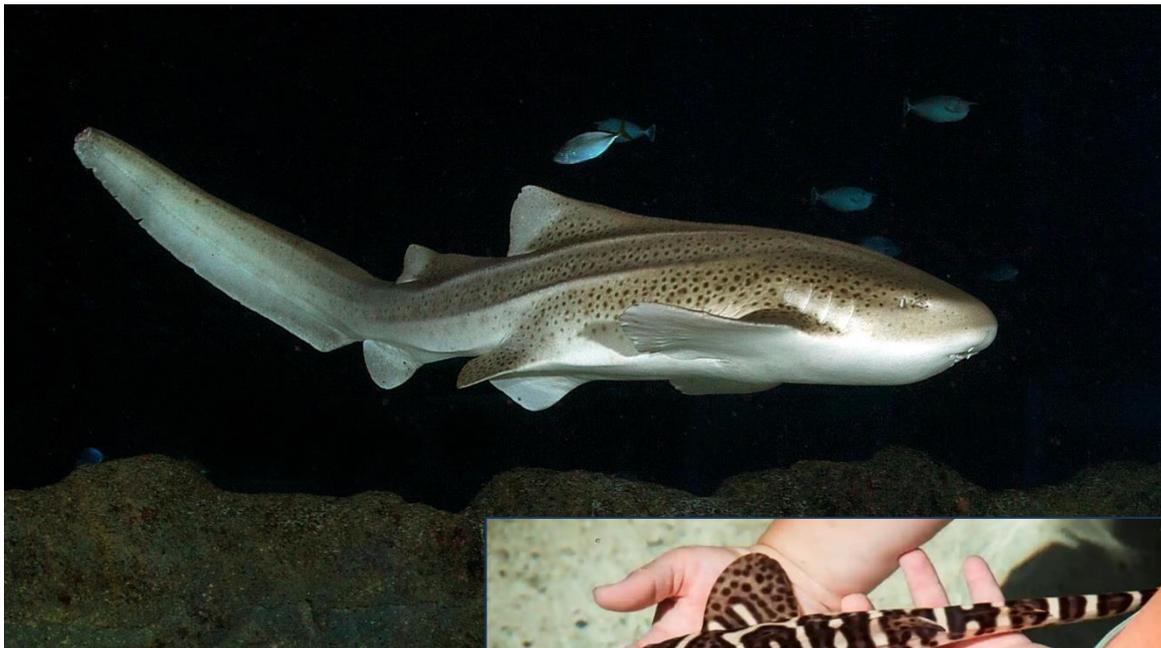# Population Viability Analysis (PVA) Report
for Population Augmentation of Zebra Sharks (*Stegostoma tigrinum*) in Raja Ampat, Indonesia

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Prepared by Kathy Traylor-Holzer, Ph.D.
Senior Program Officer, IUCN SSC Conservation Planning Specialist Group

Cover photos: Mark Erdmann (adult shark); Tourism and Events Queensland (juvenile shark)

A contribution of the IUCN SSC Conservation Planning Specialist Group, in collaboration with the StAR Project.

Table of Contents

Executive Summary

The zebra shark, *Stegostoma tigrinum* is a broadly distributed shark species of the Indo-West Pacific thought to be locally extinct throughout much of Indonesia and functionally extinct in the Raja Ampat archipelago. A shark and ray sanctuary with a well-enforced network of marine protected areas in Raja Ampat provides a good opportunity for zebra shark recovery, but may require management intervention. In 2020 the *Stegostoma tigrinum* Augmentation and Recovery (StAR) Project was initiated, a multi-national collaborative initiative to re-establish a healthy, genetically diverse, and self-sustaining population of zebra sharks, starting with the Raja Ampat archipelago.

The purposes of this Population Viability Analysis (PVA) were to develop a *VORTEX* population model for the zebra shark and to use this model to evaluate potential strategies for augmentation of the wild zebra shark population in Raja Ampat and identify key knowledge gaps related to this project. PVA results will inform decisions by the StAR Project in its efforts to re-establish a healthy zebra shark population in Raja Ampat.

Data and expert opinion were used to develop a reasonable stochastic population model for this species. This base model represents demographic traits expected for a healthy, self-sustaining population that is not under threat. Survival rates, especially for sharks over one year of age, have the greatest uncertainty and, along with fecundity, influence population growth and viability. Conditions in Raja Ampat are assumed to contain good habitat, prey availability and protection from exploitation. Vital rates were estimated to result in a conservative intrinsic population growth rate of 5% annually.

A large population size is necessary to maintain high levels of genetic variation and low accumulation of inbreeding. Results using the base model proposed that at least 200 sharks are needed for good demographic growth and resilience against short-term severe decline without risk of extinction, while 500 or more sharks may be needed to maintain high genetic variation and avoid inbreeding impacts.

Modeling suggested that the existing estimated zebra shark population in Raja Ampat is not likely to be viable long term due to its small size and relative isolation. While population projections are highly variable, PVA results indicated that it may take 60-90 years to reach a population size of 100-200 sharks needed for demographic stability and resilience against severe decline, with a 23% chance of extinction over this time. Projected loss of genetic diversity and accumulation of inbreeding due to small population size suggest that the zebra shark population in Raja Ampat is unlikely to remain genetically healthy without significant genetic augmentation of the population.

Population augmentation model scenarios simulating the release of juvenile sharks into Raja Ampat as proposed by the StAR Project resulted in substantially increased projected population size and genetic diversity, resilience to rebound from severe decline with little to no extinction risk, and overall good viability. A release program length of around 10 years is estimated as reasonable in terms of conservation benefit, but actual benefit will be dependent upon realized release and survival rates. Genetic lines available in the current *ex situ* potential breeding stock in the Association of Zoos and Aquariums Species Survival Plan® (SSP®) are estimated to be sufficient for beneficial genetic augmentation.

Uncertainty around demographic rates used in the model, both for wild shark vital rates and for release program success rates, prevent precise projections for future shark population size. However, this PVA is a valuable tool in assessing the relative viability of the zebra shark population in Raja Ampat with and without augmentation, the conservation value of population augmentation, and the relative augmentation effort that may be needed. Taken together, these PVA results provide strong evidence that population augmentation can be significantly valuable to the establishment of a healthy, viable population of zebra sharks in Raja Ampat. In addition, this PVA project can serve as a model for other shark PVAs that incorporate demography and genetics to explore population management options for conservation. Likewise, if successful, the StAR Project may serve as a model for the conservation translocation of captive-bred sharks into the wild.

Zebra Shark Model Development

Purpose of PVA

The zebra shark, *Stegostoma tigrinum* (formerly *S. fasciatum*), is a broadly distributed shark species of the Indo-West Pacific and is usually found within a narrow band of shallow coral reef habitat and soft bottom (Dudgeon *et al*. 2019). This species is also commonly referred to as the leopard shark, but will be referred to as the zebra shark throughout this report to avoid confusion with another leopard shark, *Triakis semifasciata*, that is native only to the west coast of North America. Two subpopulations of *S. tigrinum* are recognized based on genetic analysis of 13 microsatellite loci and mitochondrial ND4 sequence: Indian Ocean-Asian subpopulation and Eastern Indonesian-Oceania subpopulation (Dudgeon *et al*. 2009); the line of demarcation between the two approximately coincides with the biogeographic Wallace Line that separates Asian and Australian flora and fauna. This species is listed as Endangered on the IUCN Red List of Threatened Species due to overfishing and habitat degradation (Dudgeon *et al*. 2019). The East Indo-Oceania subpopulation is assessed as Near Threatened based on the number of sharks inhabiting the Australian coast; however, the species is likely to be locally extinct throughout much of Indonesia. This species is extremely rare in Raja Ampat, with only three individuals recorded in over 15,000 person-hours of surveys since 2001, and is likely functionally extinct in the Raja Ampat archipelago of Indonesia. Pressure on extant populations continues throughout parts of its range.

A shark and ray sanctuary, coupled with a well-enforced network of nine marine protected areas (MPAs) in Raja Ampat, have contributed to the recovery of many elasmobranch populations in the region. However, zebra shark populations are not rebounding. In 2014, experts began discussing the idea of augmenting zebra shark populations, which took shape as a serious multi-institution initiative during a late 2019 workshop hosted by the Georgia Aquarium. In 2020 the *Stegostoma tigrinum* Augmentation and Recovery (StAR) Project was formally initiated, centered around re-establishing a healthy, genetically diverse, and self-sustaining wild population of zebra sharks, starting with the Raja Ampat archipelago.

The purposes of this Population Viability Analysis (PVA) were to develop a *VORTEX* population model for the zebra shark (*Stegostoma tigrinum*), and then to use this model to evaluate potential strategies for augmentation of the wild zebra shark population in Raja Ampat Archipelago, Indonesia, and to identify key knowledge gaps related to this project. PVA results will inform decisions by the StAR Project in its efforts to re-establish a healthy zebra shark population in Raja Ampat.

Measures of success

The purpose of the StAR Project is to re-establish a healthy, genetically diverse, and self-sustaining population of *S. tigrinum*, starting with the Raja Ampat archipelago. Further discussion of this goal identified key quantitative measures of interest, namely positive stochastic growth rate excluding releases, resilience to rebound from short-term catastrophic decline, and low mean inbreeding in the population. Additional measures of interest include wild population size, proportion of adults and proportion of wild-hatched sharks in the wild population, and risk of extinction over time.

VORTEX simulation model

The simulation software program *VORTEX* (v10.5.4) was used to conduct the zebra shark PVA. *VORTEX* is a Monte Carlo simulation of the effects of deterministic forces as well as demographic, environmental, and genetic stochastic events on wild or captive small populations. *VORTEX* models population dynamics as discrete sequential events that occur according to defined probabilities. The program begins by either creating individuals to form the starting population or importing individuals from a studbook database and then stepping through life cycle events (e.g., births, deaths, dispersal, catastrophic events), typically on an annual basis. Events such as breeding success, brood size, sex at birth, and annual survival are determined based upon designated probabilities that incorporate both demographic stochasticity and annual environmental variation. Consequently, each run (iteration) of the model gives a different result. By running the model hundreds of times, it is possible to examine

the probable outcome and range of possibilities. For a more detailed explanation of *VORTEX* and its use in population viability analysis, see Lacy (1993, 2000) and Lacy *et al.* (2020).

Computer modeling is a valuable and versatile tool for quantitatively assessing risk of decline and extinction of wildlife populations, both free ranging and managed (Lacy 2019). Complex and interacting factors that influence population persistence and health can be explored, including natural and anthropogenic causes. Models can also be used to evaluate the effects of alternative management strategies to identify the most effective conservation actions for a population or species and to identify research needs. Such an evaluation of population persistence under current and varying conditions is commonly referred to as a population viability analysis (PVA). The usefulness of a PVA is dependent upon the quantity and quality of data available on the biology of the species, its current population status, and current and future threats and management. Limited data mean limited applicability of the results; however, PVA often can provide useful information even with a modest amount of data.

Model development process

Demographic information for free-ranging zebra shark populations is limited. Model development included gleaning relevant information for *S. tigrinum* populations both in the wild and in aquariums, demographic parameters assessed for other similar shark species, and expert opinion. Published and gray literature were compiled and examined along with the studbook database for the captive population (i.e., historical database that includes sex, pedigree, and life event information for individual sharks). Discussion of model inputs and structure took place through a series of virtual conference meetings from December 2020 to March 2021 with StAR Project Steering Committee members and additional species experts.

Since there is little available information on wild shark survival rates, researchers have used better known attributes such as lifespan, age of sexual maturity and reproductive output to estimate intrinsic population growth and survival rates for healthy wild shark populations not subjected to overfishing or similar threat (Smith *et al.* 1998; Cortés 2002; Chen and Yuan 2006; Pardo *et al.* 2016). These analyses were consulted to guide construction of a base model to represent the demographic traits for a healthy, wild zebra shark population in good habitat and free from threat (i.e., capable of natural intrinsic growth). Uncertainty surrounding model inputs was explored to identify key data gaps. This model served as a base to explore population augmentation scenarios specific to the StAR Project. Input values used in the base model are described below. This individual-based model operates on a one-year time step, with projections running for 100 years into the future (1000 iterations per scenario).

Species biology model inputs

Breeding structure

Zebra sharks are polygamous with no known pair bonds between males and females. Seasonal aggregations of breeding age adults have been observed that are initially male-biased and shift to a female-biased sex ratio of 3.8:1 (Dudgeon *et al.* 2008). However, there is no evidence of male-male competition that excludes adult males as potential breeders and no evidence of density-dependent reproduction. The mating system was modeled as short-term polygyny as the closest model approximation, with all mature individuals considered to be potential breeders and no density-dependent impacts. An upper limit of 10 female mates per adult male per year was imposed in the model to limit reproduction in the event of highly skewed sex ratio situations; however, under equal sex ratio conditions adult males would be expected to mate with 0-2 females per year in the model. All offspring produced by a female in one year were assigned the same sire, with male mates randomly assigned across years.

Parthenogenesis has been observed in this species in captive females in the absence of male mates (Robinson *et al.* 2011; Dudgeon *et al.* 2017). Experts believe parthenogenesis is unlikely to be a factor for wild or augmented populations; thus, this form of reproduction was not included in this model.

Reproductive rates and lifespan

Age of first reproduction in captivity has been reported as 6-8 years for females and 7 years for males (Dudgeon *et al*. 2019). Population growth is sensitive to this parameter for females but generally not males in polygynous species. *VORTEX* applies fecundity rates to all adult females and so this model input value should represent the *average* age of first reproduction, not the earliest observation. Data from the AZA studbook show little female reproduction for ages 6-8, with good fecundity for age 9 (Villeverde *et al*. 2020); however, age is estimated for much of this population and therefore age-specific fecundity cannot be precisely measured. Age of first reproduction (i.e., maturity) was explored at ages 7-8 for both males and females in the model.

There are no published data for age-specific fecundity rates for wild zebra sharks. While data for the AZA captive population are limited, peak fecundity rates occur in females from age 9 to 24 (Villeverde *et al*. 2020). Females have reproduced up to 22-25 years after capture (Levan *et al*. 2019). For modeling purposes, fecundity is assumed to be constant for all adults regardless of age, with no reproductive senescence.

At least two captive wild-caught sharks (one male, one female) have lived to an estimated age of 27 years (Villeverde *et al*. 2020). Personal communication from Thomas cited in Dudgeon *et al*. (2019) states that the species can live over 28 years in captivity. Maximum lifespan in the wild is estimated at 27-30 years for the purposes of this model.

Zebra sharks are oviparous, producing large egg cases over a several-month period each year. Embryos develop for 160 days before hatching, with up to 25% of eggs resulting in hatchlings in captivity (Squire Jnr, pers. comm., in Dudgeon *et al.* 2019). Hatch rates may differ in the wild. For purposes of this base model, reproduction was defined as the production of hatchlings. That is, proportion of females breeding and number of offspring (brood size) used in the model were estimated with respect to the production of hatchlings, not egg cases.

Given the mating system and lack of parental care in this species it was assumed that most adult females breed and produce offspring each year in the wild. Dudgeon and Ovenden (2015) used 13 micro-satellite loci to estimate the effective population size (N_e) for a population of 458 mature zebra sharks in Australia, resulting in an N_e/N ratio of 0.82 (SE=0.33). This supports the assumption that most adults in this population consistently produce living offspring and that the sex ratio of breeders is not highly skewed. Annual percentage of adult females producing hatchlings was explored across the range of 75-85% in the model.

While some information was available for the percent of females reproducing, less is known about the number of hatchlings produced each year by successfully breeding females. Natural reproduction in one captive female over five consecutive years resulted in 5, 3, 1, 7 and 10 hatchlings, respectively, with a mean of 5.2 (SD=3.5). Analysis of 20 years of AZA studbook data (1999-2018) identified 13 reproductive females whose offspring could be reliably attributed to them. After excluding 11 records of same day deaths (potential stillbirths), "hatchling brood size" (i.e., number of live hatchlings from the same female within one year) was compiled for 28 "broods" with a total of 152 hatchlings. Mean brood size was 5.43 (SD=6.67). All but one brood was $\leq$20, which was used as a maximum number of hatchlings per year in the model.

Generation time and intrinsic growth rate

Even less is known of survival rates for wild sharks than for reproduction. Natural mortality rates of elasmobranch populations are difficult to estimate and have traditionally relied on life history parameters (Heupel and Simpfendorfer 2002).

Given the assumption of a healthy population in good habitat and free from threat, it is possible to use reproductive and longevity information to infer generation time and intrinsic population growth rate. It is then more feasible to construct a reasonable mortality schedule that results in the expected rate of population growth. This general approach of the interplay of life history characteristics has been taken

for many shark species (Smith *et al*. 1998; Cortés 2002; Frisk *et al.* 2005; Hoenig 2005; Tsai *et al.* 2010; Hisano *et al.* 2011; Pardo *et al.* 2016), but not previously for the zebra shark.

Generation time is an important life history characteristic and is generally inversely aligned with population growth and rate of loss of genetic variation through drift. In species such as sharks with overlapping generations, generation time (T) can be taken as the average age of reproduction. T can be calculated from detailed age-specific fecundity and survival data, when available. Data from the AZA studbook produced an estimated T of 12.8 years (Villeverde *et al.* 2020), which is based on limited data, captive conditions, and uncertainty regarding the actual age of many (wild-caught) individuals.

When age-specific fecundity and survival data are not available, T can be estimated based on reproductive lifespan, as recommended for IUCN Red List Assessments by Fung and Waples (2017), as follows:

$$T = \alpha + z*RL$$

where α is age at first reproduction, RL is reproductive life span and z represents the fraction of adult lifespan that passes before the average individual has contributed half of its lifetime, age-weighted reproduction. This coefficient z may be derived from data for comparable species. Of the 78 species assessed by Fung and Waples (2017), the closest approximation in terms of life history may be the dolphin, with $z = 0.25$. Applying this value to zebra sharks, with $\alpha = 7$ and maximum reproductive age $= 27$, then $T = 7 + (0.25 * (27 - 7)) = 12$ years. The Red List Assessment for zebra sharks estimates T at 17 years based on first age of maturity at 6 years and maximum longevity at 28 years (Dudgeon *et al.* 2019); this implies that $z = 0.5$ (i.e., average age of reproduction occurs mid-point in the reproduction lifespan), which in turn would imply that reproduction is not constant with age but must increase with age in some way to account for adult mortality. The highest z value calculated by Fung and Waples (2017) across 66 taxonomically diverse animal species was 0.27, suggesting that $T = 17$ yrs may be an overestimate for zebra sharks.

Shark species are generally characterized as having 'K-selected' life history traits such as being late to mature, long-lived, and having small brood sizes, which result in relatively long generation times and low intrinsic growth rates (Smith *et al*. 1998; Cortés 2002; Pardo *et al*. 2016). Smith *et al*. (1998) investigated the intrinsic rebound (population growth) potential of 26 species of Pacific sharks based on age of maturity (α), maximum age (w) and average fecundity (F, number of pups). Growth was most sensitive to age of maturity (α), followed by fecundity, and was not sensitive to maximum age. Species assessed by Smith *et al*. that most closely matched these parameters for zebra sharks are:

Species	Max. size	α	w	F	Intrinsic r
Blacktip (*Carcharhinus limbatus*)	191cm	7	18	5.2	0.054
Gray reef (*Carcharhinus amblyrhynchos*)	255cm	7	18	5.0	0.054
Mako (*Isurus oxyrinchus*)	364cm	7	28	8.0	0.051
Galapagos (*Carcharhinus galapagensis*)	370cm	8	24	8.0	0.048

Estimates of intrinsic population growth rate for the seven shark species in this study fell between 0.048 to 0.054, using $\alpha = 6\text{-}8$ and $w = 16\text{-}35$, with the exception of blue sharks that have high fecundity (F = 23.2; r = 0.061). Demographical modelling of 38 shark species by Cortés (2002) found that population growth rate is not correlated with body size but is influenced primarily by age at maturity, reproduction (fecundity) and juvenile survival. Cortés also concluded that generation time is highly positively correlated with age at maturity and negatively correlated with population growth for these shark populations. Taken together, these data suggest that a generation time of 12-13 years and an intrinsic growth rate of approximately 5% may be reasonable estimates for wild zebra shark populations.

Mortality rates
Multiple methods are available for estimating natural mortality rates from life history data, most of which provide an estimate of constant lifetime mortality. Mortality rates, however, are not likely to be constant across all ages/sizes for sharks, and indirect methods have been used to estimate age-specific mortality rates.

Heupel and Simpfendorfer (2002) monitored neonatal blacktip sharks (*C. limbatus*) in a small Florida bay and estimated natural mortality to be about 50% for the first six months, almost all of which occurred in the first 15 weeks. A similar trend in early mortality was observed in young bull sharks (*C. leucas*) (Heupel and Simpfendorfer 2011). Driggers *et al*. 2008 estimated annual natural mortality in young tiger sharks *Galeocerdo cuvier* as approximately 30-32% for first year (but starting at ~2 months old) and 20-22% for juveniles in their second year (age 1-2). Young sharks that develop in nursery (hatching) areas with abundant food resources can grow more quickly and may have lower early mortality rates due to predation (Wourms 1977, in Driggers *et al*. 2008). First-year mortality in captive-hatched zebra sharks in the SSP was 49% between 1999-2019, with substantially better survival in the last five years (~28% mortality) and thus lower survival in earlier years.

Smith *et al.* (1998) estimated natural mortality M for 26 Pacific shark species from w using Hoenig's (1983) formula (ln $M = 1.44 - 0.982$ ln w), derived from both fishes and mammals. Applying this calculation to a maximum age of 27-30 years estimated for zebra sharks results in an estimated constant natural mortality of 0.15 to 0.165. Assuming a higher mortality in smaller, immature sharks, this would result in a lower adult annual mortality rate to produce the same annual growth rate.

Exploration of demographic inputs to estimate mortality rates
Information compiled on reproduction, mortality, lifespan, generation time and growth rates was used to explore combinations of demographic values to determine the best match for zebra sharks and to identify key uncertainty. Intrinsic population growth r and generation time T were deterministically calculated using *VORTEX* while varying all combinations of five factors: 1) age of first reproduction (7, 8yr); 2) maximum age (27-30yr); 3) annual proportion of adult females reproducing (0.75, 0.80, 0.85); 4) first-year mortality (40%, 50%, 60%); and 5) annual adult mortality (8%, 10%, 12%, 14%). The results were examined for combinations of these inputs that were similar both in estimated growth rate (falling within 0.050-0.056) and generation time (within 12-12.8yrs) for zebra sharks. In all cases, annual mortality for immature sharks (age1-6yrs) was held at 20%.

As expected, maximum age had little effect, while first age of reproduction was more important (see Table 1). There were 15 combinations that resulted in growth rates and generation time within the target ranges, which can be categorized into two general input combinations:

  Category 1: Lower and later reproduction; higher hatchling survival; lower adult survival
     o   First reproduction: 8 years
     o   Female breeding: 75-80%
     o   First-year mortality: 40%
     o   Adult mortality: 14%

  Category 2: Higher and earlier reproduction; lower hatchling survival; higher adult survival
     o   First reproduction: 7 years
     o   Female breeding: 80-85%
     o   First-year mortality: 60%
     o   Adult mortality: 10-12%

Of these two categories, the second combination is closer to parameter values identified earlier for zebra sharks. Guided by these results, the following values were selected for the base model inputs representative of a large wild zebra shark population not under threat (also see Appendix II):
     o   Average age of first reproduction: 7 years
     o   Maximum age: 28 years

- o Adult females breeding: 80%
- o First-year mortality: 60%
- o Immature annual mortality: 20%
- o Adult annual mortality: 11%

Using this combination of demographic input values results in a deterministic intrinsic growth rate = 0.052 (λ=1.053) and generation time = 12.55 years and is consistent with expectations for large, unexploited populations of zebra sharks in the wild, based on the best available information. Shark experts involved in model parameterization acknowledged substantial uncertainty surrounding these values. For example, there are reports of wild zebra sharks living longer, and at least one captive population experienced higher fecundity. The philosophy adopted in this modeling exercise was to use the best available and most realistic estimates of demographic parameters, while avoiding overly optimistic projections in the face of any uncertainties, i.e., to adopt a conservative but realistic estimate of population growth.

Table 1. Demographic input value combinations tested that result in deterministic r (0.050-0.056) and generation rate (12.0-12.8yrs) as a best estimate for zebra sharks. Red box indicates Category 1 combinations; blue box indicates Category 2. AFR-age of first reproduction; %Breed=percent of adult females producing hatchling(s); MaxAge=maximum age; FirstYMort=first-year mortality of hatchlings; AdultMort=annual adult mortality; R=deterministic r; T=generation time (years).

Case	AFR	%Breed	MaxAge	FirstYMort	AdultMort	R	T
1	7	75	27	60	10	0.051	12.74
2	7	80	27	60	10	0.056	12.67
3	7	80	28	60	10	0.056	12.79
4	7	85	27	60	12	0.052	12.16
5	7	85	28	60	12	0.052	12.25
6	7	85	29	60	12	0.053	12.65
7	7	85	30	60	12	0.053	12.41
8	8	75	27	40	14	0.050	12.60
9	8	75	28	40	14	0.050	12.68
10	8	75	29	40	14	0.050	12.74
11	8	75	30	40	14	0.050	12.80
12	8	80	27	40	14	0.055	12.55
13	8	80	28	40	14	0.055	12.63
14	8	80	29	40	14	0.056	12.69
15	8	80	30	40	14	0.056	12.75

Variation in demographic rates
Actual reproductive and mortality rates vary from year to year in the real world and can impact population viability, especially for small populations. The *VORTEX* model incorporates stochastic (chance) variation in four ways that represent the sources of stochasticity outlined by Shaffer (1981). First, the actual proportion of sharks surviving and reproducing each year varies around the mean rate due to chance based on population size (demographic variation). Second, annual variation in environmental conditions (EV) can lead to good or poor survival and/or reproduction from year to year. This was incorporated into the mortality rates in the model by adding EV as a standard deviation of 10% of the mean rate for all mortality rates, and SD=5 for reproductive rate. EV for reproduction and survival were partially correlated (0.5), such that model years that are good for survival are also somewhat correlated with those good for reproduction and vice versa. A third source of variation is catastrophic events, which could be natural (e.g., disease) or anthropogenic (e.g., toxin contamination). No catastrophes were added to the base model, but this issue was explored separately (see below).

Genetic processes are also incorporated into the model, both as the random loss of genetic variation (genetic drift) and as inbreeding depression (lower viability of inbred individuals). *VORTEX* models inbreeding depression as reduced survival in inbred juveniles; the severity of the effect is determined by the number of lethal equivalents (LE) in the model. No inbreeding depression was added to the base model but was explored separately (see below).

Regulation of population size
No density-dependent reproduction or mortality was incorporated into the model. When population size (N) exceeds carrying capacity (K) at the end of a model year, population size is controlled by the probabilistic removal of each individual across all age and sex classes designed to, on average, bring the population size down to K.

Impact of Population Size on Viability

Populations may not achieve intrinsic growth rates, even when free from additional threats, due to variation in realized demographic rates resulting from annual variation in mean rates (environmental variation and catastrophes), genetic impacts (inbreeding), and demographic variation (random fluctuations influenced by sample size). Even in the absence of inbreeding and catastrophes, demographic variation can reduce the viability of small populations.

A series of scenarios was developed using the base model to explore the impact of population size on growth rate, rate of genetic drift and extinction risk. As inbreeding impacts and catastrophic events were not included, these results represent optimistic projections especially for small populations. For these scenarios, the model was initiated with the starting closed population (using a stable age distribution) at the habitat's carrying capacity. All scenarios were run with N=K for the following values: 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 75, 50, 25. Demographic and genetic results are given in Table 2. These results represent a closed population free from threats such as overexploitation.

A second set of scenarios was run, identical to the first except that a single-year catastrophe was forced in Year 10 that killed 50% of the population. Population size was evaluated 10 years post-catastrophe to evaluate the degree of recovery to the pre-catastrophe numbers, along with extinction risk over 100 years (see Table 2, *With Catastrophe* columns).

Demographic measures

Small closed populations of 50 or fewer showed reduced growth and some extinction risk over 100 years (Table 2), and populations under 100 were not able to fully recover from a short-term catastrophe event (Figure 1). Populations of at least 200 were able to fully recover from a one-year catastrophic decline of 50%, had no risk of extinction over 100 years due to demographic or environmental variation, and were able to maintain a population size close to K. Populations of 500 or more show strong growth close to the deterministic growth rate (r_{det}=0.052) and resilience against catastrophic events. These results do not incorporate potential inbreeding depression impacts, which would lower growth and resilience further as inbreeding accumulates in the smaller N populations.
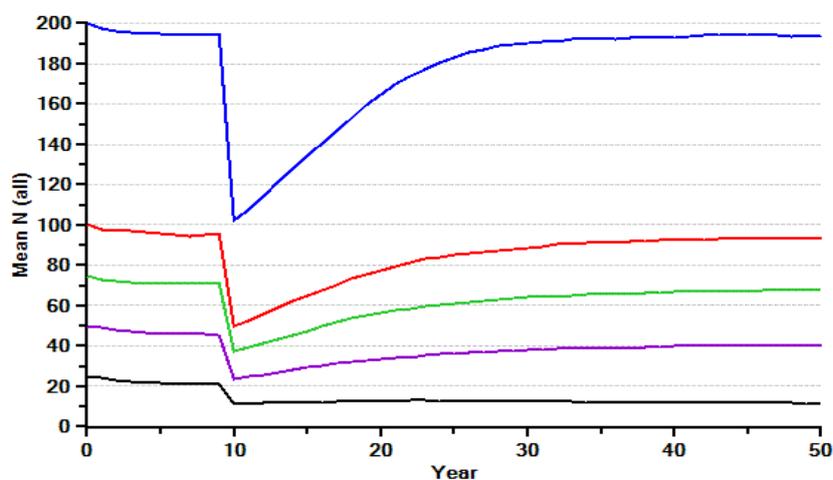

Figure 1. Mean population size over time before and after 50% reduction in Year 10, for N=K=200, 100, 75, 50, 25.

Genetic measures

Small populations show substantial loss of genetic variation and accumulation of inbreeding over 100 years (Figure 2; Table 2). Populations of 200 or more retain at least 90% of starting gene diversity, while 500 or more individuals are needed to keep additional inbreeding under 3% and an effective population size of at least 100, recommended to limit loss in total fitness (Frankham *et al.* 2014). Note that these model scenarios report additional inbreeding starting from Year 0 and so actual inbreeding levels over time may be higher in real populations depending upon current relatedness.
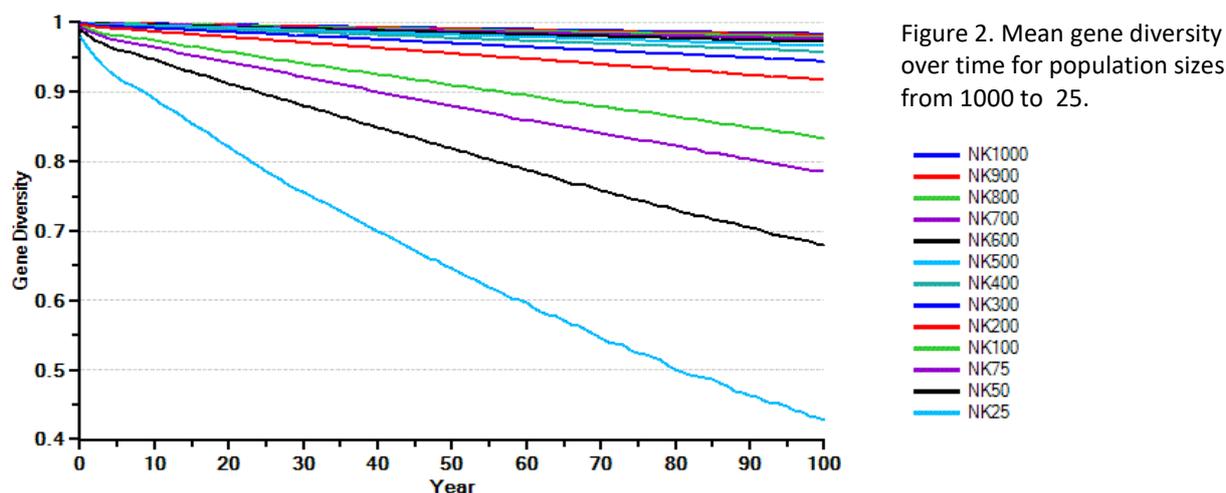
Figure 2. Mean gene diversity over time for population sizes from 1000 to 25.

Table 2. Demographic and genetic results for populations held at constant size with no catastrophe (mean values at year 100). N=mean population size; r_{stoch} = stochastic growth rate; PE=probability of extinction; GD=gene diversity; N_e=effective population size; F=mean inbreeding coefficient. Last two columns give results with catastrophe at Year 10: Rec10Yr=% recovery 10 years post catastrophe.

N=K	Demographic measures				Genetic measures			With Catastrophe	
	r_{stoch}	N_{100}	N_{100}/K	PE_{100}	GD_{100}	N_e	F_{100}	Rec10yr	PE_{cat}
1000	0.051	989	0.99	0	0.983	242.2	0.0147	87%	0
900	0.051	889	0.99	0	0.981	216.6	0.0164	87%	0
800	0.051	789	0.99	0	0.979	193.1	0.0178	87%	0
700	0.051	689	0.98	0	0.976	169.3	0.0207	87%	0
600	0.051	591	0.98	0	0.972	145.3	0.0241	86%	0
500	0.051	492	0.98	0	0.966	120.4	0.0291	86%	0
400	0.050	392	0.98	0	0.957	95.6	0.0366	87%	0
300	0.050	294	0.98	0	0.944	72.0	0.0478	85%	0
200	0.050	194	0.97	0	0.917	48.1	0.0708	85%	0
100	0.048	94	0.94	0	0.834	23.0	0.1435	82%	0
75	0.048	69	0.93	0	0.785	17.4	0.1839	*80%	0.005
50	0.044	43	0.86	0.011	0.679	10.9	0.2789	*77%	0.060
25	0.032	13	0.51	0.331	0.428	5.1	0.5101	*61%	0.534

*population never fully recovers to pre-catastrophe size.

Overall viability in relation to population size

Large population size is necessary to maintain high levels of genetic variation and low accumulation of inbreeding, suggesting that the actual viability of the smaller populations might be lower than suggested in Table 2, if inbreeding impacts were added to the model. Populations of $\geq$500 individuals showed strong growth (r_{stoch}=0.051) close to the deterministic growth rate (r_{det}=0.052), resilience against catastrophic events, high retention of gene diversity (>0.96) and relatively low accumulation of inbreeding (< 0.03). Larger populations modelled had Ne/N ratios of ~0.24, suggesting that ~2,000 individuals would be needed to meet the recommended Ne $\geq$ 500 to maintain evolutionary potential (Frankham 1980). More recent estimates (Frankham *et al.* 2014) suggest that Ne >> 1000 may be a better estimate of the size required to retain evolutionary potential for fitness in perpetuity, which is estimated to require at least 4,000 individuals. In general, model results suggest that a larger population size may be required to meet long-term genetic goals than demographic sustainability goals in a closed population free from overexploitation or similar threat.

Modeling the Zebra Shark Population in Raja Ampat

Current situation in Raja Ampat

Habitat and carrying capacity

The target area in Raja Ampat for this project includes the area in Figure 3 indicated by the red outline marked as 'c' and includes sandy bottom with patch reefs and potential crossing areas. The current best estimate by species experts for the potential carrying capacity for this area for zebra sharks is 1,000-2,000 sharks. This area is surrounded by deep water that zebra sharks are unlikely to cross and includes nine MPAs. It is assumed that most zebra sharks would remain within this area outlined in red, but that there could be infrequent immigration or emigration of individuals.

Figure 3. Map showing potential habitat distribution for zebra sharks in Raja Ampat (developed by Conservation International).
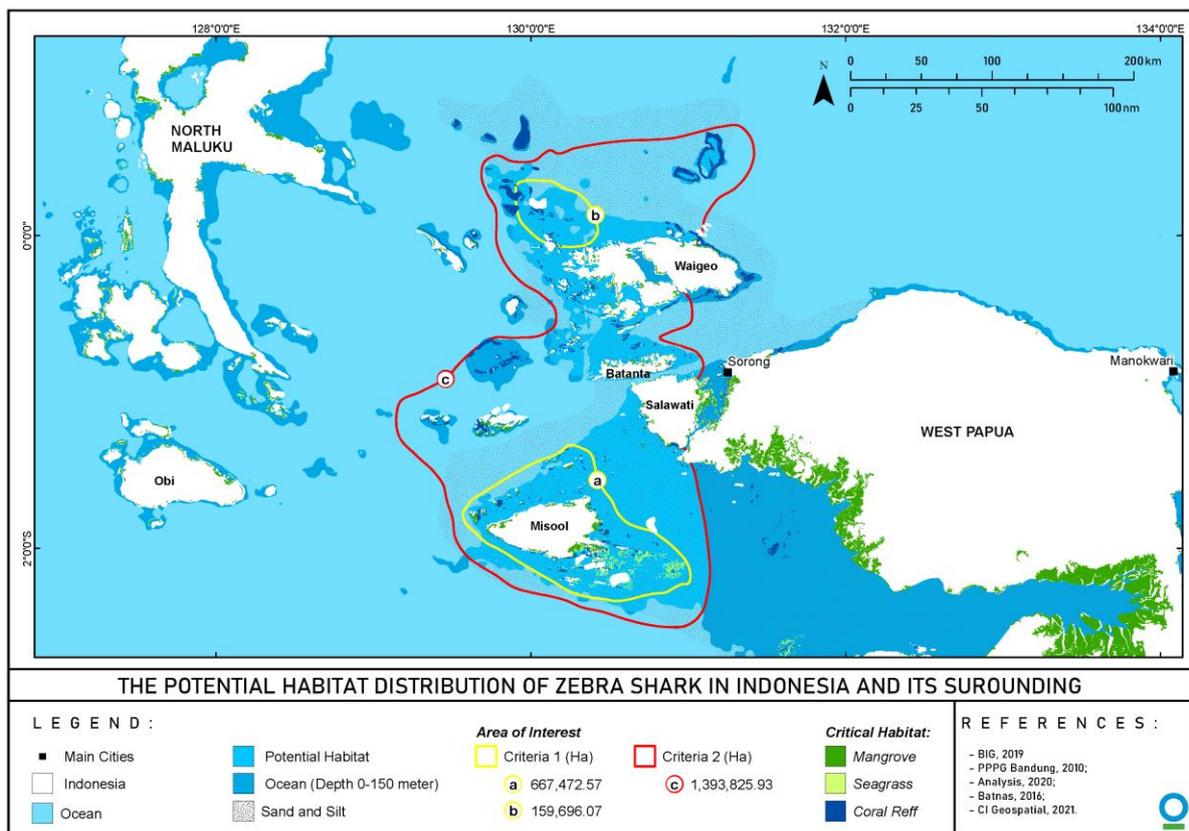
Current zebra shark population

The high level of SCUBA diving activity results in occasional zebra shark sightings throughout this area. Biodiversity assessments and routine reef monitoring conducted by Conservation International over the past 20 years similarly report an extremely low rate of sightings – a total of three individuals in over 15,000 person-hours of in-water surveys. While no population estimates are available, these sightings are infrequent and suggest a very small remnant population at low density. There is no evidence of regular reproduction or detectable population growth. A reasonable estimate by species experts involved in this project is about 20 individuals spread throughout the approximately six million hectares of the Raja Ampat archipelago.

Viability of current zebra shark population

Model inputs

Demographic values from the base model were used to develop a model for the current zebra shark population in Raja Ampat, with the following adjustments.

Given the low density of existing sharks in a relatively large area, density-dependent reproduction was added to the model by lowering the proportion of adult females breeding when the adult population is at low density. Figure 4 indicates the relationship used in the Raja Ampat model as an estimate of density-dependent reproduction. The mean percentage of adult females breeding was adjusted to 51% at 10 adults, 75% at 100 adults, and 78.5% at 270 adults. This translates to an intrinsic growth rate of 0.0155 at low density (10 adults) up to 0.050 at high density (1000 sharks consisting of ~270 adults based on a stable age structure).

The initial population size at Year 0 for each iteration was drawn from a beta distribution with a mean of 20 (range of 15-28) as shown in Figure 5. This represents the uncertainty in the current population size. An even sex ratio was used for the initial population, with an altered stable age distribution conscribed to ages 4-16 years with no juveniles or old individuals, i.e. biased toward young breeding adults. All initial individuals representing the current population were assumed to have a low level of relatedness (i.e., initial inbreeding of ~2%).
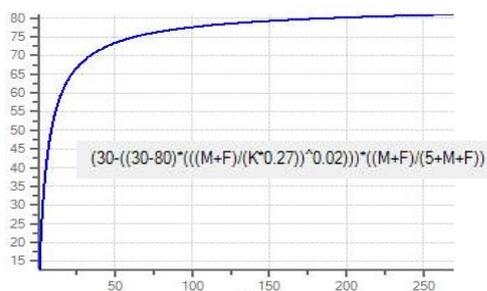

Figure 4. Percent adult females breeding (y-axis) vs adult density (x-axis).

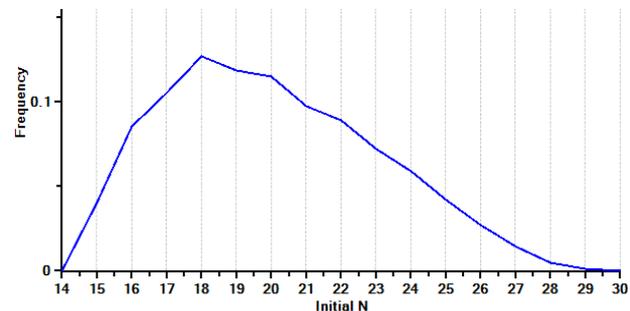$$(30-((30-80)*(((M+F)/(K^0.27))^0.02)))*((M+F)/(5+M+F))$$

Figure 5. Beta distribution used for initial population size.

While the population is generally thought to be isolated, a small amount of migration were added in the model. This was included as one unrelated adult shark immigrating into the population once every five years, and one adult emigrating at the same rate. An equal sex ratio was used for migrants. Immigrants were assigned the same demographic rates (reproduction and survival) as resident sharks; thus, immigrants had a high probability of breeding but reproduction was not assured.

Inbreeding impacts may be a factor for small populations such as the zebra shark population in Raja Ampat. While inbreeding depression is well known and has been estimated for some wild mammalian and avian populations (O'Grady *et al*. 2006), little information is available on inbreeding depression in sharks. Mourier and Planes (2013) did not find evidence of inbreeding depression (i.e., reduced survival of inbred individuals) in a blacktip reef shark population in French Polynesia, despite evidence of inbred individuals. O'Grady *et al*. (2006) estimated mean inbreeding sensitivity in wild mammal and bird populations to be ~12.29 lethal equivalents (LEs) spread across survival and reproduction. The default setting in *VORTEX* recommended as a conservative estimate of inbreeding impacts is 6.29 LEs, with 50% due to recessive lethal alleles and subject to purging. For the zebra shark model, a more conservative estimate of 3 LEs was used to impose a small but non-zero impact of inbreeding.

While Raja Ampat is anticipated to be protected from exploitation, its zebra shark population may be at risk from unknown future catastrophic events, such as disease outbreaks, prey decline or human-cause events. Reed *et al*. (2003) assessed 88 vertebrate populations (mammals, birds, poikilotherms) and found the risk of severe population decline ($\geq 50\%$) to be approximately 14% per generation. This is the default recommendation for PVA modeling. Scenarios were developed for zebra sharks that

included a non-specific single catastrophic event resulting in the loss of 50% of the population, either forced in Year 10 or occurring at a low probability of 1% (i.e., occurs about once in 100 years, or 1% risk per year), which approximates the suggested rate of 14% risk per generation (i.e., 14/12.55=1.1).

Demographic viability projections
In the absence of a catastrophic event, and assuming a low level of migration in and out of the population, the zebra shark population in Raja Ampat was projected to likely persist in low numbers over the next several decades. While the population was projected, on average, to grow, there is substantial variation in projected population size due to stochastic processes (Figure 6), with ~2% risk of extinction over the next 20 years. This projection assumes that the current resident sharks are not transient, consist mostly of prime age breeding adults, and are able to find mates such that at least 50% of adult female produce hatchlings each year. Projected growth rate was higher initially due to the biased age structure modeled and slows after five years as the population age structure stabilizes. If the current sharks are older, growth would be expected to be slower.

Similar projections were explored for a current population of 30 or 40 sharks, assuming a similar initial growth rate, to assess potential viability if the current population size is underestimated; mean projected population size is under 100 sharks for the next 15 years (mean$_{Y15}$= 67 and 98, respectively).

Under the model assumptions, the population was projected to remain under 50 sharks for the next 20 years (90% chance of being under 100 sharks), making it vulnerable to catastrophe events (Table 2). If this population were to suffer a severe catastrophic event, as illustrated in Figure 7 occurring in Year 5, the population would take on average over 50 years to recover to pre-catastrophe levels, although this is highly stochastic. Under this scenario with a severe population decline, the risk of extinction by Year 40 increases from 6% (with no catastrophes) to 28% with a Year 5 catastrophe.

The best estimated projection for the current zebra shark population, assuming a low risk (1% per year) of severe decline and a low level of inbreeding depression, suggests that it would take, on average, about 60 years to reach a population size of 100 sharks and about 90 years to reach 200 sharks, potential benchmarks for demographic stability and resilience. Extinction risk is 18% over 60 years, increasing to 27% by 100 years; thus a growing, self-sustaining population is not assured without conservation intervention.
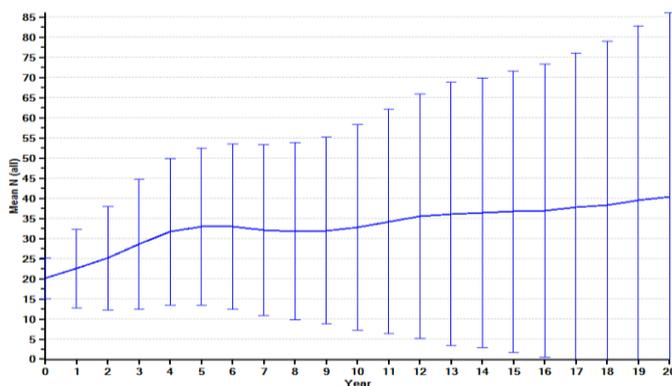

Figure 6. Projected mean population size (N) for 20 years, provided no severe declines or other threats occur. Bars indicate 90% of model iteration results.
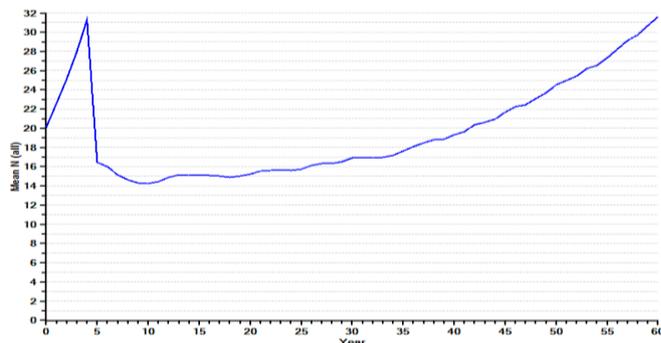

Figure 7. Projected mean population size (N) for 60 years, with a 50% one-time decline in Year 5.

Genetic viability projections

Small populations lose gene diversity faster due to genetic drift, which reduces evolutionary potential, and accumulate inbreeding faster, which may reduce survival and/or reproduction. Genetic augmentation either through natural immigration and/or through conservation translocation (population augmentation) can help to offset these genetic impacts.

The low rate of migration used in this zebra shark model has little direct effect on population size in short-term model projections, as the model includes the same number of immigrants as emigrants. However, low levels of immigration provide some genetic benefit. After about four generations (50 years), projections for a closed (isolated) population vs an open (with migration) population indicate lower gene diversity and higher levels of inbreeding without migration (Figure 8). After 50 years, mean inbreeding is expected to be about twice as high in a closed population (0.15) as one with one immigrant per five years (0.075). When a modest level of inbreeding depression is included, this degree of inbreeding can slow growth and increase extinction risk (38% extinction risk over 100 years) even in the absence of catastrophic decline.

While the gene flow provided by occasional migrants is genetically beneficial, it may be insufficient to maintain a genetically diverse and healthy population. Under the migration rate modeled, gene diversity was projected on average to drop from 95% to 84% by 100 years, which approximates a population related at the level of half siblings or more. Mean Ne calculated from loss of gene diversity is ~36. This suggests that the current zebra shark population in Raja Ampat is unlikely to remain genetically healthy without significant genetic augmentation of the population.
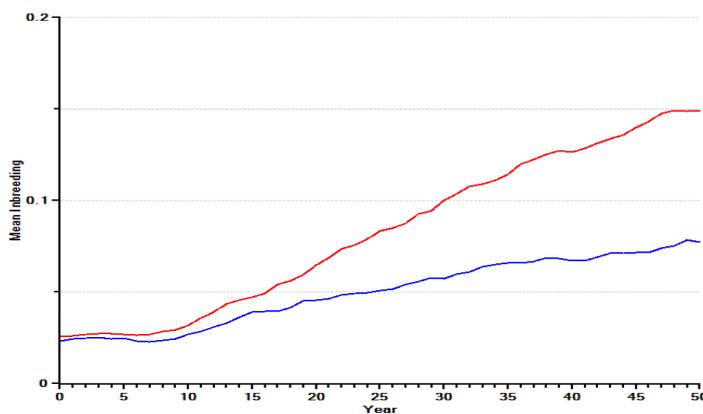
Figure 8. Projected mean inbreeding (F) for 50 years, with migration (blue line) and as an isolated population with no migration (red line).

Projected viability in the absence of releases

The best projection for the current zebra shark population was developed, assuming that the current individuals are skewed toward prime breeding age and are relatively unrelated, that there is a low level of new unrelated adult migrants, and assuming a low risk (1% per year) of severe decline and low sensitivity to inbreeding depression. While highly variable, the results suggest that it may take 60-90 years to reach a population size of 100-200 sharks needed for demographic stability and resilience against severe decline, and that there is a 23% chance of extinction over this time.

Additionally, gene diversity is projected to decline and inbreeding to increase substantially while the population remains small. The resulting effective population size is far below that recommended to offset inbreeding and preserve evolutionary potential. This suggests that the current zebra shark population in Raja Ampat is unlikely to remain genetically healthy without significant genetic augmentation of the population.

Taken together, these results suggest that population augmentation could provide both demographic and genetic benefits toward building a healthy, genetically diverse, and self-sustaining population of zebra sharks in Raja Ampat.

Impact of population augmentation on viability

Description of StAR Project augmentation proposal
The StAR project is a multi-national, collaborative initiative to re-establish a healthy, genetically diverse, breeding population of zebra sharks within the Raja Ampat archipelago. This is believed to be the world's first conservation translocation of captive-bred, threatened or endangered elasmobranchs. The source population will be the *ex situ* population managed by the Zebra Shark Species Survival Plan® (SSP)® of the Association of Zoos and Aquariums (AZA). Egg cases produced by genetically appropriate adults in US aquaria will be translocated from the US to Raja Ampat for hatching, rearing and release at two sites. Being an oviparous (egg-laying) species, the zebra shark can serve as a feasible model, as egg cases can tolerate trans-Pacific air shipment with little or no ill effects.

Once in Indonesia, egg cases will be distributed to two release sites (indicated approximately by 'a' and 'b' on Figure 3). Local facilities will hatch and rear juvenile sharks onsite, releasing them at approximately 6 months of age, depending upon growth rate. One to two shipments are anticipated each year over the course of a multi-year program.

Model scenarios were developed to simulate the StAR Project augmentation program. Virtual discussions with StAR Project species experts led to the development and parameterization of release scenarios. All scenarios were run for 100 years with 1000 iterations per scenario.

Demographic model inputs
The model developed for the current zebra shark population described above was revised to include the release of juvenile zebra sharks in the population as proposed by the StAR Project. All scenarios include the current estimated (primarily adult) shark population and a low rate of migration as well as the release of juveniles.

Released sharks are assumed to have the same survival and reproductive rates as the resident and migrant wild sharks once they reach one year of age. While it is believed that released sharks will move beyond their release area once mature, a small preference for breeding with sharks from the same vs different release site (60:40) was added to the model.

All scenarios were modeled as a 10-year release program (unless otherwise indicated), with the number of releases increasing over the first three years and reaching full release efforts in years 4 through 10. This schedule would allow sufficient time for juveniles released in year 4 to reach breeding age around the time of program cessation. It was assumed that captive breeding efforts, shipping protocols, and rearing and release techniques may be adjusted and improved over the first three years of the program.

Three scenarios were explored:
The *Best Guess* scenario represents the best estimates by the StAR Project species experts for the number of eggs shipped per year (10 initially to 80% by year 3), estimated hatch rate (70-80%), survival of hatchlings to release at approximately 6 months (50-60%), and post-release survival of juveniles to age 1 year (30% initially to 75% by year 4).

The *Optimistic* scenario represents more ambitious, but believed to be achievable, results in eggs shipped (100 maximum), hatch rate (80-90%), survival to release (50-75%) and post-release survival to age 1 year (35-80%).

The *Pessimistic* scenario represents a conservative estimate of eggs shipped (maximum of 64), hatch rate (60-75%), survival of hatchlings to release (50-55%) and post-release survival to age 1 year (30-70%). Table 3 gives the estimated mean number of eggs shipped, number of resulting hatchlings, and number of juveniles released based on the rates used for each of these scenarios. These estimates project a total of about 214 to 585 juvenile sharks released over the entire program, with between 144 to 442 expected to survive to age 1 year.

Table 3. Parameter values used for the Best Guess, Optimistic and Pessimistic scenarios for population augmentation through release of juvenile sharks into Raja Ampat.

Scenario	Rate	Year 1	Year 2	Year 3	Years 4-10	Total
Optimistic	# eggs shipped	15	100	100	100/yr	915
	# hatched	12	83	87	90/yr	812
	# released	6	49	58	68/yr	585
Best Guess	**# eggs shipped**	**10**	**50**	**80**	**80/yr**	**700**
	# hatched	**7**	**37**	**61**	**64/yr**	**553**
	# released	**3-4**	**20**	**35**	**38/yr**	**327**
Pessimistic	# eggs shipped	10	28	46	64/yr	532
	# hatched	6	18	32	48/yr	392
	# released	3	9	17	26/yr	214

Genetic model inputs

VORTEX models gene diversity using one neutral locus, with two alleles (one maternal and one paternal) assigned per individual. Individuals in the initial population or entering the population through release or immigration can have their alleles drawn from a specific distribution, while offspring from reproduction modeled in the population inherit maternal and paternal alleles through a simulated gene drop process.

In order to estimate the genetic impact of resident sharks, immigrants and population augmentation through the release of juveniles from the *ex situ* population, independent allele frequency distributions were created for each of these three sources (initial residents, immigrants and releases), and two alleles for each shark created in the model were assigned by randomly drawing from the relevant distribution. A total of 120 alleles were created for one neutral locus, with 40 unique alleles of equal distribution for the resident sharks, 40 different alleles of equal distribution for the creation of immigrants, and 40 additional alleles of unequal proportions to represent the 20 wild-caught founders represented in the captive breeders used to produce eggs for population augmentation.

As of March 2021, there were 67 living sharks in the AZA regional studbook (Levan 2021) that could be identified as genetically appropriate to serve as a source of progeny for Raja Ampat. Of these, 22 (9 males, 13 females) currently are at a US facility with a potential mate. These consist of 14 wild-caught and 8 captive-hatched potential breeders. These 22 sharks represent 20 genetic founders: 14 wild-caught founders still living and 6 additional founders represented in the 8 captive-hatched sharks. Assuming an equal probability of breeding from each of the 22 living potential breeders, the resulting distribution of the 40 founder alleles (20 founders x 2 alleles each) was used to create juvenile sharks for release in the model (see Appendix II for founder representation).

It is possible that not all potential breeders in the SSP will produce progeny for release. An alternative situation was modeled in which a limited number of breeding sharks contribute to the releases. Ten adult sharks (5 male, 5 female) from three experienced breeding facilities were used as potential breeders in a set of *Limited Reproduction* scenarios to ascertain the impact of fewer founder lines represented in the releases. All other aspects of the scenarios remained the same, including the release schedule. In these scenarios, releases descend from 12 rather than 20 founders (see Appendix II).

Overall results of augmentation scenarios

All release scenarios result in zebra shark populations with good long-term viability, i.e., self-sustaining populations with strong growth, resilience to catastrophes, low risk of extinction, high gene diversity and relatively low inbreeding (Table 4). The *Best Guess* scenario projects a mean population size of 189 sharks (SD=62) in 20 years, with >96% gene diversity and no extinction risk. Viability is projected to be higher under the *Optimistic* augmentation scenario and lower under the *Pessimistic* scenario. Projected population size is highly variable; however, projected mean projected population size is significantly larger with population augmentation as compared to that projected for the current shark population in the absence of augmentation (Figure 9). Augmentation increases and maintains gene diversity well above 90%, a common minimum goal to avoid inbreeding impacts (Figure 10).

Table 4. Model results for the Best Guess, Optimistic and Pessimistic scenarios, based on 20 or 12 founder genetic lines and with or without 1% risk of catastrophic decline per year (Cat Risk). Means are given at Years 20 and 50 for population size (N), gene diversity (GD), and inbreeding (F) and for extinction risk (PE).

| Scenario	# Fdrs	Cat Risk	N_{20Y}	N_{50Y}	GD_{20Y}	GD_{50Y}	F_{20Y}	F_{50Y}	PE_{20Y}	PE_{50Y}
Optimistic	20	No	376	970	0.970	0.965	0.023	0.032	-	-
	20	Yes	341	824	0.969	0.961	0.024	0.034	-	-
	12	No	372	968	0.955	0.950	0.039	0.046	-	-
	12	Yes	343	831	0.954	0.947	0.039	0.048	-	-
Best Guess	**20**	**No**	**212**	**744**	**0.966**	**0.957**	**0.022**	**0.036**	**-**	**-**
	20	**Yes**	**189**	**582**	**0.964**	**0.950**	**0.023**	**0.040**	**-**	**0.001**
	12	**No**	**209**	**736**	**0.954**	**0.946**	**0.035**	**0.048**	**-**	**-**
	12	**Yes**	**190**	**574**	**0.953**	**0.939**	**0.035**	**0.052**	**-**	**0.003**
Pessimistic	20	No	144	498	0.961	0.947	0.021	0.043	-	-
	20	Yes	129	362	0.958	0.935	0.021	0.049	-	0.015
	12	No	141	499	0.951	0.937	0.033	0.054	-	0.001
	12	Yes	130	379	0.949	0.929	0.033	0.055	-	0.007
Current	*n/a*	No	40	95	0.889	0.860	0.046	0.077	0.005	0.047
	n/a	Yes	37	70	0.883	0.848	0.044	0.068	0.009	0.080
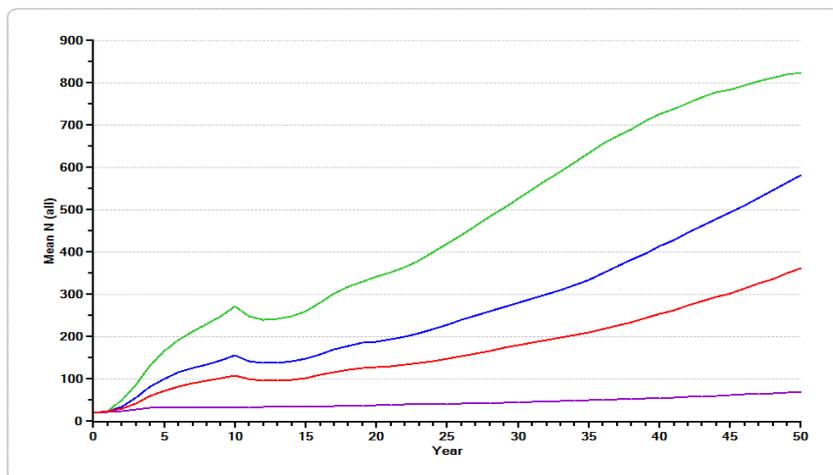
Figure 9. Projected mean population size (N) for 50 years for the Optimistic, Best Guess and Pessimistic augmentation scenarios and with no augmentation. Includes 1% annual risk of catastrophic decline.
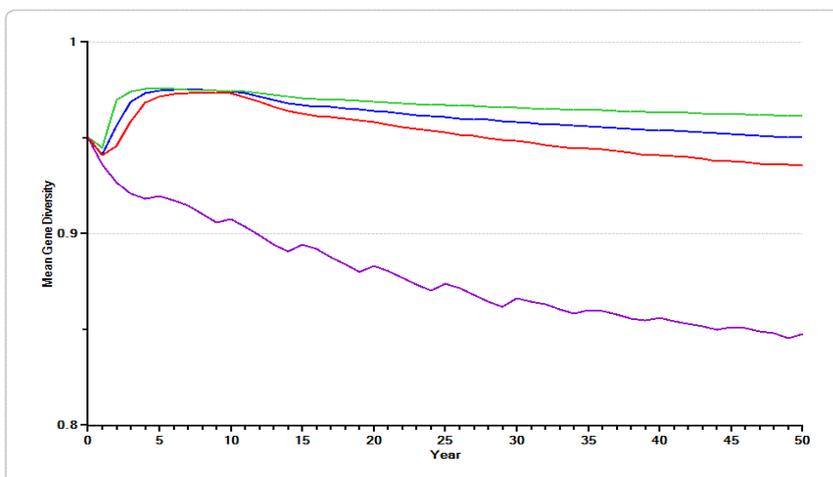

Figure 10. Projected mean gene diversity (GD) for 50 years for the Optimistic, Best Guess and Pessimistic augmentation scenarios and with no augmentation. Includes 1% annual risk of catastrophic decline.

Population growth and genetic drift during releases

As augmentation rates improve from *Pessimistic* to *Best Guess* to *Optimistic* scenarios and result in more juvenile sharks released and surviving (Table 3), population size is able to grow more rapidly. Mean population size in 20 years was projected to be 5 times higher with augmentation under the *Best Guess* scenario compared to no augmentation. Growing rapidly to 100-200 sharks lessens the impact of stochastic events such as demographic stochasticity and inbreeding depression, which in turn improves viability and reduces extinction risk.

Population growth is strong during releases, under the rates modeled, but may stall temporarily when releases cease (Figure 9). This is because most of the released sharks will not have yet reached breeding age at that point. After several years, population growth is expected to increase as most released sharks reach sexual maturity. Conversely, high gene diversity is maintained while releases continue; once releases cease, genetic drift leads to the gradual loss of gene diversity that is not fully offset by the level of immigration modeled. The larger the population is when releases are stopped, the slower the rate of genetic drift after that time; for example, the slower loss of gene diversity under the *Optimistic* scenario illustrated in Figure 10 is due to the larger population size achieved prior to ending releases.

Additional scenarios explored releases for 4, 6, 8, 12, 14 or 16 years using the *Best Guess* inputs. More release years results in more juveniles released and faster growth, which has demographic and genetic benefits, as expected. The relative genetic benefit, and to a lesser extent demographic benefit, starts to diminish proportionately as the release program is extended. Using the number of annual releases and survival rates modeled, conducting releases for at least 8-10 years was recommended as being a reasonable compromise for balancing long-term viability and program investment with required annual effort and resources.
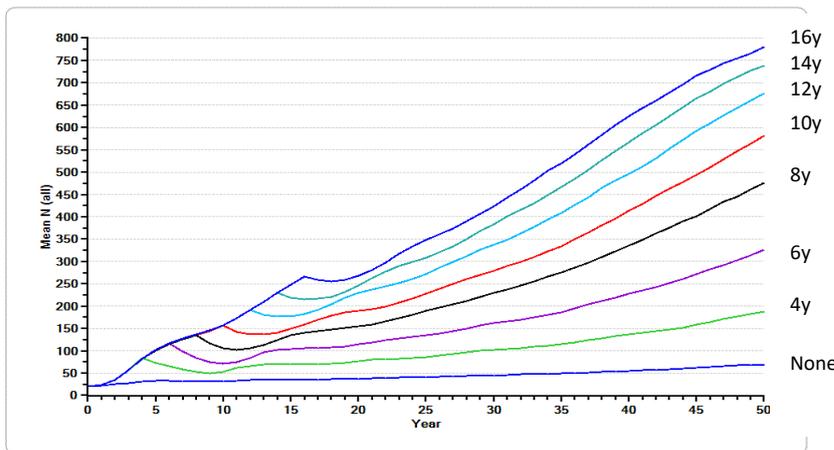

Figure 11. Projected mean population size (N) for 50 years for the augmentation scenarios for 4-16 years and with no augmentation. Includes 1% annual risk of catastrophic decline. Lines are in decreasing order of program length.
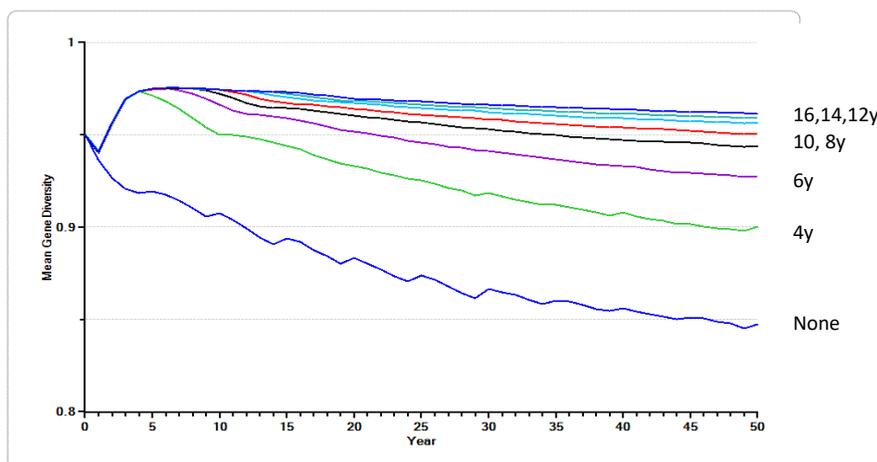

Figure 12. Projected mean gene diversity (GD) for 50 years for the augmentation scenarios for 4-16 years and with no augmentation. Includes 1% annual risk of catastrophic decline. Lines are in decreasing order of program length and match the colors used in Figure 11.

Lower founder base for releases

Lowering the genetic founder base of the sharks produced for release, from 20 to 12 founders, has no impact on projected growth rate, population size or risk of extinction. Initial gene diversity established during releases is lower with 12 founders (mean = 0.964) than with 20 founders (mean = 0.975), but the subsequent rate of loss is similar (Figure 13). Similarly, initial inbreeding is higher with fewer founders but accumulates at about the same rate. In all cases, retention of gene diversity remains high and inbreeding relatively low, even with reduced founder lines. As demography is not affected, this suggests that the additional inbreeding does not lead to inbreeding depression over the time scale modeled and under the low level of inbreeding (LE=3) in the model.
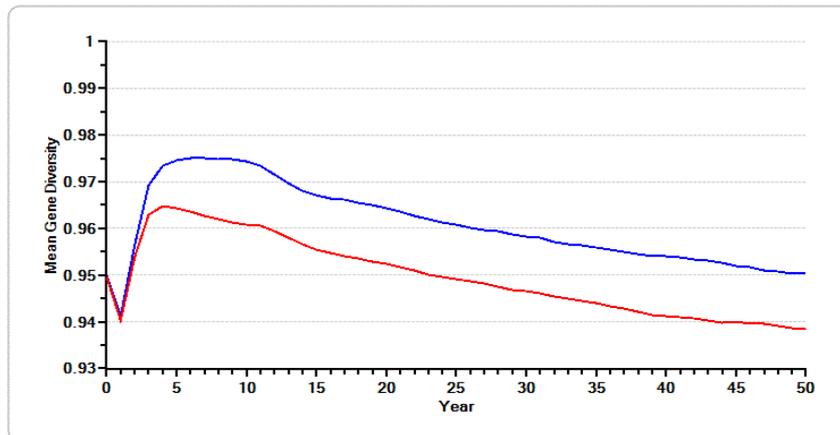

Figure 13. Projected mean gene diversity (GD) for 50 years for the Best Guess scenario with releases based on 20 and 12 founders. Includes 1% annual risk of catastrophic decline.

Conclusions regarding augmentation

The model results from the population augmentation scenarios suggested that the release of juvenile zebra sharks into Raja Ampat as proposed by the StAR Project is likely to result in substantially increased population size and viability, given the estimates of number of juveniles hatched and released and estimated survival rates. A release program length of around 10 years was anticipated as reasonable in terms of efforts and conservation benefit. The anticipated genetic lines available in the current *ex situ* potential breeding stock in the AZS SSP were estimated to be sufficient for beneficial genetic augmentation.

Conclusions

Despite the paucity of demographic and genetic data on wild zebra shark populations, existing wild and captive data, combined with studies of other shark species and general life history heuristics, enabled the development of a reasonable stochastic population model for the species. This base model represents the demography expected for a healthy, self-sustaining population that is not under threat. Survival rates for sharks over age 1 year have the greatest uncertainty and were estimated to result in a conservative intrinsic growth rate of 5% annual growth.

Large population size is necessary to maintain high levels of genetic variation and low accumulation of inbreeding. Modeling using the base model suggested that at least 200 sharks are need for good demographic growth and resilience against short-term severe decline without risk of extinction. Populations of 500 or more sharks showed strong growth, high retention of gene diversity and relatively low accumulation of inbreeding. Larger populations, perhaps 2,000 individuals or more, may be needed to meet the recommended effective population size to maintain evolutionary potential. In general, model results suggested that a larger population size may be required to meet long-term genetic goals than demographic sustainability goals in a closed population free from overexploitation or similar threat.

Best estimates of the current zebra shark population in Raja Ampat suggested that it may be insufficient to be viable long term due to its small size and relative isolation. While highly variable, model results suggested that it may take 60-90 years to reach a population size of 100-200 sharks needed for demographic stability and resilience against severe decline, and that there is a 23% chance of extinction over this time. Additionally, gene diversity was projected to decline and inbreeding to increase substantially while the population remains small. The resulting effective population size is far below that recommended to offset inbreeding and preserve evolutionary potential. This suggests that the current zebra shark population in Raja Ampat is unlikely to remain genetically healthy without significant genetic augmentation of the population.

Population augmentation scenarios were developed to simulate the release of juvenile sharks into Raja Ampat as part of the StAR Project to establish a healthy, self-sustaining zebra shark population. Model results suggested that the release of juvenile sharks as proposed is likely to result in substantially increased population size and genetic diversity, resilience to rebound from severe decline with little to no extinction risk, and overall good viability. A release program length of around 10 years is anticipated as reasonable in terms of efforts and conservation benefit. The anticipated genetic lines available in the current *ex situ* potential breeding stock in the AZA SSP are estimated to be sufficient for beneficial genetic augmentation.

Some caution should be exercised when considering the precision of these modeling results. There is substantial uncertainty around many of the demographic rates used in the model, both for wild shark vital rates and for release program success rates. Efforts were made to use the best estimates that are realistic and not overly optimistic. In addition, due to the initial small population size there was high variation especially in projected population numbers over time. Combined, these factors mean that the model results cannot be considered as precise; rather, they are more useful in comparing relative trends. That is, these projections may not provide an accurate and precise value of population size and genetic diversity at a specific future time, but they provide strong evidence that population augmentation can be significantly valuable to the establishment of a healthy, viable population of zebra sharks in Raja Ampat. In addition, this PVA project can serve as a model for similar shark PVAs that incorporate demography and genetics to explore population management options for conservation. Likewise, the StAR Project may serve as a model for the conservation translocation of other species of captive-bred sharks into the wild.

References

Chen, P. and W. Yuan. 2006. Demographic analysis based on the growth parameter of sharks. *Fisheries Research* 78: 374-379.

Cooke, R.S., T.C. Gilbert, P. Riordan and D. Mallon. 2018. Improving generation length estimates for the IUCN Red List. *PLoS ONE* 13(1): e0191770. https://doi.org/10.1371/journal.pone.0191770.

Cortés, E. 2002. Incorporating uncertainty into demographic modeling: application to shark populations and their conservation. *Conservation Biology* 16: 1048-1062.

Driggers, W.B.III, G.W. Ingram Jr., M.A. Grace, C.T. Gledhill, T.A. Henwood, C.N. Horton and C. M. Jones. 2008. Pupping areas and mortality rates of young tiger sharks *Galeocerdo cuvier* in the western North Altantic Ocean. *Aquatic Biology* 2: 161-170.

Dudgeon, C.L., D.C. Blower, D. Broderick, J.L. Giles, B.J. Holmes, T. Kashiwagi, N.C. Krück, J.A.T. Morgan, B.J. Tillett and J.R Ovenden. 2012. A review of the application of molecular genetics for fisheries management and conservation of sharks and rays. *Journal of Fish Biology* 80: 1789-1843.

Dudgeon, C.L., D. Broderick and J.R. Ovenden. 2009. IUCN classification zones concord with, but underestimate, the population genetic structure of the zebra shark *Stegostoma fasciatum* in the Indo-West Pacific. *Molecular Ecology* 18: 248-261.

Dudgeon, C.L., L. Coulton, R. Bone, J.R. Ovenden and S. Thomas. 2017. Switch from sexual to parthenogenetic reproduction in a zebra shark. *Nature Scientific Reports* 7: 40537. doi:10.1038/srep40537.

Dudgeon, C.L., K. Feldheim, M. Schick and J.R. Ovenden. 2006. Polymorphic microsatellite loci for the zebra shark *Stegostoma fasciatum*. *Molecular Ecology Notes* doi: 10.1111/j.1471-8286.2006.01442.x.

Dudgeon, C.L., J.M Lanyon and J.M. Semmens. 2013. Seasonality and site fidelity of the zebra shark, *Stegostoma fasciatum*, in southeast Queensland, Australia. *Animal Behaviour* 85: 471-481.

Dudgeon, C.L., M.J. Noad and J.M. Lanyon. 2008. Abundance and demography of a seasonal aggregation of zebra sharks *Stegostoma fasciatum*. *Marine Ecology Progress Series* 368: 269-281.

Dudgeon, C.L. and J.R. Ovenden. 2015. The relationship between abundance and genetic effective population size in elasmobranchs: an example from the globally threatened zebra shark *Stegostoma fasciatum* within its protected range. *Conservation Genetics* 16: 1443-1454.

Dudgeon, C.L., C. Simpfendorfer and R.D. Pillans. 2019. *Stegostoma tigrinum* (amended version of 2016 assessment). The IUCN Red List of Threatened Species 2019: e.T41878A161303882. https://dx.doi.org/10.2305/IUCN.UK.2019-3.RLTS.T41878A161303882.en.

Franklin, I.R. 1980. Evolutionary change in small populations. Pp. 135-140 in: M.E. Soule and B.A. Wilcox (eds.), *Conservation Biology: An Evolutionary-Ecological Perspective*. Sunderland, MA: Sinauer Associates.

Frankham, R., C.J.A. Bradshaw and B.W. Brook. 2014. Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. *Biological Conservation* 170: 56-63.

Frisk, M. G., T. J. Miller, and N. K. Dulvy. 2005. Life histories and vulnerability to exploitation of elasmobranchs: Inferences from elasticity, perturbation and phylogenetic analyses. *J. Northw. Atl. Fish. Sci.* 35: 27-45. doi:10.2960/J.v35.m514.

Fung, H.C. and R.S. Waples. 2017. Performance of IUCN proxies for generation length. *Conservation Biology* 31: 883-893. https://doi.org/10.1111/cobi.12901.

Heupel, M.R., J.K. Carlson and C.A. Simpfendorfer. 2007. Shark nursery areas: concepts, definition, characterization and assumptions. *Marine Ecology Progress Series* 337: 287-297.

Heupel, M.R. and C.A. Simpfendorfer. 2002. Estimation of mortality of juvenile blacktip sharks, *Carcharhinus limbatus*, within a nursery area using telemetry data. *Can. J. Fish. Aquat. Sci.* 59: 624-632.

Heupel, M.R. and C.A. Simpfendorfer. 2011. Estuarine nursery areas provide a low-mortality environment for young bull sharks *Carcharhinus leucas*. *Marine Ecology Progress Series* 433: 237-244.

Hisano, M., S.R. Connolly and W.D. Robbins. 2011. Population growth rates of reef sharks with and without fishing on the Great Barrier Reef: Robust estimation with multiple models. *PLoS ONE* 6(9): e25028. doi:10.1371/journal.pone.00250258

Hoenig, J.M. 2005. Empirical use of longevity data to estimate mortality rates. SEDAR33-RD17. SEDAR, North Charleston, SC. 8 pp.

Knip, D.M., M.R. Heupel and C.A. Simpfendorfer. 2012. Mortality rates for two shark species occupying a shared coastal environment. *Fisheries Research* 125-126: 184-189.

Lacy, R.C. 1993. Vortex: A computer simulation model for population viability analysis. *Wildlife Research* 20: 45-65.

Lacy, R.C. 2000. Structure of the Vortex simulation model for population viability analysis. *Ecological Bulletins* 48: 191-203.

Lacy, R.C. 2019. Lessons from 30 years of population viability analysis of wildlife populations. *Zoo Biology* 38(1): 67-77.

Lacy, R.C. and J.P. Pollak. 2020. Vortex: A stochastic simulation of the Extinction Process. Version 10.5.0. Chicago Zoological Society, Brookfield, IL.

Lacy, R.C., P.S. Miller and K. Traylor-Holzer. 2020. *Vortex 10 User's Manual*. 1 April 2020 update. IUCN SSC Conservation Planning Specialist Group, and Chicago Zoological Society, Apple Valley, Minnesota, USA.

Lee, K.A., C. Huveneers, V. Peddemors, A. Boomer and R.G. Harcourt. 2015. Born to be free? Assessing the viability of releasing captive-bred wobbegongs to restock depleted populations. *Frontiers in Marine Science* 2: 18. doi:10.3389/fmars.2015.00018.

Levan, J., R. Villaverde and J. Jewell. 2019. AZA Regional Studbook, Zebra Shark (*Stegostoma fasciatum*). Virginia Aquarium and Marine Science Center, Virginia Beach, VA. 85pp.

Mourier, J. and S. Planes. 2013. Direct genetic evidence for reproductive philopatry and associated fine-scale migrations in female blacktip reef sharks (*Carcharhinus melanopterus*) in French Polynesia. *Molecular Ecology* 22: 201-214.

O'Grady, J.J., B.W. Brook, D.H. Reed, J.D. Ballou, D.W. Tonkyn, and R. Frankham. 2006. Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. *Biological Conservation* 133: 42-51.

Pardo, S.A., H.K. Kindsvater, J.D. Reynolds, and N.K. Dulvy. 2016. Maximum intrinsic rates of population increase in sharks, rays, and chimaeras: the importance of survival to maturity. *Can. J. Fish. Aquat. Sci.* 73: 1159-1163.

Portnoy, D.S. and E.J. Heist. 2012. Molecular markers: progress and prospects for understanding reproductive ecology in elasmobranchs. *Journal of Fish Biology* 80: 1120-1140.

Reed, D.H., J.J. O'Grady, B.W. Brook, J.D. Ballou, and R. Frankham. 2003. Estimates of minimum viably population sizes for vertebrates and factors influencing estimates. *Biological Conservation* 113: 23-34.

Robinson, D.P. W. Baverstock, A. Al-Jaru, K. Hylands and K.A. Khazanehdari. 2011. Annually recurring parthenogenesis in a zebra shark *Stegostoma fasciatum*. *Journal of Fish Biology* 79: 1376-1382.

Shaffer, M.L. 1981. Minimum population sizes for species conservation. *Bioscience* 31:131-134.

Smith, S.E., D.W. Au and C. Show. 1998. Intrinsic rebound potentials of 26 species of Pacific sharks. *Marine and Freshwater Research* 49: 663-678.

Tsai, W-P., K-M. Liu and S-J. Joung. 2010. Demographic analysis of the pelagic thresher shark, *Alopias pelagicus*, in the north-western Pacific using a stochastic stage-based model. *Marine and Freshwater Research* 61: 1056-1066.

Villaverde, R., J. Levans and A. Lawless. 2020. Population Analysis & Breeding and Transfer Plan, Zebra Shark (*Stegostoma fasciatum*) AZA Species Survival Plan Yellow Program. 36pp.

Watson, L. and M. Janse. 2017. Chapter 41: Reproduction and husbandry of zebra sharks, *Stegostoma fasciatum*, in aquaria. In: *The Elasmobranch Husbandry Manual II: Recent Advances in the Care of Sharks, Rays, and their Relatives*. Ohio Biological Survey. Pp.421-432.

Yokoi, H., H. Ijima, S. Ohshimo and K. Yokawa. 2017. Impact of biology knowledge on the conservation and management of large pelagic sharks. *Nature Scientific Reports* 7: 10619. doi:10.1038/s41598-017-09427-3.

Appendix I. PVA contributors.

Table A1. StAR Steering Committee members and other species experts who contributed to PVA virtual discussion to develop and parameterize the PVA model.

Organization Name	Contact Name
Conservation International	Dr. Mark Erdmann Abraham Sianipar
Georgia Aquarium	Dr. Alistair Dove Dr. Lisa Hoopes Dr. Kady Lyons
Misool Foundation	Marit Miners
Murdoch University	Abraham Sianipar
Seattle Aquarium	Grant Abel Tim Carpenter Caitlin Hadfield Dr. Erin Meyer
Thrive Conservation	Nesha Ichida
University of Queensland	Danielle Davenport
University of the Sunshine Coast	Dr. Christine Dudgeon
Virginia Aquarium	Julie Levans

Appendix II. Input values for the zebra shark base model and founder allele frequencies used.

Table A2. Model Inputs for *VORTEX* model parameters. EV=environmental variation; SD=standard deviation

Parameter	Base value
REPRODUCTIVE RATES	
Mating system	Short-term polygyny (maximum female mates = 10)
Age of first reproduction (in years)	7 (both sexes)
Proportion of adult females reproducing per year	Density dependent; 0.51 (10 adults) to 0.785 (270 adults); EV=0.05
Mean (SD) number of hatchings per year per reproductive female	5.43 (6.67); maximum = 20
Sex ratio at hatch	50:50
MORTALITY RATES (annual)	
Neonate (hatch to age 1 year)	60% (EV=6)
Immature (age 3-6 years)	20% (EV=2)
Adult (age 7-28 years)	11% (EV=1.1)
ADDITIONAL PARAMETERS	
Maximum age (years)	28
Inbreeding	3 lethal equivalents (50% due to recessive lethal alleles)
Catastrophe	1% annual risk of 50% decline

Table A3. Founder representation used to develop allele distributions for released juveniles.

Founder Studbook #	Current Status	# descendants in 8 captive-hatched breeders	Representation (all breeders scenarios)	Representation (limited breeders scenarios)
1	Alive	3	0.113636	0.100
2	Dead	2	0.045455	0.050
3	Alive	0	0.045455	0.100
4	Alive	1	0.068182	0.150
5	Alive	0	0.045455	0.100
21	Alive	0	0.045455	0
22	Dead	3	0.068182	0.100
23	Dead	3	0.068182	0.100
46	Alive	0	0.045455	0
133	Alive	0	0.045455	0
134	Alive	0	0.045455	0
181	Alive	0	0.045455	0.100
184	Alive	0	0.045455	0.100
194	Alive	0	0.045455	0
196	Alive	0	0.045455	0
203	Dead	2	0.045455	0.050
204	Dead	2	0.022727	0.025
205	Dead	2	0.022727	0.025
358	Alive	0	0.045455	0
359	Alive	0	0.045455	0
Gene diversity			0.986	0.975
# Founders			20	12