

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

C. L. Dudgeon^{1,2} · J. R. Ovenden²

Received: 10 February 2015 / Accepted: 3 July 2015 / Published online: 15 July 2015
© Springer Science+Business Media Dordrecht 2015

Abstract Genetic effective population size (N_e) estimators are useful as applied conservation tools. Many elasmobranch (shark and ray) species are threatened at local and global scales, and tools to monitor these populations are greatly needed. This study investigates contemporary N_e and its relationship with census size (N_c) in a population of zebra sharks (*Stegostoma fasciatum*). Our N_e using the linkage disequilibrium method, 377 (95 % CI 274–584) was found to closely approximate the mark-recapture N_e for this population of mature sharks (458, 95 % CI 298–618), with an N_e/N_c ratio of 0.82 (SE = 0.33). Furthermore, we conducted a series of sensitivity analyses to examine how the numbers of samples and loci affect the precision and accuracy of the estimators. We demonstrate that for this species robust and precise estimates are obtainable with a minimum of 91 samples (approximately 20 % of the census population) and 10 microsatellite loci. These findings contribute important information to the greater body of N_e and N_e/N_c relationships in elasmobranchs and wildlife populations as well as provide important guidelines for implementing genetic monitoring in elasmobranch conservation efforts.

Keywords N_e/N_c · Leopard shark · *Stegostoma fasciatum* · Linkage disequilibrium · Microsatellites

Introduction

Effective population size (N_e) is an important evolutionary parameter that has implications for contemporary wildlife monitoring and management (Frankham et al. 2010). Theoretically N_e is defined as the size of an ideal population that is experiencing a known amount of genetic drift (Wright 1931). For the conservation and management of wildlife populations, estimates of contemporary N_e are of interest. When N_e estimates are based on individuals sampled from a single cohort, they reflect the effective number of breeders in one reproductive cycle (N_b). For mixed-age samples N_e estimates reflect the harmonic mean of the number of breeders over several generations (Lande 1995; Reed and Frankham 2003; Frankham et al. 2010; Waples et al. 2014).

For many wildlife populations, obtaining indicators of abundance is a challenging yet essential requirement for conservation planning and monitoring (Sollman et al. 2013). Genetic estimates of contemporary N_e have great potential to address this problem, particularly for rare and threatened species. Several studies have investigated the relationship between N_e and census size N_c across different taxa (e.g. Frankham 1995; Palstra and Ruzzante 2008). While no consistent relationships exist across taxa there are some patterns emerging. In particular, for species with Type 1 survivorship curves (Begon et al. 1990), i.e. large, long-lived animals with low fecundity, late maturity and high survivorship to adult stages, N_e estimates are similar to N_c estimates with ratios approaching one (Frankham 1995; Portnoy et al. 2009). This contrasts with the very low

Electronic supplementary material The online version of this article (doi:10.1007/s10592-015-0752-y) contains supplementary material, which is available to authorized users.

✉ C. L. Dudgeon
c.dudgeon@uq.edu.au

¹ School of Veterinary Science, The University of Queensland, Gatton, QLD 4343, Australia

² Molecular Fisheries Laboratory, School of Biomedical Sciences, The University of Queensland, St Lucia, QLD 4072, Australia

N_e/N_c ratios (e.g. 10^{-3} – 10^{-5}) displayed by species with Type III survivorship curves (i.e. low survivorship to adult stages, high fecundity, early maturation, high variation in reproductive success) such as bony fishes and shrimp (Hauser et al. 2002; Ovenden et al. 2007).

The elasmobranchs (sharks, skates and rays) tend towards Type I survivorship characteristics (Musick 1999). There has been increasing conservation concerns for these animals within the last decade due to their exploitation for fins, meat and other products and their inability to quickly replenish populations under high levels of exploitation. Local and global population declines in N_e are widely reported for many species of elasmobranchs (Ferretti et al. 2010; Worm et al. 2013). A recent study surveying IUCN Red List data for chondrichthyans (elasmobranchs and the ancestral group: chimeras) showed that 17.4 % of 1041 species are classified as threatened, and that almost half the species (46.8 %) are data deficient (Dulvy et al. 2014). Information on the population size and health of these species is required but is difficult to obtain, particularly for rare, threatened and non-commercially fished species.

Few studies have investigated contemporary N_e for elasmobranch populations (Ahonen et al. 2009; Blower et al. 2012; Chapman et al. 2011; Chevolut et al. 2008; Portnoy et al. 2009). Portnoy et al. (2009) found that N_e estimates generated from microsatellite data in sandbar sharks *Carcharhinus plumbeus* were similar in magnitude to N_e estimates of mature adult sharks with resulting ratios of $N_e/N_c \sim 0.5$ – 1 . To date, no other studies have directly compared N_e and N_c in an elasmobranch species, although comparison of the N_e estimates of grey nurse sharks *Carcharias taurus* with published census information for mature adults results in a ratio of similar magnitude (mean $N_e = 126$, 95 % CI 67–474, Ahonen et al. 2009; mean $N_c = 178$, 95 % CI 58–321, Otway and Burke 2004). In contrast, comparison of N_e estimates obtained using temporal comparisons (Wang and Whitlock 2003) between samples taken 40 years apart for the thornback ray *Raja clavata* with very large census sizes of 500,000–3 million adult individuals for this species, resulted in very low N_e/N_c ratios (between 9×10^{-5} and 1.8×10^{-3} , Chevolut et al. 2008). The variation in N_e/N_c ratios among elasmobranch species to date emphasizes the size of the knowledge gap that must be addressed before genetic N_e is useful for the conservation of threatened, endangered and protected species.

Recent years have seen an increase in the use of the linkage disequilibrium (LD) method for estimating contemporary N_e (Waples et al. 2014). As population size decreases, the extent of inbreeding within the population increases resulting in departures from expected genotype and gametic frequencies. The LD between the loci within the population is measured as the deviation between

observed and predicted two-locus genotype frequencies in all combinations from multilocus genotypes across a random sample of individuals from the population (Luikart et al. 2010; Waples et al. 2014). As opposed to temporal methods, which require samples to be collected at multiple points in time, LD parameters require only a single time point random sample, greatly facilitating the sampling collection. This has likely led to a rapid increase in the use of LD as well as other single time point methods to estimate N_e (Palstra and Fraser 2012; Waples et al. 2014). Furthermore, ongoing development of the parameter estimates including bias corrections (Waples 2006) as well as accessible software such as NeEstimator (Do et al. 2014) have facilitated the use of LD methods for N_e estimation. There are however, some theoretical concerns regarding the widespread application of N_e estimators using LD methods. In particular, for iteroparous species with overlapping generations, N_e derived from adults sampled during a single annual breeding aggregation is likely to reflect N_b rather than N_e (Palstra and Fraser 2012). How N_b relates to N_e across different taxa requires further investigation (Waples et al. 2014). Furthermore, like all estimation methods, LD estimates of N_e lack accuracy and precision when derived from low numbers of samples and loci and can also result in inestimable parameters and upper confidence limits (Waples and Do 2010). Inestimable confidence limits have occurred in studies on the sandbar (Portnoy et al. 2009) and white shark (Blower et al. 2012) and may be addressed by increasing the sample size as well as increasing the number of genetic loci. However, it would be ideal to know these in advance as there are practical constraints to obtaining the required number of samples and loci for obtaining these estimates (Hare et al. 2011).

This study investigates N_e in a population of zebra shark (*Stegostoma fasciatum*), a medium-sized (up to 2.5 m total length), oviparous species that aggregates seasonally at key sites in the waters of the central eastern Australian coast. This population occurs towards the temperate latitudinal extent of the distribution for this species and appears to span over 1000 km of coastline as demonstrated by acoustic telemetry studies (Dudgeon unpublished data), but shows genetic separation from the region approximately 1500 km north of the aggregation site (Dudgeon et al. 2009). Robust capture-mark-recapture (CMR) estimates of N_e are available for the population (Dudgeon et al. 2008), and in its Australian range the species has not experienced the substantial declines experienced in the remainder of its distribution (Pillans and Simpfendorfer 2003). Observations show that the aggregating individuals on the central eastern coast are almost entirely mature adults and may constitute a breeding aggregation. A large proportion of adults in the aggregation (~ 25 %) has been sampled for previous population genetic analyses (Dudgeon et al. 2009)

and a species-specific microsatellite library (14 loci) has been developed (Dudgeon et al. 2006). There are two main objectives to this study. The first was to test the expectation that in this population of Type I species the N_e/N_c ratio approaches unity. To do this, we estimated genetic N_e using the LD approach and compared the results with N_c estimates (CMR estimates of the number of mature adults). Secondly, we conducted sensitivity analyses on the numbers of samples and loci required to obtain finite N_e estimates to provide sampling guidelines for future studies based on empirical wildlife population data.

Methods

Census estimates and tissue sample collection

All sampling was conducted at The Group, a subtropical reef located 500 m from North Stradbroke Island in southeast Queensland (Australia). Adult zebra sharks aggregate at this site over the summer months every year (Dudgeon et al. 2008, 2013). Population genetic analyses demonstrate separation of southern Queensland from northern Queensland zebra shark populations (Dudgeon et al. 2009). Census estimates, N_c were obtained through visual mark-recapture surveys of adult zebra sharks conducted between the months of November through February 2003–2006. Individuals were identifiable based on body markings and photographs were used to record presence at the study site (methods described in Dudgeon et al. 2008). Tissue samples were biopsied from 105 individuals during this census time (methods described in Dudgeon et al. 2009). To increase the number of tissue samples in the dataset, further sampling of wild zebra sharks ($n = 49$) was conducted during the austral summer months of 2012 and 2013. Photo-identification was used to compare the new individuals with the original database from 2003 to 2006. A further nine samples were added to the genetic data set resulting in a total of 114 samples after photo-identification matched these new individuals with nine sharks that had been included in the census estimate from 2003 to 2006 (i.e. comprised the same generation as the original samples) but had not had genetic samples taken previously. Further, we compared genotypes to eliminate duplicate individuals. The samples were collected in accordance with the ethical approval of the University of Queensland (Animal Ethics Committee approval # SVS/323/11), and with permit approval from the Queensland Department of Primary Industries and Fisheries (General Fisheries Permit #515490) and the Queensland Department of Environment and Resource Management (Moreton Bay Marine Park Permit #QS2012/MAN153).

Genotyping

Tissue samples were genotyped at 14 microsatellite loci (see Dudgeon et al. 2006, 2009). GenePop analysis (web version 3.4, Raymond and Rousset 1995) revealed physical LD between a pair of microsatellite loci: Sfa205 and Sfa236, and one of these (Sfa236) was subsequently removed. Genetic diversity was summarised with Genalex version 6 (Peakall and Smouse 2006) and the presence of null alleles and likely scoring errors was investigated using Microchecker version 2.2.1 (van Oosterhout et al. 2004).

Linkage disequilibrium N_e estimates

We estimated N_e using the LD method (Hill 1981) with bias correction (Waples 2006), and incorporation of missing data (Peel et al. 2013) as implemented in NeEstimator V2.01 (Do et al. 2014).

N_e/N_c ratio

The ratio of N_e to N_c was derived using the estimate for N_e from the total genetic dataset and the final annual estimate for N_c (Dudgeon et al. 2008). The standard error for the ratio was estimated using the approximation variance formula for the ratio of two random variables, as presented by Kendall and Stuart (1987, p. 351, equation 10.17). An assumption of positive covariance was made between N_e and N_c . Therefore we applied a conservative estimate of covariance within the variance formula based on the theoretical upper limit of covariance between N_e and N_c .

Sensitivity analysis

To assess the effect of sample size on the N_e parameter and variance estimates, the total data set of 13 loci and 114 samples was randomly subsampled using a random number generator to shuffle the data set and select subsamples in increments of five from a minimum of 50 samples through to the total data set of 114 samples. This was replicated 100 times for each subsample. Samples were included in multiple data sets but any duplicate data sets for a given subsample number were discarded. N_e was estimated for each subsample using NeEstimator V2.01 (Do et al. 2014).

To assess the effect of number of loci on the N_e parameter and variance estimates, the total data set was randomly subsampled by restricting the number of loci in increments of 1 from 5 to 13 loci for the total 114 samples. This was replicated up to 100 times with duplicate subsamples being discarded from the analyses. For example, for the 12 loci analysis, each locus was removed from the data set once and then replaced; therefore the dataset was

subsampled 12 times. N_e was then estimated for each subsample using NeEstimator V2.01 (Do et al. 2014).

Finally, to assess the effects of reducing the number of loci and samples concurrently on the N_e parameter and variance estimates, N_e was estimated for each combination of loci and samples between 8 and 13 loci (in increments of 1) and 50 and 114 samples (in increments of 10). This was replicated 100 times and any duplicate data sets produced from the random shuffling were removed from the analyses.

As recommended by Waples and Do (2010), harmonic means of N_e were used to summarise the output of the sets of replicates. Harmonic means are less skewed by the presence of negative N_e estimates compared to arithmetic means. Harmonic means were calculated in FinCal package version 0.6 (<http://felixfan.github.io/FinCal/>) in R version 3.0.1 (R Development Core Team 2013).

The P_{crit} value, the criterion for excluding rare alleles from the analysis, was set to 0.01 for all sample sizes greater than 50 and 0.02 for the sample size equal to or less than 50 based on the formula P_{crit} greater than $1/(2S)$ where S is the number of individuals with data at both pairs of loci (Waples and Do 2010). The inadvertent inclusion of rare alleles has been shown by simulation to upwardly bias the N_e estimates (Macbeth et al. 2013).

Results

The number of alleles per locus varied from four at locus SF138 to 39 at locus SF72. Due to some loci having missing data (see Supplementary material 1), the harmonic mean sample size across all 13 loci for the total 114 samples was 109. There was no evidence of stuttering or null alleles. Summary statistics including observed and expected heterozygosity are reported in the Supplementary material 1.

N_e estimates and N_e/N_c ratio

Estimates based on the total data set of 13 loci, 114 (harmonic mean = 109) samples and $P_{crit} = 0.01$ resulted in $N_e = 377$. The 95 % CI for the N_e estimates were similar for both the parametric and jackknife methods (95 % CI_{parametric} 274–584; CI_{jackknife} 256–677). The parametric method for estimating 95 % CI of N_e is used for the sensitivity analyses. The N_e estimate showed high congruence with the N_e estimate obtained from the mark-recapture estimate of the number of mature, adult zebra sharks: $N_e = 458$, 95 % CI 298–618 (Dudgeon et al. 2008). Therefore, based on these estimates the ratio of N_e/N_c for Australian east coast population of leopard sharks was 0.82 ± 0.33 SE.

Sensitivity analyses

Reducing the number of samples used for the estimate showed a loss in precision when the number of samples dropped below 96 (Fig. 1). Due to some missing data and duplication in the sub-sampling process, the number of samples used to estimate N_e in the sensitivity analysis tended to be around 5 less than the total number included in the data set. When the average number of samples used in the analyses was 91 or below, some N_e estimates displayed inestimable upper confidence intervals, indicated as infinity in the analysis output. The percentage of estimates with inestimable upper confidence intervals increased steadily with the reduction of sample size to 50 (mean of 47). At this minimum sample size, 57 % of the 100 replicates demonstrated inestimable upper confidence intervals. When the number of samples was reduced below 80, the N_e parameter was inestimable for some of the replicates as indicated by negative N_e output values. For all sample size variations, the harmonic mean of N_e remained fairly stable between 300 and 500. However, as the number of samples was reduced the spread of the N_e estimates for the 100 replicates increased substantially (Fig. 2). At 91 samples, 26 % of the N_e estimates were outside of the 95 % CI values from the total data set, with 25 % being greater than the upper 95 % CI limit. The lower confidence intervals remained reasonably consistent across sample size, fluctuating between 200 and 300 (Fig. 2).

Reducing the number of loci used for the estimate showed a loss in precision when the number of loci dropped below 10 (Fig. 3). More specifically, when the number of loci used in the analyses was 9 or below, some N_e estimates started displaying upper confidence intervals that could not be computed. The percentage of estimates with upper confidence intervals like this increased steadily from 4 % at 9 loci to 46 % at 5 loci. Estimates of N_e that could not be computed were only generated for a few iterations of 5 (3 %) and 6 (2 %) loci. The harmonic mean of N_e for all loci subsamples remained consistently between 300 and 400 (Fig. 4). At 9 loci, 17 % of the N_e estimates were outside the 95 % CI of the total data set, with 10 % below the lower 95 % CI limit and 7 % above the upper 95 % CI limit. The lower confidence intervals remained reasonably consistent across sample size, fluctuating between 200 and 300.

When samples and loci were subsampled concurrently, inestimable upper CI values appeared with higher numbers of samples at lower loci. In particular, for 10, 11 and 12 loci, inestimable upper CI values appeared at 101 samples as opposed to 91 samples for the entire data set (Fig. 5).

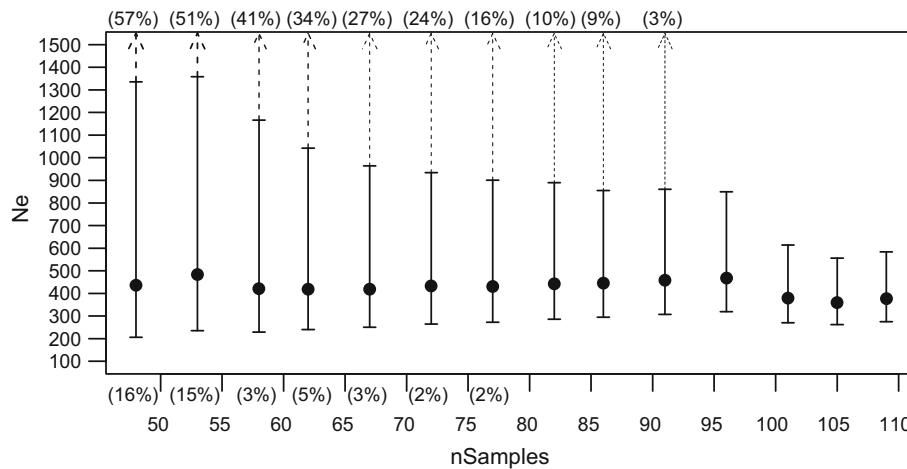


Fig. 1 Sensitivity analysis showing the effects of subsampling the number of samples ($\times 100$) for the total number of loci ($n = 13$). The harmonic mean of the number of samples (nSamples) is given on the x-axis. The harmonic mean for N_e and the upper and lower CIs are

shown. The *dashed upward arrows* indicate the percentage of upper CI with infinite values. The percentage of N_e estimates with negative values per sample size is indicated in *brackets* below the x-axis

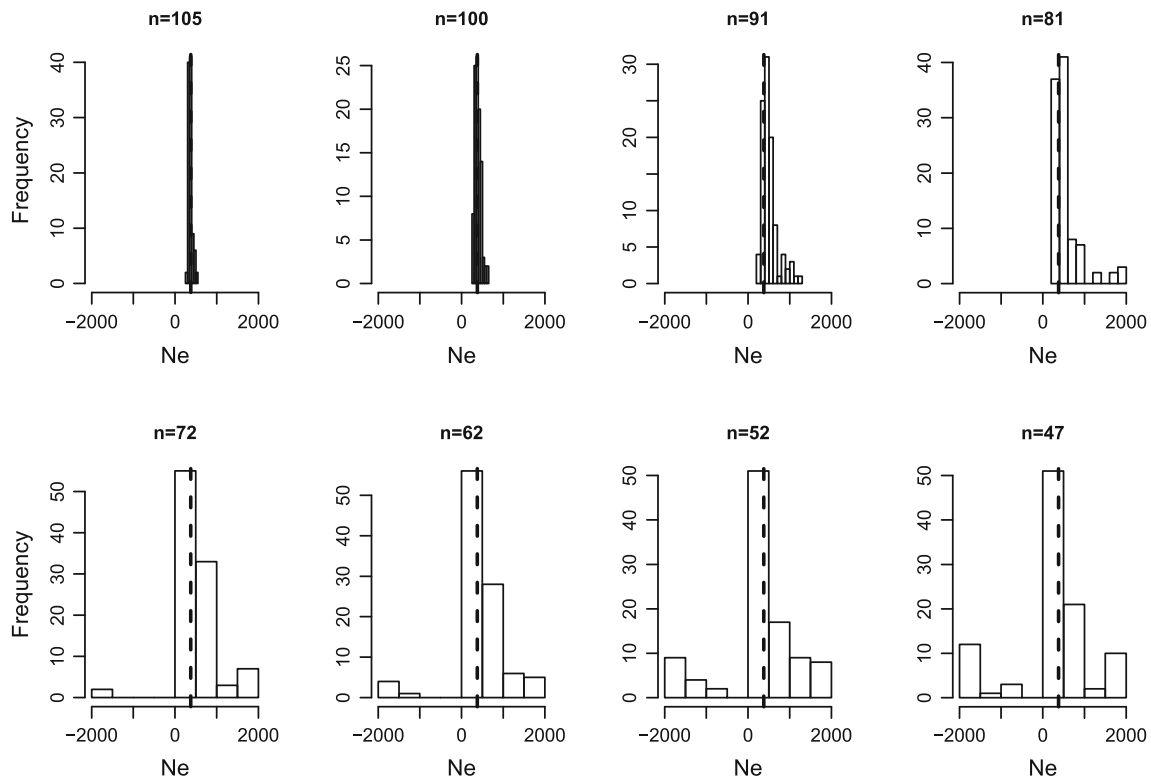


Fig. 2 Distribution of N_e estimates at each of the sample size increments for $P_{crit} = 0.01$ ($n \geq 52$) and $P_{crit} = 0.02$ for $n = 47$. The *vertical dashed line* represents the N_e estimate from the total data set

($N_e = 377$). The outer bins on the x-axis correspond to estimates $N_e \leq -2000$ and $N_e \geq 2000$

Discussion

Effective population size estimates provide useful insights into possible loss of genetic diversity and evolutionary potential (Tallmon et al. 2010). Guidelines suggest that N_e

values of at least 50 are required to prevent inbreeding depression (Franklin 1980); N_e values greater than 500 are required to retain long-term evolutionary potential (Frankham et al. 2010; Franklin 1980) and N_e values greater than 1000 are required to avoid deleterious allele accumulation (Palstra and

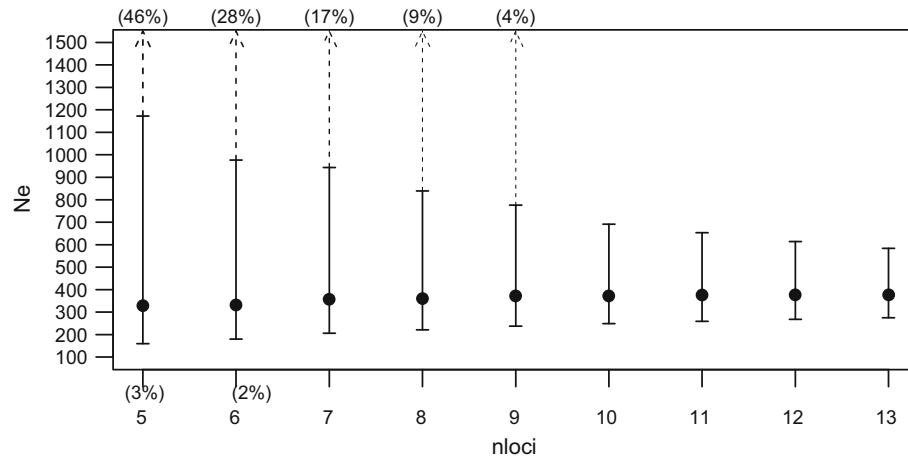


Fig. 3 Sensitivity analysis showing the effects of subsampling the number of loci ($\times 100$) for the total number of samples ($n = 114$). The harmonic mean of the number of samples (n_{Samples}) is given on the x-axis. The harmonic mean for N_e and the upper and lower CIs are

shown. The *dashed upward arrows* indicate the percentage of upper CI with infinite values. The percentage of N_e estimates with negative values per number of loci is indicated in *brackets* below the x-axis

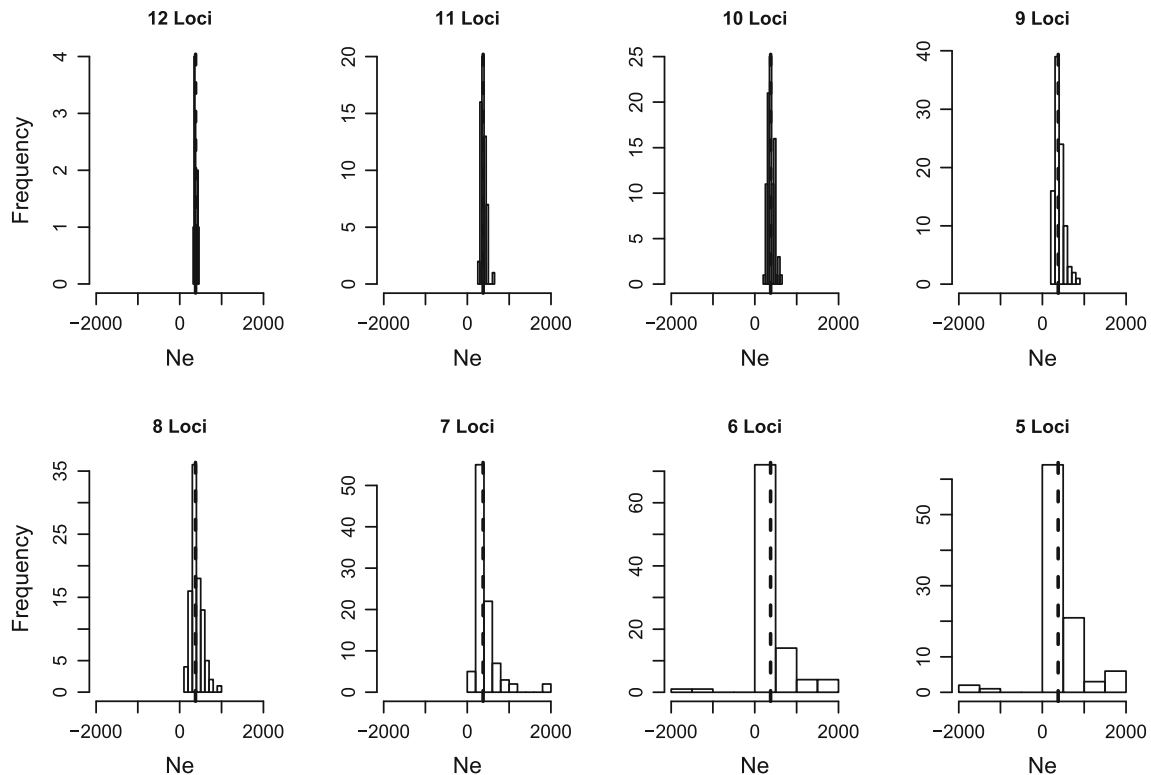


Fig. 4 Distribution of N_e estimates at each of the loci increments for $P_{\text{crit}} = 0.01$. The *vertical dashed line* represents the N_e estimate from the total data set ($N_e = 377$). The outer bins on the x-axis correspond to estimates $N_e \leq -2000$ and $N_e \geq 2000$

Ruzzante 2008). In this study we found the zebra shark population to have a mean N_e of 377 (upper confidence intervals 584–677). N_e values have been reported ranging from 126 to 1512 for populations of five other elasmobranch species (Table 1). Four of these species reported populations with N_e values < 500 suggesting these taxa may lack sufficient long-term evolutionary potential for persistence. However, a

review by Palstra and Ruzzante (2008) showed that $\sim 70\%$ of studies reported similarly low N_e values although only 25% of studies were of populations considered to have conservation concerns. Given this study population of zebra shark is largely protected with few threats, the small estimated N_e values may be indicative of a healthy population of a relatively rare species. However, this N_e estimate may also indicate that

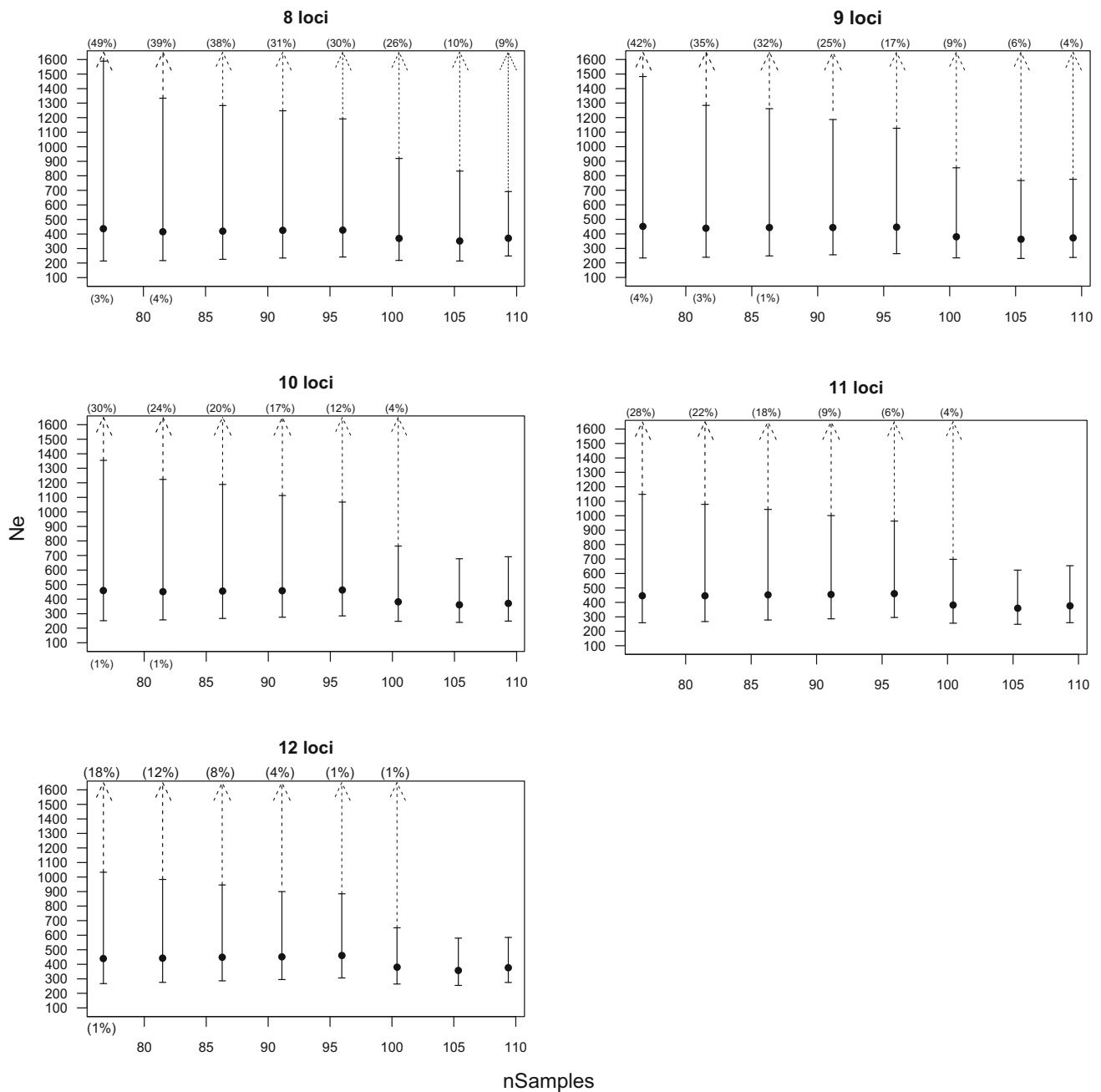


Fig. 5 Sensitivity analysis showing the effects of subsampling the number of loci and samples concurrently ($\times 100$). The harmonic mean for N_e and the upper and lower CIs are shown. The *dashed upward*

arrows indicate the percentage of upper CI with infinite values. The percentage of N_e estimates with negative values per sample size is indicated in *brackets* below the x-axis

zebra sharks are susceptible to overexploitation and have limited long-term evolutionary potential.

N_e and N_c comparison

For species with Type I survivorship curves, as displayed by most elasmobranch species including zebra sharks, N_e may also provide an indication of the census size of the

population. While no constant relationships exist between N_e and N_c across taxa, our findings support those of Portnoy et al. (2009) for the sandbar shark, where N_e closely resembles N_c ($N_e/N_c = 0.5-1$). Portnoy et al. (2009) warn that if low and similar N_e and N_c values are common across elasmobranch species this may be a cause for concern due to large amounts of exploitation and low rebound potential for many taxa. Obtaining N_c estimates for elasmobranch populations is difficult and there are few studies where a

Table 1 Comparison of genetic effective population size (N_e) and N_e/N_c (census size) ratios for different shark and ray species

Species	N_e (95 % CI)	N_e/N_c (range)	Sample size	# loci
<i>Carcharhinus plumbeus</i> Sandbar shark ^a	416–1080 (202– ∞)	0.5 (0.21–1.0)	53–201 per cohort	8
<i>Carcharias taurus</i> Grey nurse shark ^{b,c}	126 (68–474)	0.7 (0.21–1.0)	63	6
<i>Carcharodon carcharias</i> White shark ^d	1512	NA	97	6
<i>Pristis pectinata</i> Small tooth sawfish ^e	230–350 (142–955)	NA	137	8
<i>Raja clavata</i> Thornback ray ^f	283 (145–857)	10^{-3} – 10^{-5}	363	5

The sample sizes and numbers of microsatellite loci used to generate the N_e estimates are shown. The use of ∞ follows the notation from the publication and indicates inestimable upper confidence limits

^a Portnoy et al. (2009)

^b Ahonen et al. (2009)

^c Otway and Burke (2004)

^d Blower et al. (2012)

^e Chapman et al. (2011)

^f Chevolut et al. (2008)

direct comparison is possible. Ratios approaching unity were generated when combining separate studies of N_e and N_c for a population of grey nurse sharks (Ahonen et al. 2009; Otway and Burke 2004). In contrast, the N_e/N_c ratio for the thornback ray was orders of magnitude smaller than for other elasmobranchs (10^{-3} , Chevolut et al. 2008). These disparate findings raise questions about the effects of life history strategies on N_e and N_c estimates within elasmobranchs as well as potential effects due to population structuring and immigration.

Comparison of N_e/N_c ratios across a wide array of taxa demonstrated a median ratio of 0.14 with a general pattern of lower N_e/N_c ratios for species with Type III survivorship curves (such as 10^{-3} – 10^{-5}) than for species with Type I or Type II survivorship curves (e.g. 0.5–1) (Palstra and Ruzante 2008). Variation in reproductive success has been proposed as a primary influence on N_e/N_c ratios in wildlife populations (Frankham 1995; Hedgecock 1994) with fecundity and juvenile mortality two factors driving that variation. Elasmobranchs typically have late maturation, low fecundity, long life-spans and consistent reproductive output per year. In comparison, many teleosts and marine invertebrate species have high fecundity, high juvenile mortality and highly variable reproductive output (Hedgecock 1994). There is considerable variation in reproductive strategies between elasmobranch species and the potential affects on N_e and N_e/N_c relationships unknown. Elasmobranchs display a wide range of reproductive modes including two forms of oviparity and six forms of viviparity (Carrier et al. 2004; Musick and Ellis 2005). Fecundity varies across taxa from one to hundreds of pups per year. In general, skates are considered more fecund than other elasmobranchs due to their oviparous form. The greatest annual fecundity is recorded from the thornback

ray with up to 153 eggs presumed laid per year although survival rate per egg is unknown (Frisk 2010). The reproductive periodicity and fecundity for zebra sharks is largely unknown in the wild but they are popular aquarium animals that breed easily in captivity. Zebra sharks mature at around 6 years of age (D. Robinson pers. comm.) and one captive male shark is at least 28 years old (Reef HQ Aquarium, Townsville pers.comm.). A captive female shark laid 80 eggs within an annual breeding season of which 19 survived through hatching (L. Squire Jr, pers. comm). Posthatching survival in the wild is unknown but adult sharks show high survivorship (Dudgeon et al. 2008), which is typical of elasmobranchs (Cortés 2004). In contrast, sandbar sharks display viviparity with late maturation at around 13 years (from the Western Atlantic) producing 8–10 pups every 2–3 years (Sminkey and Musick 1996). Similarities in findings of N_e and N_e/N_c ratios for zebra sharks and sandbar sharks point to life history strategies such as late maturation and long life span having greater affect on N_e and N_e/N_c estimates than reproductive mode itself. In a recent study by Waples et al. (2013), N_e/N_c ratios were examined across 63 different species and age at maturity and adult lifespan were found to account for half the variation across the reported ratios.

Changes in census population sizes will also affect the relationship between N_e and N_c (Frankham 1995; Palstra and Fraser 2012). Fluctuations in N_c may occur due to changes in populations over time from natural or anthropogenic causes. Natural causes are more likely to occur in organisms such schooling bony fish and small mammals with boom and bust life history strategies, although climatic extremes can result in population devastation across taxonomic groups (Frankham 1995; Chavez et al. 2003). Temporal changes in population size (e.g. Worm et al.

2013) due to over-fishing will affect N_e/N_c ratios as changes to N_e are likely to lag behind changes in N_c depending on the number of generations over which these changes occur, the degree of generational overlap (Palstra and Fraser 2012; Waples et al. 2014), and the age composition of the individuals removed from the population. In simulated data with discrete generations, N_e estimated using the LD method was able to detect early population declines (Antao et al. 2010).

N_e will be more representative of N_c when the population size is constant and when N_c is taken as the number of reproductively mature adults in the population rather than other definitions of N_c such as the total number of individuals alive at one time (Palstra and Fraser 2012). This study provides a good model system for elasmobranchs based on these criteria as this is one of few studies to provide robust census size estimates for mature sharks within a population to compare with robust N_e estimates. The census size estimates used in this study were based on robust mark-recapture modeling of photo-identification surveys of an aggregation of mature adults conducted over a 3-year period (Dudgeon et al. 2008). Zebra sharks are unexploited in Australia, and therefore, the census size of the focal population is likely to be constant over the last several generations. Furthermore, several individuals in the aggregation have been observed between 8 and 12 years post initial sighting (Dudgeon unpublished data) supporting the long-term stability of this population.

Factors affecting N_e

The N_e estimator used in this study is based on the LD method. In contrast to temporal methods, which require multiple samples over time, LD estimators offer a major advantage in terms of cost and effort required for single time point sampling (Whiteley et al. 2012). Along with the ongoing development of the parameter estimate corrections for bias, sample size and missing values (Waples 2006; Peel et al. 2013) as well as accessible software to implement parameter estimation, there has been a rapid increase in the use of these estimators in recent years (Waples et al. 2014). However, the interpretation of the N_e estimation is restricted by some biological considerations including gene flow between populations and overlapping generations (Whiteley et al. 2012). Migration between neighbouring populations can affect both N_e and N_c depending on the net direction of movement. While immigration can increase the N_c of a population, it can also increase N_e and the resulting N_e/N_c ratio, as local N_e will approach global N_e even though the local census size may not increase (Charlesworth 2009). For example, positive bias on N_e/N_c due to immigration was detected in house sparrow

populations (Baalsrud et al. 2014). Conversely, immigration can also result in reducing N_e values based on LD. This is due to an apparent increase in the LD signal amongst a mixed sample from the recipient population when immigrants and residents are genetically differentiated (Waples and England 2011). For this study, any effect of migration on the N_e estimates is likely to be minimal. Zebra sharks are a tropical species and the population examined within this study exists at the latitudinal extreme of their distribution in subtropical waters where zebra sharks aggregate over the warmer months each year. Population genetic analysis shows low but significant F_{st} values implying separation from the northern population (approximately 1500 km to the north) (Dudgeon et al. 2009). Furthermore, acoustic tracking studies show strong site fidelity to the aggregation site over the aggregation period with tagged animals moving hundreds of kilometres but returning annually to the study site (Dudgeon et al. 2013; Dudgeon unpublished data).

Aggregations of long-lived animals such as zebra sharks are likely to be composed of overlapping generations. Inability to assign sampled individuals to cohorts raises questions as to whether N_e (the harmonic mean of the number of breeders contributing to all cohorts) or N_b (the number of breeders contributing to a single cohort) is being estimated. While N_e is the primary parameter of interest for examining evolutionary processes, N_b may be more informative for ecological time scales (Waples et al. 2014) as well as having greater promise for assessing trends in population sizes relative to management agencies (Ozerov et al. 2015). Waples et al. (2013) investigated the potential effects of overlapping generations on N_e estimates based on LD methods across a range of iteroparous taxa. They proposed that two life history traits, age at maturity and adult life span, accounted for two-thirds of the variation found amongst N_e/N_b ratios for the taxa examined. Although no elasmobranchs were included in the survey by Waples et al. (2013), of all the included taxa, dolphins showed the most similar life history traits (age at maturity = 8 years, adult life-span = 27 years) to zebra sharks (age at maturity = 6 years, adult life-span = 28+ years). Dolphins like other mammals were found to have N_e/N_b ratios ~ 1 . Similar relationships may exist between N_b and N_e for elasmobranchs, which would reduce the complexity in the interpretation of the N_e estimates with overlapping generations.

Simulation study findings

There are few guidelines available for the numbers of individuals and loci required for estimating genetic N_e in wildlife populations. The anticipated N_e is likely to dictate the number

of individuals required (Waples and Do 2010). Palstra and Ruzzante (2008) proposed sampling at least 10 % of the N_e to achieve finite point estimates of N_e with the temporal method. Simulation studies by Tallmon et al. (2010) demonstrated that for small populations ($N_e \sim 100$ –250) samples sizes of 60 (equates to ~ 24 –60 % of N_e) with 15 loci resulted in precise N_e estimates. For larger populations ($N_e \sim 500$), samples sizes needed to be increased to ~ 120 (equates to ~ 24 % of N_e). For very large populations however, genetic methods are unlikely to provide reasonable N_e estimates due to little genetic drift unless the effective size is much smaller than the census size (Tallmon et al. 2010). In this study, using an LD approach, we found finite point estimates of N_e and consistent precision in CI when the number of samples was ≥ 91 for the total 13 loci data set. This equates to approximately 25 % of the N_e estimate and 20 % of the N_e estimate.

Guidelines for the numbers of loci required for N_e estimation indicate that ≥ 10 unlinked and highly polymorphic loci are desirable (Luikart et al. 2010; Palstra and Ruzzante 2008; Waples and Do 2010). The sensitivity analyses for the zebra shark populations support these values. For the total data set of 114 individuals, the harmonic mean and spread of the N_e estimates was similar for 10–13 loci. When the number of loci dropped below 10, some iterations resulted in inestimable upper confidence limits. These findings can be informative for studies reporting N_e estimates on elasmobranch species. Estimates for sandbar (Portnoy et al. 2009) and white sharks (Blower et al. 2012) both resulted in inestimable upper confidence limits. Both studies had proportionally low numbers of samples to N_e estimates as well as the number of microsatellite loci used: 8 and 6 respectively (see Table 1). In contrast the studies on grey nurse sharks (Ahonen et al. 2009) and small tooth sawfish *Pristis pectinata* (Chapman et al. 2011) resulted in estimable upper confidence limits. While the numbers of microsatellite loci employed for these studies were similar to that for sandbar and white sharks, the relative number of samples to N_e estimates were much higher. That is samples numbers were 40–50 % of the N_e estimates for grey nurse sharks and sawfish in comparison to samples numbers of ~ 6 –12 % of N_e estimates for white and sandbar sharks (Table 1). Ideally a pilot study should be conducted to obtain preliminary N_e estimates to inform the numbers of individuals and loci needed to obtain the required statistical accuracy and precision.

There are practical constraints with the number of samples that can be collected and the number of loci that can be developed for these studies. With the continual development of high throughput DNA techniques (see Dudgeon et al. 2012), increasing the numbers of loci may be more achievable than increasing sample numbers. Tallmon et al. (2010) found a greater increase in precision for small population sizes ($N = 100$ or 250) from doubling

numbers of loci than sampling size. However, our findings support those of Luikart et al. (2010) and Antao et al. (2010) that show increasing the number of samples has a greater effect on precision than increasing the numbers of loci. Furthermore, increasing loci numbers can result in physical LD, which will negatively bias N_e estimates (Waples and Do 2010). One of the 14 loci developed for the zebra shark microsatellite library had to be removed from the dataset due to physical LD. The inclusion of this locus in N_e estimation resulted in a considerable downward bias ($N_e = 181.2$, 95 % $CI_{\text{parametric}} 153$ –219).

An interesting pattern emerging from the sensitivity analyses showed that the harmonic mean of the N_e estimates remained fairly consistent throughout different combinations of samples and loci, although the spread of the N_e estimates increased along with the proportion of inestimable N_e estimates and upper CI for reduced numbers of samples and loci. Therefore, with smaller data sets it would also be worthwhile to run a series of sensitivity analyses to examine the stability of the N_e estimates across reduced data sets.

Broader implications

This study lends support to the close coupling of N_e and N_c in elasmobranchs as demonstrated by Portnoy et al. (2009). Furthermore, we have provided guidelines for the minimum numbers of samples (91 samples or 20 % of the census population) and loci (10) needed to obtain good precision and finite values for N_e estimates. We recommend examining N_e/N_c ratios across a wider variety of elasmobranch taxa using standardized methodology including LD methods for N_e estimates and CMR or other fisheries-independent abundance surveys for N_e estimates. By including elasmobranch species with different life-history strategies such as age at maturity, longevity, fecundity and reproductive periodicity, it will be possible to more broadly examine the relationship between N_e and N_c and to assess the potential for N_e to provide information on populations for which ecological abundance estimates are not possible. Given the patterns emerging from the few taxa examined, there may be consistencies amongst taxa that will make N_e a very useful tool in light of conservation and management challenges for elasmobranchs.

Data accessibility

The complete microsatellite genotyping dataset of 114 samples and 14 loci is available for download through the eSpace at the University of Queensland (doi:10.14264/uql.2015.717).

Acknowledgements This project was funded by The Sea World Research and Rescue Foundation and The University of Queensland. We thank numerous field assistants, in particular Adam Barnett, Cynthia Awruch, Michael Williamson, Lydie Couturier, Rob Slade, Jody Kreuger and Angela Dean. Thanks to Sean Corley and the Animal Genetics Laboratory, Gatton, UQ for genotyping data; Dean Blower and Tom Kashiwagi for informative discussions; Lucy Ransome for photo-ID matching and Matias Braccini and Adrian Thomson for analytical assistance.

References

- 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
- Antao T, Perez-Figueroa A, Luikart G (2010) Early detection of population declines: high power of genetic monitoring using effective population size estimators. *Evol Appl* 4:144–154
- Baalsrud HT, Saether BE, Hagen IJ et al (2014) Effects of population characteristics and structure on estimates of effective population size in a house sparrow metapopulation. *Mol Ecol* 23:2653–2668
- Begon M, Harper MJ, Townsend CR (1990) *Ecology: individuals, populations and communities*, 2nd edn. Blackwell, Cambridge
- Blower DC, Pandolfi JM, Bruce BD, Gomez-Cabrera M, Ovenden JR (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
- Carrier JC, Pratt HL, Castro JI (2004) Reproductive biology of elasmobranchs. In: Carrier JC, Musick JA, Heithaus MR (eds) *Biology of sharks and their relatives*. CRC Press, Boca Raton, pp 269–286
- Chapman DD, Simpfendorfer CA, Wiley TR et al (2011) Genetic diversity despite population collapse in a critically endangered marine fish: the smalltooth sawfish (*Pristis pectinata*). *J Hered* 102:643–652
- Charlesworth B (2009) Fundamental concepts in genetics: effective population size and patterns of molecular evolution and variation. *Nat Rev Genet* 10:195–205
- Chavez FP, Ryan J, Lluch-Cota SE, Niquel MC (2003) From anchovies to sardines and back: multidecadal change in the Pacific Ocean. *Science* 299:217–221
- Chevolut M, Ellis JR, Rijnsdorp AD, Stam WT, Olsen JL (2008) Temporal changes in allele frequencies but stable genetic diversity over the past 40 years in the Irish Sea population of thornback ray, *Raja clavata*. *Heredity* 101:120–126
- Cortés E (2004) Life history patterns, demography, and population dynamics. In: Carrier JC, Musick JA, Heithaus MR (eds) *Biology of the sharks and their relatives*. CRC Press, Florida
- Do C, Waples RS, Peel D et al (2014) NeEstimator V2: reimplementation of software for the estimation of contemporary effective population size (N_e) from genetic data. *Mol Ecol Resour* 14:209–214
- Dudgeon CL, Feldheim KA, Schick M, Ovenden JR (2006) Polymorphic microsatellite loci for the zebra shark *Stegostoma fasciatum*. *Mol Ecol Notes* 6:1086–1088
- Dudgeon CL, Noad MJ, Lanyon JM (2008) Abundance and demography of a seasonal aggregation of zebra sharks *Stegostoma fasciatum*. *Mar Ecol Prog Ser* 368:269–281
- Dudgeon CL, Broderick D, Ovenden JR (2009) IUCN classification zones concord with, but underestimate, the population genetic structure of the zebra shark *Stegostoma fasciatum* in the Indo-West Pacific. *Mol Ecol* 18:248–261
- Dudgeon CL, Blower DC, Broderick D, Giles JL, Holmes BJ, Kashiwagi T, Krück NC, Morgan JAT, Tillett BJ, Ovenden JR (2012) A review of the application of molecular genetics for fisheries management and conservation of sharks and rays. *J Fish Biol* 80:1789–1843. doi:10.1111/j.1095-8649.2012.03265.x
- Dudgeon CL, Lanyon JM, Semmens JM (2013) Seasonality and site fidelity of the zebra shark, *Stegostoma fasciatum*, in southeast Queensland, Australia. *Anim Behav* 85:471–481
- Dulvy NK, Fowler SL, Musick JA et al (2014) Extinction risk and conservation of the world's sharks and rays. *eLife* 3:e00590
- Ferretti F, Worm B, Britten GL, Heithaus MR, Lotze HK (2010) Patterns and ecosystem consequences of shark declines in the ocean. *Ecol Lett* 13:1055–1071
- Frankham R (1995) Effective population size/adult population size ratios in wildlife: a review. *Genet Res* 66:95–107
- 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: Soule ME, Wilcox BA (eds) *Conservation biology: an evolutionary-ecological perspective*. Sinauer Associates, Sunderland, pp 135–150
- Frisk MG (2010) Life history strategies of batoids. In: Carrier JC, Musick JA, Heithaus MR (eds) *Sharks and their relatives II: biodiversity, adaptive physiology, and conservation*. CRC Press, Boca Raton, pp 283–307
- Hare MP, Nunney L, Schwartz MK et al (2011) Understanding and estimating effective population size for practical application in marine species management. *Conserv Biol* 25:438–449
- Hauser L, Adcock GJ, Smith PJ, Ramirez JHB, Carvalho GR (2002) Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). *Proc Natl Acad Sci USA* 99:11742–11747
- Hedgcock D (1994) Does variance in reproductive success limit effective population sizes of marine organisms? In: Beaumont AR (ed) *Genetics and evolution of aquatic organisms*. Chapman & Hall, London, pp 122–134
- Kendall MG, Stuart A (1987) *The advanced theory of statistics vol I*, 4th edn. Charles Griffen & Company, London, p 351
- Lande R (1995) Mutation and conservation. *Conserv Biol* 9:782–791
- 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, Buckworth RC, Ovenden JR (2013) Linkage disequilibrium estimation of effective population size with immigrants from divergent populations: a case study on spanish mackerel (*Scomberomorus commerson*). *G3* 3:709–717
- Musick JA (1999) Ecology and conservation of long-lived marine animals. In: Musick JA (ed) *Life in the slow lane: ecology and conservation of long lived marine animals*. American Fisheries Society Symposium, Bethesda, pp 1–10
- Musick JA, Eillis JK (2005) Reproductive evolution of chondrichthyans. In: Hamlett WC (ed) *Reproductive biology and phylogeny of chondrichthyes*. Science Publishers Inc., Enfield, pp 1–25
- Otway NM, Burke AL (2004) Mark-recapture population estimate and movements of grey nurse sharks. In: *NSW Department of Primary Industries Fisheries Final Report Series*. NSW Fisheries Office of Conservation, Nelson Bay, Australia
- Ovenden JR, Peel D, Street R et al (2007) The genetic effective and adult census size of an Australian population of tiger prawns (*Penaeus esculentus*). *Mol Ecol* 16:127–138
- Ozerov M, Jurgenstein T, Aykanat T, Vasemagi A (2015) Use of sibling relationship reconstruction to complement traditional monitoring in fisheries management and conservation of brown trout. *Conserv Biol*. doi:10.1111/cobi.12480
- Palstra FP, Fraser DJ (2012) Effective/census population size ratio estimation: a compendium and appraisal. *Ecol Evol* 2:2357–2365

- Palstra FP, Ruzzante DE (2008) Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? *Mol Ecol* 17:3428–3447
- Peakall R, Smouse PE (2006) GENALEX 6: a genetic analysis in Excel. Population genetic software for teaching and research. *Mol Ecol Notes* 6:288–295
- Peel D, Waples RS, Macbeth GM, Do C, Ovenden JR (2013) Accounting for missing data in the estimation of contemporary genetic effective population size (N_e). *Mol Ecol Resour* 13:243–253
- Pillans RD, Simpfendorfer CA (2003) Zebra shark, *Stegostoma fasciatum* (Hermann, 1973). In: Cavanagh RD, Kyne PM, Fowler SL, Musick JA, Bennett MB (eds) The conservation of status Australasian Chondrichthyan: report of the IUCN Shark Specialist Group Australia and Oceania Regional Red List Workshop. IUCN, Brisbane
- 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
- R Development Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J Hered* 86:248–249
- Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. *Conserv Biol* 17:230–237
- Sminkey T, Musick JA (1996) Demographic analysis of the sandbar shark, *Carcharhinus plumbeus*, in the western North Atlantic. *Fish Bull* 61:1–38
- Sollman R, Gardner B, Parsons AW et al (2013) A spatial mark-resight model augmented with telemetry data. *Ecology* 94:553–559
- Tallmon DA, Gregovich D, Waples RS et al (2010) When are genetic methods useful for estimating contemporary abundance and detecting population trends? *Mol Ecol Resour* 10:684–692
- 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
- Wang JL, Whitlock MC (2003) Estimating effective population size and migration rates from genetic samples over space and time. *Genetics* 163:429–446
- 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, 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, England PR (2011) Estimating contemporary effective population size on the basis of linkage disequilibrium in the face of migration. *Genetics* 189:633–644
- Waples RS, Luikart G, Faulkner JR, Tallmon DA (2013) Simple life-history traits explain key effective population size ratios across diverse taxa. *Proc Biol Sci* 280:20131339
- Waples RS, Antao T, Luikart G (2014) Effects of overlapping generations on linkage disequilibrium estimates of effective population size. *Genetics* 197:769–780
- Whiteley AR, Coombs JA, Hudy M et al (2012) Sampling strategies for estimating brook trout effective population size. *Conserv Genet* 13:625–637
- Worm B, Davis B, Kettner L et al (2013) Global catches, exploitation rates, and rebuilding options for sharks. *Mar Policy* 40:194–204
- Wright S (1931) Evolution in Mendelian populations. *Genetics* 16:97–159