a single reproductive cycle. This

420 is comparable to ratios inferred for other Carcharhinidae, including *C. plumbeus* (sandbar shark)
421 in Delaware Bay, North Atlantic, which ranges between 0.50(95%CI 0.45) and 0.63(95%CI 0.57)
422 (Portnoy, McDowell, McCandless, Musick, & Graves, 2009). Nb can be expected to be reduced
423 relative to Na if females with high fecundity skip reproductive cycles after giving birth, resulting
424 in different females breeding in different cycles (Waples & Anato, 2014). This should decrease
425 both lifetime Vk and Nb, while increasing Ne. The ratio reported herein appears to be consistent
426 with expectations for the breeding behaviour of *C. carcharias*, suspected to undergo intermittent
427 breeding (Bruce, 2008). Observations suggest the gestation period of *C. carcharias* females may
428 approach 18 months from fertilization to parturition (Bruce, 2008; Mollet, Cliff, Pratt, & Stevens,
429 2000), resulting in the unavailability of a portion of adult females to produce offspring each cycle.

430 Neutral genetic variation is lost at a rate of $1/2\,Ne$ per generation (Wright, 1931) such
431 that even numerically large populations can be at genetic risk if Ne is small (Waples et al., 2018).
432 Although important, due to sampling restriction (i.e. difficulty sampling across a generation as
433 required by estimators) and uncertainty of breeding histories, we could not estimate Ne directly
434 nor did we consider the linear relationship between Nb and Ne which requires either true or
435 estimated $\frac{Nb}{Ne}$ to be quantified (Waples et al., 2013). Instead, using simulations, we show $Nb >$
436 Ne using life-history information for white shark, and that $Nb >> Ne$ if intermittent breeding
437 were occurring. This aligns with expectations, where a small number of offspring, delayed
438 maturation, intermittent breeding and low lifetime variance in fecundity all act to increase Ne
439 relative to Nb or N (Waples & Antao, 2014). This is important as it suggests the study population
440 in the EAP at least exceeds the inbreeding avoidance goal ($Ne \geq 100$) (Frankham, Bradshaw, &

441 Brook, 2014). However, in relation to the long-term viable population benchmark, $Ne \geq 1000$
442 (Frankham et al., 2014) our results are less certain. We suggest any genetic effects of a recently
443 and significantly reduced population size in the EAP, such as a decline in Ne or loss of
444 heterozygosity, may not be fully realised until adequate benchmark studies can be completed (i.e.
445 historical or ancient DNA). However, genetic bottlenecks in white sharks have been recorded
446 elsewhere (O'Leary et al., 2015). Our results emphasise the importance of continued monitoring,
447 improved protections, and interventions to reduce mortality. Indeed, the vulnerability of
448 chondrichthyan fishes to exploitation has been comprehensively documented (Hutchings, Myers,
449 García, Lucifora, & Kuparinen, 2012) and relative to other marine fish, the intrinsic capacity for
450 population increase and rebound potential in white shark is low (Cortés, 2002) (i.e. long-lived,
451 late age to maturity, high juvenile survival). In addition, shark species often travel large distances
452 and use different habitats throughout their lives (Fujioka & Halpin, 2014), where they may be
453 vulnerable to environmental changes (density, food availability, climate, illegal fishing).
454 Regrettably, mortalities continue to occur in the EAP driven by action taken to mitigate human-
455 shark interactions. During the years 2018-2019, fifty-one bather-protection nets were distributed
456 across seven regions of NSW (Australia). Catches of white shark and other shark species are
457 increasing year-on-year (Dalton & Peddemors, 2019), which in some cases are lethal for sharks
458 despite catch-and-release programs (Roff et al., 2018). The recent modelling of the recovery of
459 the North West Atlantic white shark population provides a useful principal in this regard; "every
460 fish counts" (Bowlby & Gibson, 2020, p.9).

Conclusion

We have used genetic data to estimate the size of the effective breeding population (Nb) over four consecutive years (2010 to 2013) for white sharks the in an east-Australian New Zealand population, representing an indirect measure of reproductive effort over a relatively short temporal period. Our results suggest Nb has remained stable over four years and agrees with previous studies that report stability of population size in the EAP. However, longer time series of data are needed to determine the efficacy of past and present management and conservation actions on the genetic constitution of the population. In this study, *Nb* estimates were more precise using data from SNP rather than microsatellite loci and estimates from two single-sample genetic estimators were equivalent. We suggest future monitoring using *Nb* should continue given the availability of non-lethal tissue samples from bather protection programs, the ease of genomic data collection and analyses and the complementary nature of *Nb* and *Na* estimates.

Acknowledgments

Project funding and primary support for sample collection was provided through the Shark Management Strategy by the New South Wales Department of Primary Industries (NSW DPI), Australia. This project would not have been possible without the dedicated support of contracted fishers and the NSW DPI shark research team (especially Craig Brand and Chris Gallen). The authors would also like to thank Dr. Charlie Huveeners, Flinders University, South Australia for the provision of samples from south-west Australia, Jamie Wyatt for assistance with DNA-laboratory work, and Andrzej Killan from Diversity Array Technologies for his continued support for conservation genomics projects and Professor Michael Bennett for his useful comments, and we extend our sincere thanks to the reviewers of this manuscript.

References

Ackerman, M. W., Hand, B. K., Waples, R. K., Luikart, G., Waples, R. S., Steele, C. A., … Campbell, M. R. (2017). Effective number of breeders from sibship reconstruction: empirical evaluations using hatchery steelhead. *Evolutionary Applications*, *10*(2), 146–160. doi: 10.1111/eva.12433

Andreotti, S., von der Heyden, S., Henriques, R., Rutzen, M., Meÿer, M., Oosthuizen, H., & Matthee, C. A. (2016). New insights into the evolutionary history of white sharks, Carcharodon carcharias. *Journal of Biogeography*, *43*(2), 328–339. doi: 10.1111/jbi.12641

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. *Evolutionary Applications*, *4*(1), 144–154. doi: 10.1111/j.1752-4571.2010.00150.x

Bacles, C. F. E., Bouchard, C., Lange, F., Manicki, A., Tentelier, C., & Lepais, O. (2018). Estimating the effective number of breeders from single parr samples for conservation monitoring of wild populations of Atlantic salmon Salmo salar: Decennial Temporal Variation in Nb. *Journal of Fish Biology*, *92*(3), 699–726. doi: 10.1111/jfb.13537

Blower, D. C., 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. *Marine Ecology Progress Series*, *455*, 229–244. doi: 10.3354/meps09659

Bowlby, H. D., & Gibson, A. J. F. (2020). Implications of life history uncertainty when evaluating status in the Northwest Atlantic population of white shark (*Carcharodon carcharias*). *Ecology and Evolution*, ece3.6252. doi: 10.1002/ece3.6252

Bruce, B., Bradford, R., Feutry, M., Gunasekera, R., Harasti, D., Hillary, R. M., & Patterson, T. (2018). *A national assessment of the status of white sharks*. Hobart: National Environmental Science Program Marine Biodiversity Hub. Retrieved from National Environmental Science Program Marine Biodiversity Hub website: https://www.nespmarine.edu.au/document/national-assessment-status-white-sharks

Bruce, B. (2008). White sharks: the biology and ecology of *Carcharodon carcharias*. *Sharks of the Open Ocean*, 69–81.

Bruce, B., Harasti, D., Lee, K., Gallen, C., & Bradford, R. (2019). Broad-scale movements of juvenile white sharks *Carcharodon carcharias* in eastern Australia from acoustic and satellite telemetry. *Marine Ecology Progress Series*, *619*, 1–15. doi: 10.3354/meps12969

Burgess, G. H., Bruce, B. D., Cailliet, G. M., Goldman, K. J., Grubbs, R. D., Lowe, C. G., … O'Sullivan, J. B. (2014). A Re-Evaluation of the Size of the White Shark (*Carcharodon carcharias*) Population off California, USA. *PLoS ONE*, *9*(6), e98078. doi: 10.1371/journal.pone.0098078

Commonwealth of Australia. (2013). *Recovery Plan for the White Shark (Carcharodon carcharias).* Department of Sustainability, Environment, Water, Population and

Communities. Retrieved from Department of Sustainability, Environment, Water, Population and Communities. website: http://tinyurl.com/zylhrnx
Cortés, E. (2002). Incorporating uncertainty into demographic modelling: application to shark populations and their conservation. *Conservation Biology*, *16*(4), 1048–1062.
Dalton, S., & Peddemors, V. (2019). *Shark Meshing (Bather Protection) Program 2018/19 Annual Performance Report* (No. INT19/109030). NSW Department of Primary Industries.
Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillett, B. J., & Ovenden, J. R. (2014). NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Molecular Ecology Resources*, *14*(1), 209–214. doi: 10.1111/1755-0998.12157
Domeier, M. L., & Nasby-Lucas, N. (2013). Two-year migration of adult female white sharks (*Carcharodon carcharias*) reveals widely separated nursery areas and conservation concerns. *Animal Biotelemetry*, *1*(1), 2. doi: 10.1186/2050-3385-1-2
Dulvy, N. K., Fowler, S. L., Musick, J. A., Cavanagh, R. D., Kyne, P. M., Harrison, L. R., … White, W. T. (2014). Extinction risk and conservation of the world's sharks and rays. *ELife*, *3*. doi: 10.7554/eLife.00590
EPBC. (1999). Environment protection and biodiversity conservation act. *Commonwealth of Australia*.
FAO. (2000). *The International Plan of Action for the Conservation and Management of Sharks.* Rome.
Frankham, R. (1996). Relationship of Genetic Variation to Population Size in Wildlife. *Conservation Biology*, *10*(6), 1500–1508. doi: 10.1046/j.1523-1739.1996.10061500.x
Frankham, R., Bradshaw, C. J. A., & Brook, B. W. (2014). Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. *Biological Conservation*, *170*, 56–63. doi: 10.1016/j.biocon.2013.12.036
Franklin, I. R. (1980). Evolutionary change in small populations. In M. E. Soulé & B. A. Wilcox (Eds.), *Evolutionary change in small populations.* (pp. 135–150). Sunderland: Sinauer.
Fujioka, E., & Halpin, P. N. (2014). Spatio-temporal assessments of biodiversity in the high seas. *Endangered Species Research*, *24*(2), 181–190.
Georges, A., Gruber, B., Pauly, G. B., White, D., Adams, M., Young, M. J., … Unmack, P. J. (2018). Genomewide SNP markers breathe new life into phylogeography and species delimitation for the problematic short-necked turtles (Chelidae: Emydura) of eastern Australia. *Molecular Ecology*, *27*(24), 5195–5213. doi: 10.1111/mec.14925
Gore, M. A., Frey, P. H., Ormond, R. F., Allan, H., & Gilkes, G. (2016). Use of photo-identification and mark-recapture methodology to assess basking shark (Cetorhinus maximus) populations. *PloS One*, *11*(3), e0150160. doi: 10.1371/journal.pone.0150160
Gosselin, T. (2017). *radiator: RADseq Data Exploration, Manipulation and Visualization using R*. R package version 0.0.5 https://github.com/thierrygosselin/radiator.
Goudet, J., Jombart, T., & Goudet, M. J. (2015). Package 'hierfstat.' *R Package Version 0.04-22. Retrieved from Http://Www. r-Project. Org, Http://Github. Com/Jgx65/Hierfstat*.
Gubili, C., Bilgin, R., Kalkan, E., Karhan, S. Ü., Jones, C. S., Sims, D. W., … Noble, L. R. (2010). Antipodean white sharks on a Mediterranean walkabout? Historical dispersal leads to

565 genetic discontinuity and an endangered anomalous population. *Proceedings of the*
566 *Royal Society B: Biological Sciences*, *278*(1712), 1679–1686. doi: 10.1098/rspb.2010.1856
567 Heupel, M., Knip, D., Simpfendorfer, C., & Dulvy, N. (2014). Sizing up the ecological role of
568 sharks as predators. *Marine Ecology Progress Series*, *495*, 291–298. doi:
569 10.3354/meps10597
570 Hill, W. G. (1974). Estimation of linkage disequilibrium in randomly mating populations.
571 *Heredity*, *33*(2), 229. doi: 10.1038/hdy.1974.89
572 Hill, W. G. (1981). Estimation of effective population size from data on linkage disequilibrium.
573 *Genetical Research*, *38*(03). doi: 10.1017/S0016672300020553
574 Hillary, R. M., Bravington, M. V., Patterson, T. A., Grewe, P., Bradford, R., Feutry, P., … Bruce, B.
575 D. (2018). Genetic relatedness reveals total population size of white sharks in eastern
576 Australia and New Zealand. *Scientific Reports*, *8*(1). doi: 10.1038/s41598-018-20593-w
577 Hutchings, J. A., Myers, R. A., García, V. B., Lucifora, L. O., & Kuparinen, A. (2012). Life-history
578 correlates of extinction risk and recovery potential. *Ecological Applications*, *22*(4), 1061–
579 1067. doi: 10.1890/11-1313.1
580 Jacoby, D. M., Croft, D. P., & Sims, D. W. (2012). Social behaviour in sharks and rays: analysis,
581 patterns and implications for conservation. *Fish and Fisheries*, *13*(4), 399–417. doi:
582 10.1111/j.1467-2979.2011.00436.x
583 Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: a
584 new method for the analysis of genetically structured populations. *BMC Genetics*, *11*(1),
585 94. doi: 10.1186/1471-2156-11-94
586 Jones, A. T., Lavery, S. D., Le Port, A., Wang, Y. G., Blower, D. C., & Ovenden, J. (2019).
587 Sweepstakes reproductive success is absent in a New Zealand snapper (*Chrysophrus*
588 *auratus*) population protected from fishing despite “tiny” Ne/N ratios elsewhere.
589 *Molecular Ecology*, *28*(12), 2986–2995. doi: 10.1111/mec.15130
590 Jones, A. T., Ovenden, J. R., & Wang, Y.-G. (2016). Improved confidence intervals for the linkage
591 disequilibrium method for estimating effective population size. *Heredity*, *117*(4), 217–
592 223. doi: 10.1038/hdy.2016.19
593 Kilian, A., Wenzl, P., Huttner, E., Carling, J., Xia, L., Blois, H., … Uszynski, G. (2012). Diversity
594 Arrays Technology: A Generic Genome Profiling Technology on Open Platforms. In F.
595 Pompanon & A. Bonin (Eds.), *Data Production and Analysis in Population Genomics* (Vol.
596 888, pp. 67–89). Totowa, NJ: Humana Press. doi: 10.1007/978-1-61779-870-2_5
597 Last, P., & Stevens, J. (2009). *Sharks and rays of Australia*. Australia: CSIRO.
598 Lindenmayer, D., Woinarski, J., Legge, S., Southwell, D., Lavery, T., Robinson, N., … Wintle, B.
599 (2020). A checklist of attributes for effective monitoring of threatened species and
600 threatened ecosystems. *Journal of Environmental Management*, *262*, 110312. doi:
601 10.1016/j.jenvman.2020.110312
602 Luu, K., Bazin, E., & Blum, M. G. B. (2017). pcadapt: an R package to perform genome scans for
603 selection based on principal component analysis. *Molecular Ecology Resources*, *17*(1),
604 67–77. doi: 10.1111/1755-0998.12592
605 Macbeth, G. M., Broderick, D., Ovenden, J. R., & Buckworth, R. C. (2011). Likelihood-based
606 genetic mark--recapture estimates when genotype samples are incomplete and contain

typing errors. *Theoretical Population Biology*, *80*(3), 185–196. doi: 10.1016/j.tpb.2011.06.006

Mollet, H., & Cailliet, G. (2002). Comparative population demography of elasmobranchs using life history tables, Leslie matrices and stage-based matrix models. *Marine and Freshwater Research*, *53*(2), 503–515. doi: 10.1071/MF01083

Mollet, H., Cliff, G., Pratt, H., & Stevens, J. (2000). Reproductive biology of the female shortfin mako, *Isurus oxyrinchus* Rafinesque, 1810, with comments on the embryonic development of lamnoids. *Fishery Bulletin*, (2).

Nunney, L. (1996). The influence of variation in female fecundity on effective population size. *Biological Journal of the Linnean Society*, *59*(4), 411–425. doi: 10.1111/j.1095-8312.1996.tb01474.x

Nunziata, S. O., & Weisrock, D. W. (2018). Estimation of contemporary effective population size and population declines using RAD sequence data. *Heredity*, *120*(3), 196–207. doi: 10.1038/s41437-017-0037-y

O'Connor, J. (2011). *Age, Growth and Movement Signatures of the White Shark (Carcharodon Carcharias) in Southern Australia. School of the Environment, T*. The University of Technology, Sydney.

O'Leary, S. J., Feldheim, K. A., Fields, A. T., Natanson, L. J., Wintner, S., Hussey, N., … Chapman, D. D. (2015). Genetic Diversity of White Sharks, *Carcharodon carcharias*, in the Northwest Atlantic and Southern Africa. *Journal of Heredity*, *106*(3), 258–265. doi: 10.1093/jhered/esv001

O'Leary, S. J., Puritz, J. B., Willis, S. C., Hollenbeck, C. M., & Portnoy, D. S. (2018). These aren't the loci you're looking for: Principles of effective SNP filtering for molecular ecologists. *Molecular Ecology*. doi: 10.1111/mec.14792

Ovenden, J. R., Leigh, G. M., Blower, D. C., Jones, A. T., Moore, A., Bustamante, C., … Dudgeon, C. L. (2016). Can estimates of genetic effective population size contribute to fisheries stock assessments? *Journal of Fish Biology*, *89*(6), 2505–2518. doi: 10.1111/jfb.13129

Papa, Y., Oosting, T., Valenza-Troubat, N., Wellenreuther, M., & Ritchie, P. A. (2020). Genetic stock structure of New Zealand fish and the use of genomics in fisheries management: an overview and outlook. *New Zealand Journal of Zoology*, 1–31. doi: 10.1080/03014223.2020.1788612

Peng, B., & Kimmel, M. (2005). simuPOP: a forward-time population genetics simulation environment. *Bioinformatics*, *21*(18), 3686–3687. doi: 10.1093/bioinformatics/bti584

Perrier, C., April, J., Cote, G., Bernatchez, L., & Dionne, M. (2016). Effective number of breeders in relation to census size as management tools for Atlantic salmon conservation in a context of stocked populations. *Conservation Genetics*, *17*(1), 31–44. doi: 10.1007/s10592-015-0758-5

Portnoy, D. S., McDowell, J. R., McCandless, C. T., Musick, J. A., & Graves, J. E. (2009). Effective size closely approximates the census size in the heavily exploited western Atlantic population of the sandbar shark, *Carcharhinus plumbeus*. *Conservation Genetics*, *10*(6), 1697–1705. doi: 10.1007/s10592-008-9771-2

Reid, D. D., Robbins, W. D., & Peddemors, V. M. (2011). Decadal trends in shark catches and effort from the New South Wales, Australia, Shark Meshing Program 1950 - 2010. *Marine and Freshwater Research*, *62*(6), 676. doi: 10.1071/MF10162

Roff, G., Brown, C. J., Priest, M. A., & Mumby, P. J. (2018). Decline of coastal apex shark populations over the past half century. *Communications Biology*, *1*(1). doi: 10.1038/s42003-018-0233-1

Rousset, F. (2008). *A complete re-implementation of the GENEPOP software for software for teaching and research*.

Ruzzante, D. E., McCracken, G. R., Førland, B., MacMillan, J., Notte, D., Buhariwalla, C., … Skaug, H. J. (2019). Validation of close-kin mark-recapture (CKMR) methods for estimating population abundance. *Methods in Ecology and Evolution*, *10*, 1445–1453. doi: 10.1111/2041-210X.13243

Schwartz, M. K., Luikart, G., & Waples, R. S. (2007). Genetic monitoring as a promising tool for conservation and management. *Trends in Ecology & Evolution*, *22*(1), 25–33. doi: 10.1016/j.tree.2006.08.009

Shafer, A. B. A., Peart, C. R., Tusso, S., Maayan, I., Brelsford, A., Wheat, C. W., & Wolf, J. B. W. (2017). Bioinformatic processing of RAD-seq data dramatically impacts downstream population genetic inference. *Methods in Ecology and Evolution*, *8*(8), 907–917. doi: 10.1111/2041-210X.12700

Shark Advisory Group, M. (2004). *National plan of action for the conservation and management of sharks (shark plan)*. AFFA.

Shaw, A. K., & Levin, S. A. (2011). To breed or not to breed: a model of partial migration. *Oikos*, *120*(12), 1871–1879. doi: 10.1111/j.1600-0706.2011.19443.x

Spaet, J. L., Patterson, T. A., Bradford, R. W., & Butcher, P. A. (2020). Spatiotemporal distribution patterns of immature Australasian white sharks (*Carcharodon carcharias*). *Scientific Reports*, *10*(1), 1–13.

Tallmon, D. A., Gregovich, D., Waples, R. S., Scott Baker, C., Jackson, J., Taylor, B. L., … Schwartz, M. K. (2010). When are genetic methods useful for estimating contemporary abundance and detecting population trends?: TECHNICAL ADVANCES. *Molecular Ecology Resources*, *10*(4), 684–692. doi: 10.1111/j.1755-0998.2010.02831.x

Tanaka, S., Kitamura, T., Mochizuki, T., & Kofuji, K. (2011). Age, growth and genetic status of the white shark (Carcharodon carcharias) from Kashima-nada, Japan. *Marine and Freshwater Research*, *62*(6), 548. doi: 10.1071/MF10130

Tate, R., Cullis, B., Smith, S. D., Kelaher, B. P., Brand, C., Gallen, C., … Butcher, P. A. (2019). The acute physiological status of white sharks (*Carcharodon carcharias*) exhibits minimal variation after capture on SMART drumlines. *Conservation Physiology*, *7*(1), coz042. doi: 10.1093/conphys/coz042

Van Oosterhout, C., Hutchinson, W. F., Wills, D. P., & Shipley, P. (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, *4*(3), 535–538. doi: 10.1111/j.1471-8286.2004.00684.x

Wang, J. (2005). Estimation of effective population sizes from data on genetic markers. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *360*(1459), 1395–1409. doi: 10.1098/rstb.2005.1682

Wang, J. (2009). A new method for estimating effective population sizes from a single sample of multilocus genotypes. *Molecular Ecology*, *18*(10), 2148–2164. doi: 10.1111/j.1365-294X.2009.04175.x

Wang, J., Santiago, E., & Caballero, A. (2016). Prediction and estimation of effective population size. *Heredity*, *117*(4), 193–206. doi: 10.1038/hdy.2016.43

Waples, R. S. (2006). A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. *Conservation Genetics*, *7*(2), 167–184. doi: 10.1007/s10592-005-9100-y

Waples, R. S., & Antao, T. (2014). Intermittent breeding and constraints on litter size: consequences for effective population size per generation (Ne) and per reproductive cycle (Nb). *Evolution*, *68*(6), 1722–1734. doi: 10.1111/evo.12384

Waples, R. S., Antao, T., & Luikart, G. (2014). Effects of Overlapping Generations on Linkage Disequilibrium Estimates of Effective Population Size. *Genetics*, *197*(2), 769–780. doi: 10.1534/genetics.114.164822

Waples, R. S., & Do, C. (2008). LDNe: a program for estimating effective population size from data on linkage disequilibrium. *Molecular Ecology Resources*, *8*(4), 753–756. doi: 10.1111/j.1755-0998.2007.02061.x

Waples, R. S., & Do, C. (2010). Linkage disequilibrium estimates of contemporary N_e using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. *Evolutionary Applications*, *3*(3), 244–262. doi: 10.1111/j.1752-4571.2009.00104.x

Waples, R. S., Do, C., & Chopelet, J. (2011). Calculating Ne and Ne/N in age-structured populations: a hybrid Felsenstein-Hill approach. *Ecology*, *92*(7), 1513–1522. doi: 10.1890/10-1796.1

Waples, R. S., & England, P. R. (2011). Estimating Contemporary Effective Population Size on the Basis of Linkage Disequilibrium in the Face of Migration. *Genetics*, *189*(2), 633–644. doi: 10.1534/genetics.111.132233

Waples, R. S., Grewe, P. M., Bravington, M. W., Hillary, R., & Feutry, P. (2018). Robust estimates of a high ratio in a top marine predator, southern bluefin tuna. *Science Advances*, *4*(7), eaar7759. doi: 10.1126/sciadv.aar7759

Waples, R. S., Luikart, G., Faulkner, J. R., & Tallmon, D. A. (2013). Simple life-history traits explain key effective population size ratios across diverse taxa. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1768), 20131339–20131339. doi: 10.1098/rspb.2013.1339

Whiteley, A. R., Coombs, J. A., Hudy, M., Robinson, Z., Colton, A. R., Nislow, K. H., & Letcher, B. H. (2013). Fragmentation and patch size shape genetic structure of brook trout populations. *Canadian Journal of Fisheries and Aquatic Sciences*, *70*(5), 678–688. doi: 10.1139/cjfas-2012-0493

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. Retrieved from https://ggplot2.tidyverse.org

730 Wood, J. L. A., Belmar-Lucero, S., Hutchings, J. A., & Fraser, D. J. (2014). Relationship of habitat
731 variability to population size in a stream fish. *Ecological Applications*, *24*(5), 1085–1100.
732 doi: 10.1890/13-1647.1
733 Worm, B., Davis, B., Kettemer, L., Ward-Paige, C. A., Chapman, D., Heithaus, M. R., … Gruber, S.
734 H. (2013). Global catches, exploitation rates, and rebuilding options for sharks. *Marine*
735 *Policy*, *40*, 194–204. doi: 10.1016/j.marpol.2012.12.034
736 Wright, S. (1931). Evolution in Mendelian populations. *Genetics*, *16*(2), 97.
737
738

Figures

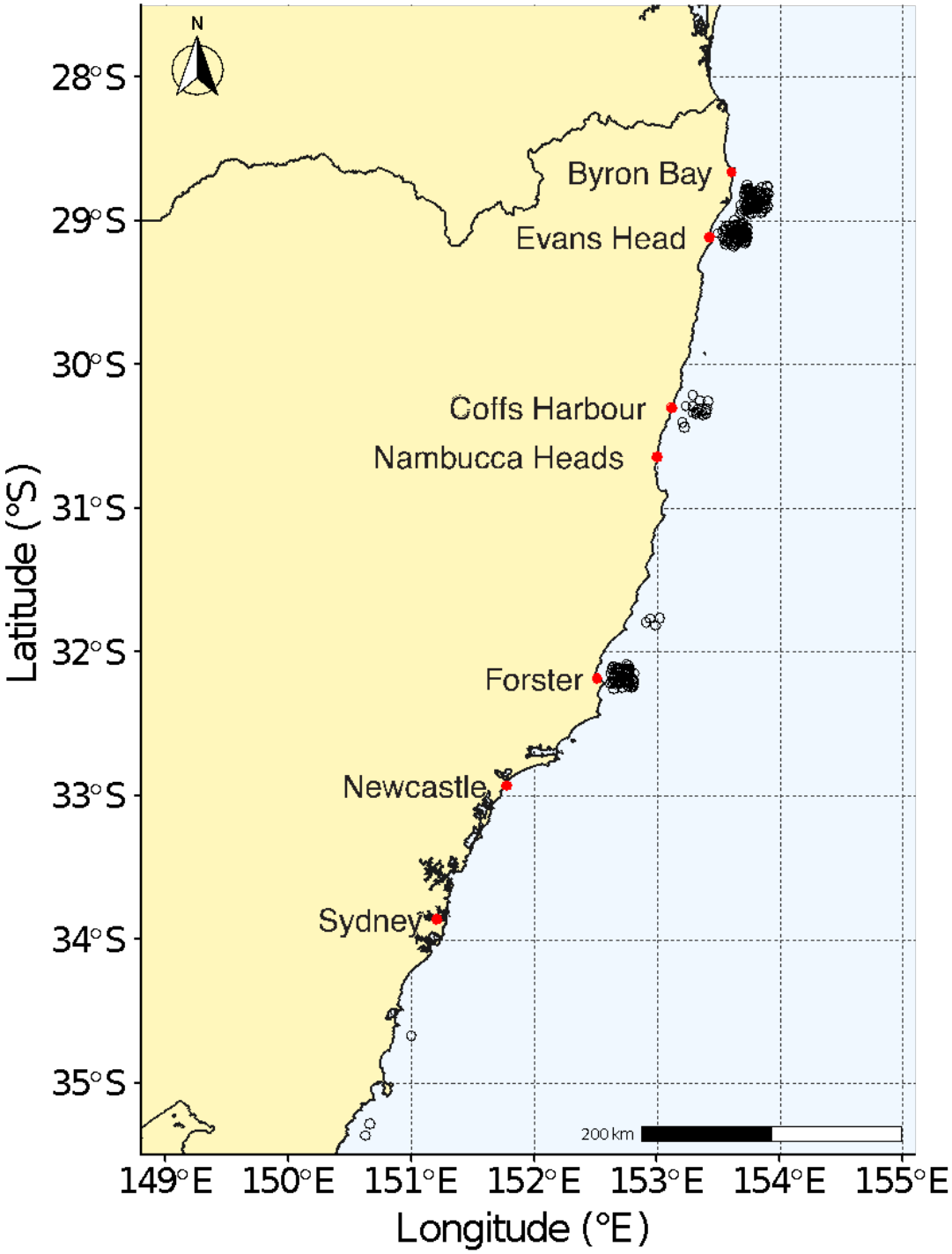

Figure 1. Map of sampling locations, where 247 EAP samples (open circles) were collected along the NSW coast and used to determine Nb.

743 **Table 1.** A comparison of empirical annual effective size (Nb) determined from genetic data
744 (microsatellites - MSAT and single-nucleotide polymorphisms - SNP) using either the linkage-
745 disequilibrium $Nb(\mathrm{LD})$ or sibship assignment $Nb(\mathrm{SA})$ method per year-of-birth-cohort for the
746 EAP of *C.carcharias*. Lower and upper confidence intervals in braces (lower CI-upper CI) and the
747 number of samples used to make the estimate (n) is reported. The standard deviation (±SD) is
748 reported for the combined estimate of $Nb\big(Nb(LD+SA)\big)$, and the number of full and half-sibling
749 pairs are reported in square braces [full-sib, half-sib in square brackets].

		2010	2011	2012	2013
MSAT	***n***	**21**	**33**	**39**	**54**
	Nb_{LD}	∞ (82.5-∞)	263.9 (51.4-∞)	128.7 (43.1—∞)	112.6 (49.3-12934.9)
	Nb_{SA}	33 (18,74)[7,56]	49 (30,84)[3,95]	51 (36-88)[5,97]	62 (41,96)[17,137]
SNP	***n***	**29**	**42**	**52**	**63**
	Nb_{LD}	193.2 (91 - ∞)	195.1 (104.2-952.9)	165.6 (104.2-359.6)	208.5 (116.4-712.7)
	Nb_{SA}	271 (136-1430)[2,2]	344 (204-923)[4,4]	241 (157-399)[8,6]	289 (200-461)[8,10]
	Nb(LD + SA)(±SD)	-	233.2(±69.5)	206.1(±45.9)	252.0(±46.7)
	$Nb/Na^{\dagger}$	-	0.31	0.27	0.34

750 † The ratio *Nb/Ne* determined using combined estimate, where *Na* represents the adult
751 population size, estimated for the year 2017 (Bruce et al., 2018)
752
753
754
755
756

Appendix A

Inverse-variance weighted mean method and worked example

This is a worked example using data from the cohort 2013. This weighted mean will give the lowest variance of any weighted mean of the values. As with nearly all Ne calculations the harmonic mean must be used as the real quantities of interest is proportional to 1/Ne.

Values

LDNe: 208.5
COLONY: 289
Sample Size: 63

The Variances

Unfortunately, neither COLONY or NeEstimator (Do et al., 2014; Jones & Wang, 2010) provides the raw variance figures required, however these can be approximated by working backwards from the provided confidence intervals.

COLONY

Colony generates 95% confidence intervals using the following formula[cite]:

$$CI\colon \frac{1}{2N_e} \pm 1.96\sqrt{V^*},$$

where V^* is the variance of the estimate of $1/2N_e$. Knowing the upper and lower bounds of this confidence interval, we can estimate V^* as,

$$V^* = \left[\frac{2L^* - {}^1/_{N_e}}{3.92}\right]^2,$$

or,

$$V^* \approx \left[\frac{{}^1/_{L} - {}^1/_{N_e}}{4}\right]^2.$$

Where L^* and L are the lower confidence bounds in terms of $1/2N_e$ and N_e respectively. An identical argument follows for the upper bounds. However, we desire the variance of N_e, which we can approximate using a first order Taylor expansion. That is, $Var[f(X)] \approx (f'E[X])^2 Var[X]$. Substituting in our particular case,

$$Var[N_e] \approx \left[\frac{-4}{2}{N_e}^2\right]^2 V^*,$$

$$Var[N_e] \approx \left[\frac{-4}{2} {N_e}^2\right]^2 \left[\frac{1/L - 1/N_e}{4}\right]^2,$$

$$Var[N_e] \approx \frac{1}{4}[N_e]^4 \left[1/L - 1/N_e\right]^2.$$

We also have

$$Var[N_e] \approx \frac{1}{4}[N_e]^4 \left[1/U - 1/N_e\right]^2$$

via the upper bound. These are now in terms of known values and we can estimate $Var[N_e]$.

Ne	U	L	$\boldsymbol{Var[N_e]}$.
289	-	200	4134.811506
289	461	-	2906.636596
Mean			3520.724051

Ne Estimator

Similar to COLONY Ne Estimator does not provide raw variances, and we need to work in terms of the confidence intervals for $(\hat{r}^2 - drift)$ which we will call here r^*. The drift term is approximately $1/S$, where S is the sample size. The 95% confidence interval for r^* is explicitly normal in the case of the jackknife confidence interval (Jones, Ovenden, & Wang, 2016). Thus, V^*, the variance of r^* is approximated by

$$V^* \approx \left[\frac{U^* - r^*}{2}\right]^2 \approx \left[\frac{L^* - r^*}{2}\right]^2,$$

where U and L are the upper and lower bounds provided for r^*. However, again, we wish to have the variance in terms of N_e. Using the same approach as for COLONY, we will approximate this using $Var[f(X)] \approx (f'E[X])^2 Var[X]$. The true function used to calculate N_e using r^* can be found in Table 1 in Waples & Do (2008), here we use the simpler original form (Equation 1 in Jones et al 2016 and others) as our $f(X)$ for this estimate, that is

$$N_e = 1/3(\hat{r}^2 - drift) = 1/3(r^*).$$

Working as before,

$$Var[N_e] \approx \left[\frac{-1}{3(r^*)^2}\right]^2 V^*$$

$$Var[N_e] \approx \frac{1}{4}\frac{1}{[(r^*)^4]}\frac{1}{9}(L^* - r^*)^2$$

$$Var[N_e] \approx \frac{1}{36}\frac{1}{[(r^*)^4]}(L^* - r^*)^2 \approx \frac{1}{36}\frac{1}{[(r^*)^4]}(U^* - r^*)^2$$

However, we still need to obtain U^* and L^* the upper and lower confidence bounds for r^*. This can be achieved by inverting the function of r^* and S from Table 1 in Waples & Do (2008). In this particular case we cannot use the simple approximation. In our case the inverted function is

$$r^* = \frac{-69S^2 + \sqrt{10000S^4N_e^2 + 4761S^4 - 248400S^3N_e} + 1800SN_e^2}{1800S^2N_e^2} - {}^1/_S.$$

Ne	CI Bound (N_e)	CI Bound (r^*)	$Var[N_e]$
208.5	116.4	0.000368638	6523.337897
208.5	712.7	6.05919E-05	10252.64749
	Mean		8387.992693

Weighted Mean and Final Variance

Now we follow the formula the weighted harmonic mean,

$$\bar{x} = \frac{1}{\sum_i w_i \frac{1}{x_i}},$$

where the weights, w_i, sum to 1. In this case we need to normalise the inverse-variances to sum to 1.

Ne	Method	Variance	Inverse Variance	Normalised Inverse Variance Weight	$\frac{1}{N_e}$
289	COLONY	3520.724051	0.000284032	0.704357394	0.003460208
208.5	LD	8387.992693	0.000119218	0.295642606	0.004796163

The final mean estimate is:

$$\frac{1}{(0.704357394 \cdot 0.003460208)+(0.295642606 \cdot 0.004796163\ )} = 259.3917339$$

The COLONY estimate has a lower variance and thus contributes around 2/3 of the final estimate (70.4%).

845