Marine Biodiversity Hub

National Environmental Science Programme

A close-kin mark-recapture estimate of the population size and trend of east coast grey nurse shark

[1]Bradford, R. W.; [1]Thomson, R.; [1]Bravington, M.; [2]Foote, D.; [1]Gunasekera, R.; [1]Bruce, B. D.; [3]Harasti, D.; [3]Otway, N.; [1]Feutry, P.

Project A9 - Grey nurse shark CK-MR Population Estimate - East coast

July 2018

Milestone 6, Research Plan v3 2017

CSIRO

NSW GOVERNMENT Department of Primary Industries

Cover Images: *David Harasti (left); Garry Warren (right)*

Enquiries should be addressed to:

Russell Bradford
CSIRO Oceans & Atmosphere. GPO Box 1538, Hobart, Tasmania, Australia, 7001
Email: Russ.Bradford@csiro.au

Project Leader's Distribution List

Lesley Gidding-Reeve/Ashley Leedman Threatened Species Commissioner	Department of the Environment and Energy: Marine and Freshwater Species Conservation Section: Wildlife, Heritage and Marine Division
Natalie Moltschaniwskyj, Alan Jordan	NSW Government (specifically NSW DPI)

Preferred Citation

Bradford, R. W. *et al.* (2018). A close-kin mark-recapture estimate the population size and trend of east coast grey nurse shark. Report to the National Environmental Science Program, Marine Biodiversity Hub. CSIRO Oceans & Atmosphere, Hobart, Tasmania.

Author Affiliations: 1. CSIRO Oceans & Atmosphere; 2. University of Queensland; 3. New South Wales Department of Primary Industries

Copyright

Acknowledgement

This work was undertaken for the Marine Biodiversity Hub, a collaborative partnership supported through funding from the Australian Government's National Environmental Science Programme (NESP). NESP Marine Biodiversity Hub partners include the University of Tasmania; CSIRO, Geoscience Australia, Australian Institute of Marine Science, Museum Victoria, Charles Darwin University, the University of Western Australia, Integrated Marine Observing System, NSW Office of Environment and Heritage, NSW Department of Primary Industries. This work could not have been completed without two previous Discretionary Grants provided by the Department of the Environment and Energy and the Queensland Department of Natural Resource Management.

Important Disclaimer

Contents

List of Acronyms

BRUV	Baited Remote Underwater Video
CKMR	Close-kin Mark-recapture
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DArT	Diversity Array Technologies Pty Ltd
DoEE	Department of the Environment and Energy
FSP	Full sibling pair
GGP	Grandparent-grandchild pair
GNS	Grey nurse shark
HSP	Half sibling pair
mtDNA	Mitochondrial DNA
NESP	National Environmental Science Program
PCL	Precaudal length
POP	Parent-offspring pair
TL	Total length
TLS	Total length stretched
TSCDG	Threatened Species Commissioner Discretionary Grant

List of Figures

List of Tables

EXECUTIVE SUMMARY

Project A9 (A close-kin mark-recapture estimate of the population size and trend of east coast grey nurse shark) of the National Environmental Science Program (NESP) Marine Biodiversity Hub was initiated to provide a contemporary population estimate of the eastern grey nurse shark (*Carcharias taurus)* using emerging genetic and statistical techniques. This project follows on from the successful NESP A3 (A National Assessment of the Status of White Sharks) project (Bruce *et al*. 2018), and uses a modification of the population-dynamics model used to derive a population estimate for white sharks (Hillary *et al*. 2018a; Hillary *et al*. 2018b). Importantly, due to the absence of age estimates as well as the unreliable length estimates of the sampled grey nurse shark (GNS), the GNS model had to be more elaborate to account for greater kinship possibilities.

The GNS dataset encompassed a wide age-range (including many adults). This, and a lack of reliable length measurements, required the assumption that the age-composition of adults had been in stable equilibrium (the quasi-equilibrium assumption: Appendix A, section A4.1). The quasi-equilibrium assumption allows for a steady rise or fall in population size over the modelled period. Although this rather long-term assumption may not accurately reflect the true history of the population over that period, some deviations from the assumed historical trajectory (for example, from long-term changes in per capita bycatch rate) would have little influence on the model, and less so the longer ago the deviation occurred. Major deviations, however, would lead to bias in the abundance and mortality-rate estimates from the model. The full model parameters and assumptions are provided in Appendix A.

Two alternative maturity scenarios were used encompassing the range provided in the literature: scenario 1 set female maturity at age 10 and male maturity at age 7; scenario 2 set female maturity at age 14 and male maturity at age 11. No information was available on juvenile survival rate, therefore, the number of juveniles in the population could not be estimated. **Using maturity scenario 1 the point estimate for the adult east Australian GNS population in 2017 was 2,167** (95% confidence interval 1,257 to 3,078) **adult animals with an estimated annual rate of increase = 3.4%** (95% confidence interval 1.2 to 5.7%)**. Using maturity scenario** 2 **the point estimate for the adult east Australian GNS population in 2017 was 1,686** (95% confidence interval 956 to 2,417) **with an estimated annual rate of increase = 4.5%** (95% confidence interval 2.5 to 4.7%). The Close-Kin Mark-Recapture (CKMR) model did not support low values of adult survival (Appendix A, Figure 7), for example, below a proportional annual survival of 90%. The model did, however, support a growing population of approximately 3.4 to 4.5% per annum (95% confidence interval 1.2 – 5.7%).

Using photographic identification (photo-ID) mark-recapture methods, CARDNO (2010) estimated the **total** eastern Australian GNS population to be approximately 2,142 (after accounting for potential site fidelity). A separate study by Bansemer (2009) estimated the **total population** to be approximately 1,941 animals (1,491-2,391). Site fidelity is known to have an influence on photo-ID methods, resulting in a potential downward bias in population estimates. The CKMR model predicted the **adult population** in 2008 was approximately 1,575 (scenario 1; CV = 0.24) or 1,131 (scenario 2; CV = 0.20) animals. The CKMR model does not provide an estimate of juvenile numbers; therefore, we were unable to provide a direct comparison of total population size using the CKMR approach without further required knowledge to develop a fully integrated population model. However, considering the rather constrained reproductive mode of the GNS, a fully integrated CKMR-

population model would likely provide a total population estimate slightly higher, but comparable, with the estimates provided by CARDNO (2010) and Bansemer (2009).

A shortcoming of this project was the derivation of age based on the reported length of the sampled sharks. All CKMR models are constructed around the idea of adult population size at time of juvenile birth. Therefore it is necessary to know, or assume, an age for each animal in order to work out when each potential offspring was likely to have been born, and whether each potential parent was likely to have been alive and mature at that time. Age was derived using a growth curve based on data from the United States population (Goldman *et al*. 2006), which may not accurately reflect the growth of eastern Australian GNS. A priority should be set on examining existing collections of eastern GNS vertebrae sourced, for example, through shark control programs where length can be accurately measured and age can be determined from counting annuli.

Half-sibling pairs (HSPs) provide a means to estimate the number of breeding adults, while Parent-Offspring pairs (POPs) provide the means to estimate total adult abundance irrespective of an individual's contribution to reproductive output. Therefore, any non-random mating (such as a male dominance hierarchy, or site fidelity) that could alter the reproductive output of a proportion of the mature animals would result in a discrepancy between the number of estimated versus observed POPs, given an estimate of the adult population based on HSPs. The CKMR model was unable to simultaneously fit to the observed values of both HSPs and POPs, but instead estimates of HSPs were lower than observed, while estimates of POPs remained too high. The threshold level chosen for the identification of HSPs was very conservative in this analysis (more so than for any other species investigated so far) in order to minimise the inclusion of false-positives (animals incorrectly identified as HSPs), and thus biasing the adult population estimates. It is possible that an, as-yet, unknown factor affecting reproductive output of a proportion of the population is contributing to the discrepancy between the number of POPs estimated versus observed (43-48 estimated: 26 observed).

Our results indicate there has been some recovery of the eastern Australian GNS population, presumably as a result of the voluntary ban on capture by gamefishers in 1979 (Pepperell 1992) followed by NSW Government protection in 1984 (Pollard *et al*. 1996), and the implementation of a critical habitat classification (DEWHA 2009). **Despite support for a degree of recovery in the eastern Australian GNS population, further work on the level of risk facing the recovering population would be required before it would be appropriate to alter the range of existing protective measures.** In parallel to developing a growth curve appropriate for the eastern GNS, continued effort should be placed on addressing the objectives of the recovery plan (DOE 2014).

The samples collected for this project (not including those from the New South Wales Department of Primary Industries archive) will be deposited with the Australian Museum's Centre for Wildlife Genomics to create a lasting archive for future research collaborations.

1. INTRODUCTION

The grey nurse shark, *Carcharias taurus* (GNS), is distributed primarily within the continental shelf zones of tropical and temperate regions of the North and South Atlantic, Indian and western Pacific oceans (Last & Stevens, 2009) to depths of at least 230 m (Otway and Ellis 2011). In Australia, two distinct subpopulations are recognised, one along the eastern seaboard of Australia, the other along the western seaboard. Within Australia the distribution of the eastern population extends approximately 2,700 km from central Queensland south to at least the New South Wales (NSW)/Victorian border (Otway *et al*. 2003; Bansemer 2009; Otway and Ellis 2011). The western population extends across a similar distance of the West Australian coastline from the North West Shelf south to at least Cocklebiddy in the Great Australian Bight (McAuley *et al*. 2002; Cavanagh *et al*. 2003; Chidlow *et al*. 2005). This project was focussed on deriving an abundance estimate of the eastern Australian GNS population.

The species is listed as "vulnerable" on the IUCN Red List of Threatened Species (Pollard & Smith 2009) as a result of observed declines in GNS numbers worldwide. The eastern Australian population has been declared "critically endangered" under the Australian *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) and as such is the subject of an on-going recovery plan. This publication addresses Objective 1 of the 2014 recovery plan: *Develop and apply quantitative monitoring of the population status (distribution and abundance) and potential recovery of the grey nurse shark in Australian waters* (Australian Government Department of the Environment 2014).

Since 2014 the CSIRO has been partnering with the University of Queensland, New South Wales Department of Primary Industries (NSW DPI), and the Office of the Threatened Species Commissioner (through the Queensland Department of Natural Resource Management – now Queensland Department of Environment and Science) to collect tissue samples and extract DNA from live GNS across its eastern Australian range. The NSW DPI provided additional tissue samples collected as part of the NSW Shark Control Program as well as targeted NSW DPI field sampling. The current project, funded through the Department of the Environment and Energy is the final component in a portfolio of projects working towards providing a contemporary estimate of adult population size for the eastern GNS population. Several estimates of eastern Australia GNS abundance have been published, the most recent of which have relied on photo identification (photo-ID) techniques (Cardno 2010; Bansemer 2009). The authors of these estimates have taken precautions to reduce the possible biases associated with non-homogeneous and non-random sampling inherent in photo-ID studies. This project used an emerging genetic technique developed by CSIRO for teleosts (Bravington *et al*. 2016a; Bravington *et al*. 2016b), and refined by the National Environmental Science Program (NESP) Marine Biodiversity Hub for euryhaline elasmobranchs and white sharks, to identify family relationships, specifically parent-offspring pairs (POPs) and half-sibling pairs (HSPs). When coupled with a species' demographics model it is possible to use genetic relatedness to derive an absolute contemporary population estimate for the adult population.

The aim of this project was to use a Close-Kin Mark-Recapture (CKMR) approach to derive a contemporary adult population estimate (and, if possible, trend) for the eastern Australian grey nurse shark. In addition, this project aimed to establish a National Archive within the Australian Museum's Centre for Wildlife Genomics (for the samples collected, through the portfolio of related projects) for use in future research collaborations.

2. APPROACH AND METHODS

This project (A9) uses CKMR, a powerful refinement of traditional mark-recapture approaches first developed for tuna (the first application; Bravington *et al*. 2016a), and then euryhaline elasmobranchs (NESP Project A1: Northern Australian hotspots for the recovery of threatened euryhaline species), and white sharks (NESP Project A3: a national assessment of the status of white sharks) (Bruce *et al*. 2018; Hillary *et al*. 2018). The refinement is that genetic fingerprinting is used rather than physical marks or tags, and "recaptures" are of an animal's close relatives rather than of the animal itself. This allows the use of samples from dead animals, and circumvents some problems of standard mark-recapture such as tag loss and tag reporting rates. Individual DNA profiles, derived from tissue samples, are compared across all samples to find POPs and, using the advanced cutting edge genotyping techniques, HSPs/ Grandparent-Grandchild Pairs (GGPs). Given adequate sampling, the number of kin-pairs found, and their spread in time, can be used in a mathematically sound and transparent mark-recapture framework (Bravington *et al*. 2016b) to estimate adult abundance, and potentially trend.

Length is used to derive an age for each animal. Length was variously reported as total length (TL), precaudal length (PCL) or total length stretched (TLS). All reported length measurements were standardised to total strength length before further analysis using formulae provided by NSW Department of Primary Industries (NSW DPI) (see Appendix A, section A1.1). The accuracy of reported length varied depending on the method used. For example, post mortem GNS were measured most accurately using a tape measure and some live GNS were measured with the aid of image analysis (paired laser or stereo video). However, the reported length of the bulk of live GNS was estimated by SCUBA divers, and was at best to within ± 20 cm. To account for this variability, we allocated length measurements to one of three categories (post mortem, stereo, and diver), which were assumed to have measurements of error of 1%, 5%, or 10%, respectively (Appendix A, sections A1.2 and A1.3).

Age-at-length data was not available for the Australian GNS. Therefore, we used raw age-at-length data from a study conducted in the United States of America (Kenneth Goldman per. comm., Alaska Department of Fish and Game) to develop a growth curve and to assess variance in age-at-length. These, along with the additional length measurement error, allowed us to construct probability distributions for age given reported length (Appendix A, section A1.3).

Age-at-maturity is an important parameter informing the CKMR model. To account for the error present in the length data and uncertainty in the true age-at-maturity, we explored two knife-edge maturity schedules. Scenario 1 modelled maturity at 10 years old for females and 7 years old for males; scenario 2 modelled maturity at 14 years old for females and 11 years old for males (Appendix A, section A4.1).

An accurate identification of sex is required for the CKMR model to take into account the sexually dimorphic life history of the GNS, where females are older and larger than males at maturity (Goldman *et al*. 2006; Otway *et al*. 2009; Otway & Ellis 2011). Male sharks are identified by the presence of claspers, which, if present, are fairly obvious when the shark is viewed from the side or below. However, for this project tissue samples were collected from the dorsal surface of the sharks and the claspers (or their absence) may have been hidden from the view of the SCUBA diver. The

genetic data provided a means to identify a genetic sex marker, present only in male sharks (Appendix A, section A2), and was subsequently used to unambiguously assign sex to the sampled animals.

The mitochondrial DNA (mtDNA) provides information on the likelihood of whether HSPs share their mother or their father, and whether the intervening individual between a grandmother and her grandchild was male or female (Appendix A, section A4.2). Therefore, the entire mitogenome was sequenced to maximise the number of discoverable haplotypes (a key element to identifying the sex of the shared parent of the half-sibling pair) while minimising their frequency (the lower the frequency, the less likely the haplotype is to have been inherited by chance) to provide the best information possible on parentage. Sequencing used a combination of long range Polymerisation Chain Reaction (PCR) amplification and miSeq amplicon sequencing (see Feutry *et al*. 2014 for more details).

3. RESULTS

A total of 514 samples were sourced over the course of this project. Of these samples, 381 were tissue samples collected from live GNS specifically for this project, six tissue samples were obtained from GNS killed in the Queensland shark control program, one sample was obtained from a GNS caught and killed in Victorian waters, and the remaining 126 were obtained from the archive held by NSW DPI, encompassing samples from both live and dead GNS.

Sampling occurred throughout the range of the eastern Australian GNS distribution (Figure 1), including 470 samples from New South Wales (404 from live animals, 66 from dead animals) and 63 samples from Queensland (57 from live animals, 6 from dead animals). The standardised TLS of the animals sampled ranged from 1.00 m to 3.01 m (average = 2.00 m), however, the accuracy of the reported lengths varied by as much as 1 m. The ratio of females to males, based on the identified genetic sex marker, was close to equal in the NSW (237 F:233 M) samples, but biased towards males in the Queensland (24 F:49 M) samples (Figure 2).

Adequate DNA was extracted from 489 tissue samples, which were then sequenced by Diversity Array Technologies Pty Ltd (DArT). An analysis of the mtDNA from 477 of those samples uncovered 61 distinct haplotypes, with an average frequency of 7.8% (standard deviation = 9.92%), to inform the CKMR analysis.

A total of 378 sharks and 2,157 loci passed all of the stringent quality control steps (including the criterion that each individual should only be sampled once) (Appendix A, section A3); resulting in a maximum of 142,884 possible pairwise comparisons to identify related individuals. In total 108 HSPs/ GGPs, 26 POPs, and 11 FSPs were identified. The number of close-kin pairs is related to the abundance of the population being estimated. For example, a small adult population would, on the whole, manifest in a high number of close-kin pairs, and vice versa.

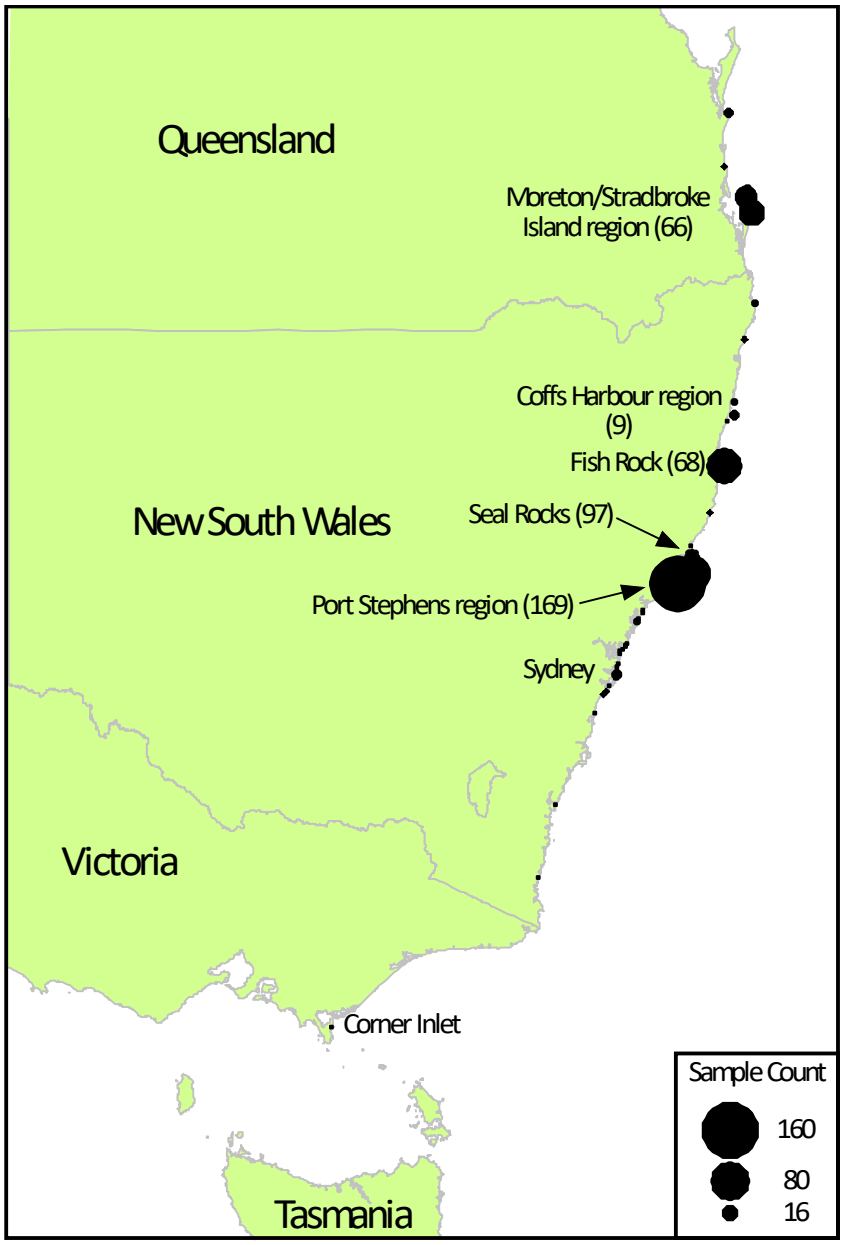

Figure 1. Distribution of sampling effort along the east coast of Australia with key regions highlighted (sample count in brackets for regions where sampling was high).

Using the CKMR approach, the total adult population size of the eastern GNS was estimated to be 2,167 (95% confidence interval: 1,257 to 3,078) adults using maturity schedule 1 (a_{mat} 10, 7), with adult survival estimated to be 0.97 per annum and the annual rate of increase = 3.4% (95% confidence interval: 1.2 to 5.7%) (Appendix A, Table 2). Using maturity schedule 2 (a_{mat} 14, 11), the adult population of eastern GNS was estimated to be 1,686 (95% confidence interval: 956 to 2,417) adults, with adult survival estimated to be 0.95 per annum and the annual rate of increase = 4.5% (95% confidence interval: 2.5 to 4.7%). A slight male sex bias (1.3:1 and to 1.1:1, respectively) was estimated (see Appendix A, Table 2). The CKMR model did not support low values of adult survival

(Appendix A, Figure 7), for example, below 0.90. The model did, however, support a growing population of approximately 3.4 to 4.5% per annum.

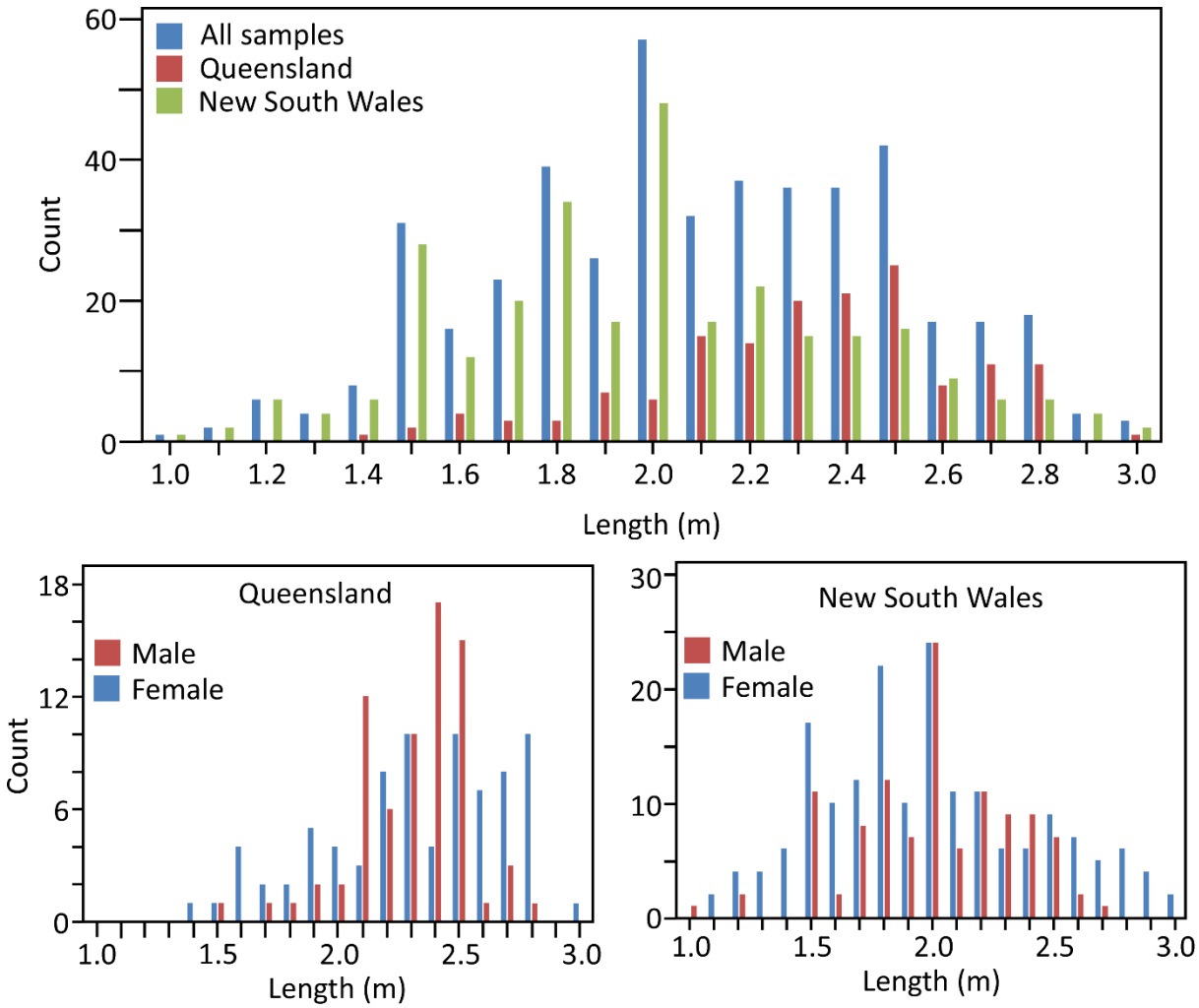

Figure 2. Distribution of GNS reported total length (standardised to total length stretched, m) for samples collected by SCUBA divers.

4. STRATEGIES TO GUIDE FUTURE MONITORING & RESEARCH

4.1.1 Improved understanding of age structure.

For most species, including sharks, an accurate estimate of age cannot be gained from external characteristics alone. Determining the age of individuals in wild populations has generally been based on lethal sampling of a proportion of the population to examine hard parts, such as otoliths and vertebrae, and then extrapolating age through age-at-length keys. Data on age-at-length is used to calculate growth curves which can be used within the context of a population model to convert lengths to probability distributions of age (i.e. to construct an age-at-length key). For a species of conservation concern, lethal sampling is obviously not ideal. Understanding the age structure of the samples would improve our understanding of the population biology and better inform the models used in a CKMR approach. The majority of the samples used in this project have been from live animals. However, a growth curve was not available for Australian GNS; hence for this project we have used data from the United States of America (Goldman *et al*. 2006) to estimate age. The NSW and Queensland Governments collect vertebrae and length measurements from GNS caught in their respective Shark Control programs and incidental captures by commercial and recreational fishers. An analysis of these vertebrae would provide length-at-age information required to derive growth curves specific to Australia's east coast GNS. Vertebral analyses are relatively inexpensive and take little time (when the samples are in-hand) to complete. Growth curves based on Australian GNS would be invaluable to improving this study.

Our model infers age from length, imputing the probability distribution for age, given the observed length and assumed growth curves for males and females. We had to account for a degree of error in the age-at-length for each of the diver, camera, and post mortem groups due to the variability between groups in measurement accuracy (Figure 3). This had to be relatively large for the samples collected by SCUBA because there was a large degree of variability in the estimates of length, presumably driven by differences in the skill levels and experience of divers. Unfortunately the reported lengths made by divers were largely uninformative with respect to age for the majority of the samples in this study. Improved methods of measurement while collecting samples by SCUBA would be highly beneficial to future applications of CKMR estimates of abundance. Figure 3 also illustrates that there were a high number of 'recaptures' in the dataset. The significance of this to future research is elaborated on in section 4.1.2 below.

An emerging epigenetic technique is being developed to estimate age based on the methylation of DNA. This technique can provide an estimate of age to within ± 3 years or better for some species of mammals (Polanowski *et al*. 2014; Jarman *et al*. 2015; De Paoli-Iseppi *et al*. 2017). If developed for GNS it would reduce the uncertainty in age estimates of sampled GNS and could, retrospectively, be applied to the existing samples to improve estimates of population size.

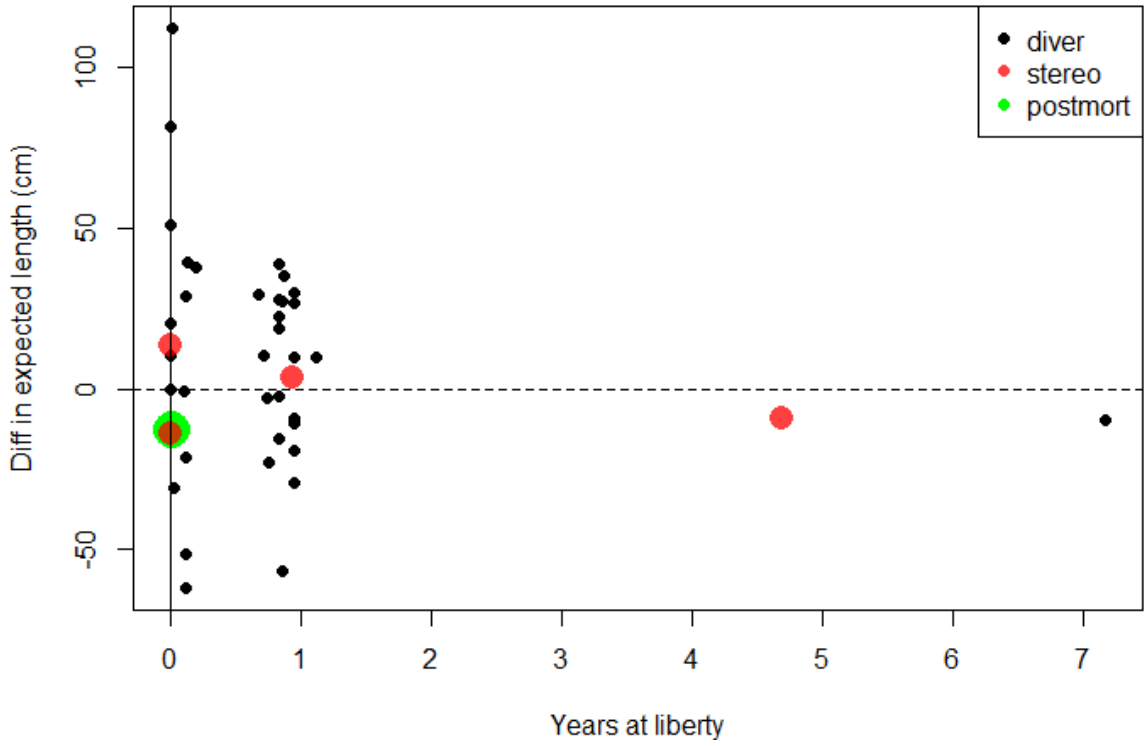

Figure 3. For each recapture of an individual GNS, the difference between the recorded and the expected length after time at liberty (expressed in years) is shown. The vertical line indicates zero time at liberty (recaptures positioned on this line occurred on, or close to, the same day) and the horizontal line indicates perfect agreement between observed and expected lengths. Length measurements were made by divers (black dots), stereo camera (red dots) or post mortem (green dot).

Two key strategies to improving our understanding of GNS age structure

1. Analyse existing vertebrae, obtained from the Queensland and New South Wales shark control programs, to derive male and female growth curves specific to the eastern Australian GNS population.
2. Investigate DNA methylation as a means to non-destructively estimate age using the same samples collected for use in CKMR (or other genetic analyses), and incorporating strategy 1 to calibrate the method. Initially a design study would determine the degree to which DNA ageing might improve the precision of the close-kin estimate. If a significant improvement would be recognised, and the approach found to be successful in other marine species (currently being assessed), then this approach could be explored for GNS. This may then allow a re-application of the CKMR method to the existing samples without the confounding issue of using poor length measurements to estimate age.

4.1.2 Tissue sampling.

It is recommended that tissue sampling of live animals be continued on a regular basis. Coupled with obtaining these samples, and in the absence of an epigenetic solution to ageing sampled sharks, a paired laser system or hand-held stereo video would ideally be used routinely to provide a better estimate of reported length. An appropriately calibrated system can result in length estimates with a very low margin of error (see for example Harvey and Shortis 1998; Cappo *et al*. 2003; Harasti *et al*. 2016). Because growth rates are highest in young animals, accurate length measurements from this demographic are likely to be highly informative if coupled with growth curves specific to Australian GNS. Sampling only the youngest cohorts would allow the application of CKMR to a more restricted and better defined age range, and would avoid the confounding effects of having to account for an extended suite of relationships (e.g. GGPs). At regular stages the DNA extracted from these tissue samples could be analysed and incorporated into an updated population estimate without going to the lengths of investigating epigenetic ageing.

This project identified several 'recaptures': 53 in total, with 48 of those recaptured twice and three recaptured three times. The high level of recapture data suggests that continued sampling on a regular basis would result in many more recaptures suitable for input into a 'gene tag' style of mark-recapture model. Such a model is being used by Preece *et al*. (2015, 2017) to estimate the abundance of age-2 southern Bluefin tuna. It is also possible that a well-designed sampling regime could inform juvenile survival/mortality rates for this species, an important component when estimating total population size, which is currently missing.

4.1.3 Expanded surveys of southern waters.

Although GNS have been recorded from all Australian waters, except Tasmania (Last and Stevens 2009), it has not been reported from the southern coast for many years (Daley *et al*. 2002). In 2017, the CSIRO identified a shark caught by a fisher in Corner Inlet, Victoria as *C. taurus*. Further, in a separate project the CSIRO incidentally caught and released a GNS in waters outside of Corner Inlet. Additionally, a sample of a possible GNS caught in South Australian waters was provided to the CSIRO for analysis. Unfortunately we were unable to extract suitable DNA to analyse and confirm species ID. It is, therefore, possible that if the GNS population is increasing, that it may be reappearing in southern waters. Dedicated surveys including the waters from the New South Wales/Victorian border to Wilsons Promontory are recommended. Initially these surveys could be based on using Baited Remote Underwater Video (BRUVs) and aerial surveys. If aggregations are identified a field program could be undertaken to obtain further tissue samples.

4.1.4 Expanded sampling of the western population.

During the course of this project samples from the western GNS population were provided. However, the number of samples was very low (6). Targeted surveys (Chidlow *et al*. 2005) along the West Australian coast were unable to identify aggregation sites. The authors postulated that an absence of identified aggregation sites may have been because the western GNS either behave differently to the eastern population, or aggregation sites may lie

in deeper water and, therefore, be undetectable by diver survey. Additionally, the surveys were conducted over a limited spatial and temporal scale.

More recently Hoschke and Whisson (2016) reported on a seasonal GNS aggregation site at the Point Murat Naval Pier in Exmouth, WA. Sixteen GNS were observed, with 10 of those returning to the site over multiple years. They also note that two other sites further south appear likely to be aggregation sites. It is recommended that these sites be surveyed (using BRUV) to determine if they are aggregation sites.

Without substantially more samples from the western GNS population it will not be possible to apply the CKMR strategy to estimate the abundance of the western GNS population. However, if additional aggregation sites are identified, it may be possible to build up sufficient sample numbers over a relatively short time frame.

4.1.5 Photo identification and Citizen Science.

Photo-ID studies have become common in studies of sharks and rays, and provide a means to non-invasively sample species under threat. Photo-ID has been used most recently as the basis for estimating abundance and movements within the eastern Australian GNS population (Bansemer & Bennett, 2008; Barker & Williamson 2010; Cardno 2010). This method also provides an excellent opportunity for the public to assist research (i.e. citizen science).

Even though care must be exercised to account for inherent biases in photo-ID studies, their use in establishing a baseline index of abundance, and with enough time possibly trend, is invaluable. Photo-ID could also be coupled with tissue sampling to assist with assessing the accuracy of photo-ID.

It is recommended that the existing photo-ID programs be continued across the geographical range of the eastern Australian GNS population (e.g. Grey Nurse Shark Research & Community Engagement Program: https://www.reefcheckaustralia.org/grey-nurse-shark-watch.html).

4.1.6 Assembly of the grey nurse shark genome.

Identifying HSPs in the GNS genome was difficult, more so than for any of the other species examined to date, and possibly linked to the demographic structure of the population. The statistical overlap between HSP and more distantly related, or unrelated, pairs of sampled sharks was fairly high. As a result the HSP-like numbers are less certain for GNS than observed for other species, even after allowing for a high false-negative rate in order to avoid false-positives as best we can. In theory, having a genome assembly would provide information about the physical linkages between genetic markers (i.e. whether they are on the same chromosome and how close together they are, allowing assessment of likely linkage disequilibrium). Siblings are more likely to share runs of markers that are close together on the genome compared with pairs that share markers by chance or due to a number of shared, but distant, antecedents. Understanding linkage disequilibrium may allow for better discrimination between HSPs and other types of relatives such as half uncle-niece

pairs and cousins for example. A genome assembly, however, is considered a very low priority amongst the strategies provided here.

4.2 Peer-reviewed publications.

Project A9 will develop a manuscript based on the findings identified above. Where possible, copies of all published material will be made available through open-source publishing. A PhD student is currently developing several manuscripts that will also incorporate aspects of output from this project. A presentation incorporating the finding of a sex marker in GNS (Section 4.3.1) was made at the Sharks International Conference 2018 in Joao Pessoa, Brazil.

Floriaan Devloo-Delva, Robin B. Thomson, Mark V. Bravington, Russell W. Bradford, Barry D. Bruce, Christopher P. Burridge, Peter M. Grewe, Rasanthi M. Gunasekera, Peter M. Kyne, Gregory E. Maes, Diana A. Pazmiño, Richard D. Pillans and Pierre Feutry. (2018). Sex-linked markers and their use for inferring sex-determination systems in sharks. Sharks International, Joao Pessoa, Brazil.

Preparatory work for the GNS CKMR NESP project (A9) was funded through two DoEE projects, including through the office of the Threatened Species Commissioner (TSCDG_grey nurse shark, titled: Grey nurse shark single-nucleotide polymorphism marker development), and has been approved in MERIT Atlas of Living Australia.

4.3 Additional Findings.

4.3.1 Sex marker.

A visual assignment of sex in GNS is based on the presence/absence of male claspers. Although in general claspers are clearly visible in male sharks, a variety of factors, including water clarity, size of shark (i.e. small immature sharks), and orientation with respect to the diver, can result in difficulty with assigning sex.

The sequencing data generated for this project provided an opportunity to identify a genetic sex marker which is only present in male GNS (Appendix A, section A2). This marker showed that, as expected, the identification of sex for sharks that underwent post mortem was highly accurate, and that for animals that were photographed using stereo cameras was largely accurate (only three out of 28 animals reported to be female were unambiguously male according to the genetic data). However more than one in three animals declared by divers to be female were unambiguously male (defined as having 5 or more detections/reads of the sex marker) and of the 147 animals declared by divers to be male, 27 (18%) were female. While the sex marker can be overlooked even if present, 18% is a significantly high error rate, indicating that divers had difficulty in accurately assigning sex. Relatively low-cost improved observation methods (e.g. incorporating photographic records of sampled animals) would be beneficial to future surveys.

The sex of three photographed individuals that were misidentified, based on the sex marker, was corrected (female to male). A further 22 individuals for which no sex was reported, and all 383 animals sampled by divers, were assigned a sex according to the presence or absence of the sex marker in their DNA.

The identification of a genetic sex marker not only assisted with correct gender assignment in this analysis, but should also assist future analyses.

4.4 Data Archive.

Field data, including sample locations, have been deposited in the Atlas of Living Australia through MERIT (https://fieldcapture.ala.org.au).

Tissue samples have been deposited with the Australian Museum's Centre for Wildlife Genomics.

5. ACKNOWLEDGMENTS

This project was funded by the Australian Government through the National Environmental Science Program's Marine Biodiversity Hub, with equal co-investment from the Commonwealth Scientific and Industrial Research Organisation, Oceans & Atmosphere. In-kind support was also been provided by the New South Wales Department of Primary Industries (NSW DPI). This work could not have been carried out without the assistance of many including Carley Kilpatrick (Queensland Parks and Wildlife Service) and through the fantastic efforts of our NSW DPI colleagues: Roger Laird, Brett Louden, Peter Gibson, Joel Williams, and Tom Davis. Our thanks go to Dr Kenneth Goldman, Alaska Department of Fish and Game, for kindly providing raw age-at-length data for the derivation of growth curves used in this study. Dr Peter Grewe designed the primers necessary for amplification of the GNS mitogenome. The sequencing of the GNS mitogenome was made possible with the assistance James Marthick (University of Tasmania, Menzies Institute for Medical Research). The Western Australian Department of Primary Industries and Regional Development provided GNS samples from the western population. Appreciation is extended to Dr Rich Hillary, Dr Toby Patterson, and Jessica Farley who provided comments on the draft; resulting in a greatly improved final version. Professor Nic Bax (Director, NESP Marine Biodiversity Hub) and Paul Hedge have provided invaluable support and guidance throughout. Samples were collected under NSW DPI Animal Care and Ethics Committee permit number 14/07 and NSW DPI Scientific Collection Permit P14/0035-1.0.

6. REFERENCES

Bansemer, C. (2009). Population biology, distribution, movement patterns and conservation requirements of the grey nurse shark (*Carcharias taurus*; Rafinesque 1810) along the east coast of Australia. University of Queensland. St Lucia, Queensland, Australia. 143 pp.

Bansemer, C. S. and Bennett, M. B. (2008). Multi-year validation of photographic identification of grey nurse sharks, *Carcharias taurus*, and applications for non-invasive conservation research. *Marine and Freshwater Research* **59**: 322-331. doi 10.1071/MF07184.

Bansemer, C. S. and Bennett, M. B. (2009). Reproductive periodicity, localised movements and behavioural segregation of pregnant *Carcharias taurus* at Wolf Rock, southeast Queensland, Australia. *Marine Ecology Progress Series* **374**: 215-227. doi 10.3354/meps07741.

Barker S. M. and Williamson, J. E. (2010). Collaborative photo-identification and monitoring of grey nurse sharks (*Carcharias taurus*) at key aggregation sites along the eastern coast of Australia. *Marine and Freshwater Research* **61**: 971-979. doi 10.1071/MF09215.

Bravington, M. V.; Eveson, J. P.; Grewe, P. M. and Davies, C. R. (2017). SBT Close-Kin Mark-Recapture with Parent-Offspring and Half-Sibling Pairs: update on genotyping, kin-finding and model development. CCSBT-ESC/1709/12. CSIRO. 29pp.

Bravington, M. V.; Grewe, P. and Davies, C. R. (2016a). Close-kin mark-recapture: estimating the abundance of Bluefin tuna from parent-offspring pairs. *Nature Communications* **7**: 13162. doi 10.1038/ncomms13162.

Bravington, M. V.; Skaug, H. J. and Anderson, E. C. (2016b). Close-kin mark-recapture. *Statistical Science* **31**: 259-275. doi 10.1214/16-STS552.

Bruce, B.; Bradford, R.; Bravington, M.; Feutry, P.; Grewe, P.; Gunasekera, R.; Harasti, D.; Hillary, R. and Patterson, P. (2018). A national assessment of the status of white sharks. National Environmental Science Program, Marine Biodiversity Hub, CSIRO. 64 pp. https://www.nespmarine.edu.au/project/project-a3-national-assessment-status-white-sharks.

Cappo, M.; Harvey, E.; Malcolm, H. and Speare, P. (2003). Potential of video techniques to monitor diversity, abundance and size of fish in studies of marine protected areas. In: *Aquatic Protected Areas – What Works Best and How Do We Know?* (Eds. J. P. Beumer, A. Grant and D. C. Smith). Pages 455-464. Cairns, Qld, Australia. Available at https://www.asfb.org.au/assets/ASFB/Member-Publications/WCAPA-2002-PT1.pdf.

Cardno Ecology Lab (2010). Development and implementation of a population protocol to provide an estimate of the size of the east coast population of grey nurse sharks (*Carcharias taurus*). Department of Environment, Water, Heritage and the Arts, Canberra. 162 pp. http://www.environment.gov.au/node/18424.

Cavanagh, R. D.; Kyne, P. M.; Fowler, S. L.; Musick, J. A. and Bennett, M. B. (2003). *Conservation Status of Australian Chondrichthyans. Report of the IUCN Shark Specialist Group Australia and*

Oceania Regional Red List Workshop. University of Queensland – School of Biomedical Sciences. 170 pp.

Chapman, D. D.; Wintner, S. P.; Abercrombie, D. L.; Ashe, J.; Bernard, A. M.; Shivji, M. S. and Feldheim, K. A. (2013). The behavioural and genetic mating system of the sand tiger shark, *Carcharias taurus*, an intrauterine cannibal. *Biology Letters* **9**: 20130003. doi:10.1098/rsbl.2013.0003.

Chidlow, J.; Gaughan, D. and McAuley, R. (2005). Identification of Western Australian grey nurse shark aggregation sites. Final Report to the Australian Government, Department of the Environment and Heritage. 36 pp. ISBN 0642551952.

Daley, R.; Stevens, J.; Last, P. and Yearsley, G. (2002). Field Guide to Australian Sharks and Rays. CSIRO Marine Research and Fisheries Research & Development Corporation, Australia. 84 pp. ISBN 1 876996 10 2.

De Paoli-Iseppi, R.; Deagle, B. E.; McMahon, C. R.; Hindell, M. A.; Dickinson, J. L. and Jarman, S. N. (2017). Measuring animal age with DNA methylation: from humans to wild animals. *Frontiers in Genetics* **8**: 106. doi 10.3389/fgene.2017.00106.

DEWHA. (2009). *Review of the Grey Nurse Shark Recovery Plan*. Department of Environment, Water, Heritage and the Arts. Canberra. 43 pp.

DOE (2014). Recovery Plan for the Grey Nurse Shark (*Carcharias taurus*). Australian Government Department of the Environment. 48 pp. http://www.environment.gov.au/resource/recovery-plan-grey-nurse-shark-carcharias-taurus.

Feutry, P.; Kyne, P. M.; Pillans, R. D.; Chen, X.; Naylor, G. J. and Grewe, P. M. (2014). Mitogenomics of the Speartooth Shark challenges ten years of control region sequencing. *BMC evolutionary biology* **14**(1), 232.

Goldman, K. J.; Branstetter, S. and Musick, J. A. (2006). A re-examination of the age and growth of sand tiger sharks, *Carcharias taurus*, in the western North Atlantic: the importance of ageing protocols and the use of multiple back-calculation techniques. *Developments in Environmental Biology of Fishes* **25**: 241-252.

Harasti, D.; Lee, K. A.; Laird, R.; Bradford, R. and Bruce, B. (2016). Use of stereo baited remote underwater video systems to estimate the presence and size of white sharks (*Carcharodon carcharias*). *Marine and Freshwater Research* **68**: 1391-1396. doi:10.1071/MF16184.

Harvey, E. S. and Shortis, M. R. (1998). Calibration stability of an underwater stereo-video system: implications for measurement accuracy and precision. *Marine Technology Society Journal* **32**: 3-17.

Hillary, R. M.; Bravington, M. V.; Patterson, T. A.; Grewe, P.; Bradford, R.; Feutry, P.; Gunasekera, R.; Peddemors, V.; Werry, J.; Francis, M. P.; Duffy, C. A. J. and Bruce, B. D. (2018a). Genetic relatedness reveals total population size of white sharks in eastern Australia and New Zealand. *Scientific Reports* **8**: 2661. doi: 10.1038/s41598-018-20593-w.

Hillary, R. M.; Bravington, M. V.; Patterson, T. A.; Grewe, P.; Bradford, R.; Feutry, P.; Gunasekera, R.; Peddemors, V.; Werry, J.; Francis, M. P.; Duffy, C. A. J. and Bruce, B. D. (2018b). Genetic relatedness reveals total population size of white sharks in eastern Australia and New Zealand: supplementary material. *Scientific Reports* **8**: 2661.

Hoschke, A. M. and Whisson, G. J. (2016). First aggregation of grey nurse sharks (*Carcharias taurus*) confirmed in Western Australia. *Marine Biodiversity Records* **9**: 17. doi 10.1186/s41200-016-0012-y.

Jarman, S. N.; Polanowski, A. M.; Faux, C. F.; Robbins, J.; De Paoli-Iseppi, R.; Bravington, M. and Deagle, B. E. (2015). Molecular biomarkers for chronological age in animal ecology. *Molecular Ecology* **24**: 4826-4847. doi 10.1111/mec.13357.

Last, P. R. and Stevens, J. D. (2009). Sharks and Rays of Australia. Second Edition. CSIRO Division of Fisheries, Hobart. 644 pp. ISBN 978-0-674-03411-2.

McAuley, R.; Newbound, D. and Ashworth, R. (2002). Field Identification Guide to Western Australian Sharks and Shark-like Rays. *Fisheries Occasional Publications* **No 1**. Department of Fisheries, Perth, Western Australia. 25 pp.

Otway, N. M.; Burke, A. L.; Morrison, N. S. and Parker, P. C. (2003). Monitoring and identification of New South Wales critical habitat sites for conservation of grey nurse sharks. *New South Wales Fisheries Final Report Series* **No. 47**. New South Wales Fisheries Office of Conservation, Nelson Bay, New South Wales. 62 pp.

Otway, N. M. and Ellis, M. T. (2011). Pop-up archival satellite tagging of *Carcharias taurus*: movements and depth-temperature-related use of SE Australian waters. *Marine and Freshwater Research* **62**: 607-620. doi:10.1071/MF10139.

Otway, N. M.; Storrie, M. T.; Louden, B. M. and Gilligan, J. J. (2009). Documentation of depth-related migratory movements, localised movements at critical habitat sites and the effects of scuba diving for the east coast grey nurse population. Department of Environment, Water, Heritage and the Arts, Canberra. 88 pp.

Pepperell, J. G. (1992). Trends in the distribution, species composition and size of sharks caught by gamefish anglers off south-eastern Australia, 1961-90. *Australian Journal of Marine and Freshwater Research* **43**: 213-225.

Polanowski, A. M.; Robbins, J.; Chandler, D. and Jarman, S. N. (2014). Epigenetic estimation of age in humpback whales. *Molecular Ecology Resources* **14**: 976-987. doi 10.1111/1755-0998.12247.

Pollard, D. A.; Lincoln Smith, M. P. and Smith, A. K. (2009). The biology and conservation status of the grey nurse shark (Carcharias taurus, Rafinesque 1810) in NSW, Australia. *Aquatic Conservation: Marine and Freshwater Ecosystems* **6**: 1-20.

Pollard, D. and Smith, A. (2009). *Carcharias taurus*. In: IUCN (2012). *IUCN Red List of Threatened Species. Version 2012.1*. http://www.iucnredlist.org/details/3854/0.

Pratt, H. L. (1993). The storage of spermatozoa in the oviducal glands of western North Atlantic sharks. In: The reproduction and development of sharks, skates, rays and ratfishes (Eds. L. S. Demski and J. P. Wourms). *Developments in environmental biology of fishes*, vol. 14. Springer, Dordrecht.

Preece, A.; Eveson, J. P.; Davies, C.; Grewe, P.; Hillary, R and Bravington, M. (2015). Report on gene-tagging design study. CCSBT-ESC/1509/18.

Preece, A. L.; Eveson, J. P.; Grewe, P. M.; Bradford, R.; Davies, C. R.; Stevenson, J. and Lansdell, M. (2017). Pilot gene-tagging project: Milestone report – December 2017. Report to the Commission for the Conservation of Southern Bluefin Tuna. CSIRO, Hobart.

APPENDIX A – GREY NURSE SHARK CLOSE-KIN MARK-RECAPTURE MODEL DESCRIPTION

This Appendix describes, in greater detail than the main body of the report, the methods used in deriving the close kin estimates of adult abundance for the Australian eastern grey nurse shark, the assumptions of the model, the processing of genetic sequencing data to identify close-kin pairs, and the identification of a genetic sex marker.

A1 Treatment of length measurement error

The GNS tissue samples used in this study were obtained from several sources, with varying degrees of accuracy in the reported length of the animal. The majority of samples were collected from live sharks by SCUBA divers in NSW and Queensland waters. Measurements of these animals varied greatly depending on the equipment available to the divers. For example, if a paired laser measuring system was available, measurements were relatively accurate. However, without the use of a measuring system, divers would provide an educated guess that could vary by 30-50 cm from true length. The remaining samples were from fish captured as bycatch or in the shark control programs. These fish were measured by trained staff and considered to be accurate. Length measurements were, therefore, allocated to three categories: “diver”, “stereo” and “postmort” with “diver” likely to be least accurate and “postmort” most accurate.

A1.1 Standardisation of reported length.

All length measurements (total length ‘natural’ TLN, fork length FL and precaudal length PCL) were converted to total length ‘stretched’ (TLS) in which the upper lobe of the caudal fin is depressed, using the following formulae in which lengths are expressed in meters (Nick Otway pers. comm. NSW DPI):

$$TLS = 1.3682\ PCL + 0.0685$$

$$TLS = 1.2212\ FL + 0.0258$$

$$TLS = 1.0211\ TLN + 0.0087$$

A1.2 Accounting for error in reported length.

As age information from Australian GNS is not available, raw data on age-at-length were obtained from a study conducted in the USA (Kenneth Goldman pers. comm. Alaska Department of Fish and Game). The length measurements in that study were converted from TLN to TLS to match the length measurements used in this study. The CV in true length-at-age was calculated for each age for which more than 3 lengths were measured. Overall, a CV of 6% was found. As Goldman’s length measurements were assumed to be without error, this reflects the variation in true length for sharks of

any given age. Our sample required additional error to reflect the inaccuracy of the length measurements themselves. An additional 1%, 5% and 10% were added to the "postmort", "stereo" and "diver" groups respectively. These values were an educated guess following examination of sharks that had been recaptured multiple times. Also, examination of the histogram of reported lengths from divers suggests a preference for particular figures, especially 1.5 m, 1.8 m and 2.0 m, which is suggestive of low accuracy.

Although selectivity may affect the length-composition of the samples we obtain (compared to the length-composition of the entire population), we assume that it does not influence age *given* length.

A1.3 Inferring age from length.

All CKMR models are constructed around the idea of adult population size at the time of juvenile birth. Thus, it is necessary to know or assume something about the probable age of each animal in order to work out when each potential offspring was likely to have been born, and whether each potential parent was likely to be alive and mature at that time. Our CKMR model for GNS needs population numbers-at-age, not numbers-at-length. However, we only have length measurements, so we have to construct probability distributions for age-given-length. The growth curve estimated by Goldman *et al*. (2006) (converted to total length 'stretched') was used to estimate the expected length-at-age for GNS. A CV of 6% + 1%, 5% or 10% depending on length measurement type (as described above) was assumed for length-at-age for each age group. A normal distribution of lengths-at-age, estimated using the error function, was assumed for each age group. For any reported ('measured') length *l*, the probability that that animal had age *a* was given by

$$P(a|l) = \frac{P(l|a)P(a)}{\sum_{\forall a'} P(a')}$$

Note that because we do not definitively know the age of any animal sampled, but instead develop a probability distribution across all possible ages given length, it is not easily possible to comment on how far back in time the model is operating. We made the assumption that population age structure has been in stable equilibrium (given some fixed rate of population increase or decrease) from the earliest birth year of the animals that were found to be offspring (i.e. the O in a POP, and either of the individuals in an HSP; or in the case of a GGP, the unobserved 'parent'). But we cannot definitively state when that year was because (a) every animal has a wide range of possible true ages, and (2) the 'parent' in a GGP is unobserved – their likely age range must be inferred, probabilistically, informed by the age of their parent (i.e. the grandparent) and the age of their offspring (the grandchild). Furthermore, we imposed a plus group at age 15, by which time the length of an individual would show little change. As a result there was little information on how much older than 15 the animals in the plus group might be and, therefore how far back in time they were born.

A2 ASSIGNMENT OF SEX

To check the accuracy of reported sex of sampled sharks, we examined the set of so called 'silico DarTs'. These are DNA sequences that are found in some, but not all, individuals and for which only one allele is ever found. They normally arise from mutations (SNPs) at the site where the restriction enzyme operates, but also result from sex-linked chromosomes. We used a technique based on log-linear regression in which, for each individual silico marker we plotted the log odds ratio for females of having that marker, against that for males. In other words, for each individual marker we calculated the log of the number of females that had the marker n_1^f (defined as having at least 5 reads) divided by the number of females that did not have the marker n_0^f:

$$\log\left(\frac{n_1^f}{n_0^f + 1}\right)$$

The '+1' in the denominator prevents division by zero. This modified log odds ratio for females was plotted against that for males. A single sex-associated marker was evident, occurring more often in animals that were reported to be male than those reported to be female (Figure 4). Note that markers that are not associated with sex will occur in the population with some frequency (some common, some rare) and will be recorded in individuals according to that population frequency, regardless of the individual's sex. These markers, therefore, show similar odds ratios for males as for females so that in Figure 4 they fall on or (due to slight deviation resulting from random chance) near the one-to-one line. A marker that consistently appears in males but not females will be seen in the upper left quadrant of Figure 4, and one that is consistently associated with females would fall into the lower right quadrant. Errors in reported sex will move the location on Figure 4 closer to the one-to-one line. This method has proved pleasingly robust even to the relatively high levels of error in reported sex found in this study – these are detailed below.

On closer examination, we found that all of the animals subjected to post mortem that were reported to be female had zero, or in a single case just 1 count of the sex marker and that all reported males had at least 5 counts of the marker (Figure 5). As one would expect, this suggests that sex is reported with 100% accuracy for post mortem sharks. It also demonstrates that laboratory error can give rise to low counts of the sex marker, even if the marker is truly absent. Similarly, a zero count could indicate that the marker is truly absent, or could simply result from low read depth.

Of the individuals that had been photographed using stereo cameras, 83% of those reported to be female had zero counts of the marker with only three out of 28 such animals having the sex marker (Figure 5). Those animals had 14, 18 and 30 counts each and were, therefore, unambiguously male, according to their DNA. Similarly, 89% of the photographed animals that were reported to be male were found to have the sex marker. However, five reported males had zero counts of the marker. Because these could simply indicate low read depth, and because cameras proved to be a relatively accurate method for indicating sex, we chose to accept the reported sex for those five animals.

Correspondence between the reported sex by divers and the 'genetic sex', as indicated by the presence or absence of the sex marker, was only 65% (or 67%) accurate for reported females and 82% (or 78%) for reported males based on 'absence' defined as having no reads, or fewer than 5 reads, respectively (Figure 5). It seems reasonable that divers would be more accurate when viewing male sharks than female sharks because claspers, when present, are quite obvious. Because the sex information provided by divers was relatively poor, we chose to rely on genetic sex. Only three out of 236 reported females and five out of 147 reported males fell into the genetically ambiguous category of having between 1 and 4 counts of the sex marker. For these animals we chose to assume that the sex marker was present and therefore that they were males.

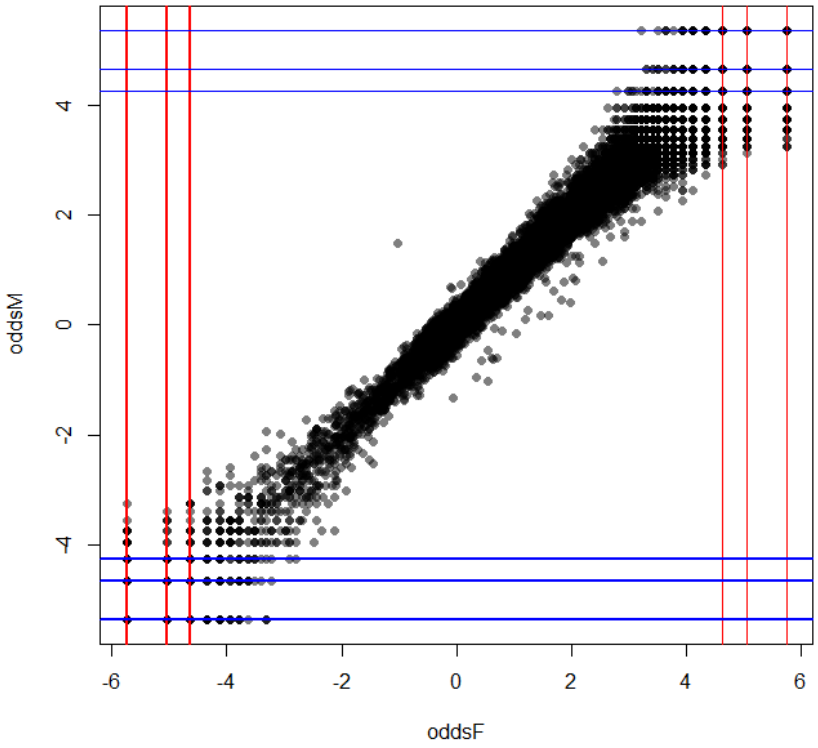

Figure 4. Modified log odds ratios for males versus females of having each of a set of 'silico' markers. The lines indicate where markers would fall if they occurred in only 1, 2, or 3 individuals of a given sex (thick lines, blue for males and red for females); or in all but 1, 2, or 3 individuals (thin lines).

The 19 photographed and six diver observed animals for which sex was not reported all had either zero counts of the sex marker and were, therefore, designated as female (11 out of 19, and three out of six), or greater than 5 counts and were designated as male. None fell into the ambiguous 1-4 count range.

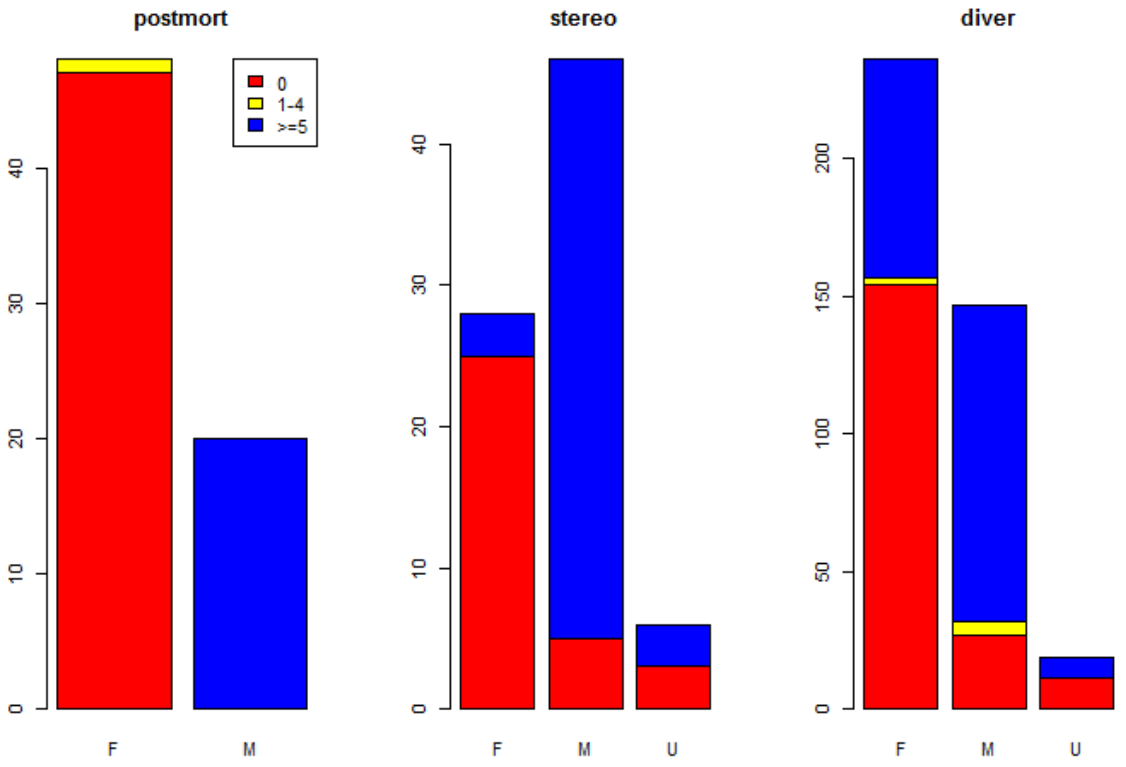

Figure 5. Number of sharks measured during post mortem (left plot), stereo-camera (middle plot), or by divers (right plot) that were reported to be female (F), male (M) or unknown sex (U), that had zero (red), between 1 and 4 (yellow) or 5 or more (blue) counts of the identified sex marker.

A3 KIN-FINDING

The genetic sequencing data were provided in the form of the number of detections ('reads') of each of a set of 102,667 sequences. Each sequence was 75 base pairs long. The 102,667 unique sequences were grouped, by DArT, into 28,234 'clusters', which are groups of sequences differing at only a few positions. These are intended to represent specific areas of the genome, hereafter called 'loci', although loci that happen to be very similar can be erroneously grouped together into a single cluster (paralogous loci). The number of times each sequence is detected in a particular individual is termed the 'read depth'. A total of 75 sharks have been sequenced twice to allow the examination of error rates.

Some GNS were sampled more than once as the biopsy mark quickly healed leaving little to no means to identify a shark as having been previously sampled. Due to this difficulty in distinguishing individual sharks, each tissue sample was assumed to have come from a different individual, and each sample was given a unique identification number (ID). On examination of the DNA sequences, however, it was clear

that some were identical (within a roughly 3% laboratory sequencing error rate). A total of 53 individual sharks were found to have been sampled more than once, with 48 sampled twice and three sampled three times. This reduced the apparent set of 489 samples by 58 to only 431 individuals, of which some were eliminated for quality control reasons, as outlined below.

A number of errors can occur, it is therefore necessary to embark on a quality control process before attempting to identify kin pairs. Such errors include the following.

1. Some of the clusters might be paralogous, meaning that they group more than one locus where sequences happen to be similar. When this occurs, it is possible for an individual to mistakenly appear to have copies of more than two of the variants contained in that cluster. However, each individual has only two chromosomes, with only one variant on each of them and, therefore, the presence of more than two variants is an indication of a paralogous loci.
2. Sequencing errors can cause a section of DNA to be mis-read.
3. Fragments of DNA from each of a large number of individuals are uniquely bar-coded and then mixed together for sequencing. Errors in the production or sequencing of the barcode can cause 'bleeding' of sequences from one fish to another.
4. Some loci and some variants at a given locus may return more reads than others, because they happen to amplify more readily under polymerase chain reaction (PCR, which creates multiples copies of the DNA), or other processes during sequencing.
5. Random chance can result in some fish or some loci having a relatively lower read depth than others.
6. DNA quality for some fish may be poor due to preservation and handling of the DNA which may result in lower reads, or even an absence of reads at a number of loci.
7. Contamination in the laboratory can cause the DNA for two individuals to be mixed together – this typically results in the detection of more than the expected number of heterozygous loci (excessive heterozygosity).

Using custom software written in the R language, we applied a strict quality control process to analyse the DArT cluster (loci) data. This software has been developed at CSIRO [work is currently underway to document the procedures and statistics used, and to make the software more widely available]. A brief description follows. First, the reported counts for each fragment for each individual were divided by the total counts over all fragments for that individual to eliminate variability due to some individuals having higher or lower total counts by chance. Seven sharks for which no length measurements were available were eliminated because without length we cannot calculate age (a requirement of the CKMR population model). Clusters whose read depth was lower than an average of 20 reads at sites that were heterozygous, were eliminated, removing 24,691 clusters. An iterative process that looked for excessive polyploidy in both fish (a possible indication of contamination) and clusters (an indication of paralogous loci) eliminated eight fish and 489 clusters. The process had to be interactive because the presence of contaminated fish can give the false impression that some clusters perform poorly, and vice versa.

For each of the remaining clusters, the two most numerous variants (in terms of average number of counts across individuals) were identified and counts for other variants were discarded. Ambiguous loci were discarded, resulting in the elimination of a further 2,709 loci.

For each cluster, individuals are expected to contain copies for up to two of the possible variants contained within that cluster. However, 'nulls' can occur when the individual has a mutation on the genome where the restriction enzymes operate. Such mutations result in no detection of the sequence of interest, even though it is actually present in the genome. Because the restriction site mutation is heritable, we can validly use these 'nulls' as a third possible variant. Having an additional variant lends greater power when discriminating close relatives (particularly the relatively hard to identify half siblings) from unrelated individuals.

Individuals for which there were no reads of either of the main variants at a cluster (termed double null, 00) were identified next. Clusters that return low (but not negligible) reads (i.e. that might have been single nulls with low read depth, or might have been double nulls with sequencing errors) were identified as ambiguous. Clusters that were ambiguous for an unacceptably large proportion of fish were removed (349 clusters eliminated).

Next, heterozygosity was investigated, with five fish and 179 clusters eliminated due to excessive heterozygosity. Excessive heterozygosity is another indication of potentially contaminated samples, and of loci that are not in Hardy Weinberg equilibrium, either because they are undergoing selection, or as a final test for clusters that group more than one locus. Hereafter we will use the term 'locus' instead of 'cluster'. The statistics that we use to identify kin pairs assume that all loci are in Hardy Weinberg equilibrium in the population order to estimate the expected frequencies of matching genotypes between pairs of individuals that share specific kin relationships, as opposed to those that share genotypes by chance.

Each locus for each fish was then scored based on whether either one or both of the main variants (labelled A and B) were present. All loci can be genotyped to one of the four categories: AB; AA/A0; BB/B0; or 00 (double null), where A0 and B0 are fish that contain a copy of the A or B allele on one chromosome but returned a null from the other. Fish that have either A0 or B0 are expected to show half as many reads, on average, as those that have AA or BB. Therefore, it is possible to score some loci to these six alleles instead of to only four, giving greater HSP finding power. Random variation means that, across fish at a particular locus, there is some blurring (Figure 6) between counts for fish that actually have A0 and those that are AA (and similar for B0 and BB). Some loci are relatively unambiguous, but others do not allow discrimination of AA from A0 and BB from B0. The decision was made, based on p-values from chi-squared tests comparing the expected with observed numbers of animals having each genotype, whether each locus could be scored at the 6-way level (AA, AO, BB, B0, AB, 00) or only the 4-way level (AA/AO, BB/B0, AB, 00). Loci that can be scored at the 6-way level provide greater power when it comes to distinguishing HSPs from unrelated (or more distantly) related individuals.

Finally, loci that performed poorly in tests of Hardy Weinberg equilibrium were eliminated (removing a further 466 loci). In total, 378 GNS passed the extensive quality control process; leaving 2,157 loci for the identification of POPs and HSPs/GGPs.

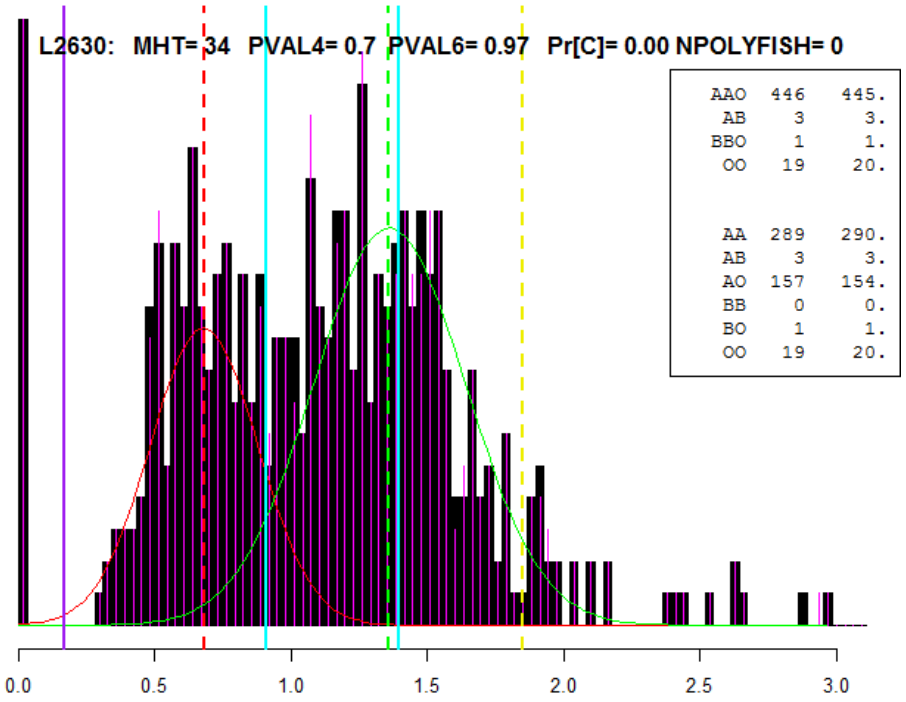

Figure 6. Histogram showing normalised read depth, across all fish, showing the fitted distributions of those for which two copies of alleles (either A or B) were found (green curve) and those for which only one copy was present (red curve). Note that this figure is created by the CSIRO software and contains more detail than is described or required here.

A3.1 Identifying kin and recaptures

In the final step, statistics (which have yet to be documented) were calculated that were designed to identify POPs, FSPs and HSPs. These were the "weighted pseudo-exclusion" ('wpsex'), which is the weighed proportion of loci at which both animals have the same genotype, optimised for POPs; the 'wtsame' statistic optimised for FSPs; and the pseudo likelihood ratio (PLOD), optimised for HSPs (Bravington *et al*. 2017). For every possible pairing of individuals, we plotted each of these statistics against one of the others (Figure 7). Colour coding indicates the resulting assumed kin relationship. Note the pair (green dot) that is situated close to the FSPs (cloud of blue dots). The mitochondrial haplotypes for these two animals differ, indicating that they are a paternal half-sibling pair (i.e. they share a father but not a mother). Clearly they are more closely related than the average HSP, suggesting

that their mothers must have been close relatives. The presence of such a pair suggests a relatively small reproductive population.

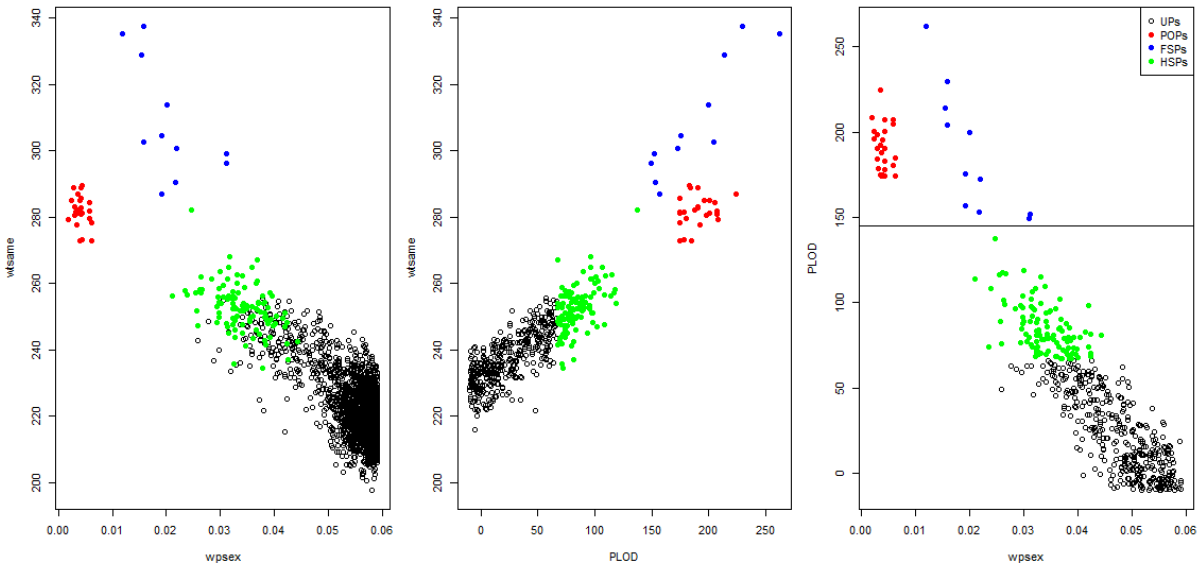

Figure 7. A subset of all possible pairings of grey nurse shark sampled for this study. Those found to be most closely related are shown. Three statistics (wpsex, wtsame and PLOD – see text for details) that examine relatedness are shown on the axes. Based on these statistics, pairs have been allocated as POPs (red), FSPs (blue) or HSPs/GGPs (green); the remainder are unrelated pairs or more distant relatives as well as the 50% of HSPs that fell below a chosen PLOD threshold (open circles).

A4 CKMR MODELLING

For modelling the population dynamics of the eastern Australian GNS, we have used the same underlying population-dynamics model that was used for white sharks (Hillary *et al*. 2018b) with some structural changes to account for the different life history and available dataset. Although the biology of the two species have some similarities when modelling for CKMR purposes, the GNS data differ qualitatively and quantitatively. The differences in the CKMR model for GNS are explained below.

The CKMR model relies on the identification of kin-pairs. The GNS dataset contained a wide age-range (including many adults) and lacked reliable length measurements for the majority of the samples. This (i) forced us to develop a more elaborate CKMR model than used previously to allow for more kinship possibilities (e.g. POPs and GGPs), and (ii) required us to assume that the age composition of adults had been stable for a relatively long period. Although this assumption (the quasi-equilibrium assumption (see A4.1)) may not accurately reflect the true history of the population over the modelled period, slight deviations from the assumption of stable age composition over an extended period (for example, from long-term changes in per capita bycatch rate) would have little overall influence on the model. Major deviations from the assumption would, however, lead to bias in the abundance and mortality rate estimates from the model.

A4.1 Assumptions.

The assumption is made that all the rates and probabilities have been stable for several generations, in other words, long enough for the age-structure of the population to have stabilized (i.e. total abundance may be rising or falling exponentially over time, but the relative proportions-at-age are constant from year to year). This is also known as the "quasi-equilibrium" assumption. This assumption covers not just annual proportions-at-age, but also any density-dependent effects on reproductive parameters; for example, the quasi-equilibrium assumption would be invalid if the population had started the period in an undepleted state, where per capita reproductive rate is lower than in an already-depleted stock.

The structural assumptions of the CKMR GNS population-dynamics model are:

1. all females mature at a fixed age, and all males at a lower fixed age (knife-edge maturity);
2. after maturity, average annual reproductive output is equal for all adults of given sex;
3. annual survival probability is constant over time, sex, and age for ages 7+;
4. annual survival probability varies log-linearly with age, between ages 0 and 7;
5. average fecundity (female offspring per female adult per year) is fixed over time;
6. male-to-female ratio at birth is fixed over time.

In the absence of published data on the age at maturity of eastern Australian GNS, two alternative sets of values were considered (to account for uncertainty in true age-at-maturity) based on the growth curves of Goldman *et al*. (2006): knife-edged maturity at 10 years old for females and 7 years old for males, or 14 years old for females and 11 years old for males. Fecundity was assumed to be 0.5, based on a presumed average litter size of 1 female and 1 male every other year (though see next paragraph). All other parameters are estimated from the data using the CKMR model. These parameters include:

- adult abundance (by sex) in some arbitrary reference year;
- ratio of adult males to females
- annual rate-of-change of abundance;
- adult survival rate per year.

The CKMR probability calculations only estimate the number of adults, and we have no direct data about juvenile survival, thus there is no reliable way to estimate total abundance in the absence of having enough information to estimate juvenile abundance. The question then arises why any assumptions/modelling of juveniles are needed at all for GNS. There are two reasons:

1. Since juvenile survival probabilities cannot exceed 1, the low fecundity of GNS places some constraints on the range of population dynamics, in part on the rate-of-increase/decrease of the population. Therefore, using an explicit model for juvenile survival prevents the model from estimating population growth rates that imply higher birth rates than are biologically possible;

2. We have to make estimates of the ages of the sharks. Those estimates are *mainly* driven by the probability distribution of length-given-age, which is assumed known from the growth curve

(see Appendix A, section A1.3). However, the age-given-length estimates are also somewhat affected by the underlying distribution of numbers-at-age within the population, which is determined by survival-probabilities-at-age among the juveniles.

In all, the structural assumptions about juvenile survival, fecundity, and age-at-maturity should not— even if slightly wrong— have much effect on our estimates of adult dynamics because we estimate juvenile survival given our chosen values for fecundity and age-at-maturity. The model is able to vary its estimate of juvenile survival in order to give the best fit to the information that the close-kin data provide regarding the adult population. The main question is whether the quasi-equilibrium assumption is appropriate for modelling all our samples. If we had reasonably accurate age estimates for our GNS sample, we could avoid the problem by omitting kinship comparisons on pairs where the relevant dates-of-birth are uncomfortably far back in time. However, because most of the length measurements are inaccurate, we cannot exclude the possibility that those individuals were born a long time ago; thus the more complicated model and more ambitious assumptions are inevitable.

At face value, the structural assumptions seem either uncontroversial given GNS biology, or unlikely to have much impact on the final answer even if the details are wrong. For example, *some* assumption has to be made about juvenile survival rates, similar but not necessarily identical to #4 above (annual survival probability varies log-linearly with ages 0-7 years), in order to make the model internally coherent; but the details of that particular assumption should only have a small impact on the abundance estimates, compared to sampling noise, including uncertainties in the dataset. Furthermore, whether maturity happens to all female GNS at exactly the same age (knife-edge), or whether it is spread out over a few years, should have little effect on the final answer.

A4.2 Population dynamics equations and CKMR probabilities.

Table 1 provides the notation for the equations used to describe the CKMR model.

Table 1. Notation used in the equations describing the Close-Kin Mark-Recapture model. Note that subscripts (e.g. regarding age, sex, and sample) have been suppressed for clarity.

Symbol	Meaning
$\mathrm{I}[], \mathbb{P}[]$	indicator function (1/0) and probability of some event
i, j	"labels" denoting particular animals being considered
x	whether the animal was sampled DEAD or ALIVE
s	sex
y, y_{ref}	year (of sampling, or generally); y_{ref} is an arbitrary "reference year" for abundance
a	age
b	year of birth, i.e. $y - a$
α	age at maturity
a	age when survival probability reaches its adult value. $a \leq \min(\alpha_{♀}, \alpha_{♂})$
N_a; N^{ad}	number of juveniles aged a; number of adults
p_a; p^{ad}	annual survival probability of juveniles aged a; and of adults
r	annual rate-of-increase/decrease of the population (1→constant)
b	fecundity (age-0 offspring per female per year). Also determines sex ratio.
β	slope of stable-age-composition in adults
π	stable-age-composition probabilities in juveniles
v	"nuisance parameters" required for handling same-cohort comparisons

The population-dynamics model is entirely age-based, with knife-edge maturity. In terms of the notation above (Table 1), we have:

$$N^{ad}_{s,y+1} = p^{ad} N^{ad}_{s,y} + p_{\alpha-1} N_{s,\alpha-1,y}$$

$$N_{s,a+1,y+1} = p_a \, N_{s,a,y} \qquad 0 < a < \alpha$$

$$N_{s,0,y+1} = b_s \, N^{ad}_{♀,y} \qquad 0 < a < \alpha$$

$$\log(p_a) = \frac{a}{\mathrm{a}} \log\left(p^{ad}\right) + \frac{(a - \mathrm{a})}{\mathrm{a}} \log(p_0)$$

In addition, the assumption of quasi-equilibrium leads to an implied solution for the rate-of-change, r, and the population age-composition of juveniles and adults (given by π and β) in terms of the other parameters, as the eigensolution of the Leslie matrix (see Hillary *et al*. 2018b for details). The setup for GNS is simple enough in that that there are in fact closed-form expressions for r, π, or β, but the formulae are omitted here for brevity. In the notation of this paper, we have:

$$N^{ad}_{s,y+1} = r \, N^{ad}_{s,y} = r^{y - y\mathrm{ref}} \, N^{ad}_{s,y\mathrm{ref}}$$

$$N_{s,a+1,y} = \beta \, N_{s,y,a}; \qquad a \geq \mathrm{a}$$

$$N_{s,a,y} = \alpha \, \pi_a \, ; \qquad a \geq \alpha$$

Note that the stable-age-composition of juveniles, $\{\pi_a; \quad a < \alpha\}$, is not a geometric progression because survival probability varies with age; it is used only in computing posterior probabilities of age given measured length.

All of the CKMR probabilities follow the expected relative reproductive output (ERRO) principles given in Bravington *et al*. (2016a), specialized to the circumstances assumed for GNS. The equations below are expressed purely in terms of age, which is assumed to be the fundamental biological variable; in practice we only have length estimates for GNS, so the equations need to be adapted as described in section A1.3.

For POPs we have:

$$\begin{aligned}\mathbb{P}\left[Kij = \mathrm{PO}\middle| s_i, x_i, y_i, a_i, b_j,\right] \\ = \mathrm{I}\left[a_i - \left(b_j - y_i\right) \geq \alpha_{s_i}\right] * \frac{1}{N^{ad}_{s_i, b_j}} \\ * \begin{cases} \mathrm{I}\left[y_i \geq b_j\right] & x_i = \mathrm{ALIVE} \\ \prod_{a=a_i}^{a_i + \max(0,\, b_j - y_i) - 1} (p_a) & x_i = \mathrm{DEAD} \end{cases}\end{aligned}$$

The three terms represent, respectively:

- *i* must be mature when *j* is born;
- all the adults of *i*'s sex that are alive when *j* is born have an equal chance of being the relevant parent;
- *i* still has to be alive at *j*'s birth.

For HSPs where the shared-parent's sex is *s*, we have:

$$\mathbb{P}\left[Kij = s - \mathrm{HSP}\middle| b_i, b_j; b_j > b_i\right] = \frac{1}{N^{ad}_{s_i, b_j}} * p^{ad(b_j - b_i)}$$

where "s-HSP" indicates that the possible kin types are maternal-HSP or paternal-HSP. The point here is that the parent was clearly alive and mature when *i* was born. To have a chance of being *j*'s parent it needs to survive the intervening period until *j*'s birth (the rightmost term) and then has an equal chance of parenthood as any other living adult of that sex at that time. When $b_i = b_j$ (i.e. same-cohort HSPs) this needs to be multiplied by an additional parameter v_s, as explained in section A4.3.

For GGPs where the sex of the "intervening" parent is *s*, we have to consider all possible ages, a^*, of the unobserved intervening parent, given that (s)he was evidently mature at *j*'s birth. Given some possible age, a^*, the question is whether his/her parent was actually animal *i*— which requires *i* to be alive and mature at the birth of the intervening parent, i.e. at $b_j - a^*$. Conceptually, this entails summing the POP probability over an infinite range of possible intervening-parent birth-years. The result is:

$$\begin{aligned}\mathbb{P}\left[Kij = s - \mathrm{GPGC}\middle| s_i, x_i, y_i, a_i, b_j\right] \\ = \sum_{a^* \geq \alpha_s} \mathbb{P}[a^* | a^* \geq \alpha_s] * \mathrm{I}\left[a_i - \left(b_j - a^* - y_i\right) \frac{1}{N^{ad}_{s_i, b_j - a^*}}\right] \\ * \begin{cases} \mathrm{I}\left[y_i \geq b_j - a^*\right] & x_i = \mathrm{ALIVE} \\ \prod_{a=a_i}^{a_i + \max(0,\, b_j - a^* - y_i) - 1} (p_a) & x_i = \mathrm{DEAD} \end{cases}\end{aligned}$$

Note that, although GGPs and HSPs cannot be individually distinguished just using DNA, there is a *statistical* difference in the probability of sharing mtDNA. A GGP will have identical by descent (*ibd)* mtDNA if-and-only-if the elder is female *and* the intervening parent is also female. For HSPs, *ibd* only

requires that the shared-parent be a mother, so the sex of the two kin is irrelevant. Of course, mtDNA can be shared even if not *ibd*, so this is not individually definitive, but it does help in the context of a CKMR model. In other words, there is useful information in how the proportion of shared mtDNA in HSPs/GGPs varies with the likely birth-gap between the members of the pair. The problem with GGPs, though, is that to compute the probability, we need to assume that the population-dynamics-assumptions have applied for a very long time.

A4.2.1 When age is uncertain.

When age is uncertain, as with GNS, all of these equations need, for each pair, to be summed over the possible age-distributions of the two animals, given their lengths. For ages in the plus-group (after which growth is assumed negligible in the sense that length provides no useful information about age), the sums can be computed analytically under the quasi-equilibrium assumption, i.e. that the age-distribution of adults follows a geometric progression. Using plus-group sums avoids the need to keep track within the model of age-classes into the 100s (for grandparents in a long-lived species). The relevant equations, although simple enough in principle since they are all based on finite and infinite sums of geometric progressions for which there is a well-known formula, have been omitted here for simplicity.

A4.3 Full-sibling pairs and same-cohort sibling comparisons.

CKMR models can only derive useful information from cross-cohort sibling pairs (i.e. animals born in different years), as same-cohort sibling pairs have different dynamics that are not informative about population size. If age is known accurately enough, same-cohort comparisons can be excluded *a priori*, but for our GNS sample that was not possible. Therefore, some extra parameters were needed to account for same-cohort pairs. In other species, the main concern with same-cohort sibship is the possibility of "lucky litter" effects, whereby early-life-stage survival rates vary substantially from litter to litter. This may result in a disproportionate excess of same-cohort siblings to different-cohort siblings compared to what a "naive" model might predict. Because litter sizes are so small in GNS (usually a maximum of 2), the scope for lucky-litter effects is very low, but there is still the complication of full- as opposed to half-sibling pairs to account for.

Many same-cohort sibs will be FSPs rather than HSPs (i.e. sharing both a father and a mother). However, multiple paternity within a litter, which is common in sharks, has been reported in GNS by Chapman *et al*. (2013). Chapman *et al*. (2013) found that 60% of 15 pregnant female GNS carried pups from multiple fathers. Forty percent of these females were expected to give birth to HSPs; the remainder producing FSPs (Chapman *et al*. 2013). Multiple paternity means that there will be same-cohort maternal HSPs. There will also be some same-cohort paternal HSPs where the same male has fathered offspring by different females. Cross-cohort FSPs should be rare because, with an adult population likely to number in at least hundreds, the chance of a female mating twice with the same male as opposed to a different male is negligible.

To reduce the number of parameters that had to be estimated, we chose to ignore FSPs altogether in the GNS model (they can be reliably identified genetically), and our CKMR probabilities only address POPs and HSPs/GGPs. To cope with same-cohort effects on HSPs, we estimate two extra parameters v_s=($v_♀$, $v_♂$) that modify the nominal HSP probabilities (maternal/paternal for $v_♀$/$v_♂$) if the comparison is same-cohort. In other words:

$$\mathbb{P}\left[Kij = s - \text{HSP} \middle| b_i = b_j = \text{y}\right] = \frac{v_s}{N_y^{ad}}^{\text{ad}}$$

One possible complication could be sperm-storage, whereby a female uses stored sperm from a previous mating to impregnate successive litters. This is known to occur in several elasmobranch species (Pratt 1993), and we have preliminary but direct confirmation from our other CKMR studies (*Glyphis* spp and school sharks). A high rate of sperm-storage in GNS would lead to our model over-predicting the number of cross-cohort HSPs (because some will actually be FSPs instead, and thus ignored by the model); in turn this would presumably lead to positive bias in abundance estimates (to "explain" the actual observed number of HSPs) and also to positive bias in survival (the "missing" HSPs would only be from nearby cohorts, since sperm storage cannot last indefinitely). To work out whether this is actually important for GNS, we would need good age data (which is currently unavailable) to establish whether cross-cohort FSPs are common.

A5 RESULT - POPULATION ESTIMATE

The age at maturity of east Australian GNS is unknown, therefore, two alternative sets of values were considered: scenario 1, knife-edged maturity at 10 years old for females and 7 YO for males (a_{mat} 10, 7), or scenario 2, maturity at 14 YO for females and 11 YO for males (a_{mat} 14, 11). The *v* parameters that account for 'litter effect' and multiple paternity were not well estimated and were, therefore, both fixed at 0.5. The parameters that were estimated were the number of adults present in the population at the beginning of the modelled period (N_0^{ad}), the ratio of adult male to female sharks in the population (M:F) , adult survival rate (p^{ad}), and the population growth rate (*r*).

The total adult population size of the eastern GNS was estimated to be 2,167 (95% confidence interval: 1,257 to 3,078) adults using maturity schedule 1 (a_{mat} 10, 7), with adult survival estimated to be 0.97 per annum and the annual rate of increase = 3.4% (95% confidence interval: 1.2 to 5.7%) (Table 2). Using maturity schedule 2 (a_{mat} 14, 11), the adult population of eastern GNS was estimated to be 1,686 (95% confidence interval: 956 to 2,417) adults, with adult survival estimated to be 0.95 per annum and the annual rate of increase = 4.5% (95% confidence interval: 2.5 to 4.7%). A slight male sex bias (1.3:1 and to 1.1:1, respectively) was estimated to exist from birth (Table 2).

Table 2. Estimated quantities of interest (adult population size in 1975 'N_{1975}' and 2017 'N_{2017}', estimated adult male to female ratio 'M:F', population growth rate r and adult survival rate p^{ad}, negative log likelihood, and expected numbers of POPs, HSPs and GGPs as well as the proportion of HSP/GGPs that share the same mitochondrial DNA haplotype) for eastern Australian grey nurse shark for a range of model scenarios. An asterisk indicates a parameter value that was assumed (fixed), not estimated. Observed numbers of kin pairs and the ratio of those that have the same, to different, mitochondrial DNA, are shown in italics. Model scenario age-at-maturity setting denoted by a_{mat} F, M.

Model Scenario	N_{1975} (CV)	N_{2017} (CV)	M:F	r *(95% CI)*	p^{ad}	-lnL	#POPs	#HSPs	#GGPs	% same mtDNA
							26	*108 together*		*41%*
p^{ad}, r free a_{mat} 10, 7	489 (0.72)	2,167 (0.21)	1.3	3.4% (1.2-5.7)	0.97	1,118.6	48	58	28	48%
p^{ad}, r free a_{mat} 14, 11	262 (0.50)	1,686 (0.22)	1.1	4.5% (2.5-4.7)	0.95	1,113.8	43	73	18	49%
p^{ad}, r fixed a_{mat} 10, 7	1,278 (0.09)	1,333 (0.09)	1.3	0.1%*	0.95*	1,123.7	57	55	22	49%
p^{ad}, r fixed a_{mat} 14, 11	1,100 (0.09)	1,147 (0.09)	1.2	0.1%*	0.95*	1,120.7	54	64	17	49%

While the model was able to simultaneously estimate values for all of the parameters listed above, the likelihood profile for p^{ad} demonstrated that while low values of adult survival were not supported by the data, there was little discrimination between values above 0.9 (Figure 8). By contrast, the likelihood profile for the rate of population growth, r, is narrower and indicates that the data support a growing population. The 95% confidence interval for r (based on the minimum negative log likelihood value plus 2) is 1.012 to 1.057 i.e. 1.2% to 5.7% growth per annum (Figure 8) for the scenario that used lower ages-at-maturity. The higher scenario yielded a 95% CI of 2.5% to 4.7%. The best fit parameter estimates for the two scenarios were a population growth rate of 3.4% or 4.5% (Table 2). Note that because males mature younger than females, it is expected that there will be more adult males in the population than females.

The numbers of observed versus expected POPs and HSPs/GGPs were not as close as might have been expected, suggesting the possibility of skewing due to the small population size. This might result in skewed model estimates, such as for population growth rate. For this reason we considered scenarios in which population growth was fixed at the low rate of 0.1%. These scenarios estimated adult population sizes of the order of 1000 sharks across the modelled period but estimated fewer HSPs and GGPs: 77 and 81 as opposed to 108 observed (Table 2).

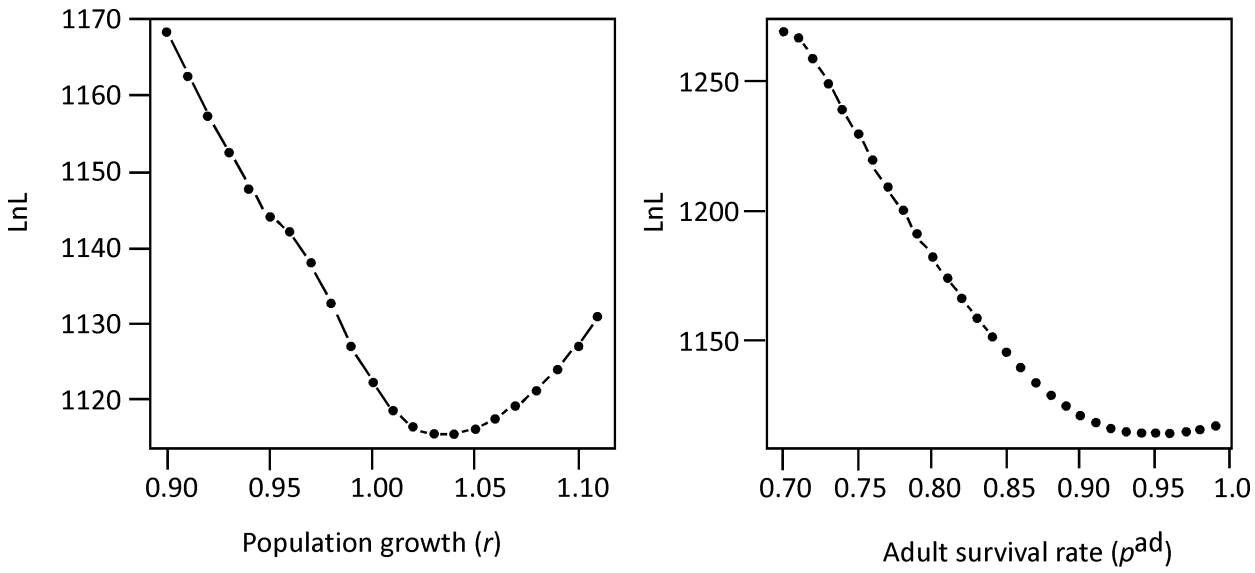

Figure 8. Likelihood profiles for population growth rate r (left plot) and adult survival rate S^{ad} (right plot) for the scenario that assumes age at maturity of 10 years old for females and 7 years old for males.

A6 DISCUSSION OF FIT

Half-sibling pairs provide a means to estimate the number of breeding adults, while POPs provide the means to estimate total adult population abundance irrespective of an individual's contribution to reproductive output. Therefore, any non-random mating (such as a male dominance hierarchy, or site fidelity) that could alter the reproductive output of a proportion of the mature animals would result in a discrepancy between the number of estimated versus observed POPs, given an estimate of the adult population based on HSPs. The CKMR model was unable to simultaneously estimate the correct numbers (observed values) of both HSPs and POPs, but instead underestimated the number of HSPs (86-91 estimated:108 observed) while overestimating the number of POPs (43-48 estimated: 26 observed).

Half-sibling pairs were particularly difficult to discriminate for GNS, with the distribution of unrelated pairs merging into that for HSPs. There appeared to be an unusually large number of animals that were kin of some sort, but that were less closely related than true HSPs (e.g. cousins). This could result from a somewhat inbred population (recall the HSP that appeared to merge with the FSPs (Figure 7), but whose differing haplotypes clearly marked it as an HSP). It is very important in a close kin study not to allow false positives when identifying HSPs. For this reason a threshold value is chosen for the statistic (PLOD) that is used to diagnose HSPs; only pairs that score above the threshold are regarded as clear HSPs, those that fall below are 'sacrificed'. The proportion of true HSPs lost in this way is quantified and accounted for in the close-kin model. For GNS we set the threshold at the theoretical mean PLOD value, thus allowing 50% of true HSPs to be 'lost'. In context, other close kin studies to date have used thresholds that imply losses of the order of 10-15%. Despite the difficulties in discriminating HSPs in

GNS, the conservatively high PLOD threshold value minimised the chance of including non-related pairs and thus biasing the adult population estimates.

It is possible that an, as-yet, unknown factor affecting reproductive output of a proportion of the population is contributing to the discrepancy between the number of POPs estimated versus observed. Any non-random mating (such as a male dominance hierarchy, or site fidelity) that could alter the reproductive output of a significant proportion of the mature animals could result in a discrepancy between the number of estimated versus observed POPs, such as was observed in the modelling of GNS.

www.nespmarine.edu.au

Contact:

Russell Bradford
CSIRO Oceans & Atmosphere

GPO Box 1538, Hobart, Tasmania, 7001

email | Russ.Bradford@csiro.au

tel | +61 3 6232 5222