Independent Expert Peer Review of the Close Kin Mark Recapture Assessment for School Shark

Review panel:

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Executive summary

The review panel was tasked by the Australian Fisheries Management Authority (AFMA) to review the Close-Kin Mark-Recapture (CKMR) based stock assessment for school sharks (*Galeorhinus galeus*), as well considering an associated industry-funded review of the assessment and the response of the stock assessment team to that review. This review was centred on three Terms of Reference:

ToR 1. Is there an inherent likelihood of consistent under-estimation or over-estimation of school shark abundance and productivity that would be expected to result from the:

a. sampling design, in particular the assumptions made in the design, the sample size and the distribution of samples given current knowledge of the range of the school shark population and its movement patterns;

The panel considered issues related to sampling design (i.e., sample size and sample distribution) and concluded that the methods used in the CKMR assessment should not lead to substantial bias in the estimation of abundance or productivity of school sharks.

b. close kin data inputs (i.e. genetic sequences), in particular the methods used in genetic sequencing, their associated uncertainties and assumptions;

The panel did not identify substantial concerns about the close kin genetic inputs to the assessment.

c. fishery dependent data inputs (i.e. landed catch and discards), including their associated uncertainties and assumptions;

The Panel concluded that there is no inherent likelihood of consistent under-estimation or over-estimation of school shark abundance and productivity that would be expected to result from the fishery dependent data inputs (i.e., landed catch and discards) used.

d. biological and selectivity parameters, including their associated uncertainties and assumptions;

The panel identified three issues that they recommended work on to further improve the accuracy and precision of assessment outcomes. The first was in relation to the ability to precisely and accurately age individuals included in the study. In the current assessment, age uncertainty could cause considerable bias in the results. Inaccurate age estimates affected a number of aspects of the assessment, including significant age differences between full sib pairs. The panel recommended improved ageing techniques to increase confidence in the results. The panel concluded that inter-litter sperm storage and repeated mating between individuals were unlikely to be explanations for age differences between full sib pairs, but could be fully eliminated as possibilities by further research on mating systems in school sharks. The second issue was the occurrence of skip breeding (females not producing litters every year). With the current approach skip breeding could introduce significant bias (estimated to be 16% above current estimates if females produce litters every 3 years and males reproduce annually). The panel recommended that research into the periodicity with which females produce litters and how that periodicity affects sibling probabilities would assist in better incorporating skip breeding into future assessments. The third issue identified was the stock structure of school sharks caught by Australian fishers. Knowledge of stock structure will not affect the outcomes of the assessment, but is important in the interpretation of the results and their use in making management recommendations. The panel recommended that further work to understand

the historical and contemporary stock structure be undertaken to assist in setting management arrangements.

e. statistical methods and assumptions used to incorporate the close-kin data inputs (i.e. genetic sequences) into the assessment model, including the methods applied to kin-finding.

The panel identified that the occurrence of skip breeding was not fully accounted for in the close kin mark recapture assessment. The panel recommended that the close kin mark recapture method be modified to account for skip breeding, and that simulation work be considered to investigate whether the potential bias from not accounting for skip breeding is reduced as more cohorts are included, and, if so, how many cohorts would be required before it fully attenuates.

2. Based on the response to question 1, do the methods employed in the CKMR assessment provide sufficiently precise, accurate and unbiased estimates of productivity and absolute school shark abundance and trends upon which to base management advice?

The panel concluded that the methods used in the CKMR assessment of school sharks are suitable for providing management advice. However, it noted a number of areas where improvements in outcomes of the assessment could be made. There is a need to i) improve the accuracy and precision of age estimation, and ii) to account for skip breeding. Addressing both of these issues will reduce bias in the estimation of abundance and productivity of school sharks. Beyond the actual results of the assessment, further consideration needs to be given to how the assessment results fit within the Commonwealth Harvest Strategy Policy, and how different hypotheses of stock structure might affect the interpretation of the results. The panel suggested that management strategy evaluations would help inform a possible harvest strategy incorporating CKMR abundance estimates.

3. What revised or alternative methods could be used to improve the precision, accuracy and level of bias associated with the CKMR assessment? In answering this question consideration should be given to how any potential improvements should be scheduled, noting the current assessment schedule for school shark (2021).

The panel concluded that the close kin mark recapture approach to assessing school sharks is likely to be the most appropriate way to understand the status of the population and to make management recommendations into the future. Precision and accuracy of the assessment results will be increased with improvements in the ability to accurately and precisely age school sharks, and by improving how the model accounts for skip breeding. The panel recommends that this work be undertaken as soon as possible and incorporated into the next assessment.

Background

School sharks (*Galeorhinus galeus*) have been an important component of southern Australian fishery catches for almost a century. Targeted in the 1940s as a source of vitamin D, and subsequently for flesh, the species has been a focus of research and assessment. Significant increases in catches, especially in South Australia in the 1980s and 1990s led to the population becoming overfished, with estimates of abundance in the 1990s around 16% of original biomass[1]. As a result of the overfishing of the species fisheries management arrangements changed, and the species was listed as Conservation Dependent under national environmental legislation. The species currently remains overfished in southern Australia.

Stock assessments for school shark in southern Australia traditionally used age, sex, area and stock structured models that incorporate catches, effort, fishery-dependent CPUE, and tagging and length data, to estimate depletion levels and future catches that would comply with the Commonwealth Harvest Strategy Policy. However, changes to management arrangements (e.g. that resulted in active avoidance of school sharks) meant that over time the traditional assessment approach became less suitable for producing useful outcomes. To overcome these issues, the Australian Fisheries Management Authority (AFMA), with advice from the Shark Resource Assessment Group (SharkRAG), implemented a Close Kin Mark Recapture (CKMR) assessment that used a new method of estimating absolute abundance using related pairs of individuals in the population[2].

The first CKMR school shark assessment was released in draft form in 2019 (Appendix D). Subsequently, the fishing industry had the assessment reviewed by a consultant, Patrick Cordue, because of concerns about its ability to provide appropriate management advice (Appendix E). The authors of the CKMR assessment responded to the review by Cordue, addressing a wide range of concerns (Appendix F). Subsequently, AFMA implemented the current review because of ongoing industry concerns about the CKMR assessment and the novelty of the approach. Details of the review and more detailed background is provided in Appendix A.

Approach taken in this review

AFMA appointed a review panel consisting of four members with a variety of experience relative to the school shark assessment:

Professor Colin Simpfendorfer (Chair). An Adjunct Professor at James Cook University with a long history of research on sharks, including shark fisheries in southern Australia. He is also a previous chair of SharkRAG.

Professor Sean Cox. A Professor at Simon Fraser University in Canada with extensive experience in fisheries stock assessments, including those using CKMR.

Dr Kevin Stokes. A fisheries consultant based in New Zealand with extensive experience in fisheries stock assessments, including those using CKMR.

Dr Robin Waples. A Senior Scientist with the US National Marine Fisheries Service with extensive experience in population genetics and its application to estimating population size.

The panel were provided with the Terms of Reference (ToR) for the review and examined the material provided by AFMA (original school shark CKMR assessment, Cordue review, CSIRO response to Cordue review, reviews of the draft final to the funder [FRDC]). The panel met several time via online platforms to discuss the material. The panel developed a series of questions for the CSIRO assessment team (see Appendix B). The CSIRO team provided a written response to the panels questions, and also met virtually with the panel (see Appendix C).

In the following pages the panel provides their responses to the Terms of Reference, including making recommendations where appropriate.

ToR 1. Is there an inherent likelihood of consistent under-estimation or over-estimation of school shark abundance and productivity that would be expected to result from the:

a. sampling design, in particular the assumptions made in the design, the sample size and the distribution of samples given current knowledge of the range of the school shark population and its movement patterns;

Sampling design

Cordue: "The three most important things to consider when collecting data are: design, design, and design. In the case of the close kin DNA data there was very little design (although this appears to be deliberate). There were three main areas that were targeted for collection of sharks but other than that there was no attempt to stratify or randomise the sampling."

The Bravington and Thompson (BT) response provides a clear and precise rebuttal of Cordue's criticism for lack of sampling design. First, "no attempt to stratify or randomise" is incorrect, as the sampling design (sampling distribution/areas and sample sizes) was deliberately chosen to achieve target levels of CKMR precision given catch sampling across multiple fishing areas, with further considerations for uncertainty in age, size, and mortality[3]. The final choice of spatial distribution of sampling (across three main fishing areas) is in proportion to fishery activity in an attempt to also account for possible sub-stock structure if it exists (potential stock structure issues are reviewed below).

Second, the conditional probability approach, as opposed to the unconditional approach combined with random sampling as suggested by Cordue, is well-documented in the CKMR literature[2]. The key feature needed for unbiased parent-offspring pairs (POPs) sampling is that capture of individuals (i.e., samples) must be independent of their kinship. Clearly, related individuals may have similar capture probabilities, for example, because they are of similar size and/or age; however, these covariates are taken into account in the conditional probability approach used in the CKMR assessment.

Our conclusion:

The Panel concluded that there is no inherent likelihood of consistent under-estimation or over-estimation of school shark abundance and productivity that would be expected to result from the sampling design.

Implications of movement

Movement patterns of individuals might not have large impacts on CKMR as long as related individuals are adequately mixed within the population, such that sampling in any area is representative of the population distribution of half sib pairs (HSPs). If this condition is met,

movement patterns such as emigration from Australian waters have no effect on CKMR estimates since emigration is embedded within the estimated mortality rate, which is common to all individuals. Immigration (e.g., from NZ in particular), if not accounted for, could cause positive bias in the estimated breeding population unless it is accounted for, since incoming individuals will result in lower prevalence of HSPs.

Our conclusion:

The Panel concluded that there is no inherent likelihood of consistent under-estimation or over-estimation of school shark abundance and productivity that would be expected to result from the current understanding of movement within the school shark population.

b. close kin data inputs (i.e. genetic sequences), in particular the methods used in genetic sequencing, their associated uncertainties and assumptions;

The CSIRO laboratory that produced the draft report has led global efforts over the past ~15 years to develop and refine laboratory procedures that are precise and repeatable enough for the demanding requirements of CKMR. Largely as a consequence of some unfortunate wording in the draft report from CSIRO, Cordue concluded that the genotyping and close-kin identification were not sufficiently reliable. The CSIRO response clarified these issues and the Panel believes that the genetic data used in the school shark report are reliable.

Our conclusion:

The Panel concluded that there is no inherent likelihood of consistent under-estimation or over-estimation of school shark abundance and productivity that would be expected to result from genetic data inputs.

c. fishery dependent data inputs (i.e. landed catch and discards), including their associated uncertainties and assumptions;

Cordue notes that the school shark stock assessment assumes a single stock in a single area and starts in 2000. Given the difference in estimated abundance for 2000-2017 between the CKMR stock assessment and the previous stock assessment model, and the speculation by the CKMR assessment of a major shift in productivity, Cordue recommends the development of a purpose-written model that can include multiple areas, non-random mating, litter size variation, and the full catch history as used in previous assessments.

The response of Bravington and Thomson to the Cordue review (see Appendix C) effectively repeats what is already in the CKMR assessment report. That is, that previous assessments have also not been able to reconcile pre-2000 catch rates and catches and the hypothesis of two or more stocks, one of which is functionally extirpated, is a plausible explanation; they do not say it is the explanation, though they provide strong arguments in support of this hypothesis. Bravington and Thomson reiterate original stock assessment report comments that the assumption of a single stock, different to that previously assumed, for which CKMR is used to estimate absolute abundance has implications for management, with a need to consider reference points and rebuilding criteria.

The review ToRs explicitly exclude an examination of the stock assessment model previously used for school shark and do not include consideration of how the RAG decided to recommend use of the

CKMR model and management implications. Given the explicit exclusion in the objectives, we do not consider whether use of pre-2000 fishery dependent data and alternative model structures might be feasible and what estimates of abundance might eventuate. Nor do we consider management implications in detail. However, the Panel notes that improvements to ageing to allow the use of fish older than 11 years would increase the kin pairs available such that the CKMR model could extend to pre-2000, though even with ageing to (say) age 20, not to pre-1990 and with few samples. While improved ageing is of course desirable for future CKMR modelling, it is unclear if extending the model further back in time would be helpful for informing management. The further the model is extended backwards into the 1990s, the greater is the potential to conflict with the assumption of a single productivity period for the assumed single stock currently exploited and fishery in need of management. If future management is based solely on CKMR-derived estimates of abundance, and possibly trend, then exploratory modelling of earlier periods to include multiple areas and stocks would be interesting, but not necessarily of practical management use.

ToR (1c) asks specifically whether the catches, discards, and sex ratio as used in the CKMR assessment could lead to consistent under-estimation or over-estimation of school shark abundance and productivity by the CKMR assessment.

The original stock assessment report describes in some detail at section 4.2 how landed catches were revised for use in the assessment. The total catches as used in the stock assessment have been revised by exclusion of catch from some WA and NSW zones, effectively consistent with ensuring a single stock. Within those slightly revised catches, reallocation by gears was made but with no significant impact due to consistency of size-selectivity between gears. The resulting catches seem well justified. Discards have not previously been included in school shark assessments. The CKMR assessment provides a clear rationale for the annual multipliers used. While sex ratio of catches clearly varies by area and year, the overall assumption of 50:50 again seems well founded, most importantly for 2004 onwards for “mesh nets” (Table 4.3, CKMR assessment report).

The population model described in appendix C of the CKMR assessment report uses data from 1989 onwards, assumes equilibrium in 1989, and estimates fishing mortality (by sex and gear) for pre-1989 as constant, 1989-1999 as constant, and then annually from 2000. The structure allows calculation of a starting population structure in 2000 after which F is estimated to ensure catches by sex, gear and year are fit exactly. The link to the CKMR calculations is of course through numbers by sex, year and age. No uncertainty is allowed for in the catches by number, allocation by gear and sex, or discard multiplier. Nor is any uncertainty regarding selectivity accounted for (which is fixed externally by gear).

Our conclusions:

The Panel concluded that there is no inherent likelihood of consistent under-estimation or over-estimation of school shark abundance and productivity that would be expected to result from the fishery dependent data inputs (i.e., landed catch and discards), including their associated uncertainties and assumptions. The panel also does not see an inherent likelihood of over- or under-estimation to result from the sex ratio assumption.

d. biological and selectivity parameters, including their associated uncertainties and assumptions;

FSP age gaps

The Cordue review posits that because of large age gaps between full sib pairs that repeat mating between the same male and female is likely.

The CSIRO response indicates that the original work assumed a low CV for age (8%) whereas in reality it is much higher (~20%) and demonstrated that with a higher assumed CV the putative age gaps between FSPs is much less problematic. The response also noted that sperm storage is another possible explanation as to why the age gaps are so large. Sperm storage would allow for repeat mating between cohorts. The response suggests that both issues could be acting in combination.

Our conclusion:

Sperm storage – it is well known that female sharks store sperm after mating, at times for years. However, more commonly it is stored for shorter periods (months). Sperm is stored in the oviducal gland, which is also the site of egg fertilisation[4]. There is no information available in the literature on the storage of sperm between litters that would support or refute the occurrence of sperm storage that allows for FSPs that appear to be the result of repeat mating. To better understand this issue we contacted Dr James Gelsleichter from the University of North Florida who is one of the few scientists globally who has investigated sperm storage in detail for live bearing sharks (mostly using the bonnethead shark, *Sphyrna tiburo*). We asked whether he was aware of any evidence of inter-litter storage of sperm in live bearing sharks. His response was:

"One thing I am very confident about – at least in bonnets [bonnethead sharks] – is that it is very unlikely that sperm can be retained in good condition over multiple years. In my flushings of the oviducal gland from recently mated or sperm-storing females, I normally have to dilute the samples by a factor of 1,000 just to get the sperm concentration down low enough to count. On the other hand, shortly after ovulation, I would struggle to find a single sperm cell in a pure undiluted flush. In general, I think there is some adhesion between the sperm and oviducal epithelial cells during storage and "release" at ovulation (which occurs in other vertebrates). I do know that there are some minor amounts of residual spermatozoa left over in the gland after ovulation from histology data but it doesn't look viable.

On the basis of this response we think it is unlikely that sperm storage leads to FSPs between litters and is thus not a plausible explanation for the large apparent age gaps between FSPs.

Repeat mating – another possible explanation for the large apparent age gap between FSPs is that the same male-female pair mates more than once (i.e. pair bonding occurs). We find this unlikely in school sharks for a couple of reasons. Firstly, there is no evidence in sharks for pair bonding, although there is probably a remote probability that repeat mating occurs by chance. Secondly, females do not breed every year (likely every 3 years), while males likely breed every year[5]. Given the difference in frequency of mating between sexes it is unlikely that pair bonding would occur.

Ageing uncertainty – given that sperm storage or repeat mating (pair bonding) is highly unlikely in school sharks, it is likely that the apparent age gaps between the FSPs is the result of uncertainty in ageing. The CSIRO response demonstrates that assuming a larger CV for ages can account for much of this discrepancy.

We therefore conclude that the most plausible explanation for the large apparent age gap between some FSPs is ageing imprecision.

Recommendations:

- Implement methods to improve the precision of age estimates (see Age Uncertainty section below for more details).
- Research to fully eliminate the possibility that sperm storage between litters and repeat mating occurs in school sharks should be considered. It would be possible to confirm this in at least different two ways:
    - Investigation of mating dynamics of school shark, and specifically the occurrence of sperm storage after mating and before and after fertilisation to investigate the quantity and quality of stored sperm. Examining oviducal glands for sperm storage in pregnant females would also provide for evidence of sperm being retained from one litter to the next. If this was done it would be possible to use genetic techniques to compare the sperm to the embryos to determine if the stored sperm was a match and hence if this would lead to possible production of FSPs between different litters.
    - Significant improvement in the precision of age estimates. This would allow more accurate estimation of the age gaps between FSPs. If there was a high level of confidence in age estimates and there remained relatively large age gaps between FSPs then this would be relatively strong evidence for repeat mating.

Pupping interval (also referred to as "skip breeding"; see information also provided in response to ToR 1e)

The Cordue review suggests that the model should have made specific allowance for the periodicity of litter production in female school sharks.

The CSIRO response argues that the inclusion of multiple cohorts in the model results in this effect being cancelled out.

Our conclusion:

The review panel considered that the CSIRO response to the Cordue review had not adequately dealt with this issue because it was not clear whether these effects would cancel out, and if so how many cohorts would be required. It is well documented that female school sharks do not pup each year, and most likely pup every three years (one year pregnant and two years resting). However, there is uncertainty about this periodicity and how it may vary by individual and age. The panel formulated a question for CSIRO (see Appendix B and Appendix C for CSIRO response) to further explore this issue for the school shark CKMR. After further discussion with CSIRO, the panel concluded that the skipped breeding effect was a significant issue for the school shark CKMR that led to bias in the overall estimation of population size. The level of effect, however, is positively correlated with the period between litters for individual females. The issue is also confounded by the imprecision of ageing for the samples. With perfect ageing it would be possible to more easily quantify and account for this issue.

Recommendations:

- The skipped breeding effect needs to be accounted for in the CKMR calculations for school sharks to minimize bias in estimates of population size. The CSIRO response to the review

panel's question indicates that there are numerical approaches to deal with skip breeding in CKMR statistical methods. Careful consideration needs to be given to developing and implementing such an approach for school sharks where this phenomenon is known to occur.

- There are also two, non-mutually exclusive, research approaches that can help inform on this issue:
    - Significant improvement in the precision of age estimates.
    - Research on the periodicity with which females produce litters, and whether this changes with size/age. The faithfulness of females to a fixed skip breeding period is likely to be an important research topic as changes in periodicity would affect the level of bias that skipped breeding causes. There is evidence of changes in the periodicity with which litters are produced by females of the whiskery shark (*Furgaleus macki*) a close relative of the school shark. In this species younger mature females produce litters every second year, while older mature females produce litters annually[6].

Selectivity parameters

The school shark assessment uses size selectivity parameters that were estimated using multiple mesh size nets and standard techniques[7]. While there are a variety of ways to determine size selectivity of the different gears used in the fishery, there is no reason to believe that the current size selectivity parameters are not appropriate.

Our conclusions:

The current size selectivity parameters used in the school shark CKMR assessment are adequate to provide accurate outcomes and do not lead to bias in the estimates of abundance or productivity.

Age uncertainty

The original assessment report and the subsequent review and response make it clear that there is a high level of uncertainty in the ages of the samples, and that this has led to significant uncertainty in the outcomes. The ages of the samples were determined from vertebral samples that were aged by counting bands that are formed with some known periodicity. In the case of Australian school sharks there are published works on the age and growth[8] and periodicity of band formation[9,10]. There are also published works on the age and growth of school sharks from New Zealand[11] and Brasil[12]. All provide similar estimates of age and growth. There were at least two issues related to age estimates of school sharks used in this assessment:

i. Low level of precision in age estimates for individuals <11 years of age. In other words, there appears to have been a poor relationship between the number of bands counted and true age. Little information is given in the original report on the exact ageing methods used, whether multiple reads were completed or if metrics that describe the age uncertainty were calculated. There are a range of metrics that could be used to express the uncertainty in age that it would be useful to calculate and present with the age data (e.g. Index of Average Percent Error and Chang's coefficient of variation[13]). These metrics require multiple reads of individual vertebrae, as well age validation techniques[14] to provide information on the level of precision. The limited information and age data presented in the CKMR assessment report

makes it difficult to evaluate the methods used. However, it is clear that methods to document and improve the precision of age estimates would be of great value to the school shark CKMR assessment.

ii. Uncertainty in the age of individuals >11 years. It is well known that school shark vertebral bands after maturity are laid down less frequently than the annual bands laid down before maturity. This is not unique to school sharks, being a phenomenon observed in many shark species[15,16]. There is some information available on the periodicity of band formation in older school sharks, but this is sourced from a small number of individuals and makes it difficult to make solid conclusions. Further work on the ageing of mature school sharks is required to enable their inclusion in future CKMR assessments.

The age uncertainty had multiple impacts on the school shark assessment:

i. Required the removal of all individuals older than 11 years of age (i.e. all adults), which reduces the number of kin pairs included in the assessment (see ToR 1c). This disproportionately affected samples collected in the Central South Australian region, with most (72%) of the 721 samples being excluded as they were >11 years old (See Tables 6 and 7 CSIRO response to panel questions; Appendix C). There was minimal exclusion of large individuals from other regions.
ii. Created large putative age gaps between FSPs (FSPs should have been the same age)
iii. Made it difficult to deal with the skip breeding effect in females

Our conclusions:

Age uncertainty leads to increased uncertainty in abundance and productivity of school sharks in the CKMR assessment. Addressing this issue is key to providing confidence in the continued use of the CKMR technique. The panel was unable to quantify the magnitude of this uncertainty, or if it was likely to introduce bias. This is because age uncertainty effects the calculations in a number of ways (e.g. skip breeding, lucky litter effect) for which the effects of age uncertainty cannot currently be predicted.

The assessment report would benefit from greater detail on the exact methods used to age sharks and presentation of data on the reliability of age estimates. This will inform on the current situation with age estimation, but not provide for any improvement.

There is a critical need for improved age estimates for future assessments. Several approaches could be considered:

i. Improvements in vertebral ageing techniques, such as use of different methods to enhance band visibility and readability
ii. Genetic approaches to ageing (e.g. epigenetic estimates of age). Discussion with CSIRO suggest that this approach may have utility and is being trialled for future use in school sharks.
iii. Other indirect methods of ageing, such as Near Infra-Red Spectroscopy which has been shown to provide age estimates rapidly from whole vertebrae[17].

We do not wish to be prescriptive with the approach that should be used; however, we do note that both epigenetic and NIRS approaches require calibration with known age individuals, and these are normally based on ages derived from vertebrae. This means that the ability to produce precise

estimates of true age from at least a subset of vertebrae may still be required even if other methods are also employed.

Recommendations:

- More information on ageing techniques and metrics of precision be provided for the school shark CKMR assessment with future assessments.
- Accurate length data be collected with samples wherever possible to help improve validation of age estimates.
- Improved methods for precise estimation of age be developed and validated.
- Research is carried out to understand the periodicity with which bands are deposited in vertebrae of mature school sharks.

Stock structure

The CKMR school shark assessment assumes that the current fishery takes individuals from a single stock. The original assessment document reports the work of Devloo-Delva et al[18] that is based on genetic analysis of juveniles from nurseries in Australia and New Zealand. The panel queried CSIRO about the possible effects of multiple cryptic stocks on the CKMR calculations (Appendix B). The response demonstrated that multiple stocks should not affect the estimation of population size using CKMR (see Appendix C). The panel is comfortable with this response. However, the panel notes that stock structure has an important role in the interpretation of the assessment results (see ToR 1c). Thus, while knowledge of stock structure is unlikely to have implications for the CKMR part of the assessment, it is important to understand the stock structure for interpreting the current status of the population and for making management recommendations. This is because the current assessment suggests the most likely reason that the historical catches cannot be accounted for is that one or more stocks have gone extinct subsequent to these large catches having been made. The assessment report suggests that it is a stock that had nursery areas in Victoria that may have gone extinct.

Genetics can provide useful information on stock structure; however, it can only reflect stock structure over evolutionary time scales. Thus, while the current assessment has assumed a single stock because of the genetic evidence, the possibility of structure within the population at shorter time scales (i.e. those indicating demographic independence between sections of the population rather than genetic independence) cannot be ruled out. In fact, the assessment explicitly acknowledges the likely existence of multiple stocks to account for why historic catches cannot be accounted for. Assuming that this explanation is true, it has important implications for the determination of the status of the population relative to fishery reference points used to develop management recommendations. This issue is explored further in ToR 2.

Our conclusions:

That knowledge of stock structure is not limiting to the CKMR calculations in the assessment, but the existence of multiple stocks is likely (at least historically). The panel considers that an improved knowledge of stock structure (both historic and contemporary; and why historic catches cannot be accounted for) would provide greater certainty to the assessment results.

Recommendations:

- Information on stock structure (historic and contemporary) should be collected using a variety of methods (e.g. genetics, tag recapture, tracking, life history, parasites).
- To provide greater evidence that extinction of this stock has occurred, Victorian nursery areas for school sharks be surveyed to determine if they continue to not be used. This may support the conclusion of a stock extinction.
- If the evidence for stock extinction is limited, then more investigation of why historic catches cannot be accounted for in the assessment would be required.

e. statistical methods and assumptions used to incorporate the close-kin data inputs (i.e. genetic sequences) into the assessment model, including the methods applied to kin-finding.

As discussed above, CSIRO has now acknowledged the validity of one of the claims in Cordue's review – that their draft report underestimated the magnitude of errors in ageing. Uncertainty associated with ageing errors has important, interacting consequences for two types of analyses required to produce unbiased CKMR estimates.

Skip breeding

Skip breeding (in this case presumably only by females) can create either positive or negative correlations between the reproductive success of individuals over time, depending on the form of skip breeding and the difference in ages between potential siblings. These correlations in turn affect sibling probabilities and abundance estimates. There are two general options for dealing with this issue. The optimal approach is to incorporate the expected correlations into the overall likelihood framework. This, however, requires accurate ages for the offspring and a good understanding of the distribution of breeding intervals, neither of which currently applies to school sharks. In addition, CSIRO has indicated that adopting this option would substantially complicate the estimation process and require significant new time for coding. An alternative approach is to ignore the issue, under the assumption that the positive and negative correlations will cancel out, provided that data for a sufficient number of cohorts are available. The latter approach is the one CSIRO has taken with school shark.

The Panel asked for a worked example to support this key assumption by CSIRO (see Appendix B), which CSIRO addressed in their 24 Nov response (see Appendix C). The new CSIRO analyses showed that bias associated with ignoring skip breeding could be substantial, especially if the typical breeding interval is 3 years rather than 2. The CSIRO analysis included two scenarios, but the one that only uses cross-cohort comparisons is the most relevant. The Panel agrees that the reported biases in abundance estimates could be halved, assuming that only females skip breed. Furthermore, it is possible that bias associated with skip breeding is smaller than other uncertainties associated with the assessment (e.g. age uncertainty). Nevertheless, a potential upward bias in abundance estimates on the order of 16% is not trivial.

Recommendations:

- Conduct additional modelling under plausible scenarios for school shark to determine the extent to which the bias attenuates (if at all) as data for more cohorts become available (either through new collections or improved methods to age existing samples). This information is necessary to optimize experimental design.

- Given that greater precision of age estimates would improve how the close kin methods account for skip breeding improved ageing techniques would have benefits. See recommendations on improved ageing in ToR 1d.

Lucky litter effect

In discussion with the Panel, CSIRO confirmed that by this term they mean variance in offspring number among individuals of the same age and sex. This sort of variation affects sibling probabilities within a single cohort and is not accounted for by standard CKMR covariates (e.g., by modelling changes in fecundity with age). For this reason, sibling-based CKMR analyses typically focus on comparisons of individuals from separate cohorts. But this requires accurate ageing. For school sharks, because the CKMR assessment do not exclude within-cohort comparisons to avoid the lucky litter effect, it instead tried to account for the consequences of within-cohort comparisons, by a) estimating their relative frequency, and b) estimating how large the effect could have been for within-cohort comparisons. Given the nature of the data and what is known about school shark biology, this is probably the best that can be done at this time. However, until such time that improvements in ageing might allow reliable exclusion of within-cohort comparisons, continually refining the estimates of the magnitude of the lucky litter effect will be important as new samples and new data become available. In general, underestimating the lucky litter effect will tend to downwardly bias abundance estimates, while overestimating it would have the opposite effect.

ToR 2. Based on the response to question 1, do the methods employed in the CKMR assessment provide sufficiently precise, accurate and unbiased estimates of productivity and absolute school shark abundance and trends upon which to base management advice?

CKMR presents at least one significant challenge in terms of providing management advice in that it does not provide stock status in the same way that traditional stock assessments do (i.e. abundance relative to original stock size). Because of this, the results of this assessment (and other CKMR assessments) are difficult to use with the Commonwealth Harvest Strategy Policy (CHSP), which is based on relative stock depletion levels. As a consequence, there is a need to develop clear guidance on operationalising the results of CKMR in the CHSP. It is the Panels understanding that AFMA and Shark RAG are aware of this issue, and we believe that there is work underway to address this. Thus while CKMR assessments can be used to generate management advice on the size of sustainable catches, it is not possible to provide guidance about the status of the stock relative to the standard limit and MEY-related target reference points used for Commonwealth managed fisheries.

The CKMR assessment method applied to school shark provides, however, a direct estimate of absolute abundance and is therefore able to derive estimates of productivity and temporal trends that are in principle suitable for supporting management of the fishery. While there are some CKMR inputs and modelling issues that could be further developed (see below), the approach should provide the most reliable abundance estimates available at the current time.

The ability to estimate absolute abundance directly is a major advantage of CKMR over other forms of indirect abundance estimation, especially model-based stock assessments that depend on fishery-dependent data. In principle, the use of direct estimates of abundance in informing management, could reduce the over-fishing risk compared to, for example, CPUE-based assessment models.

Simulation studies consistently show that even model-based assessments that are structurally and dynamically identical to the true fish stock and use well-designed fishery-independent surveys require long (e.g., 30+ years) and precise (<20-25% CVs) index time-series to provide abundance estimates precise enough to maximize long-term yield[19], assuming also the consistent application of optimal decision rules. Pacific halibut provides a comparable practical example in which a halibut-specific, fishery-independent survey operating over 22 years and consistently achieving <10% annual CVs in survey indices results in a CV~18% on spawning abundance estimates from a detailed age-structured assessment model. Trends in these abundance estimates form the basis for annual quota advice.

Translating CKMR results into management advice remains a key challenge for school shark given it is not accommodated in the CHSP. However, it is an enviable problem to *only* have an accurate and direct measure of abundance. Many fisheries, including the Pacific halibut case mentioned above, spend enormous amounts of money, time, and intellectual power trying to find or improve upon methods of estimating absolute abundance.

In moderately data-rich fisheries, including in Australia, management advice typically takes the form

$$TAC = F \cdot B$$

where TAC is a total allowable catch, F is the target fishing mortality or exploitation rate, and B is the estimated exploitable biomass. Various rules exist in most national harvest policies to adjust F to account for various precautionary and/or economic considerations. The problem is that in most of these contexts both F and B are highly uncertain, with B being especially problematic because it is sensitive to monitoring data quality (as mentioned above) plus assumptions about linearity of abundance indices, stock structure, recruitment, etc. On the other hand, the target F is less of an issue because simulation studies also show that reasonable guidelines for F can be derived from the natural mortality rate M. This has led to the use of M as a basis for control rules, for example in New Zealand orange roughy (see, for example: https://cert.msc.org/FileLoader/FileLinkDownload.asmx/GetFile?encryptedKey=gEjdiaOYA2YqvEjToY6fS+9QvGm5EV1dlbXIC/tZsFMyvc2aklFi+o9sDfubBvFp). The point here is that CKMR potentially reduces the uncertainty in B substantially and could therefore be used in the above formula to provide management advice in the context of TAC-managed fisheries.

As noted elsewhere in this report, there are potentially some interactions between fishing mortality (as it contributes to total mortality) and CKMR abundance estimates because total mortality affects the age distribution of spawners. Therefore, it is not exactly clear how an F-based harvest strategy, such as the example above, might create feedbacks that affect future CKMR estimates. Therefore, we recommend some straightforward (at least conceptually) management strategy evaluation to help inform a possible harvest strategy incorporating CKMR abundance estimates. This is certainly not available in the current scientific literature and could help to motivate more fisheries to make a transition to CKMR where possible. Simulations could also include scenarios related to key uncertainties such as ageing error and skip breeding.

On ageing error and skip breeding specifically, we have made a number of recommendations on how the assessment could be improved relative to these issues. If further assessments of school shark are undertaken using CKMR then we expect that the improvements recommended and the additional data will further improve the results and their ability to be used for providing management advice.

The interplay between the CKMR assessment and stock structure also provides some challenges for providing management advice. Issues related to stock structure and the assessment are covered, in part, in ToR 1c and 1d. This relates mostly to the conclusion of the school shark assessment that the reason that the historic catches cannot be accounted for is that at least one stock has become extinct (see Stock Assessment report, p. 64). The evidence for this stock extinction is reports that newborn school sharks have disappeared from Victorian bays (mostly Port Phillip Bay and Western Port Bay) when the last directed surveys were undertaken. The review panel does not have any additional data to further elucidate this issue. However, we do make the following observations:

i. Research, and discussions of stock structure at SharkRAG and in other forums, over many years has failed to provide certainty about stock structure and whether/how some stocks have gone extinct.
ii. Genetics associated with the current CKMR assessment has indicated a single stock in Australia and New Zealand. While genetic approaches to determining stock structure are widely used and powerful, they can fail to identify stocks that are demographically separated, but which have small amounts of movement between them at generational time scales.
iii. Recent research has demonstrated evidence for nursery areas in South Australia[20] and how this should be interpreted in relation to stock structure is currently unclear, but should be examined further.
iv. It is unclear how the current uncertainty in stock structure should be interpreted in terms of one or more stocks going extinct in the explanation of why historic catches cannot be accounted for.

If the extinction of one or more stocks is the true reason that historical school shark catches cannot be accounted for in the CKMR assessment, then this could present significant issues in terms of the provision of management advice. Since the original size of the extinct stock(s) is not known it is impossible to know when the remaining stock(s) reaches suitable limit or target reference points. This could be overcome by using an M-based harvest strategy as indicated above.

Given the challenges in terms of providing management advice outlined above, serious thought needs to be given to how the current and future results will be interpreted and used to provide sound management advice in relation to the CHSP and stock structure.

Our conclusion:

The panel concludes that the methods used in the CKMR assessment of school sharks are suitable for providing management advice. However, it is noted that we have identified a number of areas where improvements in outcomes of the assessment could be made. In particular, the need to improve the precision or age estimation and to account for skip breeding. Addressing both of these issues will reduce bias in the estimation of abundance and productivity. Beyond the actual results of the assessment, further consideration needs to be given to how the results function within the CHSP, and how different hypotheses of stock structure might affect the interpretation of the results.

ToR 3. What revised or alternative methods could be used to improve the precision, accuracy and level of bias associated with the CKMR assessment? In answering this questions consideration should be given to how any potential improvements should be scheduled, noting the current assessment schedule for school shark (2021)

The first question is whether the CKMR assessment is the best of the available options for assessment in the future. The major limiting factor in any assessment for school sharks is the availability of data on abundance trend through time or absolute numbers of individuals. The previous version of the assessment used historical (up to ~2000) CPUE data from the fishery. However, changes to management meant that CPUE no longer provides informative data on trend in abundance, besides the fact that CPUE indices always carry substantial risks of under-estimating stock declines. Huveneers et al[21] examined a range of approaches to providing data on abundance or abundance trends to the assessment, including the use of CKMR. Thus, while there are a range of alternatives, we are of the view that CKMR provides the best alternative available because of the time that it would take for the other methods to provide data suitable to enable an accurate and precise assessment. The advantage of CKMR is that it provides information on absolute abundance and trend relatively quickly and without the potential severe biases arising from fishery-dependent CPUE indices. Furthermore, data can continue to be collected and analysed at relatively low cost compared to alternatives. We therefore conclude that persisting with the CKMR approach to assessing school sharks is the best way to proceed.

If CKMR is to be used in the future, then there are a number of issues that should be addressed through research or methods development. The Panel is of the view that if these issues are addressed then the accuracy and precision of the assessment will be improved. Addressing these issues the panel concludes that they will lead to improved accuracy and precision of the assessment.

Two high priorities for improvement are recommended:

i. Accounting for female skip breeding. . Since female school sharks do not breed every year, likely producing a litter every three years. A three-year breeding cycle in females (the most commonly reported in the scientific literature) would lead to an estimated 16% of upwards bias in the results. Accounting for female skip breeding could reduce this bias, thereby providing appreciable improvement in the outcomes of future assessments.

ii. Improved estimation of ages. This will provide increased certainty in relation to a number of parts of the assessment, including FSPs and the inclusion of significantly more individuals from the Central South Australia region. It was not possible to estimate the level of improvement this would provide, but given it was identified as a limiting factor for several aspects of the assessment it should provide considerable benefits. To be most effective it would be necessary to apply the improved ageing approach (see ToR 1d for more information on possible approaches) to the historical data (i.e. individuals used in the initial CKMR assessment) and new data. Application of improved ageing methods to the individuals in the current CKMR assessment would potentially lead to reduced bias prior to the addition of more samples in a new assessment. However, this would require a re-running of the assessment to generate the outcomes.

There are some additional issues that could be improved upon, but are likely to provide less improvement in the accuracy and precision of the assessment:

i. Improved information on stock structure of school sharks in Australian waters to help with the interpretation of the assessment results.
ii. Research on the mating system of school sharks. This will help better understand issues such as the time between litters produced by individual mature females, and the likelihood of sperm storage in females between litters.

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Appendix A. Review requirements

Independent Expert Peer Review of the Close Kin Mark Recapture Assessment for School Shark

Terms of Reference

Background

Historically, school shark (*Galeorhinus galeus*) was targeted using both hooks and gillnets in what is now the Gillnet, Hook and Trap (GHAT) sector of the Southern and Eastern Scalefish and Shark Fishery (SESSF). Low levels of school shark catch have also been recorded in the Commonwealth Trawl sector, although school shark has not been targeted using trawl methods and catches have been sporadic in nature (Patterson *et al*, 2019).

Catch of school shark in the GHAT sector peaked at more than 2,500 tonnes in 1970 and then declined rapidly to around 500 tonnes in 1973. Catch in the sector again increased to around 2,000 tonnes in 1986, before declining steadily through the late 1980s and 1990s. Assessments for school shark indicate that the stock has been overfished (below the biomass limit reference point) since approximately 1990 (*Patterson et al*, 2019).

Under the Commonwealth Fisheries Harvest Strategy Policy (2018), rebuilding strategies are required to be developed for all species which are below their biomass limit reference point (DAWR, 2018). In 2009, school shark was listed as conservation-dependent under the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act). A rebuilding strategy was implemented just prior to this in 2008 and was subsequently revised in 2015. A strategy will be required until the school shark stock is above the limit reference point with a reasonable level of certainty – the strategy aims to achieve this within three times the mean generation time (66 years from 2008) (AFMA, 2015).

A key challenge with school shark is measuring the abundance of the stock and monitoring rebuilding. Recent gillnet catch per unit effort (CPUE) data is not a reliable indicator of abundance, as a result of the introduction of management measures from 1997 onwards to protect the stock and prevent targeted fishing. The resulting changes in fishing practices have caused the CPUE to break down as an index of abundance. Recognising this constraint, a fishery independent method of estimating school shark abundance commenced
in 2014 using close kin genetics techniques (AFMA, 2015).

Close-kin mark recapture assessment

During the 1990s and 2000s, management of school shark in the SESSF was based on an age structured stock assessment model that relied on CPUE as an index of abundance. This stock assessment model was most recently updated in 2009 and was used in 2012 to make forward projections under different future catch scenarios. It is also this model upon which the current rebuilding strategy is based (Thomson *et al*, 2019). The stock assessment model uses gillnet fishery CPUE data up to 1996, after which the model extrapolated abundance using landed catch data. The most recent assessment showed school shark abundance (expressed in terms of pup production) to be below 20% of unfished biomass in 2008.

A workshop in 2012 identified candidate indicators of abundance for school shark. A subsequent investigation of the feasibility of each these resulted in the recommendation by an external reviewer that the close kin mark recapture (CKMR) method be applied to school shark (Dunn, 2014).

In 2014, the Fisheries Research and Development Corporation (FRDC) commissioned CSIRO to undertake a research project to examine the use of CKMR as a method to calculate absolute estimates of school shark spawning stock abundance with sufficient precision to inform an update to the stock assessment and to update the rebuilding strategy.

In August 2018, the Shark Resource Assessment Group (SharkRAG), which makes recommendations to the AFMA Commission regarding the management of school shark, recommended the development of a CKMR assessment model including the incorporation of additional data sets, to replace the stock assessment model. In taking this approach, SharkRAG noted the stock assessment model was based on CPUE data which no longer provides an accurate index of relative abundance for the stock, used complex stock structure assumptions and lacked more recent tag-recapture data past 2005 (SharkRAG minutes, August 2018).

This recommendation meant that an estimate of depletion relative to unfished biomass is currently not available and therefore the reference points detailed in the SESSF Harvest Strategy, which are defined relative to unfished biomass, cannot be assessed. SharkRAG advised a longer term strategy needs to be developed to address this issue (SharkRAG minutes, August 2018).

In December 2018, SharkRAG accepted the CKMR assessment model, noting high confidence in the absolute estimate of abundance produced by the model, and lower confidence in the estimates of trend. Based on the model's projections, SharkRAG recommended setting an incidental total allowable catch (TAC) for the subsequent three fishing seasons (2019-20 to 2021-22) and for the model to be updated in 2021 (SharkRAG minutes, December 2018). The scheduling for the next assessment will need to considered in light of the findings of this review.

Requirement for a review

It is good practice to conduct an independent expert peer review outside of, or in addition to, the normal RAG process when (Penney *et al*, 2016):

- the research is novel, complex, or contentious, exceeds the technical expertise of existing science working groups, or requires review beyond the capabilities of established scientific work groups;
- there is substantial uncertainty and a range of conflicting scientific opinions regarding the interpretation of results;
- attempts at peer review using existing committees or panels (e.g. RAGs) have resulted in adversarial debate and irreconcilable opposing views;
- there are strong conflicts of interest relating to potential impacts of fisheries management decisions on organisations, industries or groups with whom some participants in regular peer review processes are affiliated;
- the findings are controversial or implications for fisheries management decisions are substantial.

In this case, the need for an independent expert peer review of the CKMR assessment for school shark has been precipitated by concerns expressed by some industry stakeholders that the abundance estimates from the CKMR assessment may not be adequately representing the true status of the school shark stock. The Southern Shark Industry Alliance, one of two fishing industry associations in the GHAT sector, commissioned a review of the CKMR assessment in 2019 (Cordue, 2019). CSIRO supported the conduct of the review through the provision of the necessary data and other information. CSIRO also provided a separate response concerning the findings of the review following its finalisation (Bravington *et al*, 2019). The Fisheries Research and Development Corporation (FRDC) is also conducting a review of the original stock assessment report, as part of its normal project review process.

Other important drivers for this review are that CKMR research is novel and complex, and technical assessment of the method currently exceeds the expertise of the SharkRAG and is outside the group's scope. The findings will also have substantial implications for upcoming decisions concerning the assessment and management of school shark as well as the potential to be more broadly applicable to the use of the CKMR method in other Commonwealth managed fisheries.

Of note, the CKMR method has previously undergone review by a multi-disciplinary panel during its development and implementation to Southern Bluefin Tuna.

Review terms of reference

The review should be restricted to evaluating the CKMR assessment for school shark, including data inputs and statistical methods used. It should not include an examination of the stock assessment model previously used for school shark, noting however that the

CKMR assessment uses some of the same data, biological parameters and assumptions as in the stock assessment model.

The key purpose of the review is to evaluate whether there is anything inherent in the methods or assumptions used in the CKMR assessment for school shark, which is likely to lead to consistent under-estimation or over-estimation of school shark abundance and productivity (noting abundance and productivity are aliased), which form the basis of estimates of sustainable catch.

Key questions to address are:

1. Is there an inherent likelihood of consistent under-estimation or over-estimation of school shark abundance and productivity that would be expected to result from the:

    a. sampling design, in particular the assumptions made in the design, the sample size and the distribution of samples given current knowledge of the range of the school shark population and its movement patterns;

    b. close kin data inputs (i.e. genetic sequences), in particular the methods used in genetic sequencing, their associated uncertainties and assumptions;

    c. fishery dependent data inputs (i.e. landed catch and discards), including their associated uncertainties and assumptions;

    d. biological and selectivity parameters, including their associated uncertainties and assumptions;

    e. statistical methods and assumptions used to incorporate the close-kin data inputs (i.e. genetic sequences) into the assessment model, including the methods applied to kin-finding.

    In addressing the above question the reviewer/s should include, but not limit the scope of their response to, an assessment of the analyses and assertions by Cordue (2019) and the corresponding responses by Bravington et al (2019). Any relevant findings of the FRDC review should also be assessed.

2. Based on the response to question 1, do the methods employed in the CKMR assessment provide sufficiently precise, accurate and unbiased estimates of productivity and absolute school shark abundance and trends upon which to base management advice?

3. What revised or alternative methods could be used to improve the precision, accuracy and level of bias associated with the CKMR assessment? In answering this question consideration should be given to how any potential improvements should be scheduled, noting the current assessment schedule for school shark (2021).

The reviewer/s will be provided with relevant data (under appropriate confidentiality arrangements), information, historical analysis results and reports. The reviewer/s will be expected to conduct reasonable additional analysis to answer the key questions

specified above. The scope of this analysis is to be discussed prior to commencement, noting there may be timing constraints for the delivery of the review.

A review report is to be prepared and provided to AFMA as per agreed timeframes. The report will be made public subject to relevant confidentiality requirements and privacy legislation.

Other review requirements

AFMA is looking to convene a panel of experts to undertake the review, comprising of individuals with expertise in one or more of the following areas:

- stock assessment modelling;
- shark population dynamics;
- genetics (kin finding);
- close kin mark recapture.

The reviewer/s must:

- adhere to, requirements for scientific quality assurance as detailed in *AFMA Fisheries Management Paper 16: Fisheries Research and Science Quality Assurance Policy* (October 2018);
- not have contributed or participated in the development of the research under review;
- have the appropriate expertise and experience to review the research and scientific information and analyses concerned – refer to above regarding the formation of a panel;
- be able to provide an impartial and objective review;
- declare all interests relating to any of the research under review.

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Appendix B. Questions to CSIRO

Stock structure

Please elaborate on assumptions regarding connectivity with the population of school sharks in New Zealand, which is thought to be relatively large. In traditional mark recapture, emigration after marking does not lead to bias, provided propensity to emigrate is not associated with mark status. However, immigration of unmarked individuals before the second sampling event reduces the fraction of recaptures and downwardly biases the estimate of abundance, unless accounted for. Immigration can also bias CKMR estimates, but with a twist. Immigrants from New Zealand are also genetically marked (with shared genes, by their parents), but at a different rate that is inversely proportional to adult abundance in New Zealand. If a mixed group of sharks is sampled in Australia and evaluated for HSPs, three factors will influence the estimate: 1) comparisons of sharks both born in Australia will provide a signal related to adult abundance in Australia; 2) comparisons of sharks both born in NZ will provide a signal related to adult abundance in NZ; 3) comparisons of two sharks born in different areas cannot produce a HSP match, except very rarely if some adults manage to reproduce in both areas. Only #1 is relevant to the Australia stock assessment; how were contributions of #2 and #3 accounted for in this work?

Genetic data have not found significant differences in pups produced in Australia and New Zealand. However, it is well known that the amount of migration required to genetically homogenize populations is small compared to the amount required to produce important demographic linkages, and this is especially true in large pops. The migration threshold required to allow demographic independence (roughly speaking, when population dynamics are driven more by local births and deaths than by immigration) has received surprisingly little study, but some work (Hastings 1993 AmNat) suggests it is around 10%. This is not necessarily the threshold that would cause bias in CKMR estimates. So, a relevant question is: How have Thomson et al. determined that the rate of immigration from NZ is NOT high enough to substantially affect their estimates? And secondly, what was the specific assumption about whether the population estimate made in the study included New Zealand, and what information supported this assumption?

Skip breeding

The response to Cordue says the following about skip breeding: "There is no need to explicitly model a pupping interval, mainly because enough cohorts are involved that the 'skip-spawning' effect cancels out." However, this is not supported with any quantitative analysis. Consider a simple model where a population of N adults alternates breeding in 2-year cycles, with half the population breeding every year. Comparisons of individuals born an odd number of years apart cannot possibly be siblings (so point estimate of N is infinity), while comparisons of those born an even number of years apart provide a signal related to N/2. The relative proportions of these kinds of comparisons will depend on annual mortality, among other factors. The review team would like to understand how this issue affects the results, so some more information on this would be helpful. At a minimum, a simple worked example is needed to provide confidence that potential biases are small enough that they can be ignored.

Ageing errors

Ageing errors are clearly more prevalent than originally assumed, and that issue gets some discussion in the response to the Cordue review. However, perhaps the most important

complication created by ageing errors is not treated in any detail. When ageing is not reliable, it is impossible to exclude same-cohort comparisons in the search for sibling pairs. Doing so, however, is essential to provide an unbiased estimate of abundance (or TRO), because the expected proportion of siblings in same-cohort comparisons depends on effective population size rather than census size. A relevant question thus is: How have Thomson et al. accounted for potential bias caused by same-cohort comparisons, since ageing uncertainty does not allow them to exclude such comparisons?

Random sampling

Cordue was not impressed with what he perceived as the lack of any experimental design for sampling. The response indicated that random sampling is rarely feasible in the ocean and certainly not when dealing with fishery-dependent sampling. Fair enough, but then it is important to document how potential biases related to sampling have been accounted for. In the response it simply say that:

> *Random sampling (presumably meaning equiprobable sampling, i.e. an equal probability of being sampled for all living sharks— but, within what age range? over what time period?) is not possible in a commercial fishery; just about all fishing gear is selective, and it is rare to have really good estimates of selectivity. Our conditional model bypasses that problem entirely; it simply requires knowing the facts about each sample (date, ring count, etc).*

The question then becomes: How exactly does accounting for covariates magically make all difficulties associated with non-random sampling disappear?

Sample numbers

Cordue pointed out that there were a significant number of problem samples (e.g. double sampling of the same animal). The response noted that 58 of these were from other species (likely gummy sharks). However, the original report (4.1.1) says that only 13 animals were other species. Can the research team please clarify the numbers of individuals that were other species (13 or 58) and how many were from resampling of the same individual? Given that samples were normally collected with a vertebral sample, it is hard to imagine how resampling would have occurred without the sampler noticing.

The review team would like to get a more detailed breakdown of the sample sizes by year, location (i.e. region) and sex; both for the overall sample, but also for the samples used in the final CK model.

Distance between recaptures

Figure 4.12 of Thompson et al shows the binned distances between capture locations of kin pairs. Cordue has some criticism of this, noting that it appeared that recaptures were more likely to occur at less than 50 km (but this should be 100 km based on the figure). The response shows a more detailed breakdown of the distance between recaptures. However, the text notes "This shows no indication of a tendency for kin pairs to come from samples collected closer….." (p12). Would it be possible to provide a more quantitatively rigorous answer to this concern than visual interpretation of the figures? WE would also like to see the same figures and analysis for just those samples used in the final CK model.

Historic catches

For the CKMR work, the stock assessment is run only from 2000, missing the large earlier catches, possibly taken from a different stock component but very different to catches used in the old

assessment. For the CKMR assessment, though run only from 2000, new catch and discard series have been constructed going back to 1989 and these are very different to those used previously. These differences may be explained in the two references by Cavillo-Jordan et al to AFMA - are these readily available online somewhere or can they be supplied by AFMA? The new catch constructions are notably different for trawl and GN 7 and 8 but especially for trawl from 2000-2015. Given the CKMR assessment estimates absolute abundance this may not matter but it would be good to understand why these differences exist. Further elaboration would be welcome.

Appendix C. Response from CSIRO

Response to AFMA Review Panel

Mark Bravington and Robin Thomson

30 November, 2020

Contents

1 Introduction

This document provides answers to questions posed by the School Shark CKMR Review Panel. The panel posed seven questions, each under a brief heading, which is repeated below. The panel's questions are given in Appendix A.

All Table, Figure, and page references are to our FRDC report, unless otherwise stated. "BSA2016" is the paper *Close-Kin Mark-Recapture* by Bravington, Skaug, and Anderson (*Statistical Science* 31, pp259–274, 2016).

2 Stock structure

This is a deep question with ramifications both for interpretation of CKMR estimates, and for Australian SHS management policy regardless of CKMR. Before getting into details, and without overloading the text with caveats, there are two general points to make:

1. Australia manages and assesses its SE school shark fishery on the assumption that it covers a self-contained stock, i.e. which "self-recruits" and also is largely unaffected by catches elsewhere. We have followed that basic assumption. Although there certainly is connectivity with NZ, one indirect piece of supporting evidence for largely separate dynamics is that the Australian school shark fishery collapsed in the 1990s/2000s but the NZ one did not and has not (NZ still catches around 3000T pa, vs Australian 2000T pa in 1980s but only 200T pa now).[1]

2. At some fundamental level, HSP CKMR has to be informative about the size of the *long-term breeding group* that produced the *juvenile group* from which the samples are taken[2]. Each juvenile had one mother and one father, so the chance that two juveniles have the same mother must be "roughly" the reciprocal of the number of female adults in the long-term breeding group. Now, that "roughly" can for sure be modified substantially through mortality, variations in fecundity, etc— which is why one needs to build a proper ERRO-based CKMR model, which is where bias might creep in if one is not careful— but the fundamental point is that HSP CKMR is looking at the "right" group of adults for management (assuming that the juveniles being sampled-and-managed are going to recruit into the same group that birthed them).
   And there is a further point, which while not part of the ToR of the review panel is probably more important than "absoluteness of abundance" for current Australian school shark management: if the size of the breeding group goes *up* (which is the intention of the restrictive TAC), then the rate of HSP-finding basically must go *down*. In other words, HSP CKMR must (eventually, once enough cohorts are covered and enough samples collected) be informative about trends in adult abundance.

There is also one important point specific to our results: our estimated adult abundance is quite small. Given constraints on early-life-survival etc, it is large enough to cope with Australian juvenile catches, but only leaving a small margin for potential increase. {[}Note that we got similar estimates from our rough-and-ready calculations around p38 which do not take any account of catch.{]} In particular, our estimate is nowhere near large enough to relate to a combined Aus/NZ population— a point we return to below.

An "aggregate HSP CKMR model", which in effect is what we used[3], could behave differently depending on the particular mixed-stock scenario in question: cryptic stocks, immigration, emigration, differential exploitation, and so on. The case that is probably of most interest here, is "NZ spillage", whereby a proportion of NZ-born juveniles happen to make their way to Australia, *independently* of what their later/earlier siblings

[1] That is certainly sensible historically, although the Australian-born-juvenile abundance has nowadays dropped greatly, so a fixed number of NZ-born juveniles could *in principle* make a larger contribution to "Australian" dynamics now. See later discussion.

[2] Wording chosen to steer clear for now of value-laden terms like "stock" and "population"— and the reason for saying "long-term" is that HSP CKMR only works when applied to multiple cohorts.

[3] While we did also include POPs, there are too few to have much effect on the answer, which is almost entirely driven by the HSP data.

might do[4]. Whether those juveniles subsequently return to NZ to pup or stay in Australia, would be an issue for management, but not so much for CKMR per se; the question is what CKMR will conclude about the "long-term breeding group" responsible for the juveniles in the Australian fishery within the cohorts sampled. The point here is that, from the perspective of contributions to the Australian fishery and the CKMR samples, the adult females in NZ would in effect all have lower-but-non-zero fecundity than the Australian adult females. However, the HSP equations that we used assume all adults (of given sex and size) have equal fecundity.

Appendix B here contains simple formulae to show what can happen with HSP-CKMR abundance estimates in a single fishery ("Our fishery"), applied in an aggregated fashion (i.e. not trying to allow for population structure) to what is actually a mixture of juveniles from different populations that remain fairly distinct demographically (for breeding purposes). The upshot is that:

- HSP-Aggregate-CKMR gives the obvious right answer if there are cryptic substocks (i.e. equally available to Our fishery, and available to Our fishery alone). That includes the case where some (presumably female) adults pup in NZ but persistently send juveniles to Australia.
- It also gives the obvious right answer if either population makes the dominant contribution of juveniles, i.e. roughly the abundance of that adult population.
- If one small population is fully available, but the other larger population is only partly available, and the total contributions of juveniles from the two are similar, then HSP-Aggregate-CKMR gives an abundance estimate which is below the total combined abundance of adults, although bigger than the *equivalent* adult abundance if all the juveniles came from a single adult population; that "bigger" factor cannot exceed about 2. Actually, as Appendix B notes, the expected CKMR estimate might be a reasonable compromise from a management perspective (given that management is pretending it's all one stock), although it is certainly not an unarguably ironclad "right answer". In the absence of external estimates of migration etc, any stock assessment, whether CKMR-based or not, would struggle with this situation. The real question is: what would the "right" answer actually be, when the assumptions of management are so mis-specified? (In particular, it would be harsh to claim that "CKMR was biased" here, if the appropriate reference for "unbiased" cannot even be specified!)

Then there is a question of how far any of those cases are relevant to Australian school sharks. Cryptic subpopulations certainly are likely (i.e. "maternal philopatry groups" from different pupping grounds in Australia), but do not matter for CKMR. A small contribution to SE Aus from NZ is possible but should not matter much, in that the estimate would mostly reflect Australian abundance. A larger but *strongly heritable* contribution from NZ— i.e. pupping in NZ, consistently spending the juvenile years in SE Australia, then returning to NZ to pup— would already be accommodated in our analysis, because those animals would effectively be Australian anyway, regardless of where they happen to pup; they would be vulnerable only to the Australian fishery, and so would their offspring, so they would constitute a cryptic stock within Australia. And if NZ spillover made the numerically dominant contribution to Australia, then our estimate would reflect NZ abundance; but our estimate is clearly far too small to do that. The numbers seem to rule out the possibility of a fully-mixed shared stock, or a stock driven by NZ spillover.

What if NZ spillover is not dominant, but still makes a contribution similar to Australian-born juveniles? Then the NZ stock, which is clearly far larger, must have low availability relative to Australian-born juveniles. The analysis in Appendix B here then shows that our estimate might approach 4 times the Australian abundance— so the Australian-born abundance would then be 1/4 of our estimate, but 1/2 the catch would still be coming from Australian juveniles (because contributions are equal). As noted earlier, though, there really isn't much headroom for manoeuvre with population dynamics given our estimates— certainly not enough to accommodate double the fishing mortality. This seems to restrict NZ spillover to being substantially smaller than Australian self-recruitment.

Clearly, we know that some spillover[5] must occur, and our CKMR analysis places some bounds on it (e.g. it

[4]The case where cross-cohort sibling movements are correlated is different, and closer to the notion of a "cryptic substock"; see Appendix B here.

[5]And/or cryptic substock pupping in NZ but regularly moving to Australia— which we argue should count as Australian

is less than the Australian-born component), but we do not have any data to estimate a low-level rate of spillover. Certainly, a 10% contribution from spillover— to arbitrarily pick a small number— might well be consistent with our estimates. While we would not claim that our Aggregate-HSP-CKMR estimate is absolutely perfect in that situation, it might not be an unreasonable approximation for management— and in any case the implications for management as a whole (e.g. in long-term population dynamics) would perhaps be rather larger than merely "what is N".

It would certainly be interesting to investigate this directly in future. The obvious way is to extend CKMR to NZ! Sample sizes would presumably need to be fairly substantial in order to have any chance of detecting enough kin-pairs (between and within Aus/NZ) to draw a useful conclusion. Proper power analysis and design would be needed beforehand to ensure money is not wasted on a substantial-yet-inadequate sampling program.

3 Skip breeding

Skip-breeding is definitely the case for female school shark, though we do not yet know whether it is on a 2-year or 3-year cycle, or indeed a mixture. (If the age data were more precise, we could have worked that out directly from the birth-gaps of MatHSPs, i.e. maternally-linked HSPs; more pairs and better ages will eventually resolve it.) Ignoring it, as we deliberately did, could in short studies (i.e. few cohorts) certainly cause some bias, as the question says. CKMR models can be built to explicitly allow for skip-breeding, but at the cost of significantly complicating the code, so we hoped it could be avoided— given that the effects really *do* cancel out mathematically when enough cohorts are compared. The question is whether there is enough of a spread of juvenile cohorts in our current sample to achieve reasonable cancellation.

One relevant observation concerns sex-specific effects. Skip-breeding (presumably) applies only to female not male school sharks, so if our no-skip-breeding approximation was leading to serious bias, then we would expect a substantial difference in "apparent abundance estimates" between males and females. Some insight can be gleaned from Table~4.9 p50 (base case and sensitivities); the most relevant comparison is probably base-case vs *est-*q_{father} (where, in effect, PatHSPs do inform on trend and mortality, but not on *absolute* abundance)[6]. The *est-*q_{father} version leads to about a 14% drop in estimated abundance ($\hat{N}_{1989}$): this appreciable in absolute terms, though certainly not surprising in the context of an overall CV of about 25% (Table~10). *If* one was to heroically assume that the entire difference between the base-case (which "averages" the paternal estimate and the maternal-ignoring-skip-breed estimate) and the est-q_{father} (which omits the paternal estimate) was due to bias in the maternal-ignoring-skip-breed part of the model— as opposed to statistical noise or any other phenomena— and to do some very crude maths, *then* the implication would be that the maternal-ignoring-skip-breed estimate alone is biased downwards by 28% and the *base-case estimate* itself by 14%.

Clearly, the above argument is pretty crude, and only really serves to rule out massive bias; point estimates always move around when data is removed/added to a limited dataset. Note that there is actually *no* statistically significant evidence of different overall Mat/PatHSP rates in our ignore-skip-breeding model; even on an inappropriate null hypothesis of equal expected proportions, the observed split 38/27[7] is not significant ($p \approx 0.2$) and in fact we expect somewhat more MatHSPs anyway, because females mature later and show an age-related post-maturity fecundity schedule (p23).

As suggested, we have also tried to give a simple worked example here. Unfortunately, interpretation gets complicated because of interactions with ageing-errors and with the lucky-litter effect (as per next question).

The general idea is to work out what the overall expected number of maternal sibs would be, given the age distribution of samples used in the model, and the estimated (or input) age-specific demographic parameters

anyway.

[6]Note *all* model variants assume (based on plenty of data) a 50::50 true sex ratio at birth; the ratio is allowed to change slightly with age due (only) to sex-specific fishing mortalities.

[7]Overall: 38 MatHSPs and 27 PatHSPs. The models exclude ring-counts above 11, so only use 29 MatHSPs and 13 PatHSPs directly.

for female adults: abundance; mortality-at-age; fecundity. Expected numbers are calculated as function of birth-gap, under different skip-breeding scenarios (every year, every 2 years, every 3 years); the odd-gap probabilities are of course zero in the 2-year case. Total expected numbers are a weighted sum based on the likely number of comparisons at each birth-gap. There is a complication in that we do not know the birth-year precisely, because of ageing error. Appendix C here gives details of the calculations.

The results for bias are shown in (Table 1).

Table 1: Percent expected female abundance estimate from no-skip model relative to correct skip model of either 2 or 3 years (100=perfect; 50=too low by half). See text for further explanation.

	All	OnePlus
rel to 2yr	99	115
rel to 3yr	98	133

The reason for showing two columns, is that the bias depends substantially on whether same-cohort comparisons are included or excluded. If there was no concern about lucky-litter effect (see next question) and we simply included all same-cohort comparisons, then the bias would be trivial; cancellation would be almost perfect. However, if we could exclude same-cohort comparisons altogether (in order to eliminate the lucky-litter effect) and then average only across comparisons with gaps of 1 year or more, then the bias would be substantial, certainly if the breeding interval is 3 years. In practice, and as explained in our next response, we cannot exclude same-cohort comparisons, but we do attempt to "model them away"; hence it is not clear to us which column of the Table might really apply. Even if the OnePlus column (with more bias) is more relevant, we still know that males are not subject to skip-breeding issues, so the bias on total adult abundance would be roughly halved. Thus, an overall upward bias in our estimates of *up to* 16% might be expected if the female breeding interval is 3 years, or *up to* 7% if 2 years.

Relative to an overall CV of around 25%, such biases are not really problematic, but they are big enough to be worth trying to fix in future. When we return to school shark CKMR modelling (with substantially more samples; plus we expect to have better age estimates and reliable length estimates), we will need to develop a full skip-breeding formulation— partly for skip-breeding in its own right, and also to allow for exotic possibilities such as sperm-storage. It's certainly possible to devise ERRO equations for skip-breeding, but (from experience of working with state-space models of breeding cycles) it can take a lot of modelling effort to fix all the details in a way that is computationally robust as well as biologically plausible, so this is not something that can be done quickly.

4 Ageing errors / within-cohort

We explicitly do allow for ageing error and for the possibility of same-cohort comparisons— the "lucky litter effect" (in our parlance) which tends to inflate the number of within-cohort sibs. This is for precisely the reason given in the question: that we can't take the easy route of simply excluding same-cohort comparisons a priori, because we don't know for sure whether each comparison is same-cohort or not, because of ageing error. So the only way to handle this is to introduce extra parameters (ν_1 and ν_2, p96); we make no effort to exclude possible within-cohort comparisons, and instead estimate the extra parameters alongside the "normal" CKMR parameters of more direct interest. The extra parameters deal with the observably higher rate of sibship among samples that are more likely to be same-cohort based on ring-counts. The probability of kinship for a comparison (given imprecise ages) is computed by (i) calculating the probability for each possible combination of true ages of the two animals— which (depending on ring-counts and sample-years) may include the possibility of same-cohort, and thus involve parameters related to lucky-litter effects— and (ii) forming a weighted sum of those probabilities, based on the statistical distribution of measured age given true age (the ageing-error matrix) and the numbers-at-age. Page~47 and Appendix C of the report give details.

This is the same approach taken for white sharks in Hillary et al 2018, and for the same reasons.

One important and helpful factor, is that most within-cohort sibs turn out to be FSPs (Full-Sib) not HSPs. If there was no multiple paternity within litters, then we could completely identify within-cohort sibs just by whether the pair is FSP or HSP (the chance of FSPs thru random repeat matings being negligible). Unfortunately, this doesn't quite work for school shark because there is *some* multiple paternity within-litter, though our data suggest that most littermates do share a father as well as mother (and there was little quantitative evidence from other sources). So we are still stuck with having to introduce one extra parameter to deal with overall lucky-litter effect (ν_1), plus another for the proportion of FSPs to within-cohort MatHSPs from multiple-paternity (ν_2). Overall, quite a lot of modelling effort ends up going into something we don't care much about, in order to keep the books straight. {[}BTW we assume that male adult school shark don't have lucky-litter effects in their own right; one male might mate with several females in one year, and each of those females might have a lucky litter, but the survival rates across those several litters are assumed independent. Species with "supermales" would required different treatment, and of course "supermaleness" can span more than one year anyway.{]}

So, although ageing error has turned out to be somewhat quantitatively bigger than we expected, we did build in allowance for it in the first place. Thus there is no qualitative implication of any problem from within-cohort comparisons, because our model is explicitly designed to handle them and allow for lucky-litter effects, rather than trying to exclude them altogether.

5 Random sampling

"Randomly" is one of those words that sound simple-and-innocent but aren't, and that can cause a lot of trouble unless properly chaperoned!

Basically (more detail later), all that is necessary is that sampling should be "random GIVEN covariates of each sample". As an analogy, consider linear regression of Y on X. It is necessary that, for any given X_i in the sample, then the corresponding Y_i should be drawn "randomly" from the population of Y's *at that particular X_i*. But it is *not* necessary to sample the X_i's themselves randomly, nor to somehow the overall set of Y's randomly from the entire population of Y's over all X's. It doesn't matter (i.e. doesn't lead to bias) how you get your X_i's— by design, by "semi-design", or randomly. {[}In fact, random sampling of the X's is generally undesirable in regression. For a given total sample size, it's more efficient (lower CVs) to push the sampling out to the edges of a wide range of X's— as long as you are confident that a linear model really is appropriate.{]}

How does this connect to CKMR? Overall, CKMR is just like any model-based statistical procedure in that there is

1. some response data (was this pair a half-sib pair or not?);
2. some covariates associated with each response (e.g. dates, ages, sexes of the *pair* of animals); and
3. a model (algebraic formula) which specifies the probability of the observation *given* particular covariates and some parameters (the ERRO probabilities).

Then any unknown parameters can be estimated by maximum likelihood or Bayesian principles, with associated guarantees of large-sample unbiasedness etc. That is the "magic" of statistics at work... It doesn't really matter how the sample *covariates* were selected (designed, semi-designed, opportunistically, "randomly" whatever that means) provided that there is nothing sneaky about the act of sampling which affects the *responses* but has been left out of the chosen model formulae.

There is one subtle difference with CKMR, which is that response data (and covariates) are associated with *pairs* of animals, and "randomness" needs to be considered in a pairwise sense. The key requirement is this: the mere fact that Sam the shark happens to be sampled, should not affect the probability that Sam will turn out to be HSP to any of the other samples he will be compared to, *given* Sam's covariates and theirs (i.e. the probability as calculated by whatever formula for ERRO is being used). Sampling still doesn't have to be

equiprobable among all animals having some given date/age/sex etc, but the details of the ERRO formula, and the choice of which comparisons to make and which to not bother with, do have to consistent with that requirement.

As a practical example where one does have to be careful about "randomness": it's *possible* that a species like school shark might continue to associate with littermates for some years after birth, so that animals caught in the same fishing-set might be substantially more likely to be H/FSPs than animals caught in different sets (i.e. drawn independently). This is easily fixed by just not using the results of within-set comparisons, and that is what we have done[8]; if those comparison are not part of the likelihood then they can't affect the results! "Sam", and his set-mates, can still be compared to all the other sharks in different sets— there's no problem with the "randomness" of those between-set pairs[9], it's only the within-set pairs that violate "randomness" in the above sense. So that's the subtlety with CKMR: that "randomness" is a property of the *pair* of animals involved in each comparison used, not of each animal in its own right.

As a hypothetical example where a lazy piece of modelling could violate "randomness" and lead to some bias: suppose we were dealing with a species where: (i) we only use age in the ERRO formulae (to determine date-of-birth) but not length; and (ii) that only one or two age-classes of juveniles are sampled; and (iii) that sampling is heavily size-selective even within a given age, so that smaller animals are preferred; and (iv) that individual size is strongly parentally determined, so that if You are small for your age, then Your half-brothers and -sisters in different cohorts are very likely to be small too. Then the age-only ERRO formula would be inappropriate: Your being caught means that You are more likely to be small for your age, so Your sibs are too, so they are are more likely to be caught too. (To fix the problem, the ERRO would need to be computed allowing for length as well as age.) That example is strictly hypothetical: all four conditions would need to apply to cause any problems, and the second and third (at least) definitely don't apply to school shark.

6 Sample numbers

6.1 Duplicate samples

There were 13 samples that clearly come from another species. There were an additional 12 pairs (24 samples) resulting from the same school shark being sampled more than once at the processor (which the Processor confirmed was quite possible).

The mention in the Final Report of '85 accidental duplicates' that we suggested might have been laboratory mix-ups, is an error that we had planned to remove from the report after Cordue pointed it out (but we forgot to do so). This sentence derives from an early interim report to sharkRAG before we properly understood the apparent duplication. We have since realised that some samples that appeared to be accidental duplicates were, in fact, tissues taken from animals that were not school sharks. The 13 non-school sharks plus 12 pairs of resamples makes a total of 37 duplicated samples (not 85), to which we had initially (mistakenly) added some deliberate 'technical replicates' (more detail below) whose sample IDs in the dataset returned by DArT did not match because an 'R' had been added to the ID by DArT. DArT did not do add an 'R' to the sample IDs of all technical replicates, which is why we did not immediately realise that they had done so to some replicates. Our ID matching software therefore mistakenly identified some of the technical replicates as inexplicable duplicates. Further investigation cleared up the picture and we give greater detail on all forms of duplication, deliberate or otherwise, below. Lessons from this process have contributed greatly to the development of our internal QC pipelines for genotype data.

There were four kinds of samples that were, or appeared to be, duplicate samples in terms of having (near) identical DNA sequences (note sequencing errors occur at a rate of roughly 3% so that near duplicates are treated as the same animal). These were:

[8]Where samples were associated with a specific shot, we excluded within-shot comparisons; where the specific shot was uncertain, we excluded within-trip comparisons.

[9]Technically, the presence of within-set littermates in a sample would mean that comparisons between them and some other animal would no longer be independent, even if still "random" by the definition we use. However, non-independence only affects the variance of final estimates, rather than leading to bias; see BSA2016 section 4.

1. Known duplicates - we chose two animals whose DNA we deliberately placed in each of two wells on every DNA plate sent to DArT. We did this so that we could detect instances where a plate is accidentally placed in the DNA sequencer upside down (this did not occur during this study, but happened once in an earlier study on another species, resulting in mislabelling of the resulting DNA sequences). By matching the known DNA sequence to the returned DNA sequence from those wells, we could verify that the sequences were correctly ascribed to their sample IDs. There were a total of 234 deliberately duplicated samples (i.e. technical replicates and deliberate duplicates combined).

2. Technical replicates - deliberate repeat sequencing (by DArT) of the same animal for the purpose of identifying the sequencing error rate; these samples are easily identified because they have the same sampleID in the data file returned by DArT (notwithstanding the occasional addition an 'R').

3. Accidental re-samples - these were near identical DNA sequences from animals that had unique sample IDs but that, on investigation, were found to have been sampled within days of one another, and by the same fish processor. Selected meta-data for those samples are given in Table 2. We rang Chris Pitliangas to ask whether accidental sampling was likely to have occurred i.e. could a cross-section be cut from the trunk of one shark, more than once, without the sampler realising the mistake? Chris was very sure that it could easily happen. It is notable that most of these samples differ from one another in measured length by just a few cm (the processor measured the length of the cut trunk, rather than a standardized length).

Table 2: Selected meta-data for apparent accidental resamples made in the processing factories. Every pair of rows represents a duplicate pair of samples.

Collection contact	Collection date	Vessel	Sex	Age	Length
Chris Pitliangas, Melbourne	Jul-16	Peter Crombie	F	18	100
Chris Pitliangas, Melbourne	Jul-16	Peter Crombie	F	19	119
Chris Pitliangas, Melbourne	22/11/2016	Charisma	F	14	99
Chris Pitliangas, Melbourne	22/11/2016	Charisma	F	15	82
Philios Toumazos	Oct-15	Kiella	F	12	97
Philios Toumazos	Oct-15	Kiella	F	11	98
Philios Toumazos	Oct-15	Kiella	M	2	47
Philios Toumazos	Oct-15	Kiella	M	3	45
Chris Pitliangas, Melbourne	Jul-16	Peter Crombie	F	14	108
Chris Pitliangas, Melbourne	Jul-16	Peter Crombie	F	18	97
Philios Toumazos	Oct-15	Kiella	F	8	70
Philios Toumazos	Oct-15	Kiella	F	6	65
Philios Toumazos	Oct-15	Kiella	M	3	45
Philios Toumazos	Oct-15	Kiella	M	2	47
Chris Pitliangas, Melbourne	10/02/2017	peter crombie	F	2	53
Chris Pitliangas, Melbourne	8/02/2017	peter crombie	F	3	48
Chris Pitliangas, Melbourne	3/02/2017	peter crombie	F	2	51
Chris Pitliangas, Melbourne	8/02/2017	peter crombie	F	3	50
Chris Pitliangas, Melbourne	10/02/2017	peter crombie	F	3	51
Chris Pitliangas, Melbourne	8/02/2017	peter crombie	F	4	61
Chris Pitliangas, Melbourne	8/02/2017	peter crombie	F	2	50
Chris Pitliangas, Melbourne	4/02/2017	peter crombie	F	2	48
Chris Pitliangas, Melbourne	10/02/2017	peter crombie	M	2	51
Chris Pitliangas, Melbourne	8/02/2017	peter crombie	M	3	48

4. 'Germline' duplicates - these were 13 individual samples, with unique sample IDs, that were seemingly genetically so similar that they were all allocated, by our 'pipeline', as coming from the same animal. Although several were collected together, three appear to be accidental resampling from one individual (see first three rows of meta-data in Table 3. Initially (before human examination of the genotypes) these were thought to be laboratory mix ups because they came from several times, locations and samplers. Those collected before 2015 (Collection contact given as Fish Ageing Services in the Table) were collected by AFMA Observers. Although they were not thought to have been processed in the laboratory at the same time, no other explanation was initially found. However, as soon as we investigated the genotypes themselves, it became apparent that these individuals were not school sharks. Their sequences are mostly nulls, with some non-null loci that show almost no variation across the group. Genotypes at an arbitrarily chosen 20 loci (that are nevertheless typical of the whole) are shown for six individuals thought to be school shark (Table 4), and by contrast, those for six of these 13 apparent 'duplicates' (Table 5). We speculate that these might be gummy shark because the two species are caught together and are similar in physical appearance. Most of these samples were taken by the AFMA Observer program, who are tasked with taking vertebral samples from gummy as well as school sharks.

Table 3: Selected meta-data for suspected gummy shark sampled as school shark.

Collection contact	Collection date	Vessel	Sex	Age	Length
Philios Toumazos	Oct-15	Kiella	F	12	97
Philios Toumazos	Oct-15	Kiella	F	15	108
Philios Toumazos	Oct-15	Kiella	F	14	116
Simon Robertson, Fish Ageing Services	18/12/2010	Investigator	F	8	76
Simon Robertson, Fish Ageing Services	3/03/2011	Cape Everard	F	20	105
Simon Robertson, Fish Ageing Services	7/03/2011	Christine Claire	F	4	60
Simon Robertson, Fish Ageing Services	7/03/2011	Christine Claire	F	12	87
Simon Robertson, Fish Ageing Services	3/04/2011	Andrew K	F	10	75
Simon Robertson, Fish Ageing Services	3/04/2011	Andrew K	F	4	49
Simon Robertson, Fish Ageing Services	3/04/2011	Andrew K	F	8	69
Simon Robertson, Fish Ageing Services	3/04/2011	Andrew K	F	NA	64
Simon Robertson, Fish Ageing Services	3/04/2011	Andrew K	F	NA	63
Simon Robertson, Fish Ageing Services	3/04/2011	Andrew K	F	NA	52

Table 4: Genotype detected at each of 20 loci (labelled l1 to l20) for six arbitrarily chosen samples. Each row represents an individual sample.

l1	l2	l3	l4	l5	l6	l7	l8	l9	l10	l11	l12	l13	l14	l15	l16	l17	l18	l19	l20
AA	AA	AA	AA	AA	AA	AB	AB	AA	AA	AA	OO	AA	AA	AA	AA	AA	AA	AA	AA
AA	AA	AA	AA	AA	AA	AB	AB	AA	AA	AA	OO	AA	AA	AA	AA	AA	AA	AA	AA
AA	AA	AA	AA	AA	AA	AB	BB	AA	AA	AA	OO	AA	AA	AB	AA	AO	AA	AA	AA
AO	AA	AA	AO	AA	AA	AB	BB	AA	AA	AA	OO	AA	AA	AB	AA	AO	AA	AA	AA
AA	AA	AO	AA	AA	AA	BO	AB	AA	AA	AB	AO	AA	AA	AA	AA	AB	AA	AA	AB
AO	AA	AO	AA	AA	AA	BB	AB	AA	AA	AB	AO	AA	AA	AA	AA	AB	AA	AA	AB

Table 5: Genotype detected at each of 20 loci (labelled l1 to l20) for six individuals suspected to be gummy shark.

l1	l2	l3	l4	l5	l6	l7	l8	l9	l10	l11	l12	l13	l14	l15	l16	l17	l18	l19	l20
OO	OO	OO	OO	OO	OO	OO	OO	OO	OO	OO	OO	OO	OO	OO	AA	OO	OO	OO	OO
OO	OO	OO	OO	OO	OO	OO	OO	OO	AA	OO	OO	OO	OO	OO	AA	OO	OO	OO	OO
OO	OO	OO	OO	OO	OO	OO	OO	OO	AA	OO	OO	OO	OO	OO	AA	OO	OO	OO	OO
OO	OO	OO	OO	OO	OO	OO	OO	OO	AA	OO	OO	OO	OO	OO	AA	OO	OO	OO	OO
OO	OO	OO	OO	OO	OO	OO	OO	OO	AA	OO	OO	OO	OO	OO	AA	OO	OO	OO	OO
OO	OO	OO	OO	OO	OO	OO	OO	OO	OO	OO	OO	OO	OO	OO	AA	OO	OO	OO	OO

Table 6: Number of sharks samples from each sharkRAG zone, in each year, that passed the genetic quality control procedure. Numbers are shown separately for females and then males.

	CSA	EBS	ET	WBS	WSA	WT
2010	0 / 0	39 / 47	0 / 0	0 / 0	0 / 0	0 / 0
2011	0 / 0	60 / 57	0 / 0	0 / 0	0 / 0	0 / 0
2013	0 / 0	35 / 68	0 / 0	0 / 2	0 / 0	0 / 0
2014	0 / 0	0 / 2	0 / 0	0 / 0	0 / 0	0 / 0
2015	308 / 99	127 / 148	4 / 6	8 / 0	59 / 10	5 / 66
2016	203 / 42	31 / 28	4 / 21	125 / 118	15 / 10	0 / 0
2017	210 / 42	44 / 95	2 / 3	0 / 0	0 / 0	0 / 0

Table 7: Number of sharks samples from each sharkRAG zone, in each year, that were included in the school shark close kin model. Numbers are shown separately for females and then males.

	CSA	EBS	ET	WBS	WSA	WT
2010	0 / 0	29 / 44	0 / 0	0 / 0	0 / 0	0 / 0
2011	0 / 0	55 / 57	0 / 0	0 / 0	0 / 0	0 / 0
2013	0 / 0	32 / 64	0 / 0	0 / 0	0 / 0	0 / 0
2014	0 / 0	0 / 2	0 / 0	0 / 0	0 / 0	0 / 0
2015	94 / 82	126 / 147	2 / 5	8 / 0	38 / 8	1 / 10
2016	30 / 8	30 / 28	3 / 17	118 / 111	12 / 10	0 / 0
2017	77 / 33	41 / 94	2 / 3	0 / 0	0 / 0	0 / 0

6.2 More detailed breakdown of sample sizes

The review team asked for a more detailed breakdown of the sample sizes by year, location (i.e. region) and sex; both for the overall sample, but also for the samples used in the final CK model.

Of the 3130 'samples' (including technical replicates and other duplicates) taken for this project, 2424 were found to be non-duplicates that also passed genetic quality control procedures. We have concentrated our efforts in assigning meta-data (such as fishing trip location, and reading ages) to those samples. The number of samples by year, sharkRAG zone (Figure 1), and sex for the 2424 samples that passed all genetic quality control steps is shown in Table 6. Numbers from the reduced sample, that used only animals that had 11 or fewer age 'rings', are shown in Table 7.

7 Distance between recaptures

To improve our investigation of whether kin pairs are more likely to occur amongst samples caught closer together, we generated qq-plots (Figures 2 and 3). To do this, we calculated the distance between every possible pairing of animals sampled, and then calculated quantiles from the square root of those distances (taking the square root so that effects at smaller distances are emphasised in comparison to larger distances). We did this for all samples, either including or excluding those caught within the same fishing trip, and for only samples found to have fewer than 11 vertebral age rings (i.e. those used in the model).

When within-trip comparisons (and kin pairs) are excluded, there are no pairs at distances of less than 10km. Removing the samples that had more than 11 rings makes little difference to the result. There is some indication, at zero distance (i.e. same shot comparisons) of greater tendency towards finding kin pairs together. Removing within-trip comparisons removes that effect.

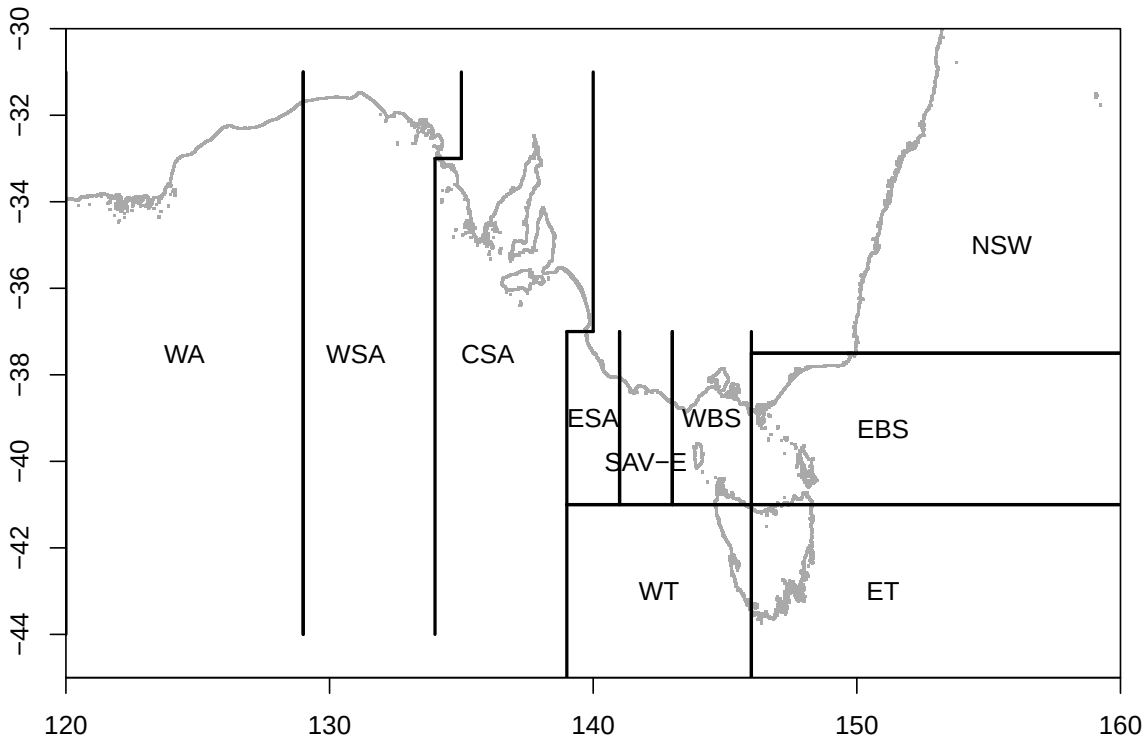


Figure 1: SharkRAG zones to which samples are allocated.

This is still a largely picture-based investigation, rather than a calculation; we would be happy to attempt other investigations suggested by the panel.

8 Historic catches

There is little difference between the total catches from the original stock assessment model and the revised catches used in the close kin model. There has been some re-allocation of catch from 7 and 8 inch gillnets to 6 and 6.5 inch gillnets. Gillnet selectivity is dome-shaped and is not estimated by either model but fixed at theoretical values derived from an experiment. Larger gillnets catch larger sharks, but the differences between gear selectivity for the different mesh sizes is slight. We do not expect this re-allocation to greatly affect our results.

The revised versus original catch time series are shown in Figure 4.4 of the FRDC final report and are reproduced here (Figure **??**). The time series shown are 'new': catch by gear generated for the close kin assessment, 'old' the catch time series used by the sharkRAG assessment conducted in 2012, and one other that is not of interest here (new no west/NSW). The new and old time series, in total, are not greatly different (bottom righthand-most plot). The greatest differences are in the catches by the 7 and 8 inch gillnets.

As stated in the final report, the original allocation to gear was in error, due to a complex data processing arrangement conducted in Fortran by Andre Punt, which was mis-interpreted by Robin Thomson and has since been re-coded. The calculations of Castillo-Jordan et al are independent of these calculations.

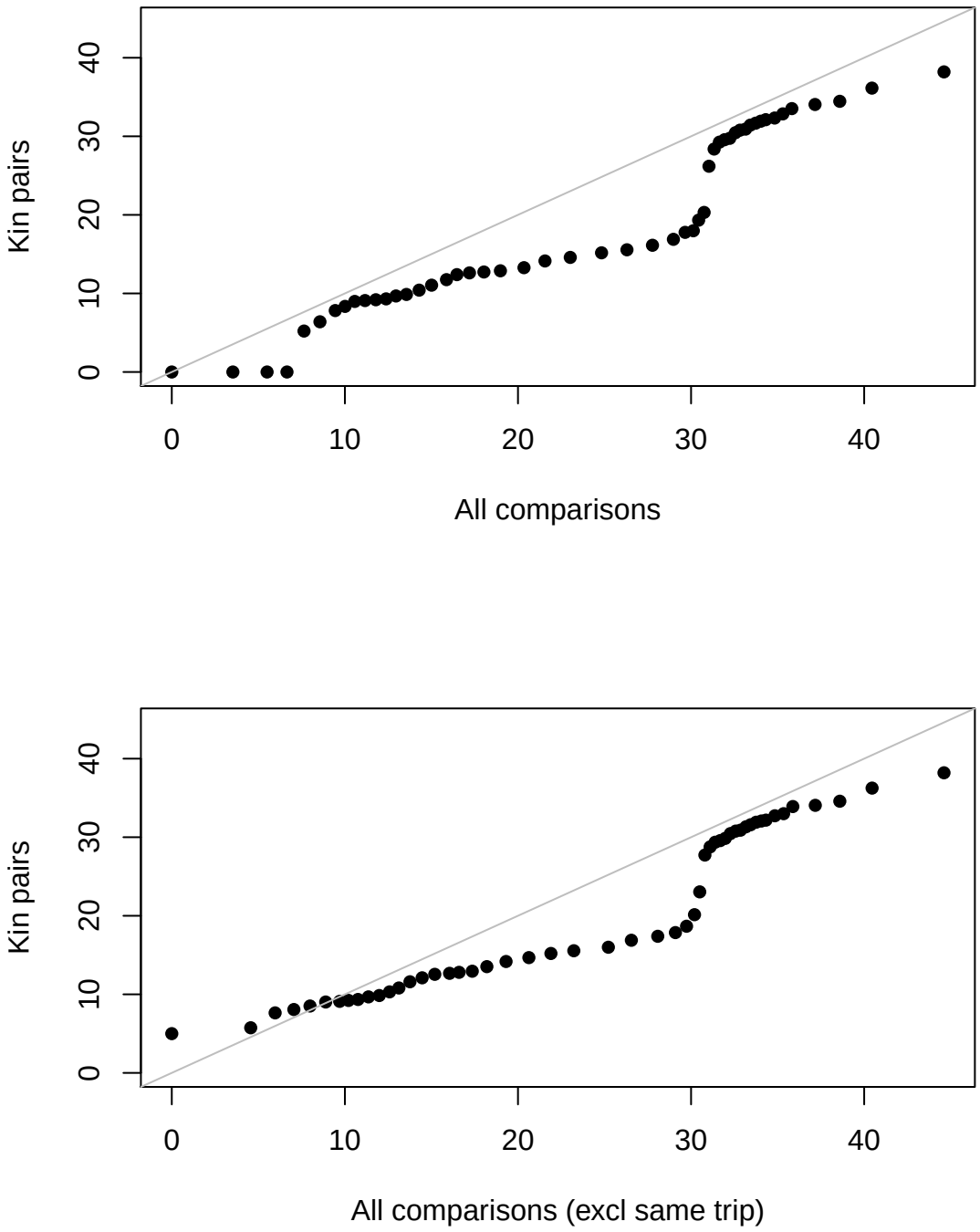


Figure 2: Quantiles for distances between all kin pairs, against those for all pairwise comparisons between samples. The upper plot excludes within-trip comparisons and kin pairs, and the lower plot allows them.

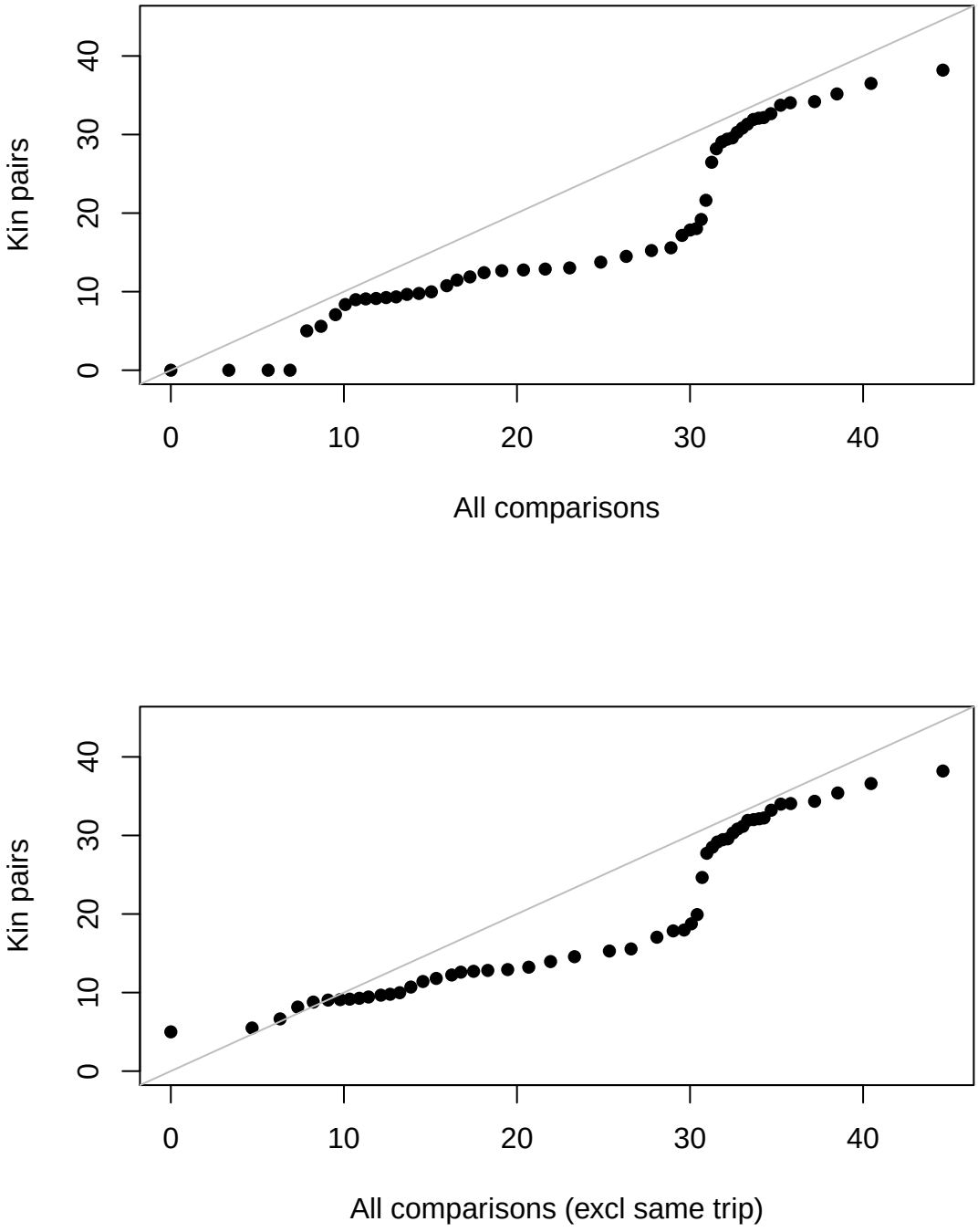
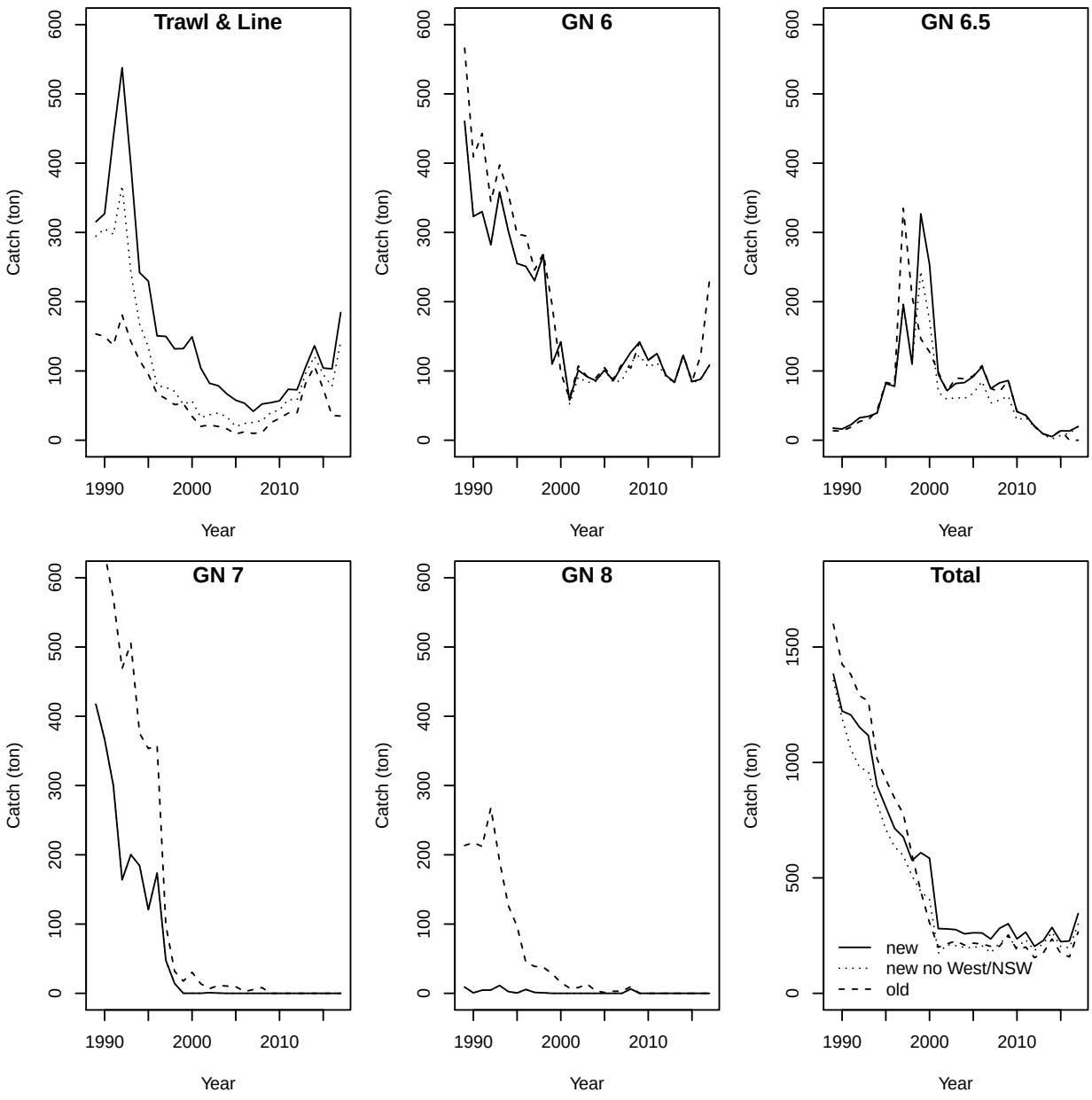


Figure 3: Quantiles for distances between all kin pairs, against those for all pairwise comparisons between samples. Animals with more than 11 reings have been excluded. The upper plot excludes within-trip comparisons and kin pairs, and the lower plot allows them.


Trawl & Line
GN 6
GN 6.5
GN 7
GN 8
Total
Catch (ton)
Year
1990
2000
2010
0
100
200
300
400
500
600
1000
1500
new
new no West/NSW
old

9 Appendix A: Questions from the School Shark CKMR review panel for CSIRO research team

Stock structure

Please elaborate on assumptions regarding connectivity with the population of school sharks in New Zealand, which is thought to be relatively large. In traditional mark recapture, emigration after marking does not lead to bias, provided propensity to emigrate is not associated with mark status. However, immigration of unmarked individuals before the second sampling event reduces the fraction of recaptures and downwardly biases the estimate of abundance, unless accounted for. Immigration can also bias CKMR estimates, but with a twist. Immigrants from New Zealand are also genetically marked (with shared genes, by their parents), but at a different rate that is inversely proportional to adult abundance in New Zealand. If a mixed group of sharks is sampled in Australia and evaluated for HSPs, three factors will influence the estimate: 1) comparisons of sharks both born in Australia will provide a signal related to adult abundance in Australia; 2) comparisons of sharks both born in NZ will provide a signal related to adult abundance in NZ; 3) comparisons of two sharks born in different areas cannot produce a HSP match, except very rarely if some adults manage to reproduce in both areas. Only #1 is relevant to the Australia stock assessment; how were contributions of #2 and #3 accounted for in this work?

Genetic data have not found significant differences in pups produced in Australia and New Zealand. However, it is well known that the amount of migration required to genetically homogenize populations is small compared to the amount required to produce important demographic linkages, and this is especially true in large pops. The migration threshold required to allow demographic independence (roughly speaking, when population dynamics are driven more by local births and deaths than by immigration) has received surprisingly little study, but some work (Hastings 1993 AmNat) suggests it is around 10%. This is not necessarily the threshold that would cause bias in CKMR estimates. So, a relevant question is: How have Thomson et al. determined that the rate of immigration from NZ is NOT high enough to substantially affect their estimates? And secondly, what was the specific assumption about whether the population estimate made in the study included New Zealand, and what information supported this assumption?

Skip breeding

The response to Cordue says the following about skip breeding: "There is no need to explicitly model a pupping interval, mainly because enough cohorts are involved that the 'skip-spawning' effect cancels out." However, this is not supported with any quantitative analysis. Consider a simple model where a population of N adults alternates breeding in 2-year cycles, with half the population breeding every year. Comparisons of individuals born an odd number of years apart cannot possibly be siblings (so point estimate of N is infinity), while comparisons of those born an even number of years apart provide a signal related to N/2. The relative proportions of these kinds of comparisons will depend on annual mortality, among other factors. The review team would like to understand how this issue affects the results, so some more information on this would be helpful. At a minimum, a simple worked example is needed to provide confidence that potential biases are small enough that they can be ignored.

Ageing errors

Ageing errors are clearly more prevalent than originally assumed, and that issue gets some discussion in the response to the Cordue review. However, perhaps the most important complication created by ageing errors is not treated in any detail. When ageing is not reliable, it is impossible to exclude same-cohort comparisons in the search for sibling pairs. Doing so, however, is essential to provide an unbiased estimate of abundance (or TRO), because the expected proportion of siblings in same-cohort comparisons depends on effective population size rather than census size. A relevant question thus is: How have Thomson et al. accounted for potential bias caused by same-cohort comparisons, since ageing uncertainty does not allow them to exclude such comparisons?

Random sampling

Cordue was not impressed with what he perceived as the lack of any experimental design for sampling. The

response indicated that random sampling is rarely feasible in the ocean and certainly not when dealing with fishery-dependent sampling. Fair enough, but then it is important to document how potential biases related to sampling have been accounted for. In the response it simply say that: Random sampling (presumably meaning equiprobable sampling, i.e. an equal probability of being sampled for all living sharks— but, within what age range? over what time period?) is not possible in a commercial fishery; just about all fishing gear is selective, and it is rare to have really good estimates of selectivity. Our conditional model bypasses that problem entirely; it simply requires knowing the facts about each sample (date, ring count, etc). The question then becomes: How exactly does accounting for covariates magically make all difficulties associated with non-random sampling disappear?

Sample numbers

Cordue pointed out that there were a significant number of problem samples (e.g. double sampling of the same animal). The response noted that 58 of these were from other species (likely gummy sharks). However, the original report (4.1.1) says that only 13 animals were other species. Can the research team please clarify the numbers of individuals that were other species (13 or 58) and how many were from resampling of the same individual? Given that samples were normally collected with a vertebral sample, it is hard to imagine how resampling would have occurred without the sampler noticing.

The review team would like to get a more detailed breakdown of the sample sizes by year, location (i.e. region) and sex; both for the overall sample, but also for the samples used in the final CK model.

**Distance between recaptures} Figure 4.12 of Thompson et al shows the binned distances between capture locations of kin pairs. Cordue has some criticism of this, noting that it appeared that recaptures were more likely to occur at less than 50 km (but this should be 100 km based on the figure). The response shows a more detailed breakdown of the distance between recaptures. However, the text notes "This shows no indication of a tendency for kin pairs to come from samples collected closer. . . ." (p12). Would it be possible to provide a more quantitatively rigorous answer to this concern than visual interpretation of the figures? WE would also like to see the same figures and analysis for just those samples used in the final CK model.

Historic catches For the CKMR work, the stock assessment is run only from 2000, missing the large earlier catches, possibly taken from a different stock component but very different to catches used in the old assessment. For the CKMR assessment, though run only from 2000, new catch and discard series have been constructed going back to 1989 and these are very different to those used previously. These differences may be explained in the two references by Cavillo-Jordan et al to AFMA - are these readily available online somewhere or can they be supplied by AFMA? The new catch constructions are notably different for trawl and GN 7 and 8 but especially for trawl from 2000-2015. Given the CKMR assessment estimates absolute abundance this may not matter but it would be good to understand why these differences exist. Further elaboration would be welcome.

10 Appendix B: Behaviour of aggregated HSP CKMR with mixed stocks

Here we ignore all demographic complexities to do with mortality, time intervals, fecundity, growth, lucky-litters, etc, and simply focus on one adult sex (females, say) where all adults have similar fecundity. The general idea is that there may be two contributing *populations* of adult female abundance N_1 and N_2 (each reproductively closed) that both contribute juveniles to a mixed-stock fishery where an aggregated CKMR analysis (i.e. that, like ours, does not try to explicitly account for population origin) is applied; what will the aggregated CKMR abundance estimate represent? {[}If the separate populations were not reproductively isolated, then they wouldn't be separate populations, and none of this would matter anyway; CKMR would simply estimate the total adult abundance. In the specific case of our school shark model, that is clearly not the situation, because our adult estimate is far too low to generate enough juveniles for the NZ fishery.{]}

The examples below are meant to be illustrative of possible CKMR behaviours for a range of species and fisheries, with no necessary implication that they are relevant to Australian school shark. However, as to the basic notion of reproductive isolation, it is worth noting that maternal philopatry (females returning to pup at the place they themselves were pupped) appears to be quite common in sharks, and it would not be surprising if it applies to *female* school shark with their discrete coastal pupping grounds. Mating in school shark happens quite separately to pupping, though, and the species is certainly very mobile from a young age, so philopatry is still consistent with one population for "practical management purposes", at least within Australia (setting aside for one sentence the question of links to NZ). Note also that the male and female (PatHSP and MatHSP) CKMR data suggest fairly similar adult abundances among the two sexes in the "breeding group that contributes to Australian catches"— whatever that group consists of— so presumably male and female behaviours in respect of movement/migration between Aus and NZ cannot differ all that much.

The basic setup is as follows. The two adult populations are size N_1 and N_2. The proportions of *juveniles* in the fishery from the two populations are p_1 and $p_2 = 1 - p_1$, which are not necessarily related to adult abundance because population #2 may be less available/vulnerable per capita than population #1. We consider only maternal sib-pairs (MSP) regardless of the father (i.e. including FSPs as well as MHSPs).

If two juveniles from population #1 are compared, then the probability they have the same mother is $1/N_1$; if two from #2 are compared, then $\mathbb{P}\left[\text{MSP}\right] = 1/N_2$; but if one is from #1 and the other from #2, then the probability is zero. The probabilities of those three events are, respectively, p_1^2, p_2^2, and $2p_1p_2$. Thus the overall probability of maternal sibship is

$$\mathbb{P}\left[\text{MSP}\right] = \frac{p_1^2}{N_1} + \frac{p_2^2}{N_2} \tag{1}$$

10.1 Cryptic substocks don't matter

Suppose that the fishery entirely covers the juveniles from population #1 and population #2, and that the juveniles are well-mixed at the ages of sampling%

— these are "cryptic substocks" that behave the same way demographically, can't be distinguished, but breed only within themselves. Then p_1 and p_2 are proportional to their respective adult abundances, so that $p_1 = N_1/\left(N_1 + N_2\right)$ and $p_2 = N_2/\left(N_1 + N_2\right)$. In eqn (1), that gives

$$\begin{aligned}
&\mathbb{P}\left[\text{MSP}\right] \\
&= \left(\frac{N_1}{N_1+N_2}\right)^2 \times \frac{1}{N_1} + \left(\frac{N_2}{N_1+N_2}\right)^2 \times \frac{1}{N_2} \\
&= \frac{N_1}{\left(N_1+N_2\right)^2} + \frac{N_2}{\left(N_1+N_2\right)^2} \\
&= \frac{1}{N_1+N_2}
\end{aligned}$$

Hence the reciprocal probability, which is the "natural" estimate of abundance in this simplified model, is $N_1 + N_2$,which is surely also the "right" definition of overall abundance. The conclusion, in this simple case, is that cryptic substocks don't matter.

It is of course possible to contrive extensions to this in which the cryptic stocks have different biological productivities or natural mortality rates etc, in which Aggregate-HSP-CKMR can sometimes lead to undesirable estimates; such situations are as much for management as for assessment.

10.2 Differing availabilities

To get "interesting" behaviour, it is necessary to assume that one population (#1, for definiteness) is fully available to Our fishery all the time, but that #2 is only partly available. That could happen if either (i) every #2-juvenile tends only to spend part of its time within Our fishery area, or (ii) some #2-juveniles randomly decide to spend all their time in Our fishery zone whereas the majority spend none of their time there *and* this decision is independent for each juvenile (see below). If θ represents the relative availability of #2-juveniles compared to #1-juveniles (where $\theta \leqslant 1$ by assumption), then

$$\begin{aligned}
\mathbb{P}\left[\text{MSP}\right] &= \frac{p_1^2}{N_1} + \frac{p_2^2}{N_2} \\
&= \frac{1}{N_1}\frac{N_1^2}{\left(N_1+\theta N_2\right)^2} + \frac{1}{N_2}\frac{\theta^2 N_2^2}{\left(N_1+\theta N_2\right)^2} \\
&= \frac{N_1+\theta^2 N_2}{\left(N_1+\theta N_2\right)^2}
\end{aligned}$$

If $N_1 \gg \theta N_2$, then this is approximately $1/N_1$; if $\theta N_2 \gg N_1$, then this is approximately $1/N_2$; and in both cases the actual value is slightly larger than the more important single-stock. This is exactly what we would hope; if the overall contribution from either stock dominates, then naive CKMR will estimate the abundance of that stock, plus a bit from the other stock.

A different scenario is if (iii) some #2-juveniles are fully available to Our fishery and others are completely unavailable— like case (ii)— but this time the behaviour is strongly heritable. In that case, we are actually back to the "cryptic substocks" situation. Even though those juveniles grow into adults that physically pup in #2, for the purposes of exploitation they are entirely vulnerable to Our fishery and to Our fishery alone, and constitute a closed reproductive (maternal) substock that is managed via Our fishery; where they actually do their pupping is not really important. Of course all sorts of ramifications could be devised to make that more complicated, to do with different biological parameters or exploitation while adult, etc.

10.3 The hypothetical worst/most-interesting case

So far, CKMR is going to behave just as anyone would hope when the populations are equally available to Our fishery, and/or when the contribution of one population is dominant. To get anything more interesting (and

less desirable), we need to consider the case where the overall contributions are similar but #2 population is less available; for the overall contribution to be similar, that means that #2 must be more abundant. To investigate this, we can specialize the above formula to $N_2 = N_1/\theta^*$ so that $p_1 = p_2 = 1/2$ (writing θ^* to emphasize that this is a very particular value of θ which happens to yield equal contributions). The number of juveniles in the fishable area at any time, is the same as would be produced by a single fully-mixed population of size $2N_1$.

Eqn (1) becomes

$$\begin{aligned}
\mathbb{P}\left[\text{MHSP}\right] &= \frac{1}{N_1}\frac{1}{4} + \frac{\theta^*}{N_1}\frac{1}{4} \\
&= \frac{1}{4N_1}\left(1+\theta^*\right) \\
\implies \frac{1}{\mathbb{P}\left[\text{MHSP}\right]} &= \frac{4N_1}{1+\theta^*}
\end{aligned}$$

When $\theta^* = 1$ we are back to equally-mixed cryptic stocks (now with $N_1 = N_2$) and get $1/\mathbb{P} = 2N_1$, which is right. As $\theta^* \to 0$, ie $N_2 = N_1/\theta^* \to \infty$ with a huge population #2 which sends only a tiny *proportion* of its juveniles to Our fishery but which still happens to contribute as many overall as does population #1, then we head towards a limit of $1/\mathbb{P} = 4N_1$ (Figure 4). Now, that is much larger than N_1 (population #1 on its own) but much smaller than $N_1 + N_2$. However, the real question then becomes: what is the "right" answer in that tiny-θ^* case? It certainly does not seem right that population #2 should count as fully as #1 does; #2-juveniles are hard to catch and generate much less yield per capita. And, depending on how density-dependence etc work, it could be quite possible to collapse population #1 while still having a viable fishery based on spillover from #2 — which might not be a great outcome for management. And if population #2 is also exploited in other fisheries, then of course those removals too should be taken into account (i.e. it would then be wrong just to assess Our fishery in terms of its own catches).

(One variant of this tiny-θ^* scenario, incidentally, would be "the fisherman's dream", which resurfaces in various settings from time to time: that there is Another Population which is huge, impossible to over-exploit, and happy to contribute in perpetuity to Our fishery. . .)

The full ramifications for management of this *hypothetical worst-case* scenario go well beyond abundance estimation, and depend on many other things. However, it is tempting to make a general comment. Even in this worst-case situation, where the "outside" population #2 contributes as much as the "local" #1 but is much less available per capita, it might be argued that Aggregate-HSP-CKMR still does a reasonable job for management: it still gives an abundance estimate that is bigger, but at *most* twice as big ($4N_1$ vs $2N_1$), as an equivalent fully-available single population that would supply the same number of juveniles— the latter presumably being the assumed basis for management. Since population #2 is certainly much more "resilient" to Our fishery (unless it is also getting exploited elsewhere), the "twice as big" is qualitatively justifiable in terms of allowing a bit more exploitation than the equivalent single population. And on the other hand, from a safety perspective, "twice as big" is the maximum inflation that can ever happen. (This turns out to be important evidence for school shark; see the main response to Q1.) There is a limit to how much the local population's abundance can be overestimated, unless the overall contribution from #2 genuinely is dominant, which *may* offer some protection against inadvertently wiping out the local population in the mistaken belief that there is a single homogenous population making all the babies. That said, we are certainly not claiming that the expected Aggregate-HSP-CKMR estimate represents the ideal definition of "the abundance" in this context— since two populations are fundamentally involved, there may not *be* any best single measure of abundance.

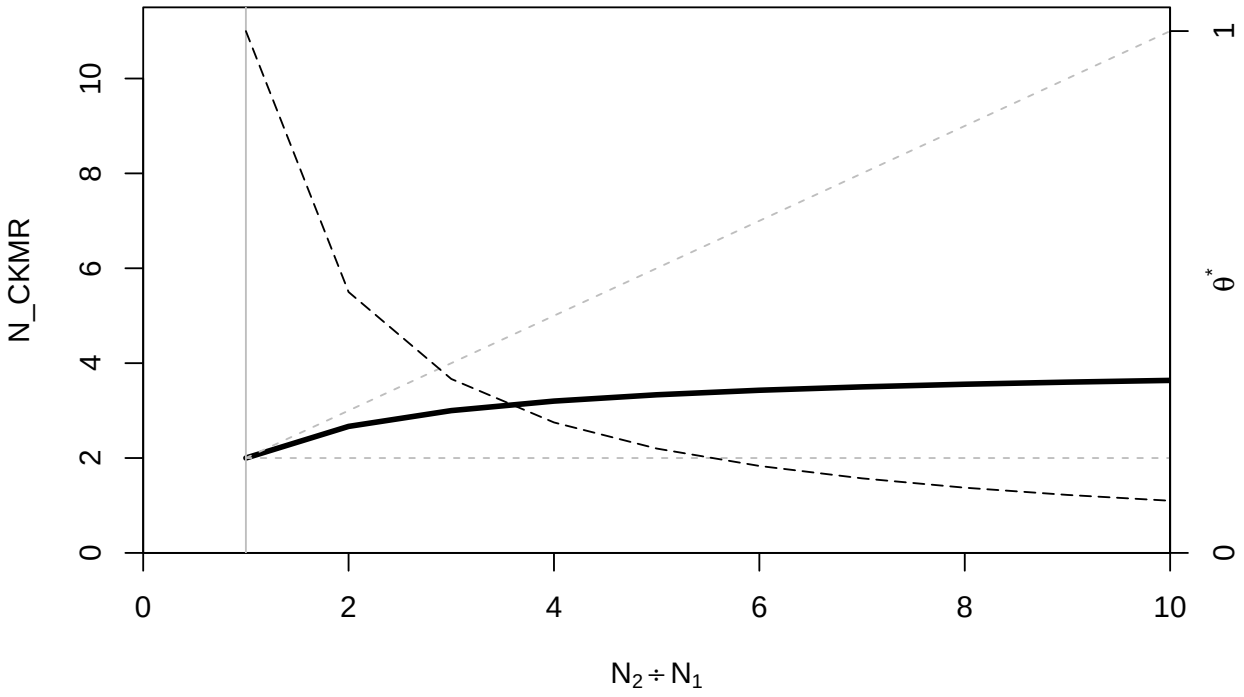


Figure 4: Aggregate HSP CKMR with two mixed stocks that contribute equally overall; stock 2 is larger but less available. Thick black line shows expected estimate from CKMR as a multiple of N1; dotted grey lines show the range of conceivably defensible definitions of total abundance. Dashed black line is the availability of stock 2 relative to stock 1. A vertical grey line indicates N1=N2.

11 Appendix C: Details of skip-breeding calculations

This is an approximate calculation based only on expected *totals* of sib-pairs under different skip-breeding scenarios, and therefore neglecting any knock-on effects on estimates of mortality and other demographic parameters.

1. Work out the likely true distribution of samples-at-age (by year and sex), given:
   (a) ring counts (only for counts up to 11, since we did not use higher-ring-count animals in the CKMR model);
   (b) assumed distribution of ring-counts-given-true-age;
   (c) estimated numbers-at-true-age in the population (from our model).

   That is a simple Bayes-theorem calculation.
2. Work out the likely number of pairwise comparisons by true birth-gap (product of number of samples).
3. Compute the true MatSP probability per comparison by true birth-gap under a steady-state assumption, given:
   (a) the estimated female adult age distribution in "year zero" (arbitrarily set to 2010, a reasonably typical year-of-birth for cohorts that we sampled). We assumed here (i.e. only for this document) that adult female numbers-at-age remained steady across the juvenile cohorts in the sample.
   (b) the estimated female mortality-at-age vector for that year, again assumed constant over time. This is used to estimate cumulative survival probability over gaps of 0, 1, 2, ... years, given starting age.
   (c) the female fecundity-at-age vector;

   We use the age-based steady-state version of the HSP formula in BSA2016 section 3.2 but for now *without* any lucky-litter (same-cohort) effect— see text. In the no-skip version, that formula is

$$\begin{aligned}&\mathbb{P}\left[\#1\text{'s mother is also }\#2\text{'s mother } g \text{ years later}\right]\\&=\sum_a \frac{n_{♀a}\mathrm{fec}_a}{\mathrm{TRO}_♀}\times\frac{\mathrm{psurv}\left(g,a\right)\mathrm{fec}_{a+g}}{\mathrm{TRO}_♀}\end{aligned}$$

   where $n_{♀a}$ is the number of age-a females and $\mathrm{TRO}_♀=\sum_a n_{♀a'}\mathrm{fec}_{a'}$. The X-year skip version is

$$\begin{aligned}&\mathbb{P}\left[\#1\text{'s mother also turns out to be }\#2\text{'s mother } g \text{ years later}\right]\\&=\sum_a \frac{n_{♀0a}\mathrm{fec}_a}{\mathrm{TRO}_{♀0}}\times\frac{\mathrm{psurv}\left(g,a\right)\mathrm{fec}_{a+g}\mathbb{I}\left[g \mod X=0\right]}{\left(\mathrm{TRO}_{♀g}/X\right)}\end{aligned}$$

   The reason for the lack of any "X" in the left-hand term, is that on average across the entire population only 1 in X females is breeding in any year, and this cancels between the numerator and denominator.
4. The overall expected number of MatSPs (for either the skip or no-skip cases) is then the sum, across gaps, of the number of comparisons at that gap times the probability-of-MSP-by-gap:

$$\mathbb{E}\left[\mathrm{MSP}\right]=\sum_{g\geqslant 0}\mathrm{ncomps}\left(\text{birth gap } g\right)\times\mathbb{P}\left[\text{MatSP}|\text{birth gap } g\right]$$

5. How to interpret this in terms of bias? Suppose that, for a given abundance, the expected overall MSPs works out higher under the no-skip scenario compared to a (presumed correct) X-year-cycle skip-breeding scenario. Then the implication is that the abundance estimate from a no-skip model would be correspondingly higher than it should be if a skip-breeding model was used— because a higher abundance would be needed in the calculation to bring the expected MSPs down to match the observed number.

The results from steps 1–3 for skip-intervals of 1yr (i.e. no skip; i.e. what we have used as an approximation), 2yr, and 3yr are shown in Table 8, and the approximate biases are shown in the Table in the main text.

Table 8: Expected number of comparisons by true birth-gap, and estimated MatSP probabilities under different breeding intervals. No additional lucky-litter effect. Comparisons divided by 1000, probabilities multiplied by 1,000,000.

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15+
nNComps	115	221	203	180	152	121	93	69	50	35	24	17	13	9	7	15
Pr noskip	42	38	35	32	29	27	25	23	21	19	17	16	14	13	12	80
Pr 2yr	84	0	69	0	59	0	49	0	41	0	34	0	28	0	23	77
Pr 3yr	125	0	0	96	0	0	74	0	0	56	0	0	42	0	0	88

Appendix D. School Shark CKMR Assessment

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DRAFT

Close kin mark recapture for school shark in the SESSF

Robin Thomson, Mark Bravington, Pierre Feutry, Rasanthi Gunasekera, Peter Grewe

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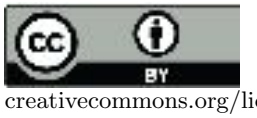

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In submitting this report, the researcher has agreed to FRDC publishing this material in its edited form.

Contents

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James Marthick kindly sequenced the mitochondrial genome for this as well as other close kin studies.

Abbreviations

AFMA	Australian Fisheries Management Authority
CKMR	Close kin mark recapture
CPUE	Catch per unit effort
CV	Coefficient of variation
DNA	Deoxyribonucleic acid
ERRO	Expected Relative Reproductive Output
FOP	Father offspring pair
FSP	Full sibling pair
HSP	Half sibling pair
MHSP	Maternal half sibling pair
MOP	Mother offspring pair
mtDNA	Mitochondrial DNA
PHSP	Paternal half sibling pair
POP	Parent offspring pair
SNP	Single nuclear polymorphism
SESSF	Southern and Eastern Scalefish and Shark Fishery

Executive Summary

Management of school shark during the 1990s and 2000s has been based on an age structured stock assessment model (Punt *et al.*, 2000; Punt, 2001; Thomson & Punt, 2009; Thomson, 2012). This model relied on commercial gillnet CPUE time series as an index of abundance. However, increasingly stringent management measures introduced to protect school shark caused CPUE to breakdown as an index of abundance, perhaps as early as the mid-1990s. Close kin mark recapture (CKMR) provides an estimate of absolute abundance that is independent of fishing behaviour. We present a first CKMR estimate of abundance for school shark and discuss the management implications of our findings.

We found 65 half-sibling pairs (HSPs), 3 parent-offspring pairs (POPs) and 34 full sibling pairs (FSPs) which were sufficient for close kin modelling. Our model estimates a school shark stock in the region of 80,000 mature individuals during the 2000. Although the CV for our abundance estimate ranges from 0.23 to 0.28 over 2000 to 2011 (and is at its most precise in 2002-2003, at 23%) the standard error on the trend in mature abundance is large relative to the trend itself so that although the median trend is slightly upwards, downward trends cannot be ruled out.

Future projections assuming varying levels of future close kin sampling for up to four years showed that standard errors on trend and abundance should greatly reduce. SharkRAG have recognised that CKMR provides a viable alternative to conventional stock assessment for school shark and have recommended that CKMR continue to be used as a monitoring tool for school shark and we scoped such continuing work.

We developed two, very simple, models that provided similar abundance estimates to those of our more sophisticated close kin model, giving us confidence that the model correctly interpreted the close kin data. Our estimate of abundance is three to four times lower than that of the stock assessment model, when that was projected forwards assuming similar levels of catch to those that have occurred (Thomson, 2012). Our model was not able to sustain the catches that occurred during the 1990s, even under optimal survival conditions for juvenile school shark. This suggests that the school shark population consists of more than one reproductively isolated stocks, and that the population that we measured is a remnant of what was present in the 1990s.

It is possible that environmental degradation of school shark nursery areas (DEWR, 2008) is the explanation for our finding. As there has been little recovery of those areas, school shark might have the capability to recover to their previous stock size. In this case, management reference points that rely on the assumption that stocks will recover to their pristine abundance in the absence of fishing, are not useful for school shark. Conventional stock assessment models give more precise estimates of relative, than of absolute, abundance but CKMR gives reliable estimates of absolute abundance. This gives managers the opportunity to manage school shark according to a more relevant quantity than abundance relative to a no longer attainable pristine state last seen in the 1920s.

Our work has advanced close kin methodology through the refinement of software developed for quality control of genetic sequencing data, and for kin finding. Our work represents a first application of CKMR to a commercially exploited shark population.

Keywords: close kin mark recapture, school shark, abundance, population dynamics, genetics, fisheries management

Chapter 1

Introduction

During the 1990s and 2000s, management of school shark in the Southern and Eastern Scalefish and Shark Fishery (SESSF) was based on an age structured stock assessment model (Punt & Walker, 1998; Punt *et al.*, 2000; Punt, 2001) that relied on catch per unit effort (CPUE) as an index of abundance. The stock assessment model was most recently updated in 2009 (Thomson & Punt, 2009) and was used in 2012 (Thomson, 2012) to make forward projections under differing future catch scenarios, on which the current recovery strategy is based. That model used landed catches to the end of 2008 (the 2012 projection also used recorded catches to 2011) and assumed that discarding was negligible. This model showed that school shark abundance (expressed in terms of pup production) was well below 20% of pristine abundance, resulting in the closure of the stock to targeted fishing. A rebuilding strategy was formulated in 2008 (DEWR, 2008) and was updated in 2015 (AFMA, 2015). The shark Resource Assessment Group (sharkRAG), which makes recommendations regarding management of school shark, recognised that CPUE no longer provides an index of abundance for the stock. This results from the increasingly stringent management measures introduced from 1997 onwards to protect the stock, and ultimately the closure of the fishery to targeted fishing. The resulting changes in fishing practices have caused the CPUE to break down as an index of abundance. The stock assessment model uses gillnet fishery CPUE data only to 1996 (Thomson & Punt, 2009; Thomson, 2012), after which the model is, essentially, extrapolating abundance using known catches. Catches of school shark have dropped to very low levels from 2000 onwards and the model predicts slow recovery of the stock. Both anecdotal reports from members of the fishing industry as well as trawl CPUE (which has never targeted school shark) support this expectation. It is important to monitor, and confirm, the recovery of the school shark stock but CPUE no longer offers a means for doing so. A workshop in 2012 (Huveneers *et al.*, 2013) identified candidate indicators of abundance for school shark. A subsequent investigation of the feasibility of each these (Thomson & Sporcic, 2013) resulted in the recommendation by an external reviewer that the close kin mark recapture method (CKMR) be applied to school shark (Dunn, 2014). We present the first application of CKMR to school shark. A stark difference between the school shark stock assessment model (Punt *et al.*, 2000; Punt, 2001) and the close kin model presented in this report, is that the stock assessment used CPUE from the fishery as an index of relative abundance, whereas the close kin model uses information on close kin pairs to give absolute abundance that is not susceptible to changes in management and fishing practices. Now that an initial close kin study

has been completed, and a data set of genotyped individuals has been established, further collection of tissue samples can be compared with that dataset to provide an ongoing index of abundance for school shark. The close kin mark recapture (CKMR) method for estimating abundance and other demographic parameters (Bravington *et al.*, 2016b) was first applied to Southern Bluefin Tuna (Bravington *et al.*, 2016a) with great success. It has since been applied to white shark in eastern Australia (Hillary *et al.*, 2018) and eastern Australian grey nurse shark (Bradford *et al.*, 2018). CKMR studies are nearing completion for western white sharks, and two populations of the endangered speartooth shark (*Glyphis glyphis*) as well as the northern rivershark *Glyphis garricki* in northern Australia.

Bravington *et al.* (2016a) describe how to properly set up close kin mark recapture models for general situations, with genetically determined 'marks' and 'recaptures' (of closely-related animals) arising from commercial landings or surveys. Depending on the biology of the species and which types of kin can be found (i,e, parent offspring pairs, POPs, and / or half sibling pairs, HSPs), it may or may not be important to have time-series of age/length compositional data. Species where adults (of given sex) do not vary much in expected reproductive output (such as whales and many sharks, including school shark) have lower data requirements. A close kin mark recapture model does not require an index of relative abundance, nor does it need to account for e.g. seasonal movement details, unless the latter affect the breeding or sampling probabilities underlying the close kin model. Catch (removals) data are useful (though not absolutely essential, unlike in conventional stock assessments), and do allow the separation of natural mortality from fishing mortality.

Whereas the close kin applications to SBT and grey nurse shark used both parent-offspring pairs (POPs) and half sibling pairs (HSPs) the bulk of the school shark catch is composed of juvenile animals, so that few POPs were expected (and indeed only three were found). There is minimal information from such low numbers, consequently we have concentrated entirely on the much more numerous HSPs, as was done for the white shark close kin projects. Close kin based on HSPs alone requires that the fecundity-at-age rates for (at least for one sex) is known, which it is for school shark.

We collected approximately 3,000 samples, all of which were aged by the Fish Ageing Service (FAS) using counts of contrasting bands of material in the vertebra. The full mitochondrial genome was sequenced for those sharks found to belong to close kin pairs, thus indicating whether the shared parent was the mother or the father. Genetic markers that indicate the sex of the sampled fish were used to verify reported sex and to indicate the sex of samples for which no sex was reported. We describe the collection and genetic analysis of school shark tissue samples; the identification of close kin pairs; the compilation of fishery dependant data and biological parameters; and finally the models used to estimate absolute population abundance using the close kin data. First, we present two simple applications of the basic principle that the number of kin pairs is inversely related to adult abundance, thus straightforwardly deriving an estimate of (absolute) abundance for school shark using close kin pair data alone. Second, we present a more sophisticated model (hereafter 'the close kin model') that accounts for four complicating biological characteristics of the species (described in Methods, Chapter 3).

In addition to the close kin data, the close kin model uses data relating to commercial catch, discards, as well as gear selectivity, and known biological parameters such as relative pup production by age (separately for males and females), to estimate parameters that describe

the stock. The model can also make use of CPUE time series and length frequencies, but their interpretation can be problematic; and certainly is for school shark. Several assumptions are adopted from the stock assessment model used in the past by AFMA and sharkRAG to manage school shark (Punt & Walker, 1998; Punt *et al.*, 2000; Punt, 2001; Thomson & Punt, 2009; Thomson, 2012); hereafter the 'stock assessment model'. The most recent update of that model used data to 2008 (Thomson & Punt, 2009). Landings, discards, and length frequency information collected between 2009 and 2017 were compiled for incorporation into the close kin model, and catch data up to 2008 were recalculated because of concerns regarding the accuracy of the original data.

The close kin model was projected into the future under a range of assumed future exploitation rates, as well as future close kin sample collection rates, to calculate median catch time series, and to assess the expected confidence intervals for estimated biomass and trend in abundance if close kin is used an ongoing monitoring tool for school shark.

The original intention of the present study was to incorporate the close kin data into the existing stock assessment model for school shark, thus providing both an absolute abundance estimate and a depletion relative to 'pristine' abundance in 1927 (the first year included in the assessment, considered to pre-date the commencement of notable levels of fishing). However, that assessment model rested on several strong, untestable assumptions. It uses a monthly time step, allows movement of sharks between each of eight spatial regions and allows movement rates to vary by sex and age as well as between two reproductively isolated populations of school shark. There is no direct evidence of multiple stocks of school shark, but earlier versions of the model were unable to explain relatively high catches that were taken from the stock without allowing multiple stocks. The incorporation of two stocks also improved fits to both CPUE and conventional tag-recapture data. The assessment model is unable to estimate abundance without the help of a Bayesian prior that was based on the opinion of those present at a shark resource meeting held during the development of the model (Punt *et al.*, 2000). The movement matrices are also based on strong priors that were constructed outside of the assessment model, using the conventional tag-recapture data and a hypothesis regarding school shark movement (Walker *et al.*, 2009).

Members of sharkRAG, invited experts and observers at a meeting held in August 2018 were unanimously uncomfortable with both the complexity of the stock assessment model and the extent to which it is driven by opinion (in the form of priors), preferring the simplicity and the data-driven aspect of the close kin model (AFMA, 2018b). SharkRAG decided not to update the stock assessment model, but instead to use the close kin model for future management (AFMA, 2018b). This choice has meant that an estimate of depletion relative to pristine abundance (in 1927) is not available and therefore that the SESSF Harvest Strategy Policy Reference Points, which are defined relative to pristine abundance, cannot be used. SharkRAG recognised that (a) the school shark stock is at a low level of depletion, below the Limit Reference Point; (b) the recovery time to the limit reference point is 66 years, giving managers ample time to devise an appropriate strategy for managing this stock; and (c) environmental conditions in the SESSF are changing rapidly, which, along with environmental degradation of some pupping grounds (DEWR, 2008), negates the concept of a return to a 'virgin biomass'. A new approach to management of this stock, one that does not reply on virgin conditions, is required.

Chapter 2

Objectives

1. Calculate an absolute estimate of spawning stock abundance with sufficient precision to inform a new stock assessment and to update the Rebuilding Strategy.
2. Update the school shark stock assessment, giving specific recommendations for future management and rebuilding. (SharkRAG chose to modify this objective, using the close kin model itself instead of updating the stock assessment model.)
3. Establish (and cost) the methods for an ongoing time series of cost effective, fishery independent, school shark abundance estimates.
4. Improve understanding of stock structure and broad scale movements of school sharks.
5. Advance close kin methodology.

Chapter 3

Methods

3.1 Samples

Our study was originally designed using a method that is relatively unsophisticated by current close kin standards (Thomson & Sporcic, 2013). That method showed that 3,000 school shark samples ought to give an absolute abundance estimate with acceptably low CV. This target was amended to 2,000, with consequent increase in expected CV, due to a funding shortfall. Fortuitously, the cost of genetic sequencing fell, allowing us to reach the full target of 3,000 samples.

School shark have been seen to move from every one of sharkRAG's shark zones to every other zone (Figure 3.1). Such intermingling does not prove interbreeding – reproductively isolated stocks could nevertheless exist – but it does show that school shark are highly mobile and therefore that sampling location is not crucially important. Nevertheless, the target sample size was broken down in proportion to fishing activity, between three broad locations (700 samples from South Australia, 900 from Bass Strait, and 400 from Tasmania) to guard against any unknown sub-structuring of the population. The target for Tasmania was inflated relative to actual landings to ensure a useable sample size from that state.

Samples consisted of a section of vertebral column taken from just behind the head (used for ageing) and a chunk of tissue (used for DNA extraction). Samples were collected by fishers (Leigh Castle, Andy Joy, Kyriakos Toumazos), fish processors (The Fish Factory: Philious Toumazos; Pitliangas Foods: Nick and Chris Pitliangas) and AFMA's Observer Program (approximately 1,000 samples), and were sent to CSIRO, mainly by refrigerated truck. Our earlier design modelling showed that more half siblings were likely to be found if we restricted the period of time over which we sampled, for that reason we did not use Observer Program samples collected before 2010 (Thomson & Sporcic, 2013). Collections made by the fishing industry were all taken between 2015 and 2018 (Figure 3.2). Information was supplied on the collection location, date, and the sex and length of the animal. Where possible, we used no more than 50 animals from any fishing trip to guard against any sampling bias that might arise from close relatives schooling together. We eliminated any such bias from our calculations by not comparing (i.e. seeking kin relationship between) animals that were caught together. In addition to the commercially caught sharks, we sourced tissue samples

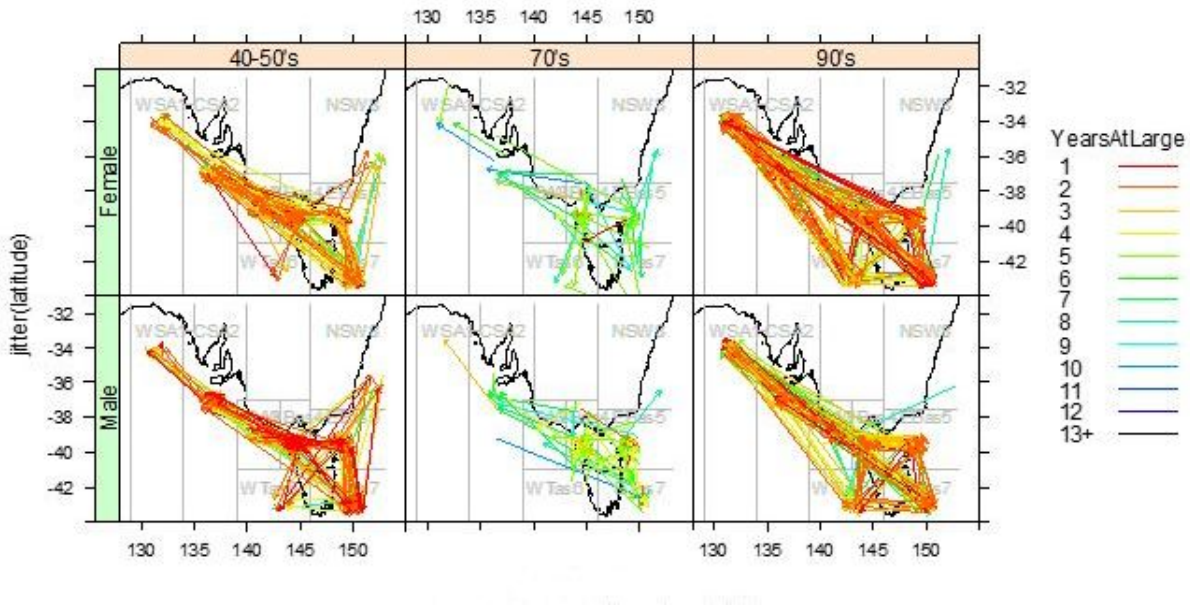

Figure 3.1: Movements of school shark from conventional tag and recapture data, organised by decade of release. Arrow colour indicates number of years at large. Arrows begin at release point and end at recapture point (arrow head).

from neonate school shark collected in Australia and New Zealand as part of two PhD projects. Sebastia'n Herna'ndez (Victoria University of Wellington, New Zealand) collected fin clips from Pittwater, Tasmania, in 2009 and from several bays in New Zealand in 2009 and 2010 (Hernández, 2013). Jaime McAllister (University of Tasmania) collected muscle samples from school shark pups in Pittwater and Norfolk Bay, Tasmania, during 2012 (McAllister, 2014). The neonate samples were used for a population genetics study of Australian and New Zealand school shark (see Chapter 4.4 and Appendix B) but were not used for the close kin study.

Initially, two plates of DNA (each representing 94 animals) were sent to Diversity Arrays Technology (DArT Pty Ltd, Canberra) for genetic sequencing using high throughput genotyping (DArTseq). One plate consisted of neonate samples and the other of commercially caught school shark. The sequences from the Australian neonates and the second plate consisting of commercially caught school sharks were used to identify 15 sex markers and 2,000 SNPs (Single Nucleotide Polymorphisms) that gave the most power for identifying half siblings. DArT then developed nucleic acid 'capture probes' that allow the targeting of regions of DNA where the SNPs of interest are located. This process reduces the cost of subsequent sequencing and increases the number of detections for the target SNPs (DArTcap). All commercial samples that had adequate tissue quality and quantity (with the exception of some that came from catches where more than 50 animals were sampled) were sent to DArT for DArTcap sequencing.

Although DArT provide a quality control and genotyping 'pipeline' their work has not been optimised for our purpose. CSIRO has developed software for close kin projects that can be used to conduct quality control, genotyping, and kin finding on genetic sequence data. This software was originally developed for SBT and was further tested and refined as a result of

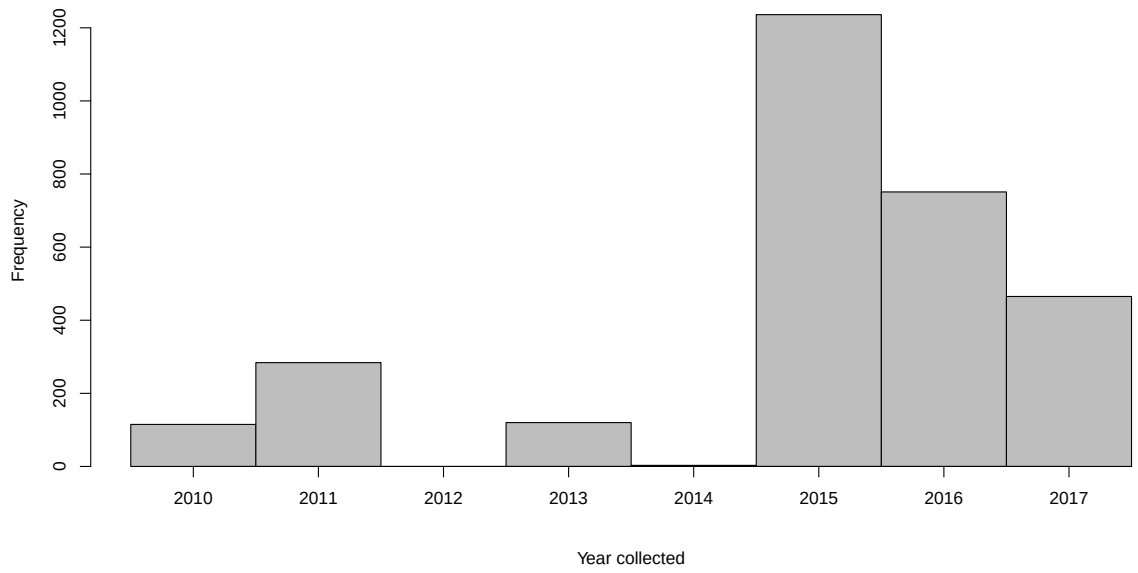

Figure 3.2: Numbers of school shark sampled by year.

its application to school shark. Our results are described in Section 4.5.

3.2 Close kin

The fundamentals of HSP-based CKMR are very simple. Here we describe the basic idea, and then list additional factors that need to be accounted for.

Suppose all female adults are 'reproductively similar' (i.e. expected to produce approximately the same number of surviving offspring per year). Now sample two fish, which for simplicity we will name Peter and Simon, born within a few years of one other (Peter is the elder). What is the probability that Peter and Simon have the same mother, i.e. are a maternal HSP (MHSP)? Simon's mother could have been any of the adult females alive at the time of Simon's birth (we will call that number N^f). The chance that she is the same as Peter's mother is therefore 'about' $1/N^f$. Thus, by making pairwise comparisons amongst a large sample of juveniles and seeing what proportion of them yield an MHSP, we can basically estimate N^f. Of course there will be some random variability in the number of MHSPs actually found, and hence uncertainty in the estimate; but if the number of MHSPs actually found is fairly big, then the relative random variability in the proportion cannot be large.

This argument would be exact, and there would be no need for 'about' and 'basically', were it not for the following four factors:

1. Peter's mother could have died before Simon was born. This reduces the probability of them being an MHSP, so mortality rates have to be allowed for.

2. Within-cohort comparisons tend to have a systematically higher proportion of MHSPs (and full sibling pairs, FSPs), because of random events that affect the survival rate of an entire litter. Same-cohort comparisons need to be excluded, or specifically allowed for, in any model using close kin data. The only reliable signal of abundance from HSPs comes from cross-cohort, not within-cohort, comparisons.

3. Adults of given sex may differ systematically in reproductive output. This is inevitable in species where body-size strongly affects fecundity, e.g. teleost fish. Variability between (female) adults will increase the proportion of (M)HSPs, as will somatic growth within any adult's lifespan. That is, if Peter's mother survives and grows bigger, then by the time that Simon is born, she will be more fecund, increasing the relative probability of also being Simon's mother. This is particularly true for teleosts whose relative fecundity changes greatly over their lifetime.

4. If there is a trend in adult abundance, then the probability depends on the total number of females alive at Simon's birth, not the "average" number of living adults. This is easy to build into a population model.

Because of point 3 above, the relative fecundity at age (or size) must be known or estimated for the population to which CKMR is applied. Parent-offspring pairs, if enough are found, can be used to estimate fecundity relationships because they provide information on the age (or size) of parents. For that reason, HSP-only CKMR cannot work unless we can assume that all adults are equally fecund e.g. white sharks and grey nurse sharks (Hillary *et al.*, 2018; Bradford *et al.*, 2018) or fecundity relationships are well known e.g. school shark (Walker *et al.*, 2005). Note that fecundity relationships that are calculated by counting numbers of eggs carried by females might not be directly measuring reproductive success; CKMR for SBT showed that older females are disproportionally successful compared to younger females, presumably for behavioural reasons (Bravington *et al.*, 2016b). For school shark, the range of fecundity is relatively constrained: small females have an average of 20 pups and the largest females only 30 pups. For this reason the exact fecundity at size (or age) relationship is less important than it is for a teleost fish.

We provide two straightforward applications for estimating abundance from close kin data, without accounting for the four 'complications' above. The first is a very simple, essentially one-line calculation (hereafter termed the 'one line calculation') and the second is a more sophisticated Generalised Linear Model (GLM) based calculation that allows for a trend over time in abundance, and for survival between years, see Section 4.6. Next we account for all four complicating factors in a full close kin model (Section 4.7).

The full close kin model has similar data and biological parameter requirements to those of a conventional stock assessment model, but does not require CPUE (or any other index of relative abundance). Fishery data (catches by gear) were calculated from logbook data and discard rates were taken from Burch *et al.* (2018). Biological parameters (e.g. number of pups per female, growth, and weight- and length-at-age) were taken from literature, and were either those used by the existing stock assessment model for school shark (Punt, 2001) or were updates, where those were available. Further details are provided in subsequent chapters.

We implemented the close kin model in C++, but run from R (R Core Team, 2017), using the 'ADT' utility (Automatic Differentiation via TAPENADE) that was developed at CSIRO

(Paavo Jumpanen, pers commn). As a result of our using ADT for this project, some bug fixes and refinements of the package have been made.

Chapter 4

Results

4.1 Close kin data

4.1.1 Sample size and distribution

Sample sizes markedly exceeded the collection targets in all locations except Tasmania (Table 4.1).

Table 4.1: Targets and collection totals for school shark close kin sample.

State	Target	Collected
South Australia	700	1,318
Bass Strait	900	1,378
Tasmania	400	339
TOTAL	2,000	3,035

We tried to avoid sampling more than 50 individuals from a fishing trip (see Methods above), and eliminated samples whose quality of DNA preservation was shown by gel electrophoresis to be lacking. We also eliminated accidental resamples of the same animals (see below) and 13 samples that were clearly not school sharks (they were presumably gummy shark, sampled in error). Of the 2,886 samples for which genetic sequences were obtained, 2,438 passed all quality control tests (see Section 4.5). In addition to tests relating to DNA quality (i.e. preservation and purity) we also eliminated samples whose genetic make-up results in ambiguity relating their kin relationships.

Examination of the genetic sequences revealed eight pairs of duplicated animals where the same animal was sampled twice on the same day. Such replication can occasionally happen by accident (Chris Pitliangas pers commn) and is easily detected and overcome by randomly eliminating one of the duplicate samples. Another four pairs of duplicates arrived (both animals in each pair coming from the same supplier) with reported sample dates that were between two and five days apart - these were also interpreted as accidental repeated sampling on the factory floor, as was one animal that was sampled twice on 8 Aug 2017 and a third

time on 10 Aug 2017 by the same supplier. It is a great convenience of genetic studies that they provide the capability to detect such accidental resampling and correct it.

4.1.2 Ageing and age error

Ageing of school shark is done my counting hyper-mineralised zones (hereafter termed 'rings') in the vertebrae. Moulton *et al.* (1992) used tag-recapture data to show that vertebral ring counts for school shark correspond closely with actual age up to roughly age 11 but thereafter underestimate true age. Walker *et al.* (2001) estimated ring deposition rate after age 11 to be 0.36 rings per year. Unfortunately their estimate is based on only five individuals, consisting of a mix of males and females. It seems probable that growth, and therefore ring deposition, slows at the age of maturity, which is 11 for females but younger for males. No sex disaggregated estimates of ring deposition rate are available, but growth curves for male and female school shark are not significantly different (Moulton *et al.*, 1992) so using the age of 11 for both sexes might be accurate. Kalish (2002) and Fenton (2001) used bomb radiocarbon dating to show that vertebral counts do greatly underestimate the ages of older school shark, but their work does not give estimates of annual ring deposition rate. We therefore used the estimate of Walker *et al.* (2001) to infer actual age, probabilistically, from vertebral ring counts including assuming annual ring deposition to age 11 for both male and females.

Sampled vertebrae were aged by the Fish Ageing Service (FAS); the largest number of rings counted was 26 (Figure 4.1). When this study was originally scoped, we assumed that the majority of our samples would come from the dominant gear type, gillnet, so that the majority of samples would be under the age of 11. However, we received a large number of our samples from line gear, giving us more older animals than we expected (Figure 4.1).

FAS randomly selected a set of vertebrae for recounts of rings, which showed that ageing error is not negligible (Figure 4.2). A CV of approximately 0.08 was found (Andre Punt, CSIRO, pers commn) when allowing for errors in both the first ('Age1') and second ('Age2') counts, as well as between individual readers (using the method of Punt *et al.* (2008)).

The full close kin model (described in Section 4.7) converts ring counts to a probability distribution of likely true ages, but for the two simpler approaches described in Section 4.6 we simply adjusted the ring count for each sample using the assumption of 1 ring per year up to age 11 and 0.36 thereafter (Figure 4.3). The older the animal the greater the likelihood of error when making this correction so both of the simpler models restrict the sample that is used to only younger animals (age ¡= 11). The oldest animals sampled had likely birth years as far back as the mid- to late-1960s.

4.1.3 Length

The reported carcass length measurements were occasionally the total length, but most often the partial length or, equally often, the dressed length (i.e. a measurement of the whole carcass that remains after the head and part of the tail are removed). Conversion of supplied length into total length was not always straightforward and some uncertainty surrounds the actual length measurement type of many of the samples. This uncertainty was such that we chose not to use the length measurements. This meant that we assumed that fecundity

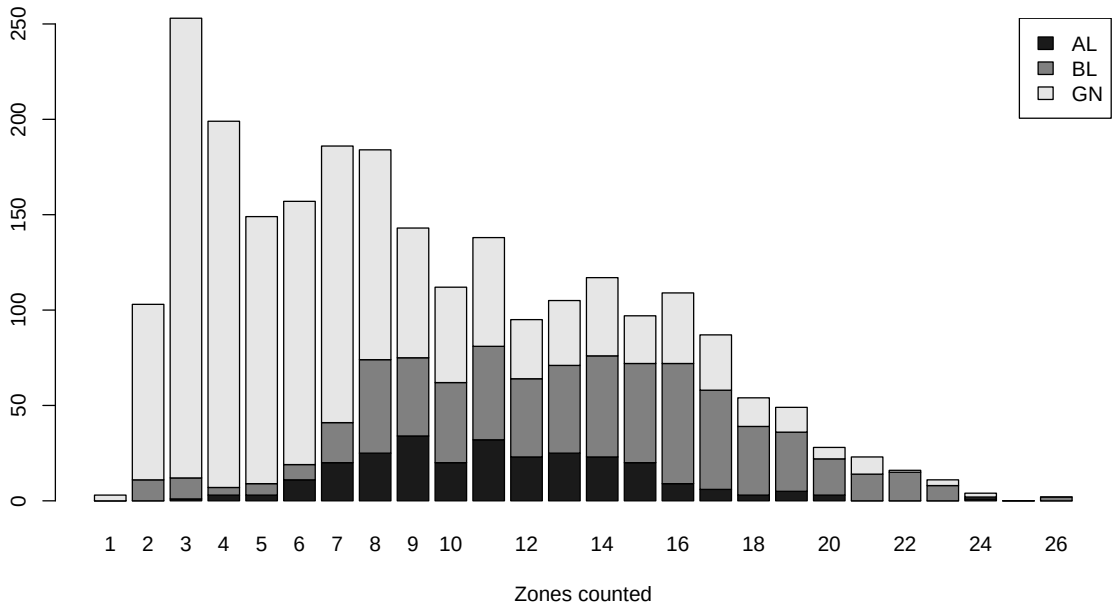

Figure 4.1: Frequency distribution of 'rings' (zones) counted in the vertebrae of the 2,438 animals used for kin finding. Samples were collected from auto-longline (AL), bottom line (BL), or gillnet (GN) fishing vessels.

was proportional to age, rather than to length, which is not an unusual assumption for a population dynamics model.

4.1.4 Trip

To reduce any bias that might arise from a tendency for close kin to swim together, we assigned every sample to a fishing trip. Trip was defined using the information supplied on the sample card: vessel name, and date. We matched this information to the Catch Disposal Record (CDR) database (which gives trip offloading date) and the logbook database (which gives shot dates). We used successive CDR records for each vessel to denote the end dates for all trips recorded for that vessel, and the intervening shot dates from the logbook to decide whether the date given on the sample card was a shot date, or whether sampling occurred in port, either on the day of offloading or shortly thereafter. This allowed us to assign almost all samples to fishing trips, and therefore to ignore any within trip comparisons (or kin pairs). Some of the older Observer Program samples pre-dated the CDR record for particular vessels, in which case the logbook, alone, was used and the fishing shot was treated as the 'trip'.

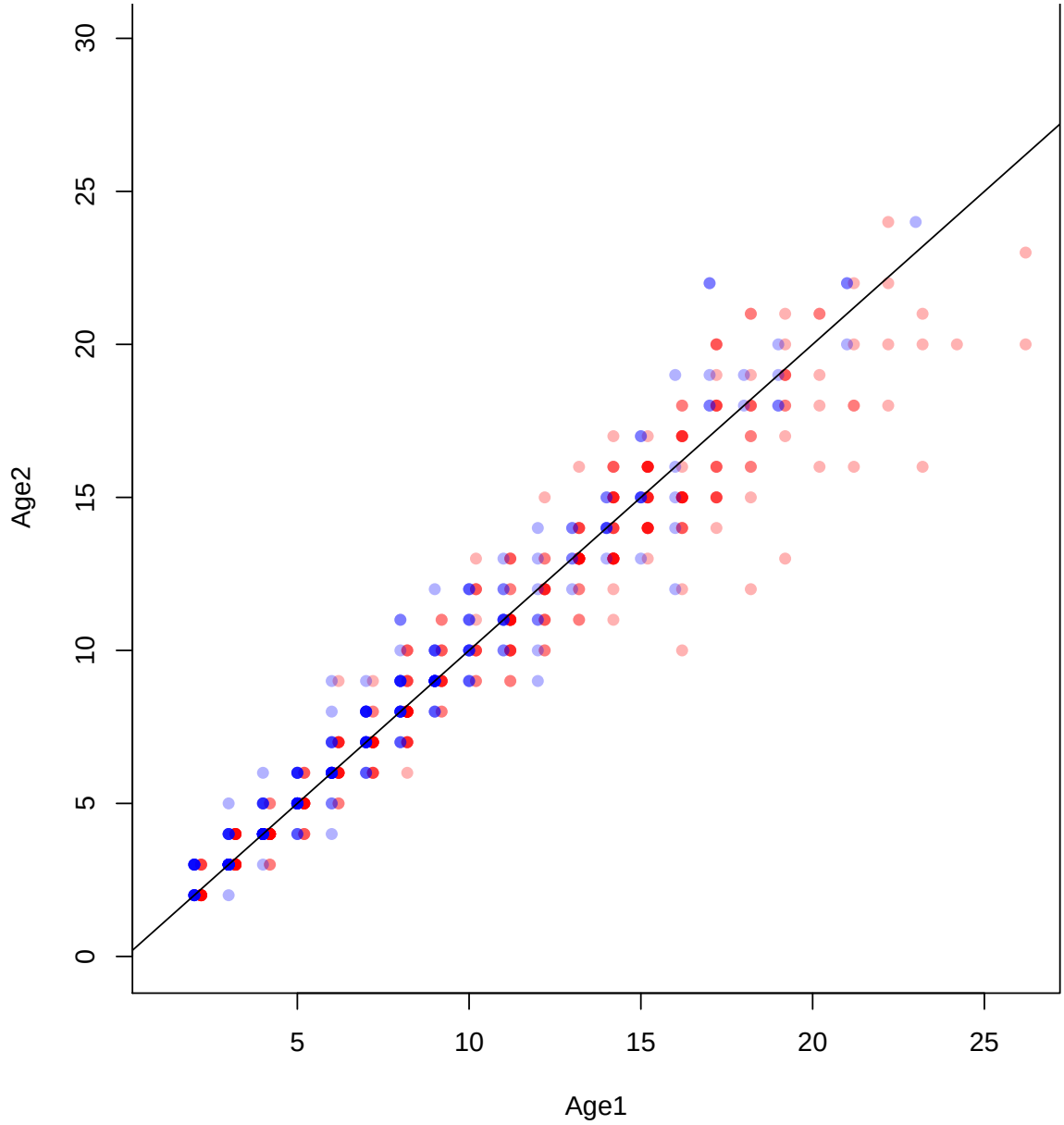

Figure 4.2: Number of vertebral zones counted (age) during a first (Age1) and second (Age2) reading of a random selection of school shark vertebrae. Ages for males sharks (blue dots) are offset slightly so that they do not overlie those for female sharks (red dots). Darker dots indicate more overlaid individuals of the same sex. Note that these are not true ages but deposition zone ('ring') counts.

4.2 Fishery dependent data

To translate the close kin data into an absolute abundance series, we constructed a population dynamics model that made use of the close kin data as well as total catch (landed catch plus discards). Earlier versions of the model used length frequency data but as that was influenced by fishing area and time of year, we effectively did not use length frequencies in the final model

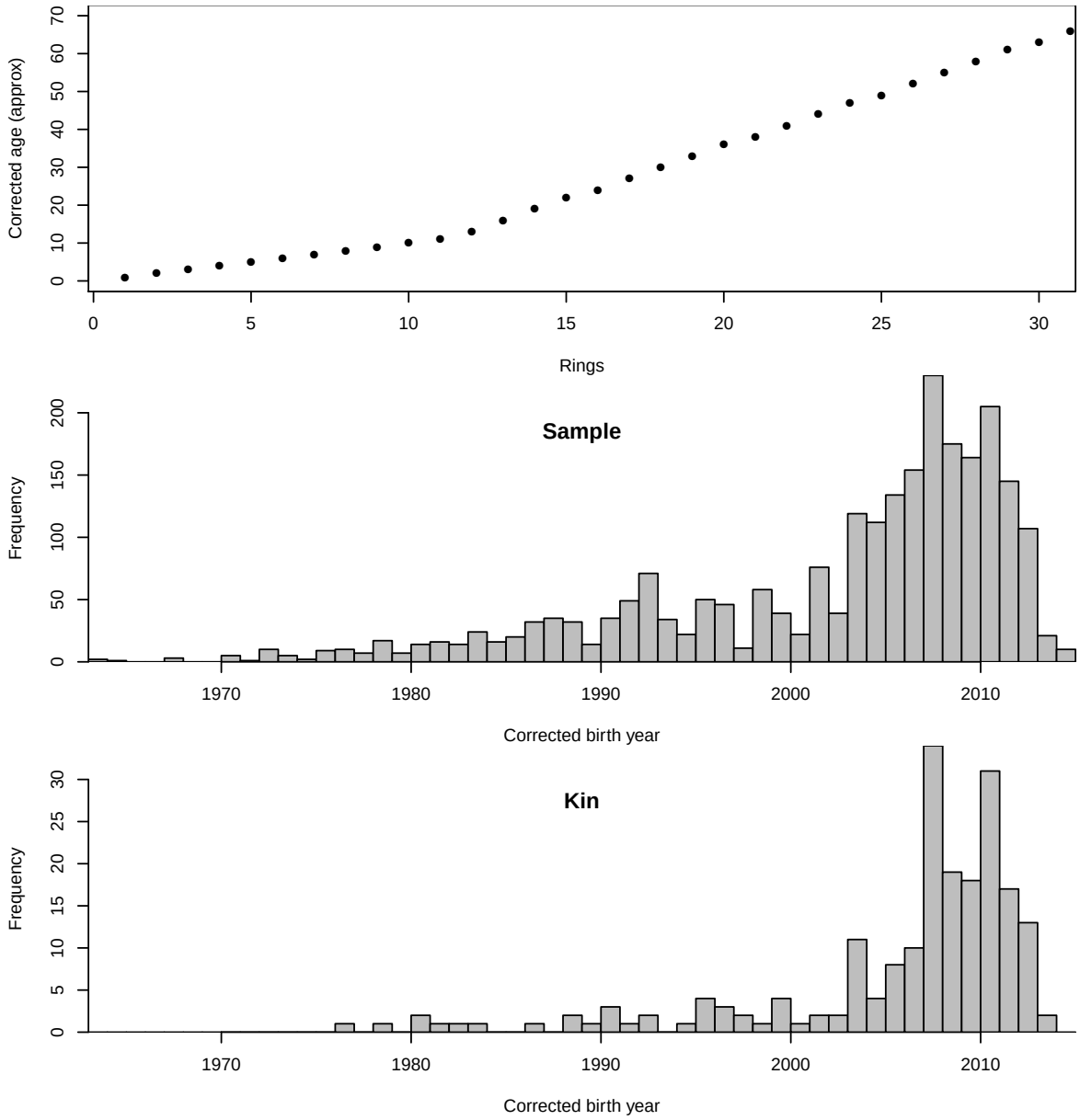

Figure 4.3: Median true age (*Corrected age*) given number of rings counted (upper plot); histogram of inferred birth years for all samples collected (middle plot) and for all animals found in kin pairs (lower plot).

and do not discuss their construction in this report.

4.2.1 Landed catches and discards

The original time series of catches used by the stock assessment model (Punt & Walker, 1998; Punt *et al.*, 2000) ('old' in Figure 4.4) seemed to have been incorrectly allocated to months and to shark zones. Although we do not use months in the close kin model, we did use zone, as a sensitivity, to remove catches from the periphery of the school shark range.

We therefore abandoned the catches from the stock assessment model, and constructed a new catch time series for school shark for 1989 to 2017 using the logbook dataset. The time series of Japanese longline catches of school shark in Australian waters that was used by Punt *et al.* (2000) was added, along with the state catch data used by those authors as well as more recent catch data (Castillo Jordán *et al.*, 2018b) ('new' in Figure 4.4). The stock assessment model treated the longline data as 8 inch gillnet catches because that selectivity was thought to better match that gear type. We instead assigned those catches to longline gear. Any differences resulting from this change ought to be small due to the similarity of these selectivities (both taking the largest animals). To investigate the influence of ignoring school shark at the periphery of their range (which might belong to isolated populations that were not sampled by this project) we excluded all catches from the shark zones NSW, WA, and WSA ('new no West/NSW' in Figure 4.4).

Discarding of school shark was considered to be negligible in the stock assessment model (Punt & Walker, 1998; Punt *et al.*, 2000). While this was probably true prior to 2009, subsequent reductions in the TAC for school shark are likely to have resulted in higher discard rates. Discard rate estimates, calculated from observer collected data, are available for 2011-2014 and show a steady increase in discarding from 9% to 15% over that period (Castillo Jordán *et al.*, 2018a). No estimates are available from 2015 onwards due to the removal of onboard observers, but work currently underway by ABARES suggest a similar discard rate during 2017 to that calculated for 2015 (ABARES, in prep). We assumed that discarding was zero up to 2009, assumed a linear trend until 2011, used the observed rates for 2012-2014 and assumed the 2014 discard rate thereafter (Table 4.2). These observed and imputed annual discard rates were applied equally to all gears and were used to inflate the landings figures (Figure 4.4).

Table 4.2: Estimated and assumed discard rates for school shark calculated using ISMP data. An asterisk (*) denotes an assumed value.

Year	Discard rate
2009	0*
2010	4.5%*
2011	9.0%
2012	11.9%
2013	14.3%
2014	15.1%
2015	15.1%*
2016	15.1%*
2017	15.1%*

Catches were assumed to consist equally of males and females (by weight). The stock assessment model used sex ratio data, collected by observers, for WSA, CSA and 6, and 7 inch gill nets. The average sex ratio for all gear types was close to 50% (1970 to 2003 in Table 4.3), (Punt *et al.*, 2000; Punt, 2001; Thomson & Punt, 2009). More recent data collections come almost entirely from port sampling by the AFMA Observer Program so that gear size and fishing zone are not easily identified. We therefore calculated the annual proportion of the catch that was female for all gillnet data combined (line collections are small) from the port

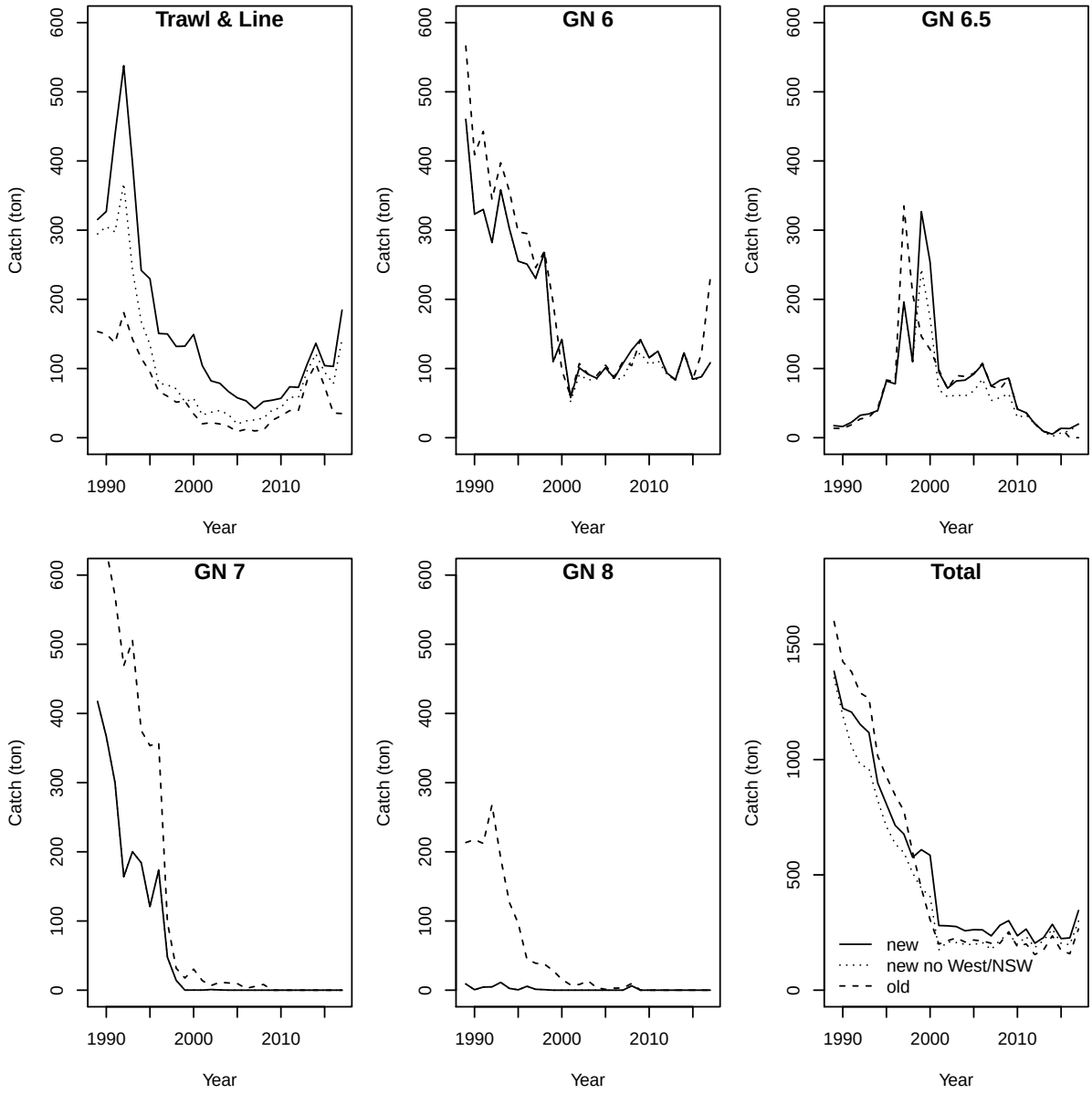

Figure 4.4: Landed catches in tonnes by trawl and line gears, and 6 (GN6), 6.5 (GN6.5), 7 (GN7) and 8 (GN8) inch gillnets as well as total catches for the original time series (*old*) and the new time series using data for all regions (*new*) or excluding WSA, WA and NSW (*new no West/NSW).*

observer data, scaled to total catch where possible (2004 to 2016 in Table 4.3).

Most sex ratios shown in Table 4.3 are close to 50% but with a relatively wide range (36% to 71%). Given the tendency of school shark to swim in same sex schools (Olsen, 1954; Walker, 1999) the CV is expected to be high – the wide range does not necessarily invalidate the assumption that catches tend to be distributed 50:50 between the sexes. For this reason we decided that it would be reasonable to assume a 50:50 sex ratio for the catch for all gears and years.

Table 4.3: Percentage of the catch that was female, as used in the stock assessment model, available for shark regions Western South Australia (WSA) or Central South Australia (CSA) and 6 inch or 7 inch mesh nets.

Year	WSA 7 inch	CSA 6 inch	CSA 7 inch	Year	Mesh nets
1970			47%	2004	59%
1971			36%	2005	55%
1972			47%	2006	56%
1973		71%	65%	2007	50%
1974		64%	61%	2008	57%
1975		57%		2009	53%
1976		52%		2010	49%
1977		55%		2011	47%
1978		57%		2012	50%
1979		61%		2013	48%
1980		62%		2014	50%
1981		55%		2015	43%
1982		56%		2016	47%
1983		57%			
1984					
1985		47%			
1986	52%	50%			
1987	55%	49%			
1988	52%	48%			
1989		47%			
1990		49%			
1991	69%	51%			
1992	58%	49%			
1993	70%	52%			
1994	58%	48%			
1995	66%	51%			
1996	68%	36%			
1997	60%				
1998		49%			
1999		44%			
2000		50%			
2001		60%			
2002					
2003		52%			
Average	61%	53%	51%	51%	

4.3 Biological parameters and selectivity

We used the same biological parameters that were included in the school shark stock assessment model, unless those could be updated using more recent work. Sex specific von Bertalanffy growth curves, calculated using tag-recapture data so that they were free from the inaccuracy of ring counts, were the same as those used by the assessment (Moulton *et al.*, 1992; Punt & Walker, 1998). Gear selectivity curves (dome-shaped for gillnets and knife-edged for a combined trawl and line fleet) were fixed (not estimated) in the stock assessment. Gillnet selectivities were calculated for the stock assessment using the method of Kirkwood & Walker (1986). The trawl and line selectivity appears to have been an educated guess.

Female fecundity (which is the product of the female proportion mature-at-age, the mean number of pups produced-at-age, and a three year pupping interval (Walker *et al.*, 2001)) were also fixed in the stock assessment model. For the close kin model we used the more recently calculated linear relationship between number of pups and length of females given by Walker (2005) which rises from roughly 20 pups per female for younger animals to 30 for the largest animals. No pupping interval was assumed, neither was juvenile survival rate; instead the close kin model was allowed to estimate a parameter that represents the product of the pupping interval, and survival during the first year of life. That parameter can also help to capture any inaccuracy that might exist in the fixed fecundity relationship.

The stock assessment model assumes a first size at maturity for females of 140cm (11 years of age) but the female maturity ogive developed by Walker (2005) allows maturity from a little over 130cm (9yo) and Olsen (1954) gave 135cm (10yo) as the minimum size at maturity for females. For consistency, we used 140cm, but future work might consider lowering that size. If more mother-offspring pairs are found in future, we should have empirical evidence of the age (and by back extrapolation, an estimate of the size) at first maturity.

Walker (2005) considered three indicators of male maturity: testis condition, seminal vesicle condition, and clasper condition. All measures suggest that males are mature from roughly 120cm, although a very small proportion of animals were mature from as little as 100cm (4 years). The smallest mature male reported by Olsen (1954) was 121cm in length. Walker (2005) gives a logistic maturity-at-length ogive for males with 50% maturity at 129.1cm and 95% at 143.9cm. We used this ogive for male maturity, but also imposed a minimum size at maturity of 120cm for males. Note that we specified biological formulae as functions of length and the converted these to age using a probability distribution that describes length at age, more details are given in Appendix C.

Length-weight relationships for males and females were taken from Walker (2005) and represent an update on the values used in early versions of the stock assessment model. The parameter values used are shown in Table 4.4.

4.4 Population genetics for Australia and New Zealand

The neonate pup samples from Australian and New Zealand were used to investigate whether there are any detectable genetic differences between school shark born in the two regions. Hernández (2013) was unable to show any genetic difference, but the more powerful sequenc-

Table 4.4: Biological, and fishing gear selectivity parameters used in the close kin model.

Parameter	Equation	Value	Source
L_{inf} female	von Bertalanffy	160.04cm	Moulton *et al.* (1992)
K female	von Bertalanffy	0.1639	Moulton *et al.* (1992)
t_0 female	von Bertalanffy	-1.2669	Moulton *et al.* (1992)
L_{inf} male	von Bertalanffy	158.33cm	Moulton *et al.* (1992)
K male	von Bertalanffy	0.1675	Moulton *et al.* (1992)
t_0 male	von Bertalanffy	-1.25	Moulton *et al.* (1992)
a female	allometric	0.699^{-8}	Walker (2005)
b female	allometric	3.276	Walker (2005)
a male	allometric	1.810^{-8}	Walker (2005)
b male	allometric	3.129	Walker (2005)
female first maturity	knife-edged	140cm	Punt *et al.* (2000)
male first maturity	knife-edged	120cm	Walker (2005)
female P_{max}	Logistic	0.333	Punt *et al.* (2000)
female LP_{50}	Logistic	134.9cm	Walker (2005)
female LP_{95}	Logistic	150.2cm	Walker (2005)
male PM_{50}	Logistic	129.19cm	Walker (2005)
male PM_{95}	Logistic	143.9cm	Walker (2005)
min size	dome-shaped	71.6cm	Punt *et al.* (2000)
max size	dome-shaped	200.0cm	Punt *et al.* (2000)
θ_1	dome-shaped	188.335	Punt *et al.* (2000)
θ_2	dome-shaped	55919.7	Punt *et al.* (2000)
minS	dome-shaped	91.1cm	Punt *et al.* (2000)
parMi	dome-shaped	71.59cm	Punt *et al.* (2000)
parMa	dome-shaped	20000	Punt *et al.* (2000)
min size (line, trawl)	knife-edged	72cm	Punt *et al.* (2000)

ing methodology used in our study might have (but largely did not) show differences. This work was presented at an Australian Society for Fish Biology conference (Hobart, 4-8 September 2016) and has been published (Devloo-Delva *et al.*, 2019). The manuscript is included as Appendix B. In summary, no genetic differentiation was detected between Australian and New Zealand-born pups.

4.5 Genetic sequencing and kin finding

DNA consists of sequences of joined amino acids (base pairs) making up a double helix. Nuclear DNA consists of pairs of chromosomes; matching strands of DNA. For the most part, these paired chromosomes will have identical sequences, but they will differ at some locations. Our investigation focuses on single nucleotide polymorphisms (SNPs) where a single amino acid differs. Individuals can have identical (homozygous) or different (heterozygous) sequences at these locations in the genome. We refer to these locations synonymously as 'loci', SNPs, or markers. We refer to the alternative sequences as alleles. The genetic sequencing method employed for our study uses restriction enzymes to cut the DNA into a large number of fragments. Only those that are 75 base pairs long are sequenced. Most fragments that belong to particular loci will have identical genetic sequences in all individuals in the population, but some will differ in one or more of the base pairs. Investigators have to decide which of those differing sequences represent alternative alleles from the same locus, and which differ so much that they are more likely to represent different loci. DArT have developed a methodology for making those decisions so that the data they provide consists of counts, for each shark, of detections of unique 75 base pair sequences, clustered into groups that are likely to have derived from the same locus (i.e. part of the chromosome). One of the quality control steps we undertake is to try to detect clusters that, in fact, are made up of more than one locus. We did this by recognising that no individual can have more than two variants (alleles) at the same locus, and by seeking excessive heterozygosity.

Sometimes an allele is present in a shark, but no detections are made. This is most often because of a mutation (such as a SNP) at the site where the restriction enzyme would have cut. A restriction enzymes cuts when it finds a particular, short, sequence of amino acids. If the cut is not made, then the fragment will be longer than 75 base pairs and will not be sequenced. We explicitly account for these non-detections (and increase the kin finding power of our statistical calculations) by treating these 'nulls' as a third allele (see Bravington *et al.*, 2015, for further details).

As described in Methods, the DArTcap method targets particular parts of the genome that include SNPs that were selected as most useful in detecting HSPs. That process nevertheless returns far more SNPs than the 2,000 that were selected. It also uses different restriction enzymes so that the rate of non-detections (nulls) can differ. We therefore had to repeat the process of estimating allele (and null) frequencies for all loci and then of identifying the loci that were most useful for detecting HSPs. POPs and FSPs are relatively easy to detect, because they share more genetic material, but HSPs have a higher genetic data requirement. Of the 81,690 loci (clusters) returned by the DArTcap process, we identified 1,757 that provided the most accurate and powerful information for finding close kin (and in particular, HSPs). This extensive data cleaning and genetic locus selection exercise, and subsequent

'kin finding', were performed using the 'gbasics' and 'kinference' R packages that have been developed at CSIRO, largely through the SBT and school shark close kin projects. It is planned that the software will be released, with documentation and a worked example, in the near future.

4.5.1 Mitochondrial DNA

The reproductive dynamics of male and female school sharks are different. Males, for example, mature earlier than females and therefore (assuming the same numbers of males and females in the population) the 'pool' of potential fathers is larger than that of mothers. It is therefore important to know whether HSPs are related through the father (a paternal HSP, PHSP) or through the mother (a maternal HSP, MHSP). This can be done by comparing the mitochondrial DNA (mtDNA) of the half siblings. mtDNA is always inherited from the mother, not the father, so that if the half sibling pair have different mtDNA sequences (known as haplotypes), then they must be related through the father (a PHSP). If they have the same haplotype, then they are probably related through the mother, but they might instead be related through the father and share their haplotype by chance. The more distinct haplotypes there are in a population, the more powerful the mtDNA is in discriminating maternal from paternal HSPs. Very small populations, or those that have been through a genetic bottleneck, can have very few haplotypes.

Mitochondrial DNA (mtDNA) is distinct from the nuclear DNA used to find kin pairs, and needs to be measured using different techniques. Individuals that were found to belong to kin pairs underwent sequencing of their mtDNA. Work by Hernández *et al.* (2015) had suggested that there is high diversity in a part of the mitogenome called the control region. We therefore, initially, sequenced only that region but found only seven haplotypes, one of which was found in roughly 50% of the animals sequenced. This renders the control region very uninformative for discriminating MHSPs from PHSPs. Several of the haplotypes reported by Hernández *et al.* (2015) were represented by only one or two animals, suggesting that those might have been sequencing errors rather than rare haplotypes. For this reason we re-sequenced the mtDNA, this time sequencing the entire mitogenome. This returned a much more informative 122 haplotypes of which the most common was found in only 5% of the sharks examined. This provides very powerful information for discriminating maternal from paternal HSPs; we estimated a mere 3% chance of the HSPs in this sample sharing their mitochondrial haplotype by chance.

We calculated haplotype frequency by excluding one animal (selected randomly) from each pair, because close relatives are more likely to share haplotypes than unrelated individuals. Among the 65 HSPs that we found, 38 had the same haplotype. This means two things. First, it backs up our HSP-finding; it is impossible that so many pairs would have the same haplotype if they were really unrelated. Second, it suggests there are substantially more 'typical' adult males than 'typical' adult females. The difference was found to be consistent with close kin model estimates made using MHSPs only based on the later age of maturity, and progressive fecundity increase post-maturity, in females. This is described in more detail in Section 4.7.3.

Since the chance of sharing a haplotype by chance is so low, we simplified the modelling

by interpreting all shared-haplotype HSPs as MHSPs and all different-haplotype HSPs as PHSPs. This might mean that one or two of our nominal MHSPs are actually PHSPs, but the overall impact on the CKMR model should be small. This is what was done did for SBT, which has similarly high diversity of haplotypes. For some other, rare, shark species for which CSIRO has applied the close kin methodology, there were very few distinct haplotypes so that more elaborate probabilistic models had to be constructed (not yet published).

4.5.2 Identifying sex from genetic data

We selected a subset of five sex markers from the fifteen found in our initial sequencing investigation. The DArTcap process can return additional SNPs that occur nearby on the genome, so instead of just five, we found six candidate sex markers in the DArTcap sequence data. Of those, four emerged as most reliable (Figure 4.5). The remaining two had lower read depths (numbers of detections of each unique genetic sequence per individual) so that it was more often difficult to distinguish between a male with very low reads of those markers, and a female who lacked those markers altogether (but might have returned a small number of reads due to laboratory error). Note that although we used the reported sex to identify the sex markers (a circular process) the method we use is surprisingly robust to errors in the reported data. The sex markers were present in male, but not female, sharks suggesting an XX-XY reproductive system.

Even using four reliable loci, there is some uncertainty in the allocation of sex, with 10% of samples considered to be unclear due to difficulty distinguishing low reads from genuinely absent alleles. A low level of leakage' occurs in the sequencing process, so that females can sometimes appear to have a small number of reads (i.e. detections) of alleles that are actually absent. On the other hand, variation in the number of reads of particular alleles can result in some males having very low counts of alleles that are actually present. Animals that had ambiguous (i.e. low) counts for all four sex markers were not re-assigned; instead, their reported sex was assumed to be correct.

Of the 2,438 animals used in the close kin model, 99 were supplied without information on sex, 31 of which were found to be genetically male and 68 female. Of those reported to be female, 90 out of 1,427 (6%) were corrected to male; and out of 912 reported males, 23 (3%) were corrected to female. The sex ratio in the final sample (of 2,438 animals) was 57% female versus 43% male.

4.5.3 Identifying kin pairs

Sequencing information was available for 2,886 samples, of which 244 were re-sequenced to allow the estimation of sequencing error rates. There were also several accidental duplicates seemingly resulting from re-sampling of the same fish at the processor, or mix ups in the laboratory. These 85 accidental duplicates were removed from the sample. An additional 36 samples were removed because of either excessive heterozygosity (an indication of DNA contamination i.e. the DNA of more than one fish was found in the sample) or too little heterozygosity (an indication of degraded DNA). Another 78 samples were removed because their gene frequencies were outside of the norm. We have found that some fish have a genetic

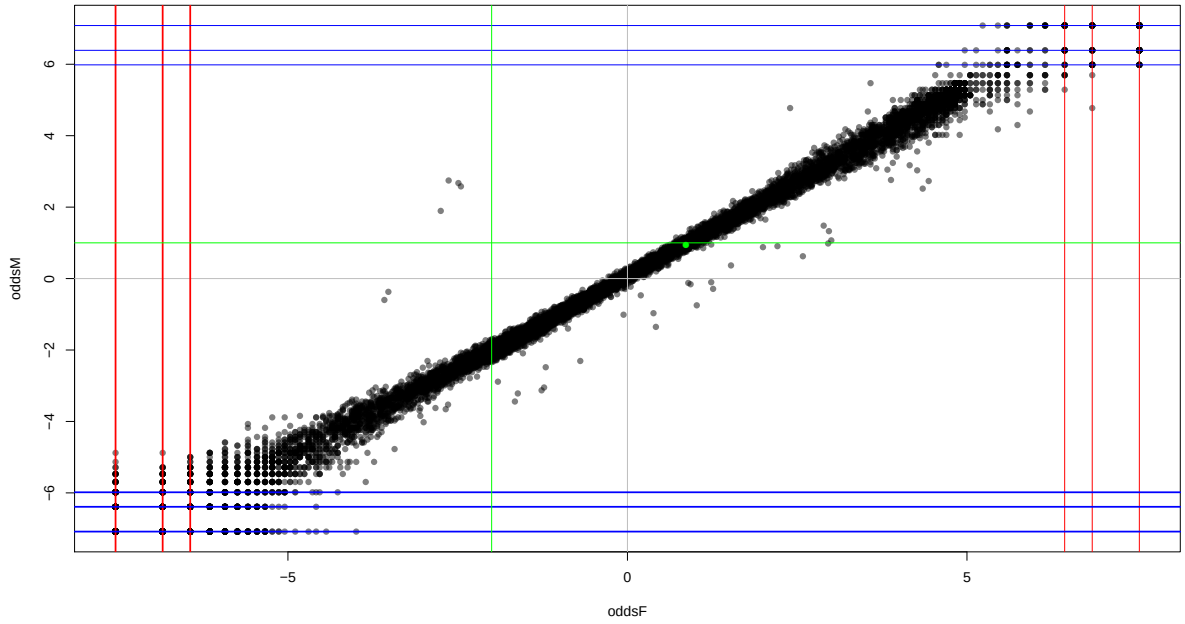

Figure 4.5: The odds ratio of having a particular genetic allele (grey dots) for male vs female school sharks. The red and blue lines show the odds corresponding with only 1,2, or 3 (thick lines) or all but 1, 2, or 3 (thin lines) animals having a particular allele – these would reveal spurious results due to low numbers of individuals. The green lines were used to select the four alleles (appearing in the top left quadrant) that proved to be reliable sex markers.

make-up that causes them to be more likely to show a higher than usual degree of kinship with other (similar) fish, at least when using our statistical methods. Further work is underway at CSIRO regarding understanding this phenomenon and altering our statistical methods to account for it. For school shark, we accounted for this by eliminating the 249 fish that showed this tendency. After determining suitable, and removing aberrant, loci and fish, a range of statistical measures were applied to determine which pairs are close relatives, and what their kin relationship is. These are not described in any detail here, work is currently underway at CSIRO to publish a description, an R package, and a worked example that will detail these methods.

Every possible pairing of samples animals was examine to see whether it was a POP, HSP, FSP or unrelated pair (UP, which includes more distant relationships such as cousins and half aunt/uncle - niece/nephews). To do this, we used a number of statistics that have been developed at CSIRO for close kin studies. These will soon be published and a worked example will be released along with the 'kinference' R package. Many of the technical principles are explained in Bravington *et al.* (2015) Section 5. Results for three statistics that are optimised to detect POPs (wpsex, nABOO), FSPs (wtsame, PLOD_FH) and HSPs (PLOD) are shown in Figures 4.6, 4.7, and 4.8. In Figure 4.6 the blue cluster of points are FSPs mixed with POPs, the green cluster are HSPs, and the grey points are unrelated pairs.

Figure 4.7 shows the distribution of the 'PLOD' statistic, which gives the pseudo-likelihood

that a pair of animals are HSPs (Bravington *et al.*, 2015). A higher PLOD value indicates closer relatedness (more specifically, a greater likelihood that the pair are an HSP). A small overlap between the distribution of HSPs (approximately, the orange curve) and those of unrelated and less related pairs (UPs) is inevitable, at least without more complete information on the genome than is available to this study. To deal with this, a threshold PLOD value (termed 'eta'; red line on Figure 4.7) is chosen (visually) as a safe threshold such that very few UPs are likely to have PLODs greater than the value of 'eta' i.e. to exclude false-positives. Pairs are only counted as definite HSPs if their PLOD is greater than eta (but less than an upper threshold denoting POPs and FSPs, grey line on Figure 4.7). Since this will lead to some false-negatives (true HSPs that are rejected by having an accidentally low PLOD), an adjustment is made in the CKMR model to allow for the likely proportion of false-negatives, which for this study was estimated to be 12%. Note that the exact value chosen for eta does not bias the results; changing it will affect the number of observed kin pairs, but this will be equally balanced by a change in the estimated false negative rate that is incorporated into the close kin model. It is important to ensure that the value of eta is not too low, because that would allow false positives, which would bias the result (to some degree).

FSPs and POPs have the same average degree of relatedness, and are easy (collectively) to separate from HSPs; they are obvious in Figure 4.6 and on the right-hand side of Figure 4.7 (to the right of the grey vertical line) and indeed a formal statistical test identifies them easily from the HSPs. Separating FSPs from POPs based purely on genetics can be done in principle (and has been observed using the statistics shown here, in SBT and grey nurse sharks), but has not worked for school shark. More discriminating statistics are under development. We are ble to use age to separate POPs from FSPs for school shark, as we demonstrate in Section 4.5.4. Figure 4.8 shows no separate clusters of POPs and FSPs. In principle, it is possible to distinguish POPs from FSPs by using enough loci and carefully-designed statistical measures (whereas, by contrast, it is impossible to distinguish between half-sibling-pairs and grandparent-grandchild pairs). The statistic we used for FSP/POP delineation was fairly easy to calculate but not the most powerful possible; more powerful versions will be sought in future. In the case of school shark, though, separation is easy, based on the age gap between individuals in each pair. FSPs will be from the same cohort whereas POPs must be separated by at least the age of maturity (see Section 4.5.4). FSPs are not much use in CKMR, because they are almost certain to be litter-mates (with an adult population of the order of 100,000, only a tiny proportion of repeat matings will occur) and same-cohort comparisons are explicitly excluded from CKMR calculations, for reasons described earlier.

4.5.4 Cohort gaps

Figure 4.9 shows the gap between estimated birth years for every kin pair (upper plot) along with corrected birth year and ring count for each animal involved in the kin pairs. The birth intervals shown in Figure 4.9 are based on corrected ring counts; the correction assumes no ageing error and no variability in the ring deposition rate (0.36 rings per year after age 11) and is used for display purposes only.

Among the FSPs and POPs (left side of upper plot, Figure 4.9), the three rightmost pairs, and only those pairs, have a gap large enough to be POPs. This very small number of POPs, relative to the number of HSPs, turns out to be roughly as expected by our model given the

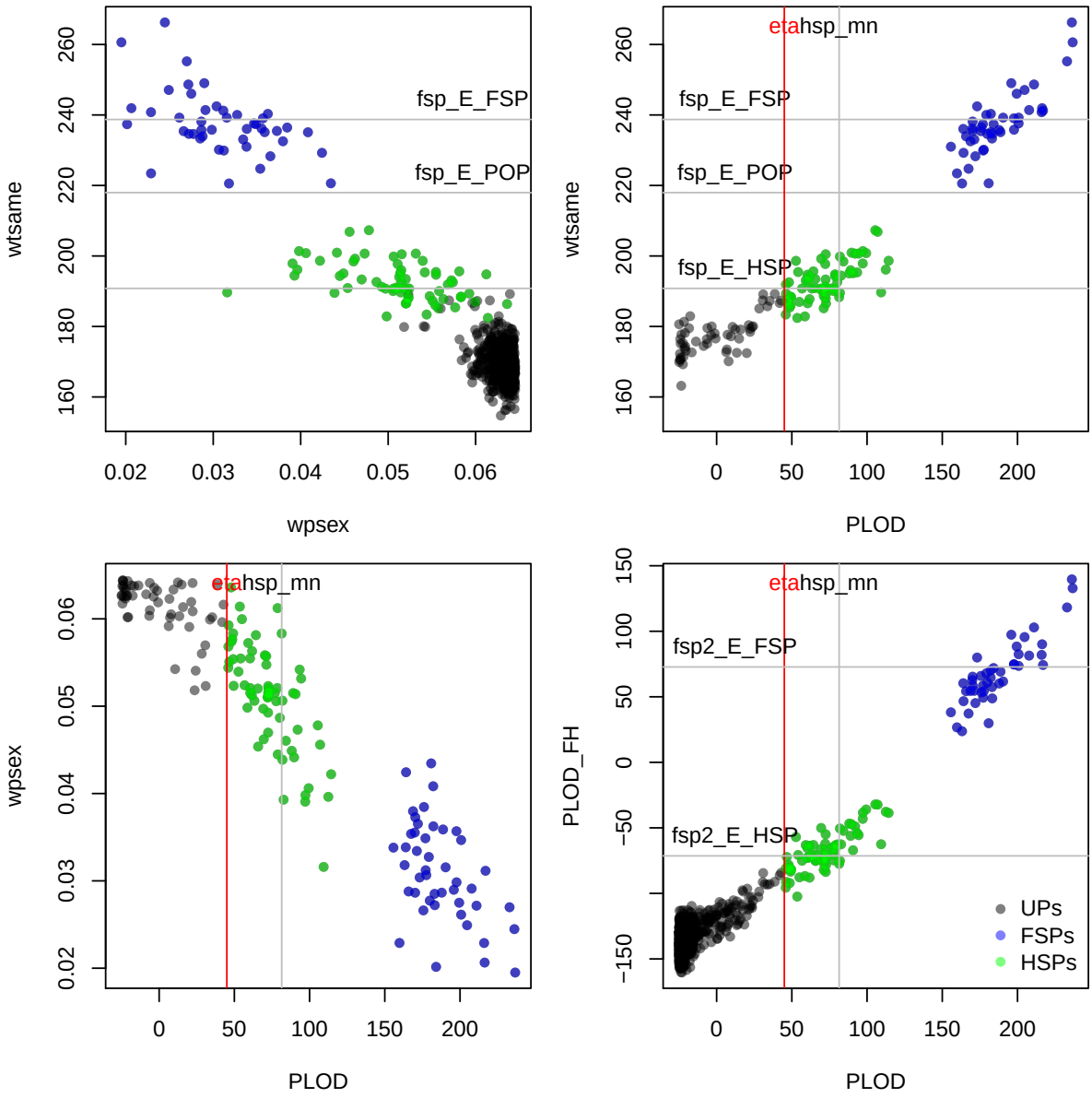

Figure 4.6: Scatter plots showing three statistics for determining relatedness 'wtsame' is optimised for finding FSPs, 'wpsex' for POPs, and 'PLOD' for HSPs. Each dot represents a pair of animals. For clarity, the majority of unrelated (and less related) pairs are not shown. Theoretical mean values for each kin type are shown (grey lines); a PLOD threshold value was chosen that distinguishes unambiguous HSPs (PLOD>eta) from those that merge into the less related pairs (PLOD<eta) (red line).

age distribution of the sampled animals (see Section 4.7.3). The remainder of the leftmost pairs must be FSPs; the maximum apparent gap is 5 years. Most of the FSPs have a gap of 0-2 years, which is entirely explicable in terms of ageing error on animals from the same cohort. The six FSPs with gaps of 3-5 years are either due to ageing error (certainly plausible), or (conceivably) to sperm storage, whereby a female uses sperm from one mating to fertilize

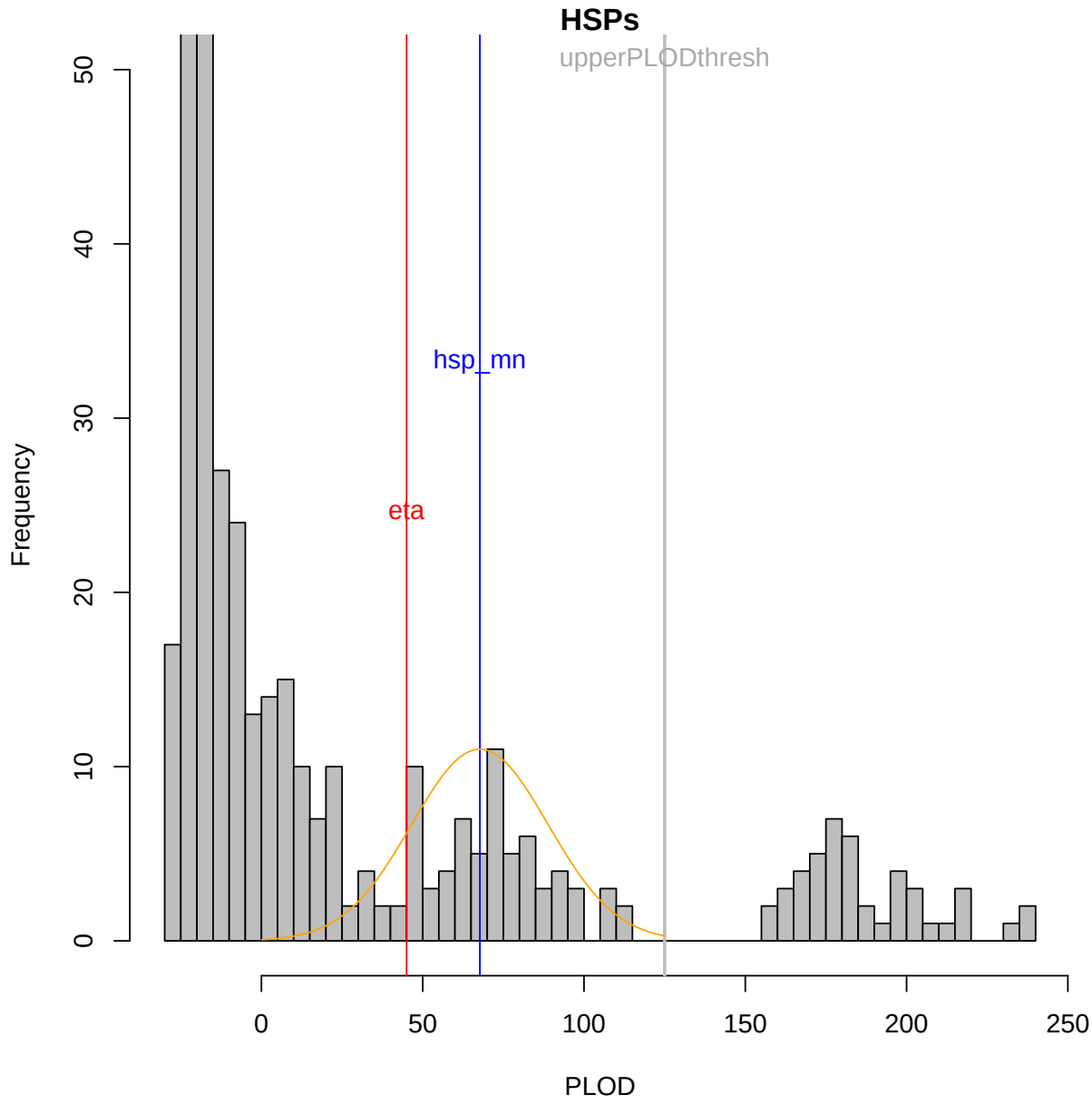

Figure 4.7: Histogram showing the PLOD statistic for more closely related pairs; 'eta' (red line) is the threshold value chosen to separate unambiguous HSPs from the mix of UPs and HSPs (PLOD<eta); 'hsp_mn' (blue line) is the theoretical mean PLOD value for HSPs. An approximation to the theoretical distribution for HSPs (probably wider than the true distribution) is shown (orange curve). The cluster between 150 and 250 (PLOD > upperPLODthresh; grey line) are POPs and FSPs.

not just one litter but also the next (two or more likely three years later). Sperm storage is known to occur in several shark species, including in school shark, at least for a few months immediately following the mating season (Walker, 2005). If school shark are storing sperm for use in litters that are two or three years apart, then apparent cross-cohort FSPs (from successive matings) should be treated demographically as if they were MHSPs; if not, they

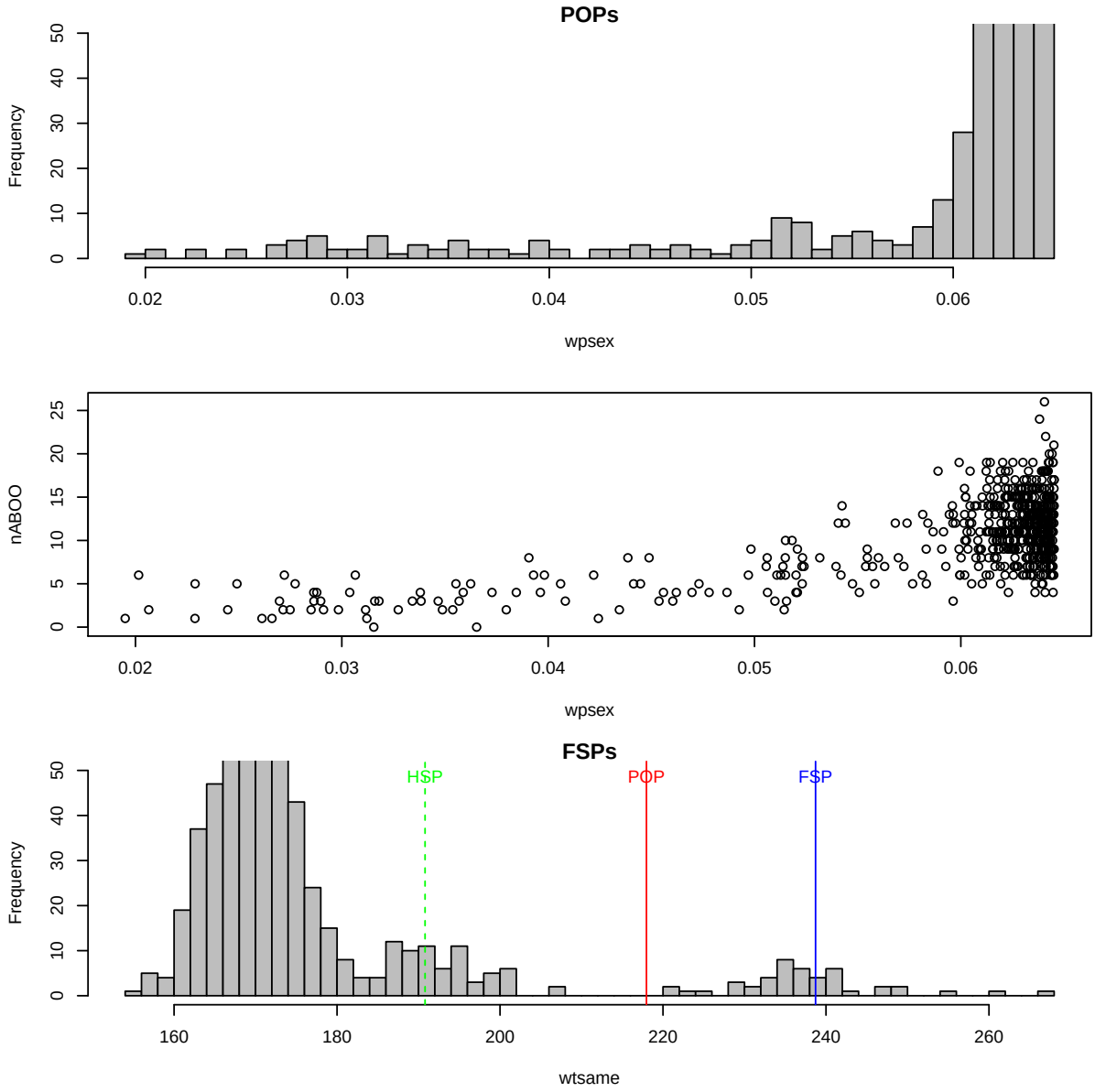

Figure 4.8: The statistic 'wpsex' is shown for the more close related pairs as a histogram (upper plot) and as a scatterplot (middle plot); 'wpsex' is optimised for finding POPs. The wtsame' statistic (optimised for finding FSPs) is shown along with the theoretical values for HSPs (green line), POPs (red line) and FSPs (blue line). The distributions for UPs have been truncated for clarity of presentation.

can be basically ignored in the CKMR model. We have chosen to assume that all the FSPs are same-cohort, and hence that the longer (3–5) year gaps in apparent birth cohort are due to ageing error. If we had chosen to assume that sperm storage occurs, we would somewhat lower the estimated abundance.

The number of FSPs found (34) is surprisingly high compared to the number of HSPs, since there are many more 'mating opportunities' for HSPs compared to FSPs, which must come

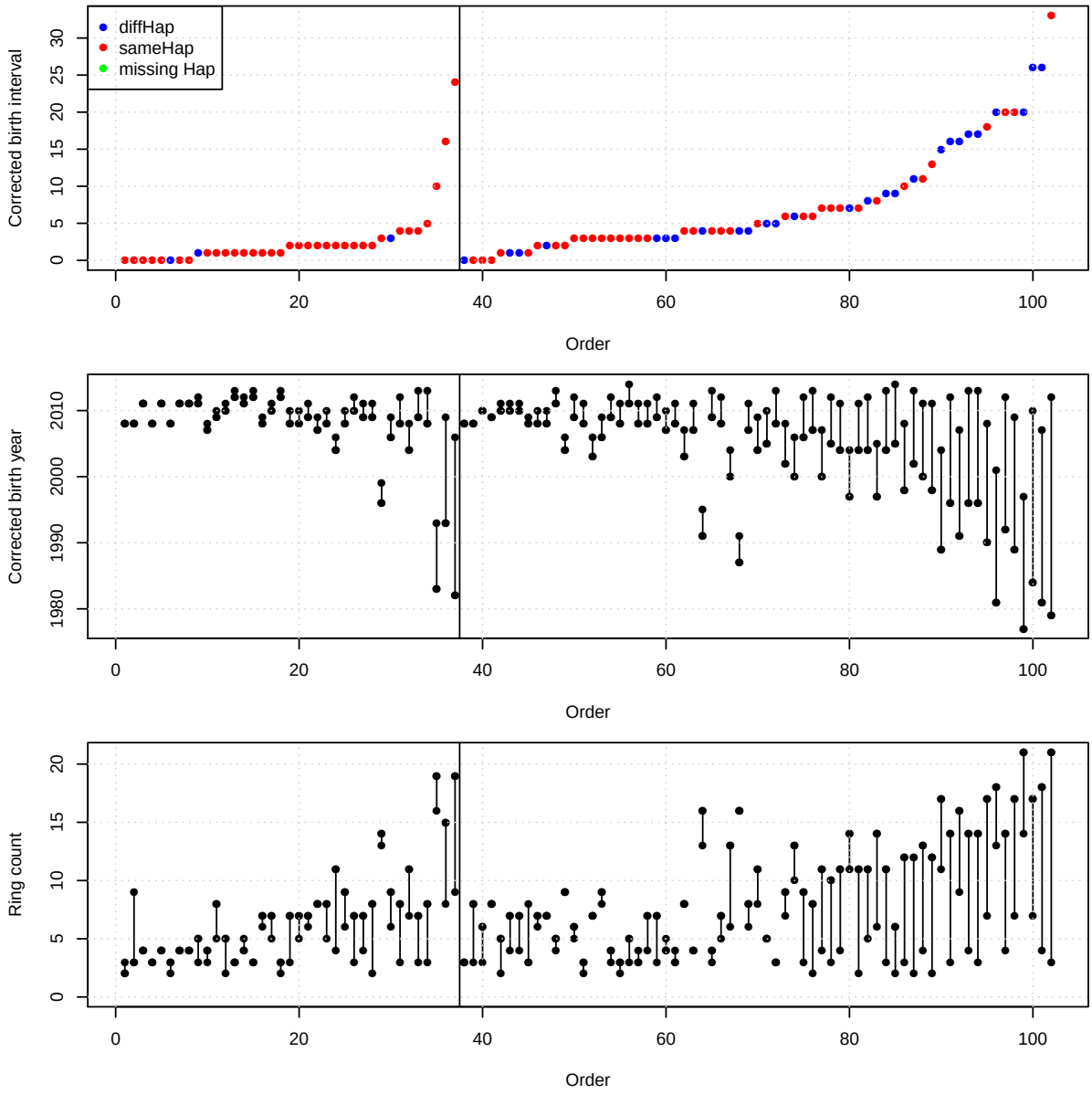

Figure 4.9: Gaps between estimated birth years of each kin pair (corrected for ring deposition rate for those over 11yo), sorted by increasing size (upper plot). FSPs and POPs are shown on the left of the vertical black line; HSPs on the right. Those with the same mtDNA haplotype are shown as black dots, differing haplotypes as red dots. Note three cases of FSPs with apparently different mtDNA haplotypes; these can only have come from mix ups during the mtDNA process – a secondary process involving numerous steps. Also, corrected birth years (middle plot) and ring counts (lower plot) are shown for the animals making up each kin pair.

from a single mating. The discrepancy suggests a substantial 'lucky litter' effect (where some litters have unusually high survival because of favourable environmental conditions, and consequently generate a disproportionate number of within cohort siblings). For this reason, additional parameters are estimated by the close kin model to quantify this 'litter

effect', as well as the proportion of full to half siblings within a litter. Note that school shark, like many animals, can give birth to litters that have been sired by more than one father Hernández *et al.* (2014).

Among the HSPs (right side of upper plot, Figure 4.9), there are some very distant gaps that could be Grandparent-Grandchild pairs (GGPs) instead of HSPs; those two types of kin are genetically indistinguishable. In our CKMR estimates, we have assumed that all detected HSP-like pairs really are HSPs, i.e. we have not incorporated the small additional probability that they might be GGPs. Including some GGPs by accident would have some impact on the CKMR model, so to mitigate that issue (among others) we excluded the oldest animals from HSP comparisons in the close kin model(s), as described later.

Among the younger HSPs, there are more black dots (MHSPs) at a 3-year gap than at 0, 1, or 2 year gaps, perhaps confirming the three year pupping interval proposed by Walker *et al.* (2001). At least some of the MHSPs found at short gaps (0-2 years) may well be same-cohort, which would indicate some low level of multiple paternity within litters (based on the proportion of short-gap MHSPs to FSPs). Hernández *et al.* (2015) provide alternative evidence of multiple paternity in school shark. No birth interval pattern is evident in the blue dots (PHSPs) in Figure 4.9, which is to be expected because males are likely to mate every year.

4.5.5 Triads / families

Interestingly, we found eight 'family groups', or 'triads' in which at least one fish was the sibling of two others. Of those eight families, six comprised one FSP and two HSPs (i.e. sharks A and B share both their mother and father; shark C has either that mother, or that father), one comprised two HSPs (i.e. A has the same mother as B; A has the same father as C; B and C are unrelated), and another comprised three HSPs (i.e. A, B and C all have the same mother but have different fathers) (Table 4.5). Mitochondrial DNA haplotype indicates whether siblings share a mother, and in every family group the haplotype matches and the type of kin pair found match expectation. For example, the fifth group in Table 4.5 comprises an HSP with matching haplotype (sharing a mother), and one with non-matching DNA (sharing a father), therefore the third kin type had to be an UP (neither mother nor father in common), as it is. The sixth family shown in Table 4.5 comprises an FSP (402 and 1782 have the same parents) and an HSP (1782 shares a mother with 2098) therefore 2098 and 402 have to be an HSP, which they are (although not unambiguously so, their PLOD was very close to the cut-off value).

The three fish involved in each family were caught in different fishing trips in all but one case. That case consisted of an FSP that were caught together, and a half-sibling that was caught in a different trip. The proportion of kin pairs involved in triads should be very low in large populations, but increasingly common in small populations; triads are rife in grey nurse shark and white shark, for example, but scarce or totally absent among the 140 HSPs that we found for SBT. However, it would be unwise to over-interpret the modest number of triads that we have for school shark. Overall, 4% of the school shark sample is included in a kin pair of some type, so it is not particularly surprising to see that in some of the kin pairs, one of the sharks happens to occur in another pair.

Triads do not particularly lead to bias in CKMR, but large numbers of them would cause the CV to be under-estimated because pairwise comparisons become non-independent. Getting the CV exactly right is not a critical concern for now, and triads are not overwhelmingly common for school shark; the variance issue will eventually be addressed in future research.

Table 4.5: Eight 'families' of inter-related school sharks, each identified by its sample number. The kin relationship between each pair of animals is half sibling (HSP), full sibling (FSP) or unrelated (UP). Bold type indicates matching haplotypes, and normal type indicates non-matching haplotypes.

	508	1654
29	HSP	HSP
508		**FSP**

	431	2347
59	**HSP**	**FSP**
431		**HSP**

	1027	1962
100	**HSP**	**FSP**
1027		**HSP**

	2077	220
1246	**HSP**	**HSP**
2077		**HSP**

	1609	1738
394	UP	HSP
1609		**HSP**

	2098	1782
402	**HSP***	**FSP**
2098		**HSP**

	1591	2219
472	HSP	HSP
1591		**FSP**

	1324	1852
774	**FSP**	**HSP**
1324		**HSP**

* PLOD=42 just below the cut-off ('eta'=45) for unambiguous HSP status

4.5.6 Location of kin pairs

The distribution of kin pairs shows no regionalization (Figure 4.10). The paucity of kin pairs that include an animal from south of Bass Strait is likely to be a function of the relatively small sample collection from the southern region (Table 4.1 and Figure 4.11). Nevertheless, there are two HSPs and one POP (the parent was caught off western Tasmania) that span Bass Strait.

Lack of regionalization is further illustrated by examining the numbers of kin pairs by shark zone, and the proportion of all comparisons between zones that yield kin pairs (Table 4.6). Only the WBS-WT and EBS-WSA pairings stand out (with 78% and 13% of comparisons yielding kin pairs, respectively), but only one kin pair was found in WBS-WT and only two kin pairs in EBS-WSA so this is probably the result of chance (and small numbers). No kin pairs included one animal from eastern Tasmania. Apart from this, no regionalization is apparent (Table 4.6). Continued sampling will solve the small number problem, and it is clear that more samples need to be sourced from western South Australia (WSA), western Tasmania (WT), and eastern Tasmania (ET). The majority of fishing trips sampled occurred in central South Australia, and eastern Bass Strait (Figure 4.11).

The distance between the capture locations of close relatives largely follows the same pattern as that shown by calculating the distances between every possible pairing of animals sampled

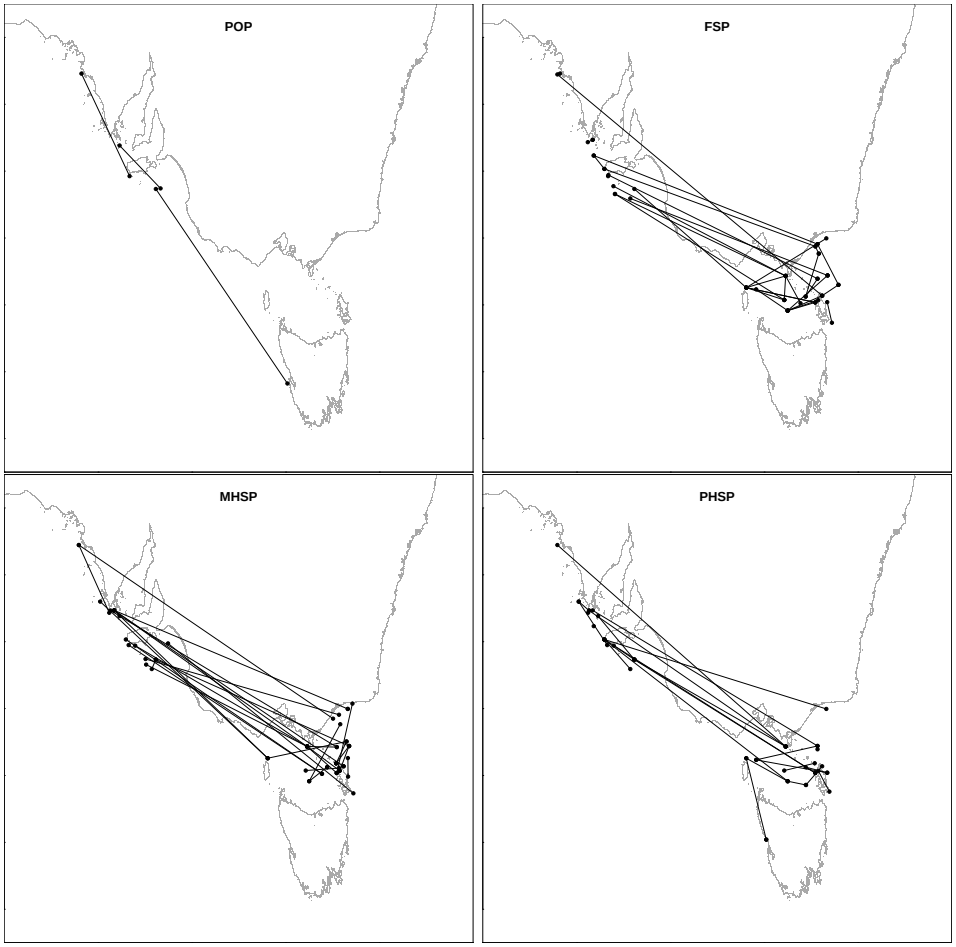

Figure 4.10: Approximate collection locations of the animals found to be parent offspring pairs (POP), full sibling pairs (FSP), maternal half sibling pairs (MHSP), or paternal half sibling pairs (MHSP).

(Figure 4.12) although there seems to be a slight tendency for pairs to be within less than 50km relative to the overall sample.

Members of the fishing industry have noticed that school shark that are caught at greater depths are a different colour from those caught in shallower waters and that they 'just look different'. They have therefore speculated that there might be separate school shark stocks in deeper and shallower waters. Very few sharks were caught deeper than 80m (Figure 4.13) and of those, 10 animals were found to have close kin – always from shallower than 80m (4.14). This seems to weakly refute the hypothesis of stock separation by depth, but more samples need to be collected for a proper investigation. Furthermore, these additional samples should cover areas deeper than 100 and 150m.

Table 4.6: Proportion of comparisons that yielded a kin pair multiplied by 10^5 (plain text); number of kin pairs found (*italic*) and proportion of the total number of comparisons that came from each pairing of zones (**bold**). The shark zones are western South Australia (WSA), central South Australia (CSA), western Bass Strait (WBS), western Tasmania (WT), eastern Tasmania (ET), and eastern Bass Strait (EBS).

Zone	WSA	CSA	WBS	WT	ET	EBS
WBS		2 *1* **3**	**1**			2 *1* **3**
CSA	**1**	3 *10* **17**	1 *1* **8**	3 *1* **1**		1 *6* **18**
WBS		4 *2* **2**	**1**	78 *1* **0.06**		6 *3* **2**
WT		**2**	**1**			**1**
ET		**1**				**1**
EBS	13 *2* **1**	6 *18* **14**	4 *6* **7**	**1**		9 *25* **13**

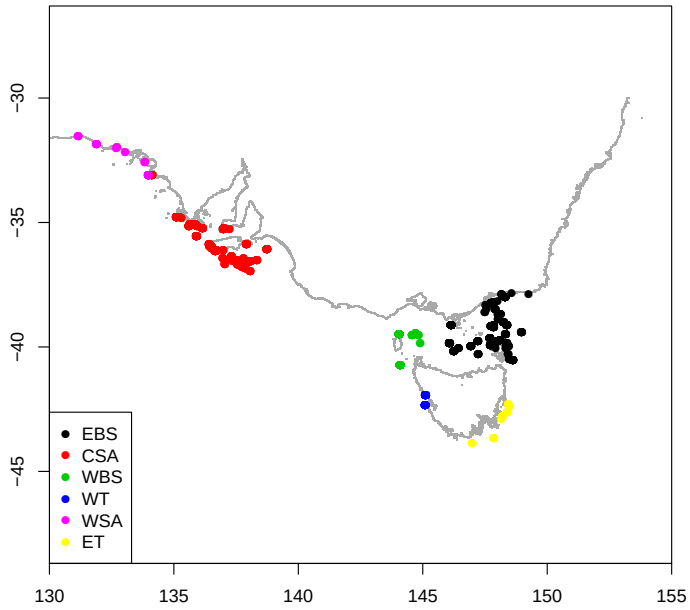

Figure 4.11: Average location of all fishing trips sampled, coloured by shark zone: western South Australia (WSA), central South Australia (CSA), western Bass Strait (WBS), western Tasmania (WT), eastern Tasmania (ET), and eastern Bass Strait (EBS).

4.5.7 Summary of kin-finding

1. The genotyping and kin finding processes worked well for school shark, and there is little ambiguity regarding the identification of the HSPs, FSPs, and POPs. We found 65 HSPs overall, which probably underestimates the true number by about 12% (and this is allowed for in subsequent modelling).

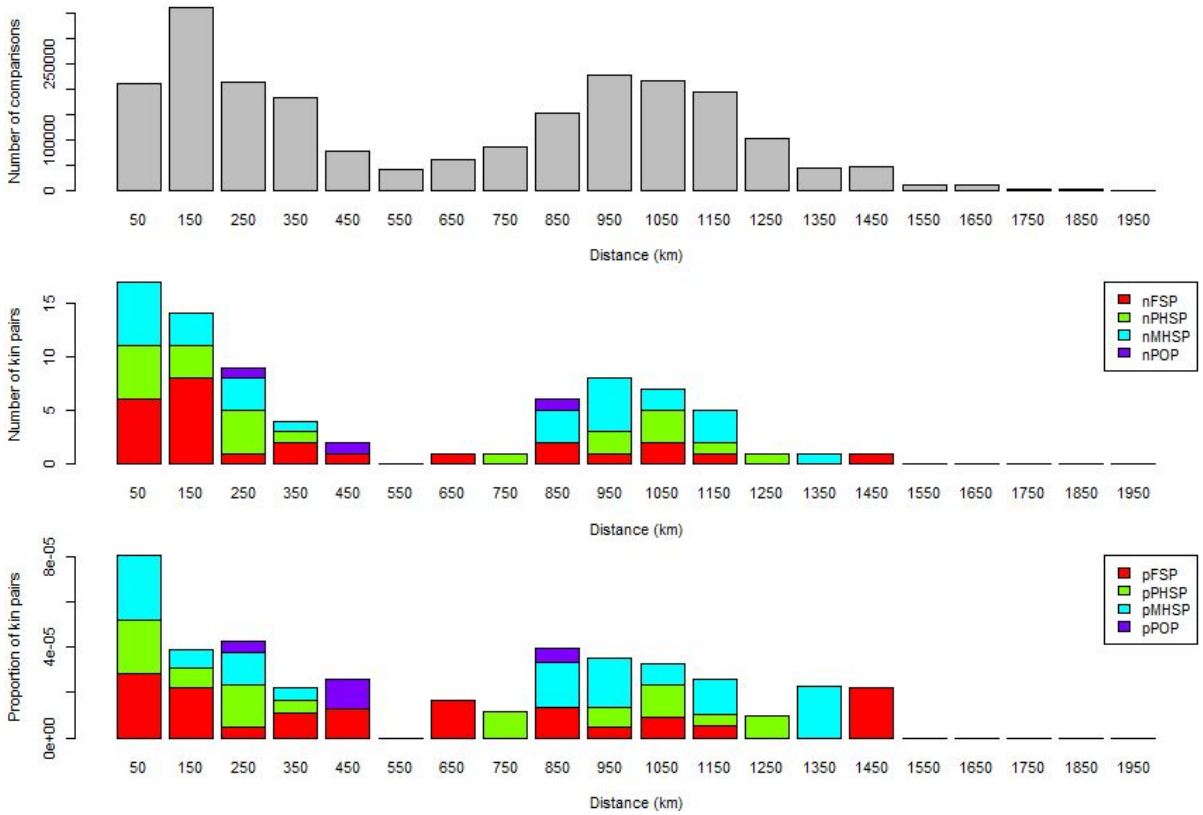

Figure 4.12: Histograms showing the distance (km) between average capture location for (upper plot) every possible pairing of animals sampled; (middle plot) close kin pairs; and (lower plot) the proportion of comparisons that yielded kin pairs.

2. mtDNA data reinforces the HSP-finding conclusion, and reveals substantially more MHSPs (38) than PHSPs (27), consistent with a larger number of adult males, which in turn is qualitatively consistent with males maturing earlier than females.

3. Birth intervals between cohorts (estimated from corrected ring counts) clearly separate three POPs from the 34 FSPs. Assuming random mate choice, the great majority, if not all, of the FSPs must really be same-cohort pairs; however, many estimated gaps are 1-2 years or more, therefore ageing errors are clearly substantial (and this is shown by repeat age readings, Figure 4.2). The ratio of FSPs (same cohort) to HSPs (mostly different cohorts) suggests a strong 'lucky litter' effect.

4. There is a modest level of multiple paternity within litters.

4.6 Simple models

Using the 'Simon and Peter' logic of Section 3.2, it is possible to make a crude estimate of recent adult abundance directly from summaries of the close kin dataset. For this crude method (but not for the more sophisticated close kin model) some assumptions must be made:

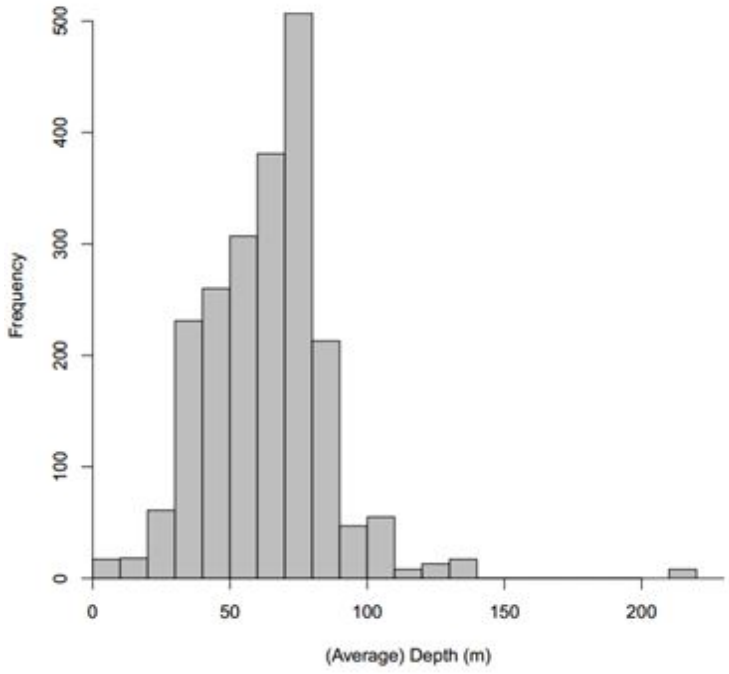

Figure 4.13: Histogram showing the depth of capture for all animals used in the close kin study.

1. that all adults of a given sex are equal in terms of fecundity (this is not far from the truthe, which is that for female school shark it changes from roughly 20 to 30 pups per litter; for males it is unknown);
2. animals that are born in the same year can be identified and, similarly, birth year can be accurately inferred from corrected ring counts;
3. mortality rates do not vary over the model time period; and
4. either there is no trend in abundance, or the log trend is linear.

For the simple models, rather than calculating probability distributions of true age given ring count, we crudely calculated a correct age, and thereby birth year, from ring count. We did this by ignoring ageing error as well as variability in ring deposition rate. We assumed an exact one-to-one correspondence of ring count to age for counts of 1 to 11 rings. For samples that had 12 or more rings, we assumed that one ring appears, roughly, every third year (corresponding to a deposition rate of 0.36 per year). Observed numbers of HSPs were corrected for the false negative loss rate as described in Section 4.5.3.

Some litters might, by chance, have higher survival rates than the norm because of favourable conditions. These will be over-represented in the kin sample because those favourable conditions will prevail for both animals in the same-cohort sibling pair (i.e. the lucky litter effect). To avoid having to estimate additional parameters to correct for this eventuality, same-cohort siblings must be removed from the sample. However, ageing errors make it difficult to identify

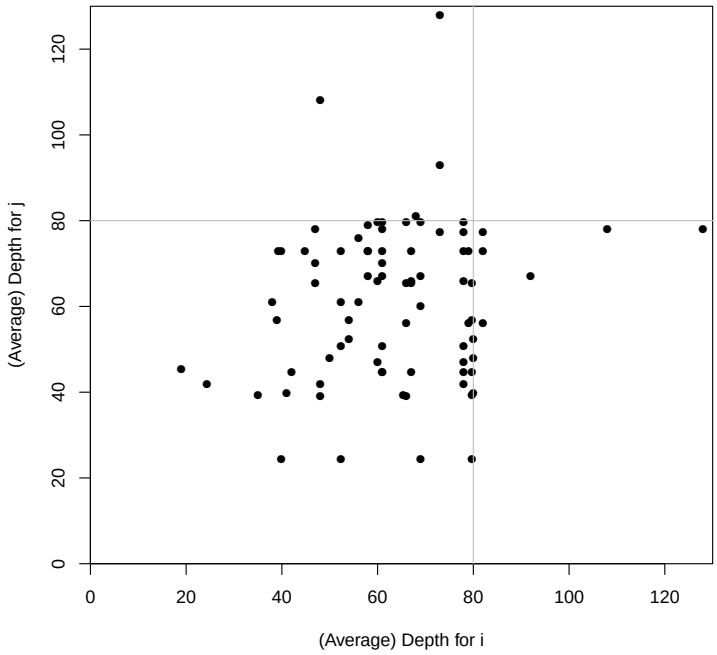

Figure 4.14: Capture depth for animals (named i and j) found to be kin pairs. Each pair is plotted only once, as a single black dot. The average depth for the fishing trip in which the animal was caught is shown (in meters).

these same-cohort pairs. We excluded kin pairs whose nominal birth years were less than four years apart. Had ageing been perfectly accurate, we would only have excluded those born in the same year, but ageing error forced us to use a wider interval. Note that when we removed these pairs, we also removed any comparisons between pairs of animals born less than four years apart.

To improve the accuracy of our corrected birth years, and to restrict the period over time over which our assumptions must apply, we removed animals with more than 15 rings and those born before 2000.

To minimise error resulting from the assumption that mortality rates do not vary during the time period of the simple model, and due to difficulty ageing animals above 11 years old, we used only those animals that had a ring count of 11 or fewer, and excluded those born before the year 2000.

4.6.1 One line calculation

Given the assumptions above, consider a particular maternal half sibling pair: the mother of the older animal is also the mother of the younger animal. The probability that the mother of the older animal would be that of the younger animal, provided she survived the interval between their births, is $1/N^f$ where N^f is the number of adult females in the population in

the year that the younger animal was born.

If there is a gap of t years between the births of the older and younger animals, and the instantaneous survival rate for adults is Z per year, then the mother survives the birth interval at rate $exp(-Z\ t)$. Note that Z is assumed to be constant (i.e. both natural and fishing mortality rates do not change during the time period of this simple model). The overall probability that the mother of the older animal is also that of the younger animal is the probability that she survives, times the probability that she, out of all the living females in the population, is the mother of the younger animal. The probability, therefore that these two animals, born t years apart, is a maternal half sibling pair (MHSP) is:

$$P(MHSP|t) = \frac{1}{N^f} e^{-Zt}. \tag{4.1}$$

In this summarized subset, consisting only of 'recent' cross-cohort comparisons:

- there are roughly 771,000 comparisons, of which 16 yielded MHSPs, and 10 yielded PHSPs;
- the mean difference in birth year within kin pairs is 6.6 years; and
- the mean birth year of the younger animal is about 2011.

Since the mean birth year difference will be biased high because of errors in ageing, we might assume that the real mean difference is closer to four years than six, and consequently that the mean birth year is roughly 2009. Assuming an average adult mortality rate of $Z = 0.10$ (the reason for choosing this value is given in Section 4.6.3 below), and ignoring trends in abundance over the 2000-2016 period, the expected number of MHSPs is *roughly*:

$$771,000 * \frac{1}{N^f}\ e^{(-0.10*4)} \tag{4.2}$$

equating this to the observed total of 16 MHSPs:

$$16 = 771,000 * \frac{1}{\hat{N}^f}\ e^{(-0.10*4)} \tag{4.3}$$

therefore

$$\hat{N}_f \approx 32,300 \tag{4.4}$$

and similarly for males, we get:

$$\hat{N}_m \approx 51,700 \tag{4.5}$$

giving a total of close to 84,000 'typical adults on average' across the 2000s.

This approximation is only possible because the variation in fecundity for female school shark is relatively small (between 20 and 30) whereas for teleost fish the change in fecundity with body size is much more profound and could not be ignored.

4.6.2 GLM model

A more nuanced treatment, allowing for a linear trend (r) in log abundance (starting from N_0^f at the beginning of the model time period), can be obtained by fitting a simple GLM to the reduced dataset. Like the one-line calculation above, the GLM assumed a constant mortality rate of $Z = 0.10$.

$$N_y^f = N_0^f e^{ry}. \tag{4.6}$$

Now the overall probability that two animals are a maternal half sibling pair, given that the older was born in the y^{th} year, and the other younger t years later, is:

$$P(MHSP|t) = \left(\frac{1}{N_0^f \, e^{ry}} \right) e^{-Zt}. \tag{4.7}$$

If samples are taken from a set of individuals, such that n unique pairings can be formed, each of which is a potential MHSP for animals born t years apart, then the expected number of MHSPs from those n pairings is n times the probability $P(MHSP|t)$ above.

This can be viewed as a series of Binomial probabilities where each pairing is a trial with success given by $P(MHSP|t)$, however these probabilities will be very low (for a population of the likely size of the school shark population) and can thus be approximated by a Poisson with expected value

$$n \, e^{-ry-Zt} \left(\frac{1}{N_0^f} \right). \tag{4.8}$$

Because the population size is relatively large, each pairwise comparison can be regarded as independent.

Similar formulae apply for paternal HSPs (PHSPs) but the number of mature males in the population N_0^m might differ from N_0^f even if mortality and birth rates are the same for both sexes (i.e. even if the number of males and females in the population is the same), simply because males mature at a younger age so that a larger proportion of the total number of males will be adults. It is also possible that mortality rates might differ for the sexes as a result of variable fishing mortality rates due to spatial segregation, or differing natural mortality rates. Nevertheless, the trend (r) in the male and female numbers ought to be similar, at least.

We used a GLM to estimate trend (r) and numbers of males and females in year 2000 (N_0^m and N_0^f) given the observed numbers of MHSPs and PHSPs by corrected birth years.

Note that ageing error is likely to lead to systematic over-estimation of birth interval (t) and corresponding under-estimation of the mortality rate Z, but the estimates of trend and absolute population should not be badly affected. Nevertheless, the results of this very approximate method should not be over-interpreted.

Figure 4.15 shows the results of the GLM model and the base case close kin model, which is discussed in Section 4.7. Reassuringly, both are of the same order of magnitude, and so is the estimate of 84,000 from the one line model. This serves as a check for the close kin model, which does not appear to have suffered from any major calculation error.

4.6.3 Caveats for simple models

Note that these simple approaches assume a constant mortality rate that subsumes both natural and fishing mortality ($Z = 0.10$), and that this rate is equal to the natural mortality rate assumed by the stock assessment (Punt *et al.*, 2000; Punt, 2001). If we assume that natural mortality is of the order of 0.1 and that it is constant, then we are effectively assuming constant (and very low, or zero) fishing pressure over the model period. Close kin data provides abundance information for only the adult component of the stock, and the bulk of the catch is taken by gillnet gear, which largely does not catch adult fish. Therefore the assumption of (very) low and constant fishing mortality is probably reasonable.

The simple model assumes that all adults are reproductively equal, which is not true for school shark, whose reproductive output varies from roughly 20 per litter to 30 per litter, so that a younger shark counts as only two thirds of an older adult from a close kin model perspective. This will lead to a slight under-estimation of abundance, but the variation in litter size, and therefore the bias in abundance, should not be huge (certainly not if compared with a teleost fish). The simple models also ignore ageing error, but the effect of that assumption (although complex) also seems unlikely to be huge.

4.7 Close kin model

We constructed a population for school shark that makes none of the four assumptions made by the one line model, nor the three made by the GLM model (Section 4.6). It therefore also requires less restriction of the sample. We

1. explicitly model the increase in fecundity with age for female sharks;
2. estimate extra parameters to account for same-cohort comparisons i.e. the litter effect, and the proportion of full to half siblings within a litter, as well as modelling the distribution of true age as a function of ring count;
3. allow fishing mortality rate to vary during 2000 to 2017 as a function of observed catches (given known gear selectivity); and
4. allow a trend in abundance that is driven by the observed catch data and the productivity of the stock and is not forced to be log-linear.

The close kin related data consisted of the ring count, collection year, sex, and (as the response variable) the relationship (kin) type for each pair of animals. The true age of each animal is imputed within the model, accounting for ageing error and ring deposition rate. We used only a subset of the close kin samples, removing those that had more than 11 rings, both to avoid the most severe ageing ambiguity and to limit the time period that had to be

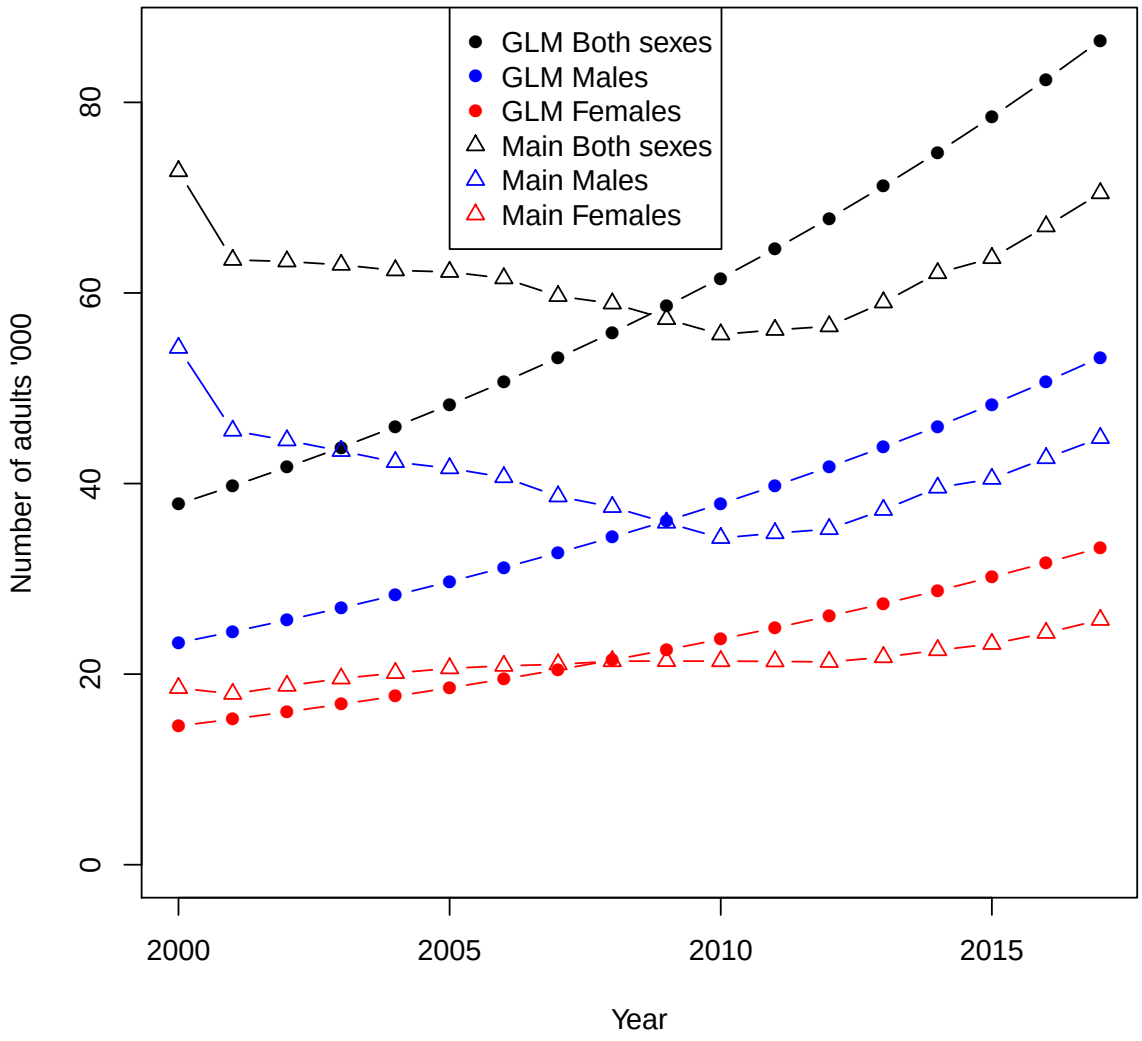

Figure 4.15: Estimated numbers of total (black), female (red) and male (blue) adult school shark from a simple GLM model (closed circles) and the base case close kin model (open triangles, *Main*).

modelled; this is described in more detail below. For all pairs of sampled animals (except within trip comparisons) the probability of the pair being a mother-offspring pair (MOP), father-offspring pair (FOP), full sibling pair (FSP), or maternal or paternal half sibling pair (MHSP or PHSP) was calculated using the idea of Expected Relative Reproductive Output (ERRO), as explained by Bravington *et al.* (2016b). We decided not to consider Grandparent-Grandchild Pairs (GGPs), because the scarcity of POPs in this study suggests that GGPs are unlikely to be common. Although the largest age gaps amongst the HSPs leaves some room for speculation, our sub-setting (i.e. no animals with more than 11 rings) effectively eliminates the possibility of GGPs altogether. Formulae for calculating each kinship type probability are shown in Appendix C.

The close kin model is more realistic than either of the simple approaches, but is nevertheless much simpler than the current stock assessment model for school shark (Punt *et al.*, 2000; Punt, 2001). The close kin model considers only one region, a single population, starts in 2000 (much later than the stock assessment model's 1927 start), does not need to model movement between regions because there is only one region, and has an annual rather than monthly time step. The model is age structured, and it computes the length distribution of the population and can compare that with observed length frequencies, but we have chosen to give the observed length data, effectively, zero weight within the model. The main reason to introduce the complications and uncertainties around seasonal and annual movement, would be to improve the realism of the fit to length frequency data. If length frequency data are not required, and if there really is only one stock throughout the period considered, then the close kin model does not need those extra embellishments. However, we assume that gear selectivity alone adequately captures the vulnerability of each length class to fishing, whereas it is likely that size-specific movement patterns coupled with differing levels of fishing effort across the species' range introduce an availability-at-length component to vulnerability, in addition to gear selectivity. In this, first, application of close kin to school shark we chose to work with a simpler model that did not consider availability in addition to gear selectivity. More elaborate models might be considered in the future (see the Discussion).

4.7.1 Close kin sample restriction

Rings more than 11

Our attempts to construct a close kin model for school shark have encountered great difficulty reconciling the recent close kin data (which support adult abundance of the order of 80,000 adults during 2000-2017) with the historical catches. Catches were very high during the 1980s and require a correspondingly large starting population to support them. If we assume that per capita pup production (i.e. pupping frequency and numbers of pups per female) has remained constant and within the bounds of known school shark biology, then the large population needed to support the catch of the 1980s is not compatible with the smaller population estimate for from the close kin data. If we allow the model the large population in the 1980s needed to support those catches, then the estimated population size after 2000 is such that the estimated numbers of in pairs is lower than the observed numbers. Alternatively, if we allow the model to fit to the numbers of kin pairs (as we did) and try to back project into the 1980s, allowing only the amount of density dependance that is biologically feasible,

then the biomass available to the fishing gear (i.e. population biomass multiplied by gear selectivity) is smaller than the actual catch. The only easy way to allow the current, single population, population model to fit both the early catches and the recent close kin data, would be to allow much greater fecundity in the earlier period than was actually observed at time. Note that much of the biological data collection that underpins the fecundity relationships used in the model were collected in the 1980s and 1990s.

Our close kin model, at least in its current form, assumes that per capita pup production is constant from 2000 to 2017. This assumption can only hold over a restricted time period (e.g. biomass dropped greatly during the 1980s), so that density dependent changes in production may have been occurring at that time. Given the age of our samples, we also have rather little direct data to inform abundance prior to 2000. It is therefore unwise to extend the current close kin model before the year 2000. We achieved this by excluding all samples for animals that were old enough to have been born before that year. The ageing error, and in particular the slow deposition of vertebral rings after age 11, mean that even sampled animals with as few as 15 rings have a non-negligible probability of having been born well before 2000. Therefore we restricted the sample to only those that were younger, and for which aging was unbiased: 11 rings or less. This allowed us to place a plus group at age 20, since there is little chance that an 11-ring individual could be older than 20, and all the age-related fecundity changes in females are thought to have stabilized by age 20. We assumed that natural mortality was constant across ages from age one right through to the plus group. It is likely that mortality is somewhat higher in the first two to three years of life, but as such young animals are seldom encountered by the fishery (and therefore by our samplers) that is of little importance to this study. It is also convenient to compress all reduced mortality into the first year of life where it can be estimated as a single parameter. The mortality rate during the first year of life is an estimated parameter (we estimate the product of first year mortality and female pupping frequency).

Trips

School shark show a tendency to school with individuals of the same size and sex (Olsen, 1954; Walker, 1999). Mark recapture studies require that (re)captures must be independent of one another, so for a CKMR study the capture of an animal must be independent of captures of its close relatives. For this reason, we assigned a trip ID to every fishing trip that was sampled for this study and excluded within-trip comparisons of samples (i.e. no looking for close relatives amongst those animals that were caught together).

Fathers

Male fecundity is of no relevance to conventional stock assessment models and has therefore been little studied in fisheries science, therefore the fecundity at age (or size) relationship for male school shark is poorly known, whereas it is known for females. Since any source of systematic variation that is not captured by the close kin model will lead to bias in a purely HSP-based close kin model, there is some risk in assuming an incorrect fecundity relationship for males. Therefore although our base case model considers fathers as well as mothers, we include a sensitivity to ignoring them, and also present a model that uses close kin pairs

that involve fathers (i.e. FOPs and PHSPs) to estimate trend in abundance, but do not allow them to influence overall abundance. These sensitivities showed similar results to the base case model, but the base case has much lower CV because all the close kin data is used (Section 4.7.3).

For the other (non-commercial) shark species where CSIRO has fitted close kin models, we assumed that there was negligible variability between males in reproductive output (i.e. all mature males have the same reproductive output) and that assumption seemed compatible with the available data. However, those species all have smaller litter sizes and are taxonomically quite different to school shark. For school shark, we assumed a male maturity ogive given by Walker (2005), see Section 4.3 of this report, but note that maturity of reproductive organs does not necessarily relate closely to successful paternity. Members of the fishing industry have noticed that larger male school shark are found at the centre of breeding aggregations whereas smaller, nevertheless mature, male sharks are on the periphery and also that the breeding males have injuries consistent with intense fighting. Smaller males, although mature, might not be successful breeders. Similarly, CKMR for SBT using sufficient POPs to estimate fecundity relationships, showed that older female SBT are even more reproductively successful compared with younger females than was suggested by counts of the numbers of eggs contained in their ovaries (Bravington *et al.*, 2016a).

Give sufficient father-offspring pairs (FOPs), we would be able to directly estimate male fecundity as a function of body size (as has been done for SBT). However, having found only one FOP, we do not have sufficient data, at this time, to estimate fecundity across the age range. We assumed a 50:50 sex ratio at birth, and that the natural mortality rate is the same for male and female sharks. Fishing mortality was imposed by the observed catches, under the assumption that the catch was made up equally of males and females (see Section 4.2.1 for justification), and we used separate (although very similar) growth curves for males and females to relate catches, via the gear selectivity function, to catches at age. The sensitivity that ignores fathers is therefore able to infer male numbers at age in the population from the information for females, given these assumptions.

Effect of sample restriction

Using only animals with 11 or more rings reduced the sample from 2,438 to 1,627 animals, and removed the three POPs as well as 9 of the 38 MHSPs as 14 out of the 27 PHSPs. By not comparing animals that were caught together, we lost 8% of all comparisons as well as 8 FSPs and 1 HSP. The model that ignores fathers does not use the 13 PHSPs that remain after the 11 ring restriction has been applied, but does use the 24 MHSPs.

4.7.2 Model structure and sensitivities

Length at age

The CV for ageing error is assumed to be 0.08 (see Section 4.1.2) until age 11 and after that we assume twice that CV (0.16) to allow for uncertainty in ring deposition rate after the age of 11. Ring deposition rate is assumed to be one p.a. up to age 11 and 0.36 thereafter.

Note that even though we excluded animals with more than 11 rings, animals that are older than 11 can have as few as 11 rings and are therefore part of the model (Figure 4.16). We use a plus group for ages 20 or greater. Even an upper limit of 11 vertebral rings resulted in a non-negligible probability that such an animal is aged 20 or more (Figure 4.16). This indicates that animals with more rings could not have been used without increasing the plus group age and therefore applying the model assumptions (chiefly that density dependence is unchanged) for a longer period of time.

We specified weight and selectivity as functions of length, and then integrated over length-at-age to derive weight-at-age and selectivity-at-age functions. The integration used the von Bertalanffy (i.e. length-at-age) relationships for males and females and required specification of variability in length for each age class. We took those CVs from the stock assessment (Punt *et al.*, 2000; Punt, 2001) but we noted that variation decreases with increasing age (Table 4.7). That suggests that the CVs were calculated using tagging data and an assumed upper length value (presumably the Linf value from the von Bertalanffy growth curve). We suggest that future work look at the sensitivity of the model to assuming a more realistic increase in CV with increasing age. If the model is found to be sensitive to this assumption, and if the original tagging data can be obtained, we recommend recalculating the CVs without the upper length constraint.

Table 4.7: Coefficient of variation (CV) assumed by the school shark stock assessment model by age group and sex.

Age	Female	Male
1	0.191	0.190
2	0.191	0.190
3	0.191	0.190
4	0.168	0.168
5	0.146	0.146
6	0.132	0.132
7	0.117	0.119
8	0.107	0.109
9	0.096	0.098
10	0.087	0.090
11	0.078	0.082
12	0.071	0.075
13	0.063	0.068
14	0.058	0.063
15	0.052	0.057
16	0.048	0.053
17	0.043	0.048
18	0.040	0.045
19	0.037	0.041
20+	0.034	0.039

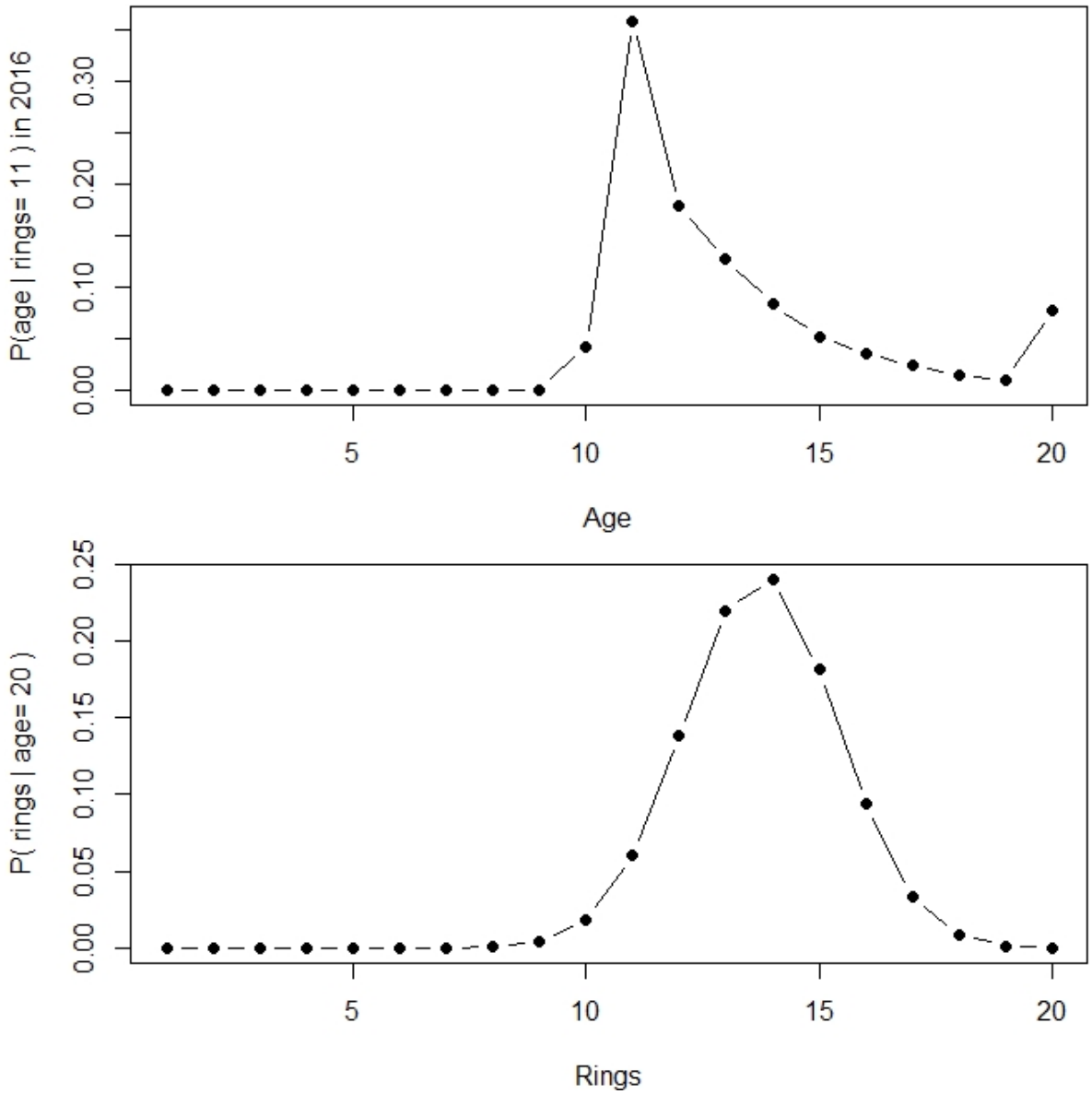

Figure 4.16: The probability distribution of true ages for an animal that has 11 vertebral rings (upper plot) and the probability distribution for the number of rings that an animal aged 20 (the youngest plus group age) will be observed to have in 2016.

Kin probabilities and likelihood

The procedure for calculating the probability that any pair of animals belongs to each kin type, and the likelihood equations, are described in words below and the equations are given in Appendix C.

When considering whether a pair of animals (where the older one is female) might be a MOP, we must first work out in which year the younger animal was born, and whether the potential mother was mature in that year. If she was, then the probability that she is the mother of the younger animal is roughly $1/N_f$ where N_f is the number of mature females present in the population in the year that the younger animal was born. To be more exact, because female fecundity varies with age, the probability depends on her Expected Relative Reproductive Output (ERRO) compared to the total ERRO across adult females; i.e., her fecundity given her age, divided by the total fecundity across all living females that year. The same procedure is used for FOPs, but using the male fecundity-at-age relationship.

Because the actual birth year for any school shark in our study is clouded by ageing error, we integrated over all possible birth years, weighted by the probability that each was the actual birth year for that animal (given the observed ring count and the degree of ageing error).

To calculate the probability that a pair of animals might be a maternal half sibling, the mother of the first animal must also be the mother of the second. Therefore she must have been mature when the older animal was born (y_1), and must have survived until the second animal was born (y_2). The probability that the mother of the first is also the mother of the second (if all adult females are reproductively equal) would be the inverse of the number of mature females present in y_1, multiplied by the survival rate for females of this age between y_1 and y_2. However, since female fecundity increases with age in school sharks, this formula must be modified to account for the likely increase in reproductive output of a female of given age in year y_1 and year y_2. As with POPs, it is also necessary to integrate over all probable birth years, given ageing error and age uncertainty.

Because of ageing error, we cannot simply exclude same-cohort comparisons based on most likely birth year. Instead, for animals born in the same year (some HSPs and, by assumption, all FSPs) we had to allow extra parameters to account for: (1) litter effect, the inflated number of surviving siblings pairs in certain litters where favourable conditions occurred, and (2) the proportion of animals within a litter that share a father. Female school sharks can mate with multiple males to produce a single litter consisting of both full and maternal half siblings (Hernández *et al.*, 2014). This 'multi-mate' parameter scales the number of full to half siblings observed. The 'litter effect' parameter scales the numbers of same- versus different-cohort siblings observed.

The log-likelihood for the close kin component of the model is straightforward, in principle. Provided that sampling is fairly sparse compared to the population size (as will be the case when population size is fairly large, see Bravington *et al.* (2016b), Section 4), then it is statistically reasonable to treat all pairwise comparisons as approximately independent, with each comparison constituting a Bernoulli trial (i.e. a yes/no outcome) whose probability is determined by the demographic parameters (those that are assumed to be known and those that are to be estimated). The complication of family groups (triads) and known FSPs does invalidate the independence assumption, strictly speaking, but does not generally cause bias

(as explained by Bravington *et al.*, 2016b).

The kin probability calculations are lengthy, particularly when age is uncertain, but the underlying biological principles are quite clear and transparent. Every animal has one mother and one father; the chance of the mother being one particular female (in particular, the parent of another specific animal) is that female's expected reproductive output divided by the total reproductive output from all females at that time and place.

The model parameters are estimated by maximizing the joint log-likelihood from all the pairwise close kin trials. See Appendix C for more detail.

Population dynamics and estimable parameters

The population dynamics model is described in detail in Appendix C but is outlined briefly here. The model starts in the year 2000, with initial age composition that year being determined by three estimated parameters. The estimated values of those parameters are not presented because their interpretation is not straightforward or particularly meaningful. Annual fishing mortality rates for males and females, for each of five gear types (line combined with trawl, and four sizes of mesh nets) are calculated from the total catches (in tonnes) for each gear type, the gear selectivities-at-age, and the weight of sharks of each sex in each age group according to the population model. We first applied half of the natural mortality for the whole population, then sequentially calculated fishing mortality rates for each gear type. We applied the fishing mortality rate for each gear before calculating the fishing mortality rate for the next gear, and finally applied the remaining half of the natural mortality. This gave us survival probabilities, by sex and age group between years, that are needed for the close kin probability calculations (Section 4.7.3).

Recruitment to the stock occurs at the beginning of each year (which corresponds to January and is consistent with school shark reproduction). Recruitment was given by the sum of the expected numbers of pups born to mature females across all ages, present in the previous year, multiplied by a joint pup survival rate and pupping frequency parameter. That parameter was estimated and was applied to all years after 2000. Note that by not using that parameter to establish the numbers at age in the first year, we effectively allow differing productivity prior to 2000. Productivity between 2000 and 2017 remains fixed.

The close kin model has eight estimable parameters (Table 4.8).

Extending the model back in time

A concerted effort was made to extend the close kin model further back in time, prior to the year 2000. It was hoped that by explicitly modelling density dependence as a function of the number of embryos produced (as was done by Punt *et al.*, 2000) the dynamics of the population during the 1990s could be more clearly described, and could then be extended further back in time. The model was unable to sustain the catches during the 1990s, and match the observed numbers of kin pairs. The catches of the 1990s are too high to be sustained by a population of the size indicated by the (more recent) close kin data.

Two alternative formulations were explored. One uses the model shown here, but with pup

Table 4.8: Description of the eight estimable parameters used in the close kin population dynamics model.

Symbol	Description
M	Natural mortality rate for all animals aged ≥ 1 year
N_{89}	Number of animals in 1989 (used to establish numbers at age in the year 2000)
τ, F_{89}, F_{90s}	Parameters that govern age distribution during the first year
δ^{2000s}	Pupping interval multiplied by mortality during the first year (which incorporates density dependence)
ν_1	Litter effect, allowing for 'lucky litters'
ν_2	Proportion of the litter that are likely to have different fathers (a value of 1 would mean that every litter has just one father)
q_{father}	if estimated, then fathers do not inform abundance; if fixed at 1 then both fathers and mothers inform the abundance estimate)

survival (i.e. the number of embryos that reach age 1) described by a density dependent relationship where more pups results in lowered overall pup survival. This assumes that pups compete with one another for food in the pupping grounds. The constraints introduced by this formulation (many variants of which were trialled; not shown) resulted in a poor fit between the observed and expected numbers of kin pairs. That mismatch invalidated those models. It was not possible, even using that formulation, to sustain the catches of the 1990s.

A second formulation, that used a much more flexible 'hockey-stick' functional form to describe the numbers-at-age in 1989, was also not able to adequately describe the school shark population. We therefore continue to use the model that, effectively, begins in 2000.

4.7.3 Base case and Sensitivities

First, we consider the model that uses MOPs and MHSPs, and not FOPs or PHSPs (*Mothers* in Table 4.9), and contrast that with the version that does allow fathers to influence abundance (*Base case*). The 29 MHSPs already used in the model are joined by 13 PHSPs; this results is lower CVs for the estimated abundance (discussed below). Note that even though the restriction to use only animals that had 11 or fewer rings eliminated all the observed POPs, the model nevertheless calculates the likelihood of any (and every) pair of animals that were sampled being a POP, and is conditioned on the observation that none of them were POPs. We used the base case for future projections (Figure 4.17).

The *Mothers* model achieves a good match between total numbers of each kin type observed and expected numbers (Table 4.9). Note the expectation that 1.7 FOPs should have been observed but none were. This is easily ascribed to chance, however it is also consistent with the idea that the young males included in our study were not as fecund as the maturity of their claspers suggests. When fathers were included in the likelihood, the match between expected and observed numbers of MHSPs degrades somewhat, but is still acceptable (*Base case* in Table 4.9). Allowing the model to estimate a constant of proportionality for PHSPs (q_{father}), which prevents that dataset from informing abundance, improves the fit and results in a value of 0.82 for the constant (q_{father} in Table 4.9 and Figure 4.17). That value seems

Table 4.9: Estimated parameters, negative log likelihood (*-lnL*) and estimated numbers of M/FOPs (*nM/FOP*), FSPs (*nFSP*) and M/PHSPs (*nM/PHSP*). Observed numbers of kin pairs are shown in parentheses in the first column. A dash indicates a parameter not included in a model, and a * indicates a fixed rather than estimated parameter value. The parentheses in the *Mothers* column indicate that kin pairs involving fathers are calculated but not included in the likelihood.

Quantity	Mothers	Base case	est q_father	CPUE	No W/NSW
M	0.11	0.09	0.08	0.09	0.09
$N_{89}('000)$	140	114	100	185	101
δ^{2000s}	0.21	0.15	0.25	0.17	0.21
ν_1	4.5	5.8	5.7	6.5	6.0
ν_2	2.2%	1.8%	2.5%	3.2%	2.1%
q_{father}	–	1.0*	0.82	1.0*	1.0*
-lnL	816.8	982.4	982.2	982.0	982.0
nMOP (0)	0.6	0.6	0.6	0.4	0.6
nFOP (0)	(1.7)	1.6	1.7	1.1	1.6
nFSP (33)	33.0	33.0	33.0	33.0	33.0
nMHSP (29)	26.4	23.7	24.8	12.7	23.7
nPHSP (13)	(15.1)	14.6	13.0	14.6	15.0

sufficiently close to 1 to justify our fixing its value at 1 for the base case model, thus reducing the CV on estimated abundance (discussed below).

Estimates of natural mortality are very close to the value of 0.1 that was assumed (but not estimated) by the stock assessment model (Punt *et al.*, 2000). The parameter δ^{2000s} is made up of the product of the pupping interval (0.5 but more likely 0.33) multiplied by survival during the first year of life (likely to be lower than that of older animals i.e. $< exp(-0.1) = 0.9$), giving a likely upper limit of $0.9/3 = 0.3$. Reassuringly, none of the estimates exceed this limit, and they correspond to survival rates during the first year of life that range from 0.80 to 0.30 (if pupping occurs every third year) which is not inconsistent with the findings of McAllister *et al.* (2015). They observed 40% initial survivorship (which included, possibly high, tag mortality) followed by 75% survivorship for young juveniles.

Estimates of the litter effect parameter were high (ν_1 ranged from 4.5 to 6.0, Table 4.9), which seems reasonable given the large numbers of sibling pairs born close together, and the large number of FSPs observed. The proportion of litter-mates that have different fathers (ν_2) is very low, which is credible given the number of FSPs observed but is lower than that implied by Hernández *et al.* (2014) who found multiple paternity in two out of five litters examined from New Zealand school shark. The sample size used by Hernández *et al.* (2014) was small, resulting in an imprecise estimate of multiple paternity rates. Also, their estimate was for New Zealand, not for Australia, nevertheless the apparent disparity is interesting and might be considered further in future school shark close kin models.

We fitted the model to the observed standardized CPUE for the trawl fleet (Sporcic & Haddon, 2018), by associating that with the combined trawl and line fleet used by the model. Trawlers have never targeted school shark so their catch rates might index abundance (al-

though questions remain regarding their difficulties in accessing quota and consequent high discard rates). Rather than estimating q_{CPUE} (the constant of proportionality that relates available biomass to standardized CPUE) we calculated the least squares estimate (Polacheck *et al.*, 1993). We assumed a standard deviation of 0.1 for the CPUE time series, based on that calculated by Sporcic & Haddon (2018, Table 27). Only the post 1999 part of the time series was used. The correspondence between the standardized trawl CPUE series and the expected series is poor, with the observed CPUE showing a steeper increase than that of the base case model (Figure 4.18). The sensitivity test that fits to the CPUE data achieves a good fit to the CPUE (Figure 4.18) but at the cost of the fit to the MHSPs (12.7 expected versus 29 observed, Table 4.9). The MHSPs are likely to be the most reliable and informative data that the model has, being more numerous than the POPs, and easier to interpret than the PHSPs due to better information on fecundity. FSPs always belong to the same cohort and are therefore subject to the 'lucky litter' effect (Thomson *et al.*, 2018); for that reason FSPs primarily inform only the estimation of the size of the 'lucky litter' effect. The model achieves a good fit to the CPUE data by assuming a plus group in 1989 that is several hundred times bigger than that of the base case, with correspondingly larger recruitment at that time (Figure 4.19). This results in faster growth in abundance due to the influence of the more fecund older fish (Figure 4.17). There is no independent evidence for the presence of those fish and an earlier attempt to fit the close kin model to length frequency data (Thomson *et al.*, 2018) suggested that older fish were in fact less abundant in the catches than suggested by the base case model, not appreciably more abundant.

Ignoring catches from the far west and NSW (*no W/NSW*) makes very little difference relative to the base case, because few catches have been made in those regions in recent years.

We also examined a sensitivity (not shown) that estimated a separate natural mortality parameter for the plus group (animals aged 20 and over) but this parameter was estimated to be unrealistically large (effectively killing all animals in the plus group) and was numerically unstable. A higher natural mortality rate, perhaps from age 30 or greater, might have been more realistic.

Adding PHSPs to the model increased the amount of close kin data and therefore reduced the CVs for the annual estimates of mature biomass (Table 4.10). CVs are higher for the most recent years because those are not directly informed by the close kin data. Standard errors (SE) for the trend, defined as the change in abundance between 2000 and 2010, and between 2010 and 2015, are very large relative to the size of the trend (Table 4.11) indicating that the model has insufficient samples from which to precisely estimate recent trends in abundance. We chose to consider population increase from 2010 because that was the year when the rebuilding plan was first implemented. We consider population increase up to 2015 because after that year the close kin data are not informative.

4.7.4 Comparison of abundance estimates

Estimated numbers of adult school shark are substantially lower than from the full assessment model (Thomson, 2012). The parameters of that model were estimated using data to 2008, and the model was projected forwards using observed catches for 2009-2011, and assumed constant 225t catches from 2012 onwards (Figure 4.20), which is similar to the actual catches

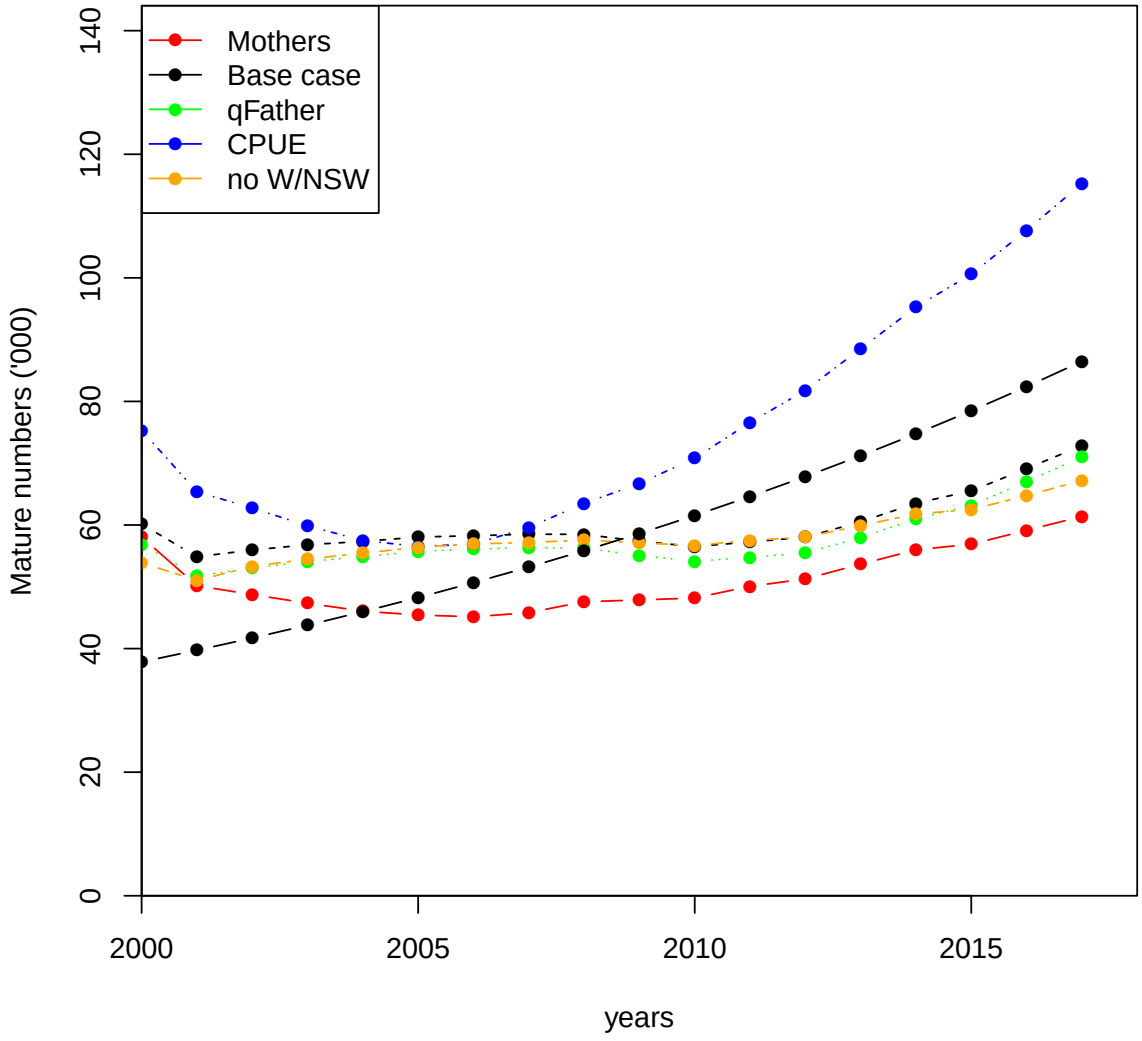

Figure 4.17: Numbers (thousands) of mature school sharks for the range of models shown in Table 4.9.

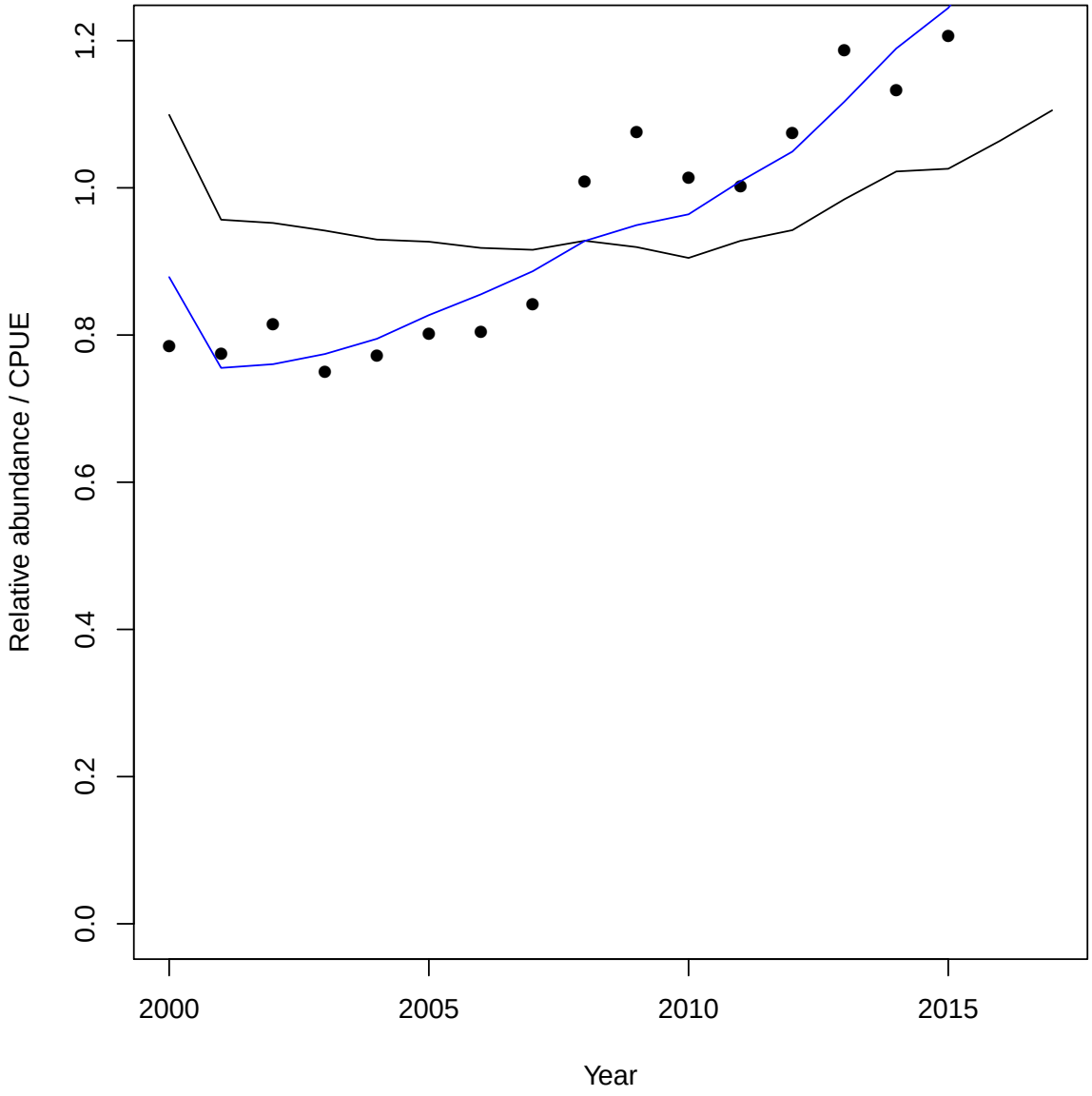

Figure 4.18: The standardized trawl CPUE index (dots) and the model expected values for the base case model (black line) and the sensitivity that is conditioned on the CPUE data (blue line).

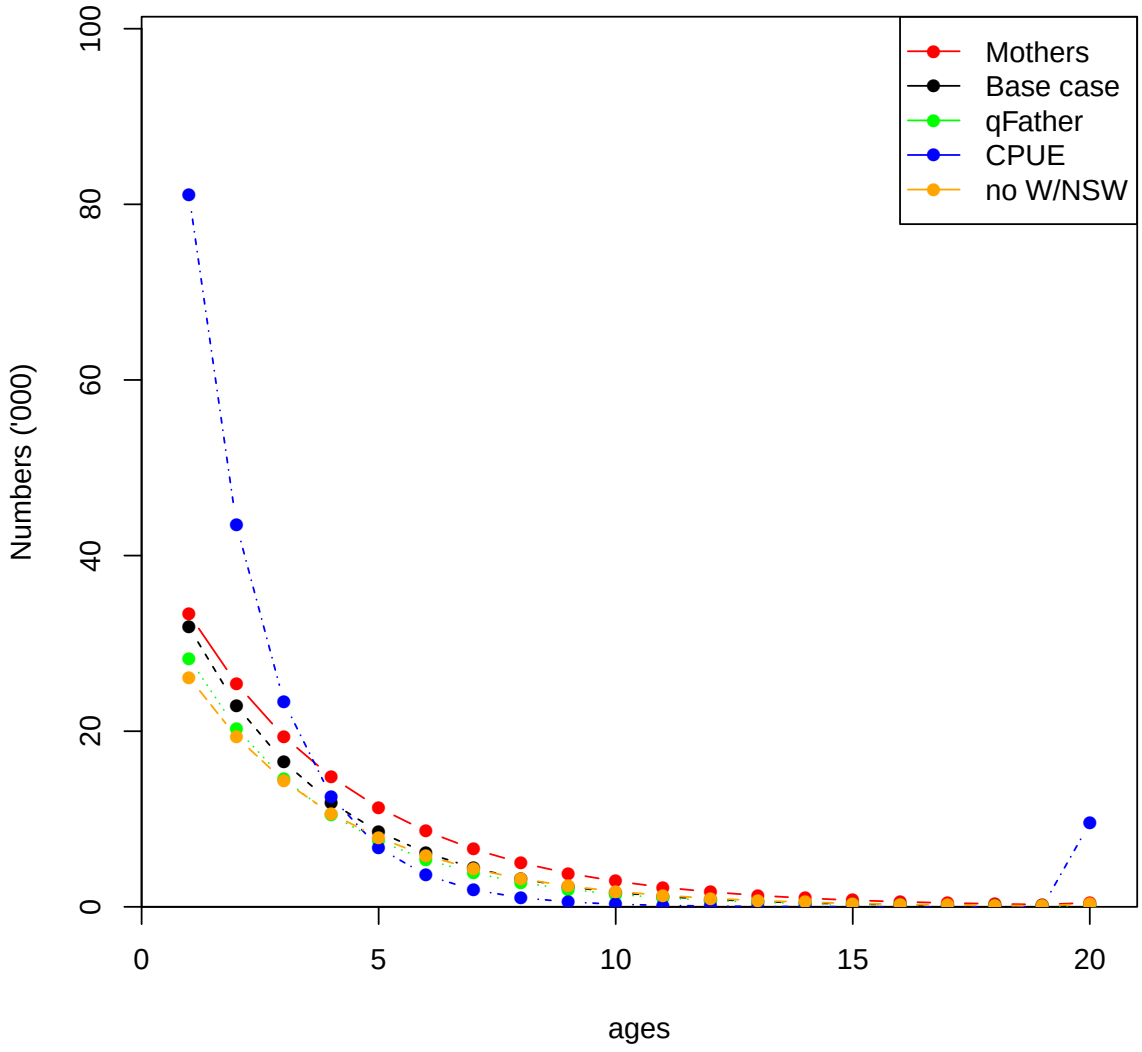

Figure 4.19: Numbers (thousands) at age in 1989 for the range of models shown in Table 4.9.

Table 4.10: CVs for the number of mature school shark between 2000 and 2017. Results are shown for the base case model without PHSPs (*Mothers*), the base case (*Base case)*, and the model that fits to trawl CPUE (*CPUE*).

Year	Mothers	Base case	CPUE
2000	0.29	0.27	0.29
2001	0.32	0.26	0.29
2002	0.34	0.23	0.28
2003	0.36	0.23	0.28
2004	0.38	0.24	0.27
2005	0.40	0.26	0.26
2006	0.39	0.27	0.25
2007	0.37	0.27	0.24
2008	0.33	0.25	0.24
2009	0.32	0.25	0.25
2010	0.34	0.26	0.27
2011	0.36	0.28	0.30
2012	0.40	0.32	0.34
2013	0.46	0.38	0.37
2014	0.52	0.44	0.42
2015	0.62	0.52	0.49
2016	0.71	0.61	0.55
2017	0.81	0.70	0.62

Table 4.11: Percentage increase in mature abundance from 2010 to 2015, and standard error (SE) in parentheses, for a subset of the models shown in Table 4.9.

Year	Mothers	Base Case	CPUE
2000-2010	-4.5% (0.54)	-2.6% (0.49)	-2.2% (0.48)
2010-2015	2.7% (0.46)	2.1% (0.40)	8.4% (0.88)

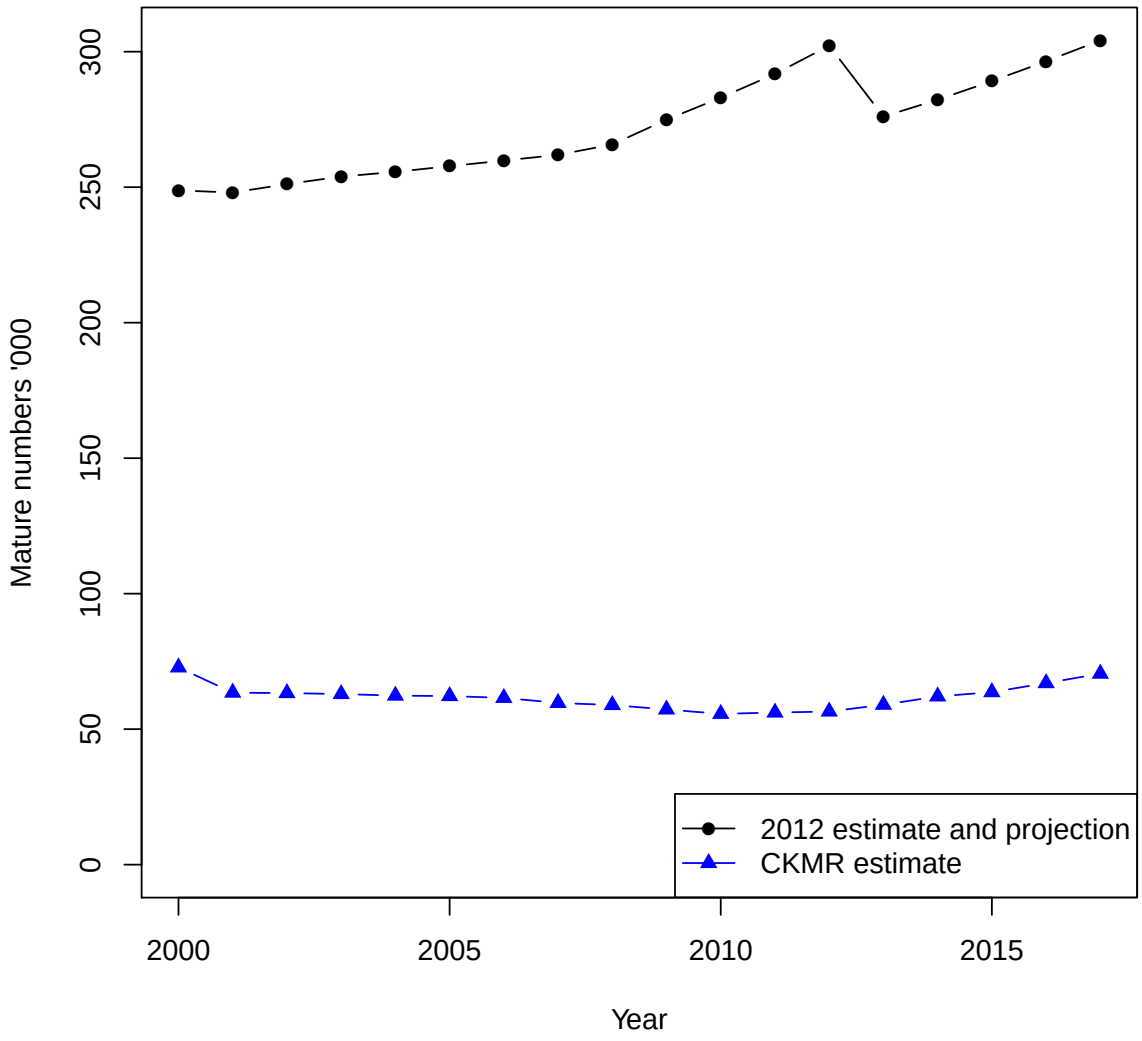

Figure 4.20: Numbers of mature school shark (females aged 11 and over plus males aged 7 and over) for the full stock assessment model's base case that assumed catches of 225t after 2011 (2012 estimate and projection; black line) and base case close kin model estimate (blue line).

taken.

The Recommended Biological Catch (RBC) for school shark is zero because its abundance is below 20% of pristine, but because some catch of school shark is unavoidable while fishing for gummy shark, a low level of catch that nevertheless permits rebuilding, is permitted. To prevent targeted fishing for school shark, they must be landed at a ratio of no more than 1:5 with gummy shark. Members of the fishing industry report great difficulty in avoiding school shark while fishing for gummy shark. This can appear to contradict the idea that school shark stocks are greatly depleted whereas gummy sharks stocks are healthy. Of course, school shark are less productive than gummy shark and the fishery initially targeted school shark, only switching to gummy shark after the high mercury content of school shark flesh became known (Kirkwood & Walker, 1986) as well as because of falling school shark abundance. It is possible for the school shark stock to be more depleted than gummy shark, but having had a larger initial population can therefore nevertheless be relatively abundant compared with gummy shark. To illustrate this point, we calculated the ratio of the numbers of school shark (from the close kin base case model) to gummy shark (from the most recent stock assessment Punt *et al.* (2016)), Figure 4.21). We calculated this ratio for those sharks that are available to 6 inch gillnet gear (using the selectivity functions in both models) and those that are aged 2 and over (a proxy for those available to the line and trawl gear). The ratio for gillnets is very close to the 20% required by management, and that for line and trawl gear ranges from 0.17 to close to 0.2 over the time series. It not surprising, therefore, that industry has to work hard to ensure that they never exceed that ratio as there is little or no margin for error.

4.7.5 Projections

Future catches

We projected the base case close kin model 20 years into the future, assuming constant future exploitation rates equal to (a) zero; (b) the 2016 exploitation rate; (c) the relatively high 2017 exploitation rate; or (d) the average exploitation rate over the most recent five years (2013-17) (Figure 4.22). By assuming fixed exploitation rates instead of fixed future catch, we allow the catch to increase each year in response to the recovery of the stock and consequent increase in unavoidable bycatch. Note that the wide confidence intervals mean that the median catches are no guarantee of sustainability (Figure 4.23, and an expanded version 4.24). Ongoing collection of close kin samples should greatly increase the precision of our estimates.

Ongoing close kin monitoring

We can use CKMR as an ongoing monitoring tool for school shark, and in so doing can continue to reduce the variance on estimates of abundance and trend. Table 4.12 shows the expected standard error (SE) on trend in the abundance of mature animals over 2010 to 2015 if we continue to monitor the stock for an additional four years, given annual samples of between zero and 700 animals. The corresponding SE for abundance in the final year is consistently higher, as you would expect, because close kin is always poorly informative for the most recent year. Nevertheless, even that SE does greatly reduce over time given

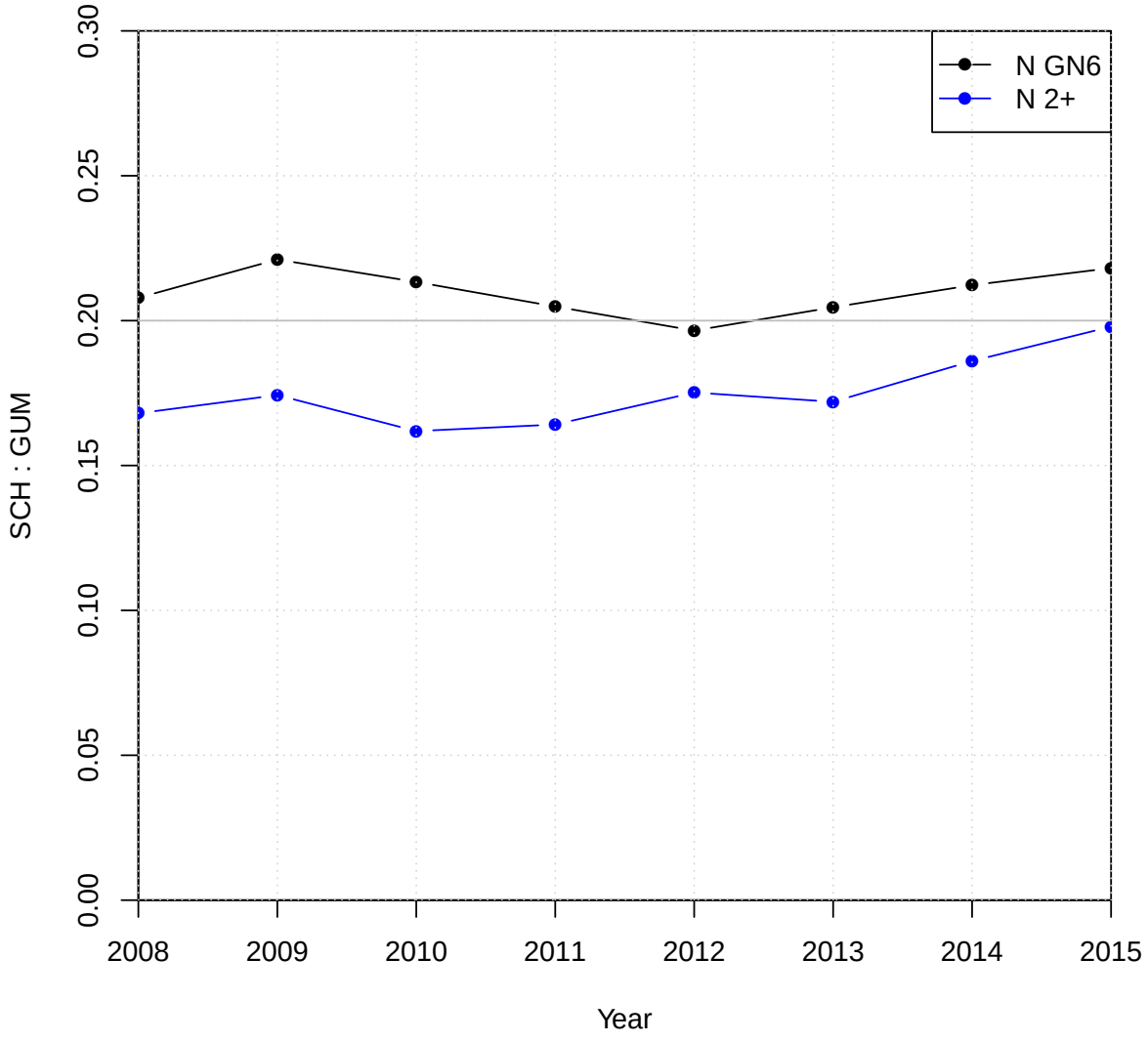

Figure 4.21: Ratio of school shark to gummy shark that are available to 6 inch gillnets *N GN6* and that are aged 2 and over *N 2+* (a proxy for those availably to line and trawl gear).

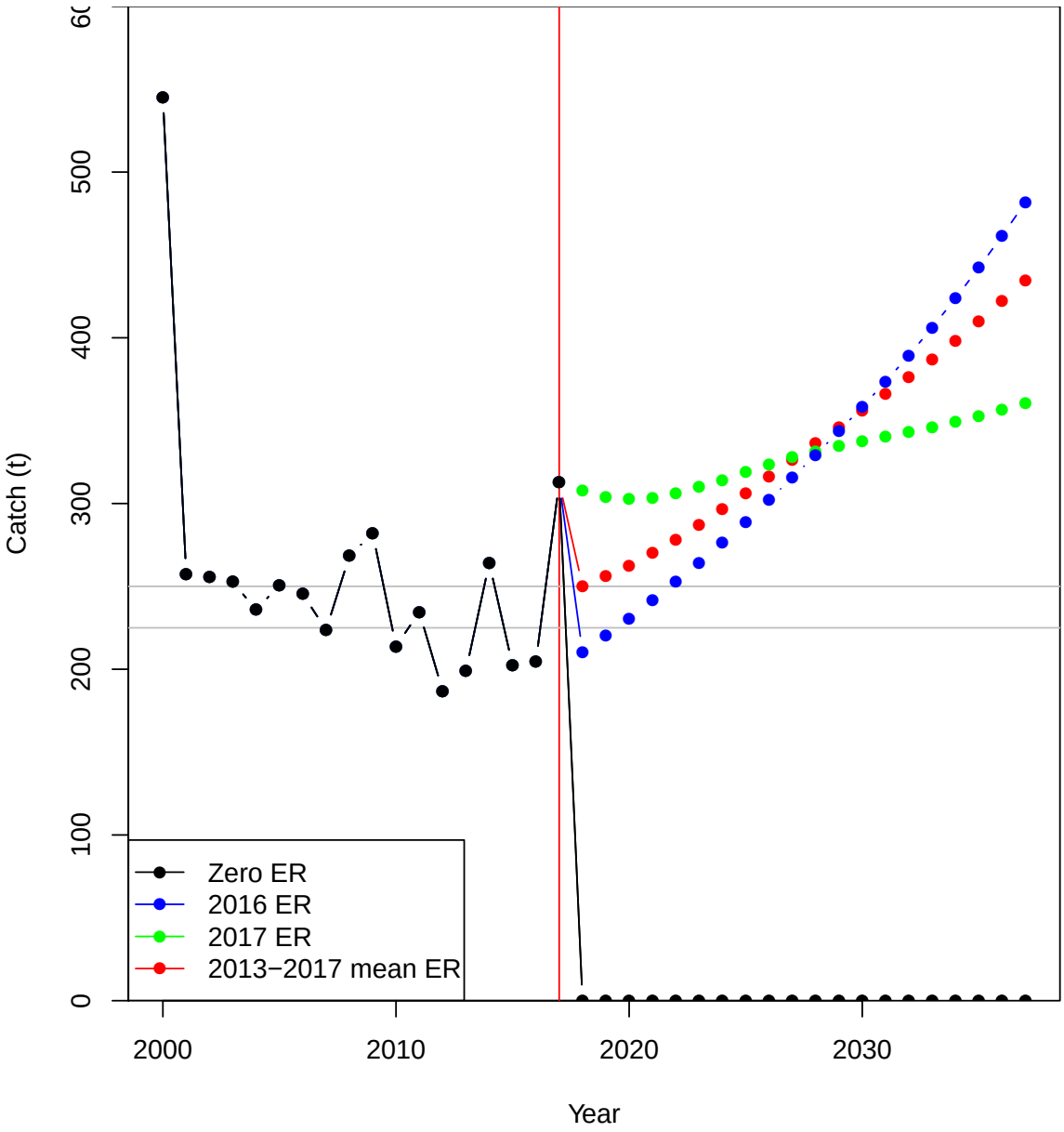

Figure 4.22: Projected catches (median) under zero exploitation (black); the 2016 (blue); the 2017 (green dots); or the average of the 2013-2017 (red) exploitation rates. Past catches are black, and the model is projecting after 2017 (red vertical line)

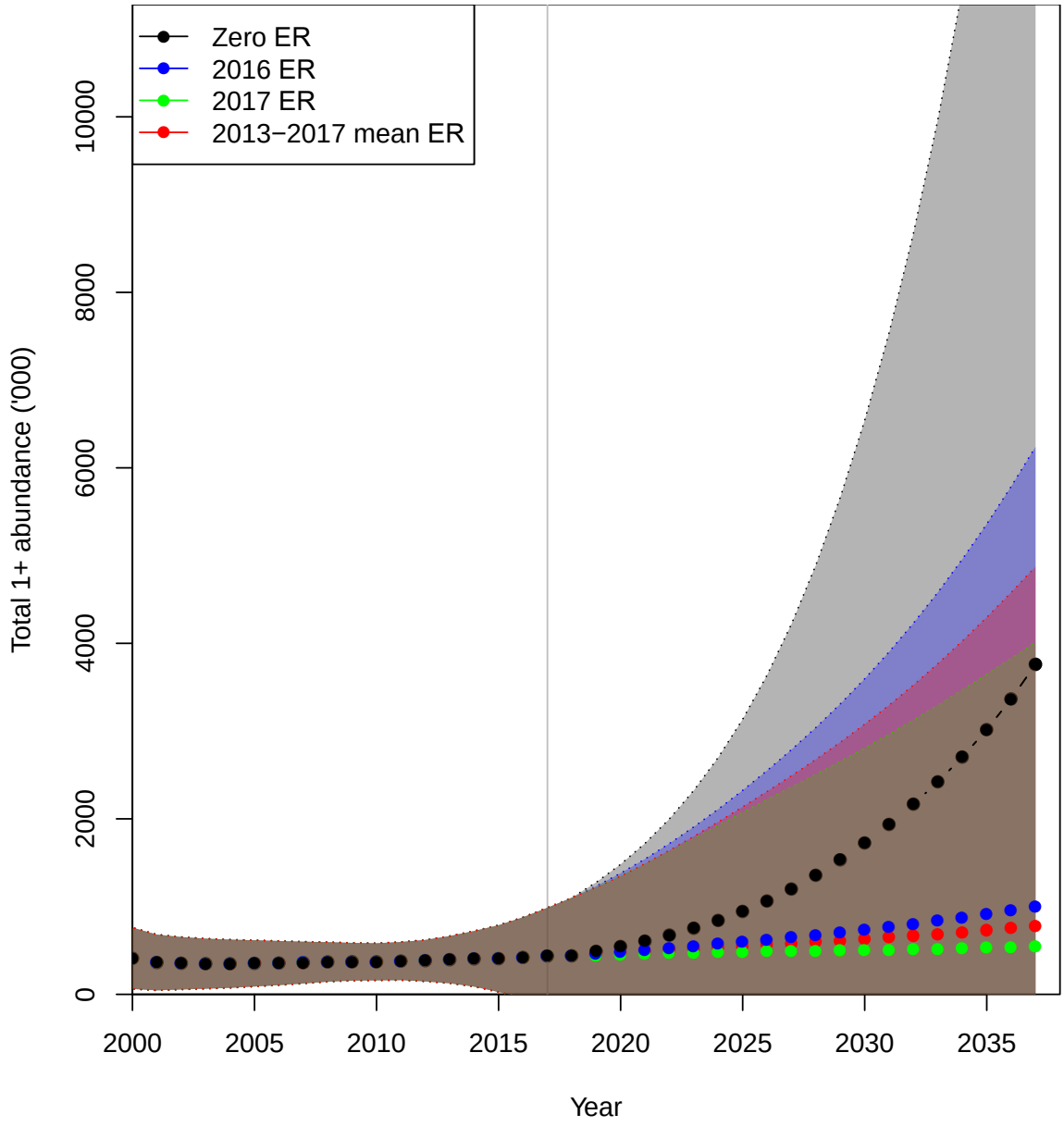

Figure 4.23: Past and projected future 1+ abundance under zero exploitation (black dots, grey shading); the 2016 (blue dots and shading); the 2017 (green dots and brown shading); or the average of the 2013-2017 (red dots and shading) exploitation rates. Shading indicates the 95% confidence interval.

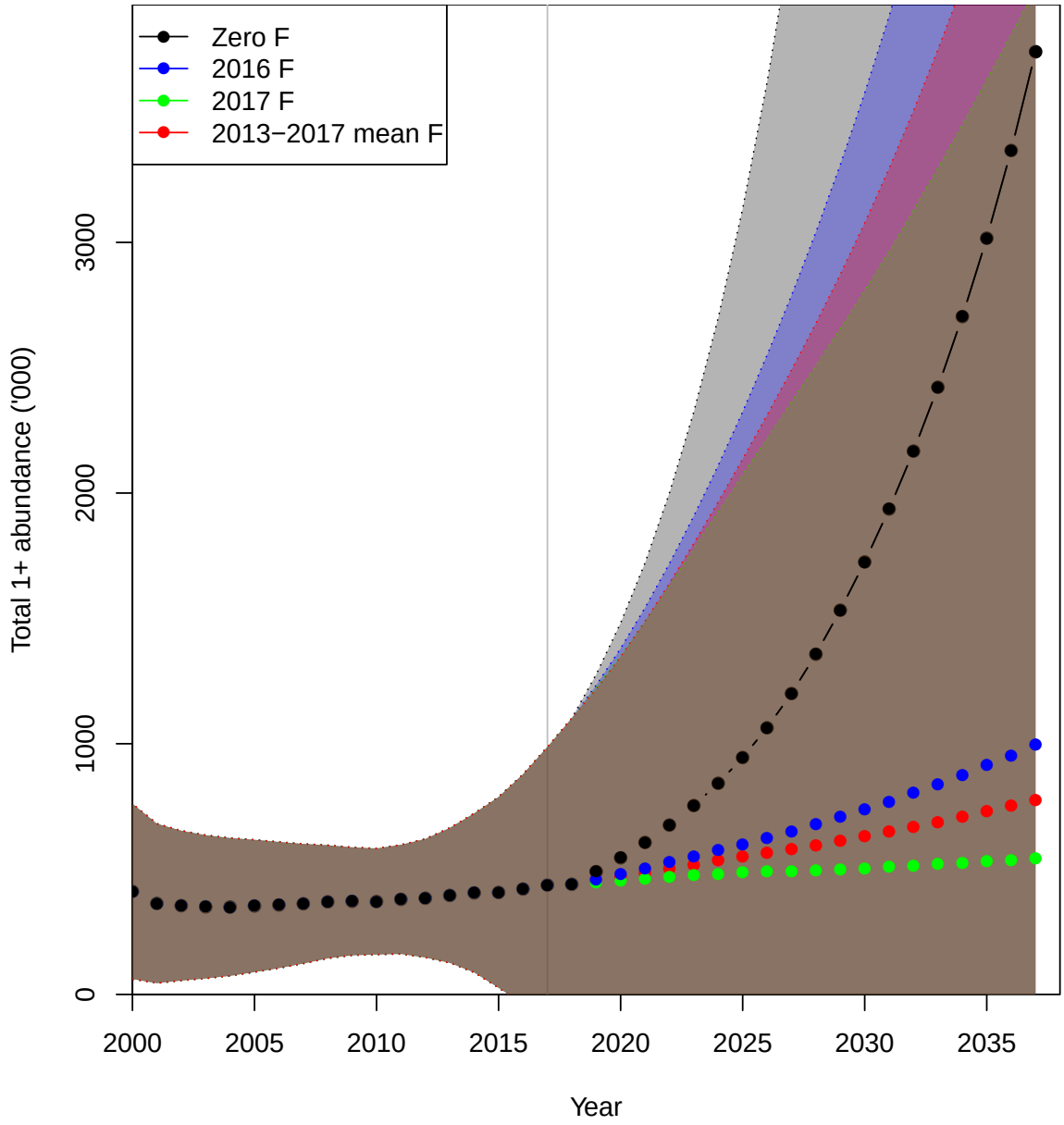

Figure 4.24: An expanded version of Figure reffig:projN1plus.

consistent sampling (Table 4.13, 700 samples p.a.). Note, however, the SE of 0.07 is still substantial compared with a trend of the order of 0.02. The first column in Table 4.12 shows an increasing s.e. over time, that is because no samples are collected in that scenario which therefore relies on only the samples already collected.

These calculations assume a 20% loss of samples during the quality control step (which is roughly the loss rate of the current study) so that a sample of 700 animals translates to only 560 animals entering the model. Note that this also assumes that all 700 animals have 11 or fewer rings and are therefore not excluded from the model.

Table 4.12: Expected standard error on trend (between 2010 and 2015) if between 0 and 700 close kin samples are collected each year from 2018 to 2022.

Last year	0	200	300	400	500	600	700
2018	0.27	0.24	0.22	0.21	0.20	0.19	0.18
2019	0.29	0.22	0.20	0.18	0.16	0.15	0.14
2020	0.30	0.20	0.17	0.15	0.13	0.12	0.11
2021	0.33	0.19	0.15	0.13	0.11	0.10	0.09
2022	0.31	0.15	0.12	0.10	0.08	0.07	0.07

Table 4.13: Expected standard error on final abundance if between 0 and 700 close kin samples are collected each year each year from 2018 to 2022.

Last year	0	200	300	400	500	600	700
2018	0.65	0.56	0.52	0.49	0.46	0.44	0.42
2019	0.65	0.47	0.42	0.38	0.34	0.32	0.29
2020	0.65	0.40	0.34	0.30	0.27	0.24	0.22
2021	0.65	0.34	0.29	0.25	0.22	0.20	0.18
2022	0.65	0.30	0.25	0.21	0.19	0.17	0.16

Chapter 5

Discussion and Conclusions

We have demonstrated two simple approaches to calculating average abundance for school shark using close kin data alone. We have confidence in the veracity of both those simple approaches and the close kin data itself. Estimated parameter values for the close kin model were within plausible ranges, and observed and expected numbers of kin pairs were well matched. The assumptions made by the simple approaches are too crude to allow their use as an alternative assessment, therefore we developed the more sophisticated population dynamics model presented here. Our results broadly match those of the simple approaches, giving us confidence in the average abundance from the more sophisticated model. The close kin data indicate that adult abundance of school shark is much lower than that suggested by the most recent stock assessment model and its 225t p.a. catch projection. This is supported by the findings of the simple model approaches which found around 80,000 'typical adults on average' across the 2000s (one line approach) and roughly 40,000 to 80,000 adults (GLM model). While estimates these are certainly crude, and really only suitable as a check that the more elaborate close kin model has been set up correctly, it is quite clear that estimates of adult abundance in the 200,000s (stock assessment model) are incompatible with the observed close kin data. However, the close kin model is inconsistent with the catches taken during the 1990s which raises the question: is the stock from which our close kin sample was taken not the stock that sustained catches prior to 2000?

Punt *et al.* (2000) observed that it was not possible, before greatly elaborating the stock assessment, to mimic the steep slope of catch rate declines in the west and at the same time match the shallower declines in the east that occurred during the 1980s and 1990s. His solution was to include two biological stocks in the model, although he hypothesized that more than two stocks were likely present. This conclusion is consistent with the difficulty we had in incorporating higher catches from the 1990s into our model. School sharks have long been known to pup in bays and inlets of Tasmania and Victoria (Olsen, 1984; Stevens & West, 1997) and have recently been shown to pup in South Australia (McMillan *et al.*, 2018). It is possible that these pupping locations represent reproductively separate populations that have their own spatial distributions and movement patterns (while at the same time undertaking large migrations and intermingling on the fishing grounds throughout their range). Such stock separation ought not to adversely impact the close kin estimate of recent absolute abundance; where that is defined as the abundance of sharks that are available to the fishing industry.

However, the existence of more than one school shark stock, at least one of which is greatly depleted, is relevant to management of the school shark population.

Key school shark pupping grounds (sea grass beds) in Port Phillip and Western Port bays (Victoria) that were identified in the 1960s, had significantly degraded by the 1990s (DEWR, 2008). The degradation occurred primarily in the mid-1970s and continued until the mid-1980s. Although the decline has been halted, there has been little recovery. The cause of the decline in these key seagrass beds has been increased water turbidity, increased nutrient loads and changing freshwater flows (DEWR, 2008). There has also been some loss of seagrass beds in Tasmania's main pupping area, Pittwater (Stevens & West, 1997). Upper Pittwater recorded the highest catches of school shark pups during a survey of all known (and possible new) pupping grounds in the 1990s (Stevens & West, 1997) making it the most important school shark pupping ground, although note that no South Australian sites were included in that study. School sharks that were pupped in Pittwater Tasmania have been shown to travel to Eastern Bass Strait and even South Australia by their second year of life (McAllister *et al.*, 2015) and similar movements of juveniles have been shown (Walker, 1999). Movement to and from New Zealand (NZ) is also known to occur (Walker, 1999), but it is clear from the relatively small absolute abundance found in this study that the correspondingly large NZ school shark population has not formed part of this abundance estimate, indicating that migration rates are low.

An alternative explanation to that of multiple school shark stocks that are differentially depleted and that have differing productivity due to degradation of pupping grounds, is that there is a single school shark stock whose productivity has changed over time. Productivity consists of natural mortality rates (for pups, sub-adults and adults with possible higher rates for the older animals i.e. senescence) the number of pups produced by females (as a function of length or age), the maturity rates of females (as a function of length or age), the pupping interval (possibly every two years but most likely every three years), and to some extent individual growth rates. Our model could not sustain the catches of the 1990s, assuming a single stock, even if females produced pups every year and those had a 100% survival rate. To explain our results as a productivity change, females would have had to produce more than the observed numbers of pups, or become mature earlier, or possibly, large numbers of mature females that are never seen by the fishing industry would have been (cryptically) producing pups (in the past, but no longer). The fecundity relationships used in our model are based on data collected during the 1980s and 1990s (Moulton *et al.*, 1992; Walker, 2005) so it is difficult to argue that they apply to the current era but not to the 1980s and 1990s. Our model estimates a natural mortality rate that is similar to the rate of 0.1 that was chosen for the stock assessment model that was developed during the 1990s. These factors all suggest that the multiple stock hypothesis is by far the more likely explanation.

The work presented here was used by sharkRAG to recommend a time series of future catches for school shark. The median catches for the projection that used average exploitation rate over the most recent five years was used. Note that the wide confidence intervals on estimates of recent abundance indicate that these median catches are no guarantee of sustainability (Figure 4.23). While the median current trend for school shark is upwards, the confidence interval is wide enough to allow a downward trend. Ongoing collection of close kin samples for an additional four years should greatly reduce these confidence intervals, but are projected to be substantial compared with the modest (median) trend.

In conclusion, school shark seem likely to consist of a number of stocks (i.e. units that are reproductively isolated, at least to some degree, and that show differing, but almost certainly overlapping, spatial distribution). It seems probable that some of those have been severely depleted, and that those that remain in sufficient numbers to dominate the close kin sample are small, but are most likely to be increasing. The absolute estimate of abundance is more accurate than the estimate of trend, but that trend is likely to be positive, indicating that current catches are sustainable and are allowing recovery, at least of the stock(s) sampled. Our results do not guarantee that current catches are sustainable, the overall trend in abundance could be downwards. The close kin samples were collected from the fishery, so the stocks sampled are likely to be those being fished. If we continue to use close kin as a monitoring tool for school shark, our estimate of trend will become more precise. Other close kin projects that are based on relatively short time series of collections, have been found to give precise estimates of abundance but imprecise estimates of trend. The estimates of trend do improve with ongoing monitoring (e.g. Hillary *et al.*, 2018).

School shark demonstrate several biological features that have not been encountered in our other CKMR analyses: high frequency of full sibling pairs (FSPs), ageing error, and ageing bias, and a long and complicated history of changing (but generally heavy) exploitation rates. These have presented some challenges in developing a suitable close kin model. The low estimate of abundance, and consequent incompatibility with catches during the 1990s, drove us into a lengthy period of model exploration in an (ultimately unsuccessful) attempt to find a single-stock model that was compatible with both the close kin data and the observed catches.

Chapter 6

Implications

The concept of pristine biomass, or B_0, is integral to the SESSF Harvest Strategy Framework (AFMA, 2017) through its use of target and limit reference points that are expressed as a fraction of B_0. The concept of B_0 implicitly assumes that before fishing began, stocks were (on average) at some virgin biomass level and that if fishing were to stop altogether, they would return to that level. However, if school shark stocks have been removed or greatly reduced, perhaps due to habitat degradation, then even if fishing were completely stopped, the stock would be expected to recover to a lower level than B_0. Similarly, if some genetic lineages have been removed from the gene pool then it is possible that some areas of habitat would not be utilised by the remaining sharks (at least not for a very long time). Reductions in productivity, resulting in consistently lower than average recruitment, have been observed in other SESSF stocks e.g. jackass morwong, silver warehou, and eastern redfish (Tuck, 2017; Day & Castillo Jordán, 2018; Burch *et al.*, 2019) possibly as a result of changing oceanographic conditions (Wayte, 2013). Management of jackass morwong has recognised a regime shift in that stock, so that it is now effectively managed using a lower B_0; but the decline of jackass morwong has continued, suggesting an ongoing reduction in productivity, rather than a sudden shift and subsequent stabilization at a lower level. Management attention has now moved towards incorporating the concept of a 'shifting B_0', at least for some stocks (Geoff Tuck, CSIRO, pers commn). Management of school shark, similarly, should consider the possibility that pre-exploitation stock sizes might not be recoverable.

The highest tier for managing SESSF stocks has been Tier 1, which uses of an age-structured Integrated Assessment model that relies on indices of relative abundance from commercial CPUE (and this might be supplemented by a less biased, but still relative, index from a Fishery Independent Survey, FIS). It is well recognised that stock assessment models of this kind are much better at estimating relative rather than absolute abundance (Punt *et al.*, 2002; Yin & Sampson, 2004; Magnusson & Hilborn, 2007). This is the result of confounding between productivity (another estimate parameter) and absolute abundance (usually parameterised as either B_0 or as R_0, which is recruitment at B_0). This means that, typically, a range of pairs of values for productivity and absolute abundance will give similarly good fits to the available data.

It has therefore been reasonable to base management on abundance relative to some early level (i.e. B_0) rather than on the more poorly estimated absolute recent abundance. CKMR,

by contrast, gives a reliable estimate of absolute recent abundance, at least of the mature component of the stock, and the size of the immature component is inferred given juvenile mortality rates. This gives the opportunity for new management strategies that are based on actual recent stock abundance, freeing us from reliance on poorly known abundance in the distant past; abundance that might no longer be achievable even in the absence of fishing. Due to intensification of the East Australia Current, ocean warming off south-eastern Australia is four times that of the global average (Ridgeway, 2007) and this will have adverse impacts on cold-water species (Poloczanska *et al.*, 2007). There is a clear need for management strategies that do not rely on the assumption of unchanging productivity between current and pre-industrial fishing eras.

The median estimated trend in school shark abundance, from both the simple GLM and the full CKMR model approaches, is upwards, but it is imprecise and a downward trend cannot be ruled out. The median annualised increase in mature school shark abundance over the 2010 to 2015 period is 2.1% p.a. If that trend is real, then the stock is showing steady growth under the existing catch scenario of roughly 250t p.a. However, the CVs for the increase in mature abundance are too high to allow confidence in the estimate. Further collection of close kin samples will narrow the confidence interval for trend.

Chapter 7

Recommendations

Ongoing monitoring using close kin
Based on the results presented here, sharkRAG recommended ongoing close kin monitoring of school shark, with sampling to occur at the rate of 700 samples p.a. (300 of those to be drawn from poorly sampled strata i.e. western South Australia, western Tasmania and deeper than 183m) (AFMA, 2018a). More samples from eastern Tasmania would also be desirable. A project proposal to do this work has been developed, and submitted (separately to this report) to AFMA.

Future close kin samples are likely to come, predominantly, from a new industry-driven data collection scheme (SIDaC) that will provide standard body length measurements made by trained observers (Ross Bromley pers commn). This will allow us to model fecundity as a function of both length and age (recognising that some individuals grow consistently faster, or slower, than the average, throughout their lives).

Inclusion of POPs
A potentially important refinement to the close kin model would be to include older animals (born before 1989) as potential parents but not as siblings or offspring. Because we have not been able to extend the population dynamics model earlier than 2000, we cannot use offspring born before 1989. Nevertheless, it is possible to use POPs where the parent was born before 1989, provided the offspring was born after that date. This is impossible because ERRO must be known for the year in which the offspring was born, but only the age (or size) of the parent must be known. The ages of parents will be known imprecisely, because they will be older animals, but we can integrate over all probable ages, given ring counts and assumed ring deposition rate. Very few POPs have been observed from our sample therefore this modification to the model is currently unnecessary, but future samples might include more POPs. Sufficient POPs would also allow estimation of fecundity relationships (for males as well as females).

The close kin model uses pre-specified CVs describing variation in length at age (Punt *et al.*, 2000; Punt, 2001) but the specified variation decreases with increasing age, which is not realistic. It is likely that those CVs were calculated using tagging data and that a hard upper size limit was used, which artificially caused smaller estimates of variation for larger animals

(which were constrained by the upper size limit and whose lower size limit approached that upper limit more closely with increasing age). We suggest that future work look at sensitivity to assuming a more realistic increasing CV in length with increasing age, and if the model is found to be sensitive to this assumption, and if the original tagging data can be obtained, that the CVs be recalculated. A model that incorporates reasonable numbers of observed POPs would conceivable be more sensitive to these assumed CVs than the model presented in this report.

More accurate catches
Catches (i.e. total removals) of school shark consists of several components, based on where data can be sourced (Castillo Jordán *et al.*, 2018a):

1. Commonwealth commercial landed catches (recorded in logbook and CDR databases held by AFMA);
2. State catches (recorded by NSW, Victorian, South Australian and Tasmanian state authorities);
3. Discarded catches (estimated using onboard AFMA Observer Program data, up to July 2015 and for 12 months in 2017-18); and
4. Recreational catches (estimated by a small number of one-off surveys, usually with high CVs).

State and Commonwealth catches are thought to be recorded accurately, but gaps exist in the recent record of discarding due to the removal of observers from gillnet and line vessels. It has been shown (Ian Knuckey, Fishwell, pers commn) that past historical observations of the mean weight of the discarded catch, coupled with logbook records of the numbers of school shark carcasses discarded, should give a sufficiently accurate estimate of discarding for recent years. These calculations should be completed with high priority.

Recreational catches of school shark have been ignored by both stock assessment and close kin models, but surveys of recreational fishing in South Australia estimated a catch of 9t in 2007-08 and a concerning 53t in 2013-14. While there is likely to be a high degree of error associated with these numbers, as there typically are for such surveys, the size of the estimate for 2013-14 is such that this merits further investigation and possible incorporation into future models.

Pups
Walker *et al.* (2001) showed that pupping frequency for females is at least two years, more likely three. The true interval would have been clear in the data from the maternal half siblings, had ageing error not obscured that signal (although it does suggest a three year interval). Genetic examination of a larger number of pups, aged 0, 1 and possibly 2 years old (where growth is sufficiently rapid for age to be clearly apparent from length) could provide clear information on the pupping frequency. Samples taken from Pittwater over at least six years ought to provide this information. Furthermore, the presence (or absence) of cross cohort full siblings amongst these pups would help in understanding whether sperm storage is occurring, and if it is, to what degree it occurs. If sperm storage is occurring, then it would

be important to incorporate it into the close kin model because it will influence the estimate of abundance.

Our model's estimated proportion of litter-mates that have different fathers (ν_2) is lower than that implied by Hernández *et al.* (2014). Ongoing tissue sampling of pups in Pittwater would help to examine the veracity of that estimate.

An intensive search for school shark pups in Victoria's historical school shark pupping grounds has not occurred since the survey by Stevens & West (1997). It would be beneficial to repeat that work, so that pup density can be compared with that found in the mid-1990s and with that found by Olsen in the 1950s. Examination of the nuclear and mitochondrial DNA sequences of pups from Victoria, South Australia and Tasmania might provide information on the likelihood of separate stocks (or at least of maternal site-fidelity to pupping grounds).

Kin
We were unable to separate POPs from FSPs using the statistics currently available for kin finding. More powerful approaches could be developed, and this work is planned at CSIRO. We were able to use age to unambiguously separate out the POPs in this study, but it would be reassuring to confirm our findings genetically. Close kin studies need not use ageing data, instead being based on length alone (as was done for grey nurse shark (Bradford *et al.*, 2018)) so that clear genetic separation between POPS and FSPs is desirable.

We found eight family groups ('triads') which ought not to bias our abundance estimate, but that would (at least if they had appeared in greater numbers) cause the CV to be under estimated, because pairwise comparisons become non-independent. This variance issue will eventually be addressed in future research.

Population dynamics model
It would be possible to model school shark as 2 or more fully intermingled populations, and, along with a strong assumption about age deposition rates and ageing error, we might be able to extend the model further back in time. There are bound to be several different hypotheses that would all make such a model possible, but that would give differing results and it is not clear that such work would be benefit management of school shark.

We have attempted to keep the close kin model as simple, and thereby as free of reliance on assumptions, as possible. We relied on known gear selectivity functions, and avoided modelling regional differences in availability (as a function of length or age) by not using length frequency data. Future modelling work could at least investigate using a spatially disaggregated model that could use a 'fleets as areas' approach to model regional availability without having to model movement patterns. Such a model would also have to separate the combined trawl and line fleet into three fleets: trawl, deep line, and shallow line as was done for gummy sharks (Punt *et al.*, 2016), because these components land distinctly different size classes of school shark. The length frequency data for school shark, particularly for recent years, is somewhat sparse and rife with difficulties in interpretation (e.g. port collected data does not include depth of fishing, or gill net mesh size, and there has been little onboard observer coverage since mid-2015). The small numbers of school shark that have been landed in recent years has also contributed to sparse data. The SIDaC scheme

should help to establish a new, reliable data stream.

Because gillnet fishing gear catch only a relatively narrow range of sub-adult school shark, only line gear can provide direct information on the mature stock. If length frequency data are incorporated into future models, we might estimate natural mortality rates (senescence) for older animals and hence develop better estimates for the most fecund, oldest females.

Chapter 8

Extension and Adoption

Verbal updates regarding the early progress of this project were provided to all sharkRAG meetings held after the commencement of the project (early 2015). In addition, written reports and / or powerpoint presentations were provided at the following sharkRAG meetings:

13 October 2016, Hobart
7 December 2017, Hobart
12 February 2018, Hobart
6-7 August 2018, Hobart, 'School shark close kin workshop'
29-30 October 2018, Melbourne
3-4 December 2018, Queenscliff

A verbal update was also given to the SESSF RAG 'RAG Chairs' meetings held in February 2018 and 2019. In March 2018, a brief summary of the project results and implications for management, was prepared at the request of Carolyn Stewardson and was widely circulated, by email, amongst relevant industry, science and management stakeholders. That document is included as Appendix D.

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Chapter 9

Project materials developed

A population genetics paper was published in *Ecology and Evolution* is attached in Appendix B.

This project contributed to the refinement of R packages for quality control of genetic sequencing data, and kin finding, that were primarily developed for SBT. These packages (*gbasics* and *kinference*) will be released, along with a descriptive publication, and a worked example, in the near future.

Appendix A

Project staff

Name	Affiliation	Role
Robin Thomson	CSIRO	Principal investigator, sample aquisition, kin finding, close kin modelling, software development report preparation
Mark Bravington	CSIRO	Project planning, software development, kin finding, close kin modelling, report editing, co-investigator
Rasanthi Gunasekera	CSIRO	DNA extraction and quality control, sample handling, preparation of samples for mitogenome sequencing
Pierre Feutry	CSIRO	Genetic support and mitochondrial genome analysis, population genetics
Peter Grewe	CSIRO	Genetic support and liason with fish processors
Paavo Jumpanen	CSIRO	Computational support, ADT programming
Claudio Castillo Jordán	CSIRO	Arranging sample transportation
Elizabeth Brewer	CSIRO	Assistance with DNA extraction
Floriaan Devloo-Delva	CSIRO	Population genetics
Simon Robertson	Fish Ageing Services (FAS)	Ageing shark vertebrae
James Marthick	Menzies Institute for Medical Research	Sequencing of mitogenome

Appendix B

Population genetics for school shark neonates from Australia and New Zealand

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ORIGINAL RESEARCH

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Accounting for kin sampling reveals genetic connectivity in Tasmanian and New Zealand school sharks, *Galeorhinus galeus*

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Abstract

Fishing represents a major problem for conservation of chondrichthyans, with a quarter of all species being overexploited. School sharks, *Galeorhinus galeus*, are targeted by commercial fisheries in Australia and New Zealand. The Australian stock has been depleted to below 20% of its virgin biomass, and the species is recorded as Conservation Dependent within Australia. Individuals are known to move between both countries, but it is disputed whether the stocks are reproductively linked. Accurate and unbiased determination of stock and population connectivity is crucial to inform effective management. In this study, we assess the genetic composition and population connectivity between Australian and New Zealand school sharks using genome-wide SNPs, while accounting for non-random kin sampling. Between 2009 and 2013, 88 neonate and juvenile individuals from Tasmanian and New Zealand nurseries were collected and genotyped. Neutral loci were analyzed to detect fine-scale signals of reproductive connectivity. Seven full-sibling groups were identified and removed for unbiased analysis. Based on 6,587 neutral SNPs, pairwise genetic differentiation from Tasmanian and New Zealand neonates was non-significant (F_{ST} = 0.0003, CI_{95} = [−0.0002, 0.0009], p = 0.1163; D_{est} = 0.0006 ± 0.0002). This pattern was supported by clustering results. In conclusion, we show a significant effect of non-random sampling of kin and identify fine-scale reproductive connectivity between Australian and New Zealand school sharks.

KEYWORDS

close kin, genetic structure assessment, population genomics, sampling bias, shark fisheries, single nucleotide polymorphisms

1 | INTRODUCTION

Among marine organisms, sharks are of the highest conservation concern; 25% of all chondrichthyan species being currently at risk of extinction (Dulvy et al., 2014). These species are particularly vulnerable to targeted or by-catch fisheries, partly because of late maturity and small litter size (Kyne, Bax, & Dulvy, 2015). School sharks (*Galeorhinus galeus*; Linnaeus, 1758) have been intensively fished throughout Australian waters since the 1920s for their oily livers and later on for their meat (Olsen, 1954). By the 1950s, there was concern that overfishing had

depleted the stock of this species with low biological productivity (i.e., 15–43 pups every 2 years; AFMA, 2015; Olsen, 1984), causing a shift toward targeting the faster reproducing gummy shark (*Mustelus antarcticus*; Günther, 1870) (Walker, 1999). However, school shark catch continued and the stock is currently estimated to lie between 8% and 17% of the pristine level (Thomson, 2012; Thomson & Punt, 2009). Consequently, school shark has been listed as Conservation Dependent under the Environment Protection and Biodiversity Conservations Act (EPBC Act, 1999). Globally, the species is recorded as Vulnerable on the IUCN Red List (Walker et al., 2006) and has recently been designated as a priority for conservation (Dulvy et al., 2017).

Management of highly migratory species, such as school shark, presents difficulties given that international agreements may be needed to properly manage shared stocks (Fowler, 2014). Consequently, straddling stocks are sometimes managed on a less appropriate national scale. Such a problem may exist for school sharks, which are managed independently in Australia and in New Zealand (Francis, 2010), despite tagging and genetics studies that have questioned the assumption of separate stocks. Individuals are reported crossing the Tasman Sea and migrating up to 4,500 km (Coutin, Bruce, & Paul, 1992; Francis, 2010; Hurst, Baglet, McGregor, & Francis, 1999; McMillan, Huveneers, Semmens, & Gillanders, 2018). Nevertheless, such tagging studies do not provide any information about successful reproduction of migrants. Note, that the level of gene flow required to overcome genetic separation is much lower than that required to assume complete mixing and, hence, joint stock management (Begg & Waldman, 1999).

A lack of apparent genetic structure between these Australian and New Zealand sharks has been reported, using allozyme, mitochondrial DNA (mtDNA), and microsatellites (Hernández et al., 2015; Ward & Gardner, 1997), thus questioning the existence of impervious reproductive boundaries in this region. However, a more recent study, with the mitochondrial and similar nuclear microsatellite markers, found a clear separation in the microsatellite data between Tasmania and New Zealand (Bester-van der Merwe et al., 2017). Single nucleotide polymorphisms (SNPs) have been shown to outperform microsatellites in population discrimination due to their random spread across the genome, lower ascertainment bias, higher accuracy and resolution, reproducibility, and comparability (Andrews, Good, Miller, Luikart, & Hohenlohe, 2016; Fischer et al., 2017; Muñoz et al., 2017; Seeb et al., 2011). Single nucleotide polymorphisms allow for a relatively cheap and easy way to obtain a full genome scan (Andrews et al., 2016). The large number of markers permits the inference of kinship with high certainty, investigation of population structure at higher resolution (Feutry et al., 2017), and accurate calculation of genetic diversity (as argued by Domingues, Hilsdorf, & Gadig, 2018).

In highly migratory species, sampling adults can introduce bias due to dispersal of individuals after birth and hence decreases the signal to noise ratio (Waples, 1998). This realized dispersal is much lower in neonate and juvenile school sharks (Olsen, 1954) and studying them should improve the power to detect fine-scale structure. However, sampling juveniles result in a higher risk of generating a false signal of genetic structure through the "Allendorf–Phelps effect" (Allendorf & Phelps, 1981; Waples, 1998), due to biased sampling toward family members. Additionally, the presence of family members within a sample set has been reported to artificially increase the number of distinct genetic pools detected by clustering algorithms commonly used in population structure studies (Anderson & Dunham, 2008). Both biases have been previously reported in sharks (Feutry et al., 2017).

This study aims at testing the hypothesis of a single panmictic population of school shark between Tasmanian and New Zealand waters using novel genomic markers, while accounting for the "Allendorf–Phelps effect." To investigate this, we genotyped neonates and juveniles from Tasmania and New Zealand. This work provides basic knowledge for the management of this commercially important species and contributes to the discussion around sampling design and data analysis when investigating the genetic structure of highly migratory species.

2 | MATERIAL AND METHODS

2.1 | Sample collection

Eighty-eight school sharks were collected between 2009 and 2013 using long lines and gillnets from Tasmania (TAS, $n = 47$) and New Zealand (NZ, $n = 41$) (Figure 1). Sampling sites in both countries were known nursery areas, and only neonates and juveniles (total length < 60 cm) were caught. Individuals smaller than 70 cm (i.e., 0–2 years old) are considered to have limited dispersal (Olsen, 1954). Muscle tissues or fin clips were collected and stored in ethanol. A modified version of the CTAB protocol (Doyle & Doyle, 1987; Grewe et al., 1993) was used to extract total genomic DNA.

2.2 | SNP genotyping and filtering

Single nucleotide polymorphism genotyping was carried out by Diversity Array Technologies (DArT, Canberra, Australia) using the DArTseq™ protocol, a method of sequencing complexity reduction representations. The DArTseq™ protocol used in this study was identical to the one previously described by Grewe et al. (2015). The DArTseq™ output consisted of 75 bp fragments containing one or more SNPs. Seventeen samples were genotyped twice to assess genotyping reproducibility.

Quality filtering was performed in R v3.5.1 (R Core Team, 2016), using the dartR v1.1.6 (Gruber, Unmack, Berry, & Georges, 2018) and the Adegenet v2.1.1 (Jombart & Ahmed, 2011) packages. Low call rate (proportion of scored loci for an individual) and high heterozygosity may indicate bad DNA quality or sample contamination, respectively. Therefore, individuals with call rate below 95% and/or heterozygosity above 20% were removed from the dataset prior to proceeding to the SNP filtering step of the data quality check process. Single nucleotide polymorphisms with a call rate (proportion of scored individuals for a locus) lower than 95%, a genotyping reproducibility below 98%, and a minor allele frequency lower than 5% were removed (Table 1). Further, loci with an average read depth lower than 15 and higher than 90 sequences per locus were filtered out. Monomorphic loci (fixed over all

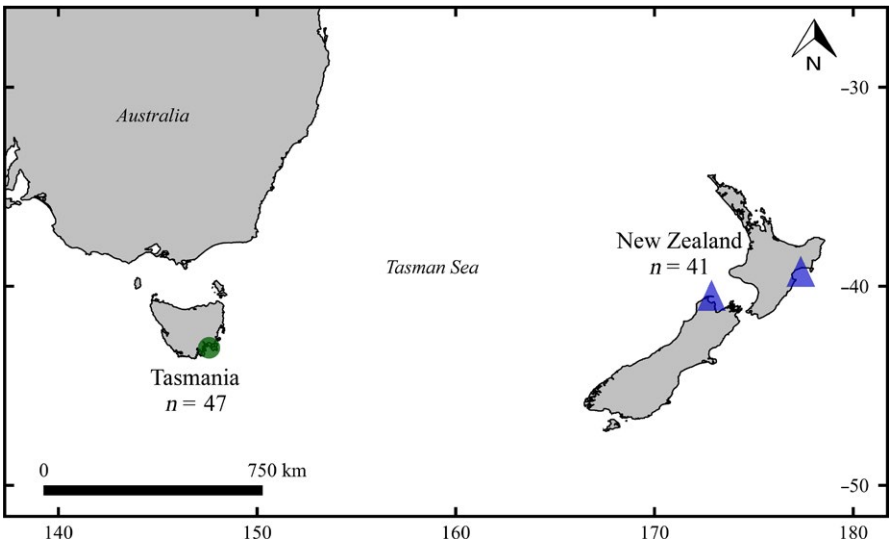

FIGURE 1 Sampling map for neonate school sharks from Tasmania and New Zealand. Green circle represents Pittwater and Norfolk Bay. Blue triangles represent Golden Bay (West, *n* = 33) and Napier (East, *n* = 8)

individuals) were deleted, since they contain no discriminating information. Outlier analysis was performed with OutFLANK v0.2 (Whitlock & Lotterhos, 2015) at a "*q* value" of 0.01, and significant outliers were removed in order to only retain neutral markers. All the cutoff values used in these filtering steps were defined after plotting the data to observe the loci/individuals' distributions (see Supporting Information S1).

Moreover, two datasets (with and without siblings) were created to test the effect of non-random sampling of siblings (Table 1). Sibship (full- and half-sibling relationships) among all individuals was checked with Colony2 v2.0.6.1 (Jones & Wang, 2010) using the initially filtered dataset (see Supporting Information S2 for the analysis parameters). To build the second dataset, only one individual per sibling group was kept prior to re-filtering all SNPs (following similar filtering steps).

TABLE 1 Quality-filtering steps for loci and sharks

	With full siblings		Without full siblings	
	Loci	Sharks	Loci	Sharks
Start	31,550	88	31,550	77
Multiple loci on the same sequence	24,504	88	24,504	77
Monomorphic loci	21,275	88	20,951	77
Locus call rate ≥ 0.95 & Shark call rate ≥ 0.95	13,931	88	13,579	77
Shark heterozygosity ≥ 0.20	13,931	87	13,579	76
Monomorphic loci	13,918	87	13,555	76
Average reproducibility ≤ 0.98	13,581	87	13,237	76
Coverage ≤ 15 reads	13,439	87	13,103	76
Coverage ≥ 90 reads	13,363	87	13,031	76
Minor allele frequency ≤ 0.05	6,768	87	6,603	76
Locus observed heterozygosity ≥ 0.6	6,763	87	6,594	76
Outlier loci	6,760	87	6,587	76

2.3 | Population diversity and structure analyses

Genetic diversity, fixation (F_{st}), and allelic differentiation (Jost's *D* or D_{est}) indices were calculated with diveRsity v1.9.90 (Keenan, McGinnity, Cross, Crozier, & Prodöhl, 2013), StaMPP v1.5.1 (Pembleton, Cogan, & Forster, 2013) and mmod v1.3.3 (Winter, 2012) packages, respectively, applying a bootstrap of 10,000. Population structuring was assessed with a Discriminant Analysis of Principal Components (DAPC, Adegenet v2.1.1; Jombart & Ahmed, 2011) and STRUCTURE v2.3.4 (Pritchard, Stephens, & Donnelly, 2000). With DAPC, the optimal number of clusters (*K*) was determined by the lowest Bayesian Information Criterion (BIC), and a successive *K*-means algorithm was used to group the sharks according to this number of clusters. The optimal number of principal components retained for the DAPC analysis was selected through cross-validation with a 10% hold-out set and 10,000 replicates. The admixture model of STRUCTURE was applied with correlated allele frequencies for 100,000 burn-in and 500,000 replicate runs. The program was set to assess structure between one to nine putative populations (*K*) with 20 iterations for each *K*. The optimal *K* was assessed based on the mean estimated natural logarithm of the probability (lnP). Except for the STRUCTURE analyses, all data filtering and analyses were performed and visualized using R v3.5.1 (R Core Team, 2016).

3 | RESULTS

3.1 | Data filtering

An average of 2,028,777 sequences per sample was obtained and the DArTsoft 2014 pipeline identified 31,550 SNPs. One individual from TAS with an excess of heterozygous loci compared to other sharks, probably due to for cross-contamination, was removed from the data. For these 87 sharks, a total of 6,760 neutral SNPs passed

all the filtering steps. Sibship analysis of this dataset revealed seven full-sibling groups (but no half siblings) among the TAS neonates. One individual from each of the seven full-sibling groups was retained (11 removed) to avoid biased clustering of family members. This resulted in a total of 76 neonate and juvenile sharks. After all filtering steps, 6,587 neutral SNPs were available for analysis.

3.2 | With full sibs

Genetic diversity indices were similar for sharks from TAS and NZ. (Table 2). The fixation and differentiation indices for the neutral SNPs indicated a significant genetic difference between TAS and NZ (F_{ST} = 0.0023, CI_{95} = [0.0017, 0.0028], p = 0.0000; D_{est} = 0.0014 ± 0.0002). However, this signal was not visible from the DAPC plot, where the BIC indicated that eight groups seemed to be the optimal solution (Figure 2a). Five of those eight groups were comprised of full siblings, and no differentiation between TAS and NZ could be found (Figure 2b). The sibling-driven clustering was not as obvious in the STRUCTURE as in the DAPC results; with a similar likelihood for K = 1, 2, 5, or 7 (Supporting Information S3).

3.3 | Without full sibs

Neutral genetic diversity decreased slightly, but non-significantly, compared to the dataset with full siblings and did not show any differences between TAS and NZ (Table 2). Pairwise F_{ST} became non-significant (F_{ST} = 0.0003, CI_{95} = [−0.0002, 0.0009], p = 0.1163; D_{est} = 0.0006 ± 0.0002) and based on the BIC of the DAPC and the mean lnP of the STRUCTURE analysis, one population seemed to be the best clustering solution (Figure 3a, Supporting Information S4). This result is supported by the lack of visible structure in the DAPC (Figure 3b) and STRUCTURE plots (Supporting Information S4).

4 | DISCUSSION

4.1 | Population structure with or without siblings?

The conclusions drawn from this study greatly depend on which dataset is interpreted (with or without full siblings). By removing full-sibling groups from the dataset, the F_{ST} value decreased by one order of magnitude and the optimal number of clusters decreased from eight to one (Figures 2a and 3a). If the sibling groups are left in the dataset, there is a risk of misinterpreting population structure for what is actually family structure. However, Waples and Anderson (2017) demonstrated that the trending common practice, consisting of purging groups of siblings prior to population genetic analyses, can introduce a bias if the presence of these groups is not a sampling artifact but rather the result of a small localized population. Removing the right amount of closely related individuals is theoretically feasible, but requires knowledge of (at least) the effective population size. Unfortunately, family structure also creates a bias when estimating this quantity (Waples & Anderson, 2017), which makes it a circular issue. In this study, all full siblings were sampled within the same year, with a maximum of four months between captures, which indicates that their presence is a sampling artifact. Another indicator of a family sampling bias is the absence of half siblings. If the presence of such a high proportion of full siblings in Tasmania was due to a small and localized population and given that males are not believed to be monogamous and that females are expected to reproduce more than once across the sampling period (Walker, 2005), one would have expected to detect half siblings too. More likely, the presence of full sibs in this dataset reflects a higher probability of sampling litter mates (individuals having the same mother and born at the same place and time). Due to interdependence between effective population size, population structure, and family structure, we suggest repetitive sampling over time can help interpret population structure in the presence of family members.

TABLE 2 Genetic diversity of 87 (6,760 SNPs) and 76 (6,587 SNPs) sharks, respectively

	With full siblings			Without full siblings		
	Overall	TAS	NZ	Overall	TAS	NZ
N	87	46	41	76	35	41
H_o	0.263	0.264	0.262	0.265	0.265	0.264
H_E	0.285	0.285	0.284	0.285	0.284	0.285
F_{IS}	0.068	0.070	0.069	0.066	0.065	0.067
A_R	1.995	1.995	1.994	1.992	1.990	1.993

Note. N, sample size; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient; A_R, allelic richness.

4.2 | Population structure compared to previous studies

Interestingly, our findings contradict nuclear DNA results from a recent study of Bester-van der Merwe et al. (2017). Potential sibling- or sex-biased sampling could explain the observed nuclear signal of structure (Allendorf & Phelps, 1981; Benestan et al., 2017; Feutry et al., 2017; Waples, 1998). School sharks are known to school by size and sex (Francis, 2010; Olsen, 1984). The nine Tasmanian and 20 New Zealand individuals from Bester-van der Merwe et al. (2017) were obtained to identify biased sampling. We were unable to test the sex-biased sampling hypothesis, because of missing sex information, but we re-analyzed the 19 microsatellites in COLONY2. Eight pairs of individuals had a probability over 75% of being either full or half siblings; settings and results are presented in Supporting Information S2 and S5. Due to the low sample size and missing alleles, a reliable estimate of allele frequencies could not be made and these results must be interpreted with caution. In addition, a recent publication from McMillan et al. (2018) described partial migratory behavior of Australian school sharks, where some females appeared to be resident. Consequently, the possibility of a small and localized population in Tasmania cannot be excluded.

This study builds on the many telemetry and genetic studies that have investigated movement and connectivity of school sharks within Oceania (Bester-van der Merwe et al., 2017; Coutin et al., 1992; Hernández et al., 2015; Hurst et al., 1999; McAllister, Barnett,

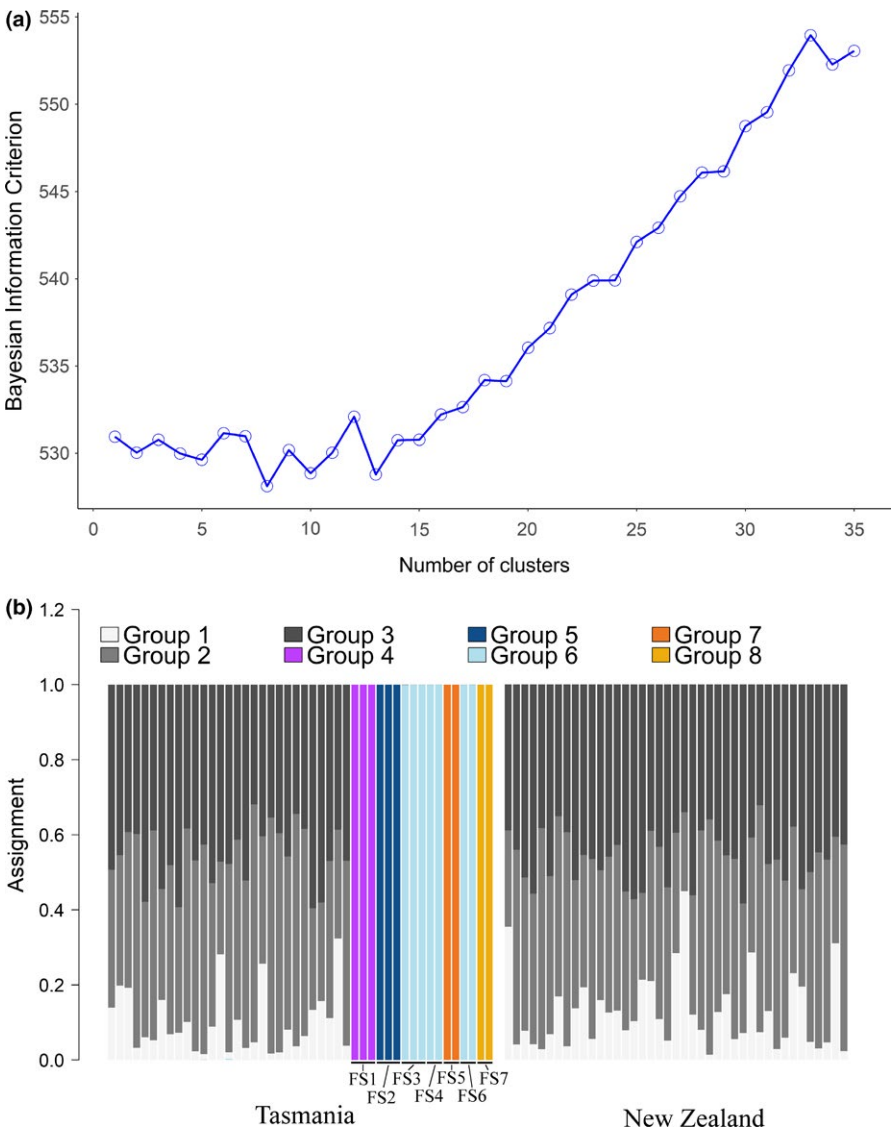

FIGURE 2 (a) Optimal number of cluster selection, based on Bayesian Information Criterion with 29 PCs. (b) DAPC assignment plot between Tasmania and New Zealand (full siblings included), based on seven PCs

Lyle, & Semmens, 2015; McMillan et al., 2018; Olsen, 1954; Ward & Gardner, 1997). Based on current results, the null hypothesis of a single panmictic population cannot be rejected. Both F_{ST} and D_{est}, as well as diversity and clustering analyses, did not detect differentiation between TAS and NZ neonates and juveniles. This is supported by the large dispersal abilities of school sharks (Coutin et al., 1992; Hurst et al., 1999; McAllister et al., 2015; McMillan et al., 2018; Olsen, 1954). Genetic diversity was similar between both sampling regions, but lower compared to previous studies (He = 0.5–0.75; Hernández et al., 2015; Bester-van der Merwe et al., 2017; Domingues et al., 2018). This discrepancy with other studies can be explained by the choice of genetic markers. This study presents the first genomic study of school sharks and in theory allows a more accurate calculation of genetic diversity (Fischer et al., 2017). Overall, our diversity measures correspond to other genomic studies in sharks (Feutry et al., 2017; Maisano Delser et al., 2018; Pazmiño et al., 2018). Furthermore, Ward and Gardner (1997) found weak evidence of genetic differentiation; however, this was based on a single allozyme and mitochondrial DNA markers. Hernández et al. (2015) showed the presence of a single genetic population in Oceania, using mtDNA and microsatellites. With increased power of genome-wide SNPs, we found similar results. The observed signal could also be attributed to other explanations that could not be identified with our current sampling design: (a) a high gene flow that dilutes existing, recent population differentiation (Bailleul et al., 2018; Waples & Gaggiotti, 2006), (b) sex-biased dispersal where one sex obscures the philopatric signal (Fraser, Lippé, & Bernatchez, 2004) or (c) temporal structure caused by their biennial–triennial pupping behavior (Waples, 1998).

4.3 | Future work

The use of neonate and juvenile samples in this study is ideal to detect population structure in highly migratory species, but our sampling design and choice of markers did not allow us to fully investigate potential temporal- or sex-biased dispersal. Regional female philopatry has been suggested by Bester-van der Merwe et al. (2017) in South Africa; however, this has not yet been observed in Oceania (Francis, 2010; Hernández et al., 2015). Hernández et al. (2015) did not detect

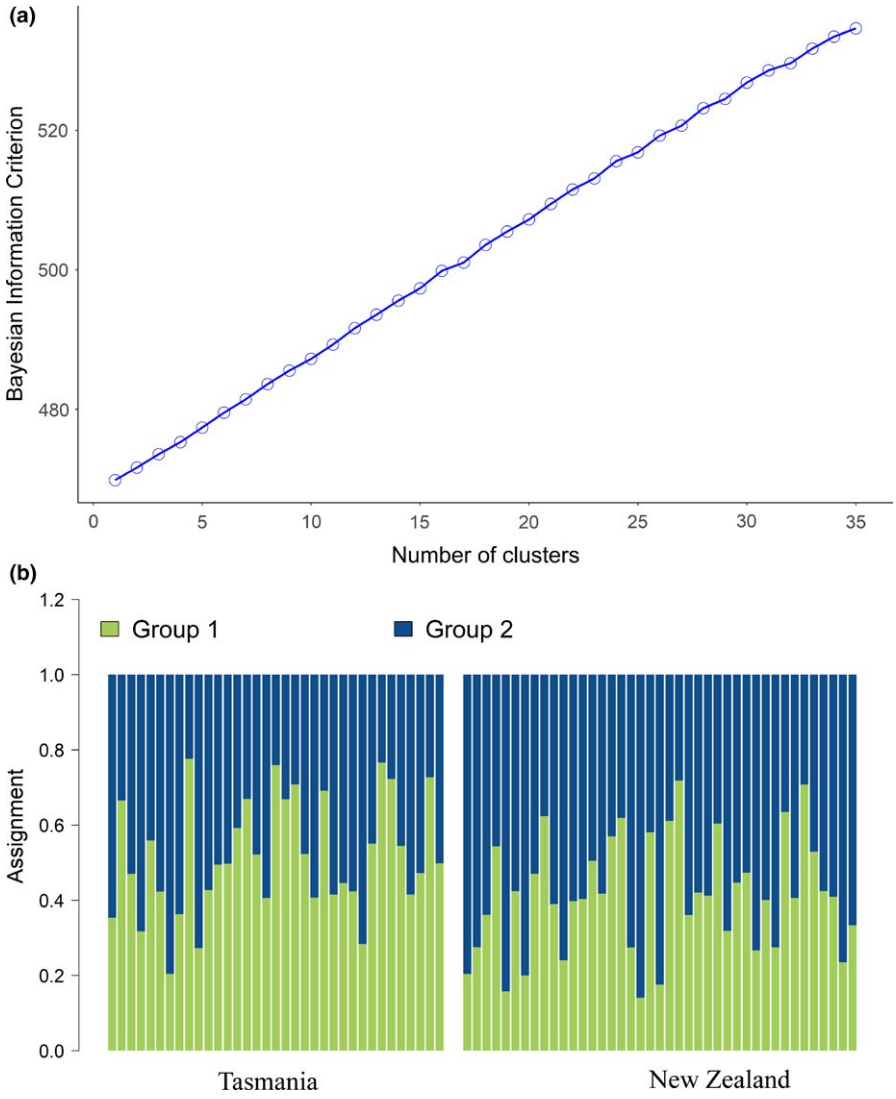

FIGURE 3 (a) Optimal number of cluster selection, based on Bayesian Information Criterion with 25 PC's. (b) DAPC assignment plot between Tasmania and New Zealand (full siblings excluded), based on 35 PCs

any sign of philopatry using mitochondrial markers, but using whole mitogenome sequences instead of the control region might provide better insight (Feutry et al., 2014). Paternally (Y-chromosome) inherited markers or the spatial distribution of siblings may also help detecting sex-biased dispersal (Feutry et al., 2017; Petit, Balloux, & Excoffier, 2002). Moreover, Pittwater, Tasmania, is currently the only known school shark nursery area in Australia where pups can reliably be caught (others in Tasmania and Victoria currently yielding few or no pups). However, samples from other nurseries closer to the mainland of Australia and multi-year sampling could possibly reveal population structure between other regions of Australia and New Zealand. In any case, given the highly migratory nature of adult school sharks, such fine-scale structure, if it existed, would only impact management practices if nurseries areas were to be targeted by the fishing fleet, which is not the case.

5 | CONCLUSION

In conclusion, this study has illustrated how kin bias can affect population structure inference if sampling is not randomly spread and proposed several measures how to identify such biased sampling toward kin. The unbiased estimates of population connectivity could not reject the existence of a panmictic population between Tasmania and New Zealand school sharks; yet possible caveats in the study have been pinpointed and the presence of small local populations may still be plausible. Overall, due to the migratory behavior of school sharks we argue that potential population structure would only form a conservation issue if nursery areas would be targeted by fisheries, which they currently are not.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTION

FD, GM, PG, RT, and PF designed the study. Samples were acquired by SH and JM. RG extracted DNA from the samples. FD analyzed the data with contribution from PF. The manuscript was drafted by FD. All authors reviewed the manuscript and gave final approval for publication. All authors agree to be accountable for all aspects of the work.

DATA ACCESSIBILITY

Raw and filtered SNPs with associated metadata. Data for this study are available at: https://doi.org/10.5061/dryad.pd8612j

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Appendix C

Technical description of model

This Appendix presents a technical description of the close kin model. The symbols used in this Appendix are detailed in Table C.1

Table C.1: Symbols used in this Appendix.

Symbol	Description
a	Age in years
A	Age of plus group (at age 20)
s	Sex, can be m for male, or f for female
f	female
m	male
g	gear
y	Year
l	Total carcass length in cm
r	Number of 'rings' or deposition zones counted in a vertebral section
a_s	Age at first maturity for sex s
a_m	Age at first maturity for males
a_f	Age at first maturity for females
b	Birth year
b_i, b_j	The birth year of individual i or j
$a_i^{b_j}$	The age of individual i during the birth year of individual j
i, j	two individuals that might form a kin pair
i, j	two individuals that might form a kin pair
z_i, z_j	Age-at-capture, year-of-capture, and sex of i and j
z_i*, z_j*	Rings-at-capture, year-of-capture, and sex of i and j
$N_{s,y,a}$	Number of sharks of sex s in year y of age a
$N^x_{s,y,a}$	Where x $\in$ (a,b,c,d,e,f) numbers-at-age arrays used within a year
N_{89}	Number of sharks of age 1 and over in 1989
R^f_a	Number of pups produced by a female of age a
R^f_y	Number of pups produced by all mature females in the population in year y
R^f_l	Number of pups produced by a females in (1cm wide) length class l

Continued on next page

Table C.1 – continued from previous page

Symbol	Description
$\tilde{R}_l^m$	Proportion of males from length class l that are mature (interpreted as relative numbers of pups)
$\tilde{R}_a^m$	Relative numbers of pups for males of age a
$S_{g,a}$	Selectivity of fish of age a by gear g (ranges from zero to 1)
$F_{s,g,y}$	Instantaneous fishing mortality for sex s by gear g in year y
$Z_{s,y,a}$	Mortality rate during year y for fish of sex s and age a
$C_{s,g,y}$	Observed catch (kg) of sex s by gear g in year y
$\hat{C}_{s,g,y}$	Estimated catch (kg) of sex s by gear g in year y
$w_{s,a}$	Weight (kg) of a shark of sex s and age a
$P(l\|a,s)$	Probability that a shark of age a and sex s belongs to (1cm) length class l
$P(r\|a)$	Probability that a shark of age a will be observed to have r vertebral rings
$P(a\|r,s,y)$	Probability that a shark that has r rings, of sex s in year y, has true age a
ϕ_{s_p,a,b_i,b_j}	Proportion of sharks of sex s and age a in year b_i that survive to year b_j
$RRO_{s,y,a}$	Relative reproductive output of sharks of sex s in and age a in year y
$U_{1,y}$	Available biomass for gear 1 (trawl and line) in year y
$CPUE_{1,y}$	Observed standardized CPUE for gear 1 (trawl and line) in year y
q_1	Catchability for gear 1
σ^2	Variance of the residuals for observed versus expected trawl catch rates
R^0	Broadly, the number of pups in 1989
δ^{89}	Broadly, the survival rate and pupping interval in 1989
δ^{2000s}	The constant survival rate and pupping interval for 2000 to 2017
τ	Broadly, an adjustment to the plus group in 1989
F^{89}	Broadly, the fishing mortality during and before 1989
F^{90s}	Broadly, the fishing mortality rate during 1989 to 1999
M	Natural mortality (an instantaneous rate)
ν_1	Litter effect, allowing for 'lucky litters'
ν_2	Proportion of the litter that are likely to have different fathers
q_{father}	Constant of proportionality that prevents fathers from informing abundance
$K_{i,j}$	The kin relationship between i and j
PO	Parent-offspring kin relationship
HS	Half sibling kin relationship
FS	Full sibling kin relationship
s_i, s_j	sex of shark i, and of shark j
a_i, a_j	age-at-capture of shark i, and of shark j
r_i, r_j	vertebral ring counts at capture for shark i, and for shark j
y_i, y_j	year of capture for shark i, and for shark j
s_p	Sex of parent ($s_p = f$ for mothers and $= m$ for fathers)
b_i, b_j	Birth year of shark i, and of shark j
$a_i^{b_j}$	Age of shark i in year b_j, the year j was born
$n_{i,j}$	Number of individuals that have the same z_i and z_j
$c_{i,j}$	Number of individuals that have z_i and z_j, that were observed to have a given kin relationship
$p_{i,j}$	Probability that individuals that have z_i and z_j, have a given kin relationship

C.1 Population dynamics

The model essentially begins in the year 2000. We need to estimate the population age distribution and abundance at the start of 2000, with reasonable flexibility. To do this, we used three estimated parameters to shape the size and age distribution of the population during those years: R^0, τ, and F^{89}. We assume equilibrium during the first year, and that allows us to calculate a fourth parmater: δ^{89}. We do not consider the model estimates for the years 1989 to 1999 to be accurate, but instead to be a means of arriving at the population structure in 2000. The value of the abundance parameter, R^0, for example, is of less interest to us than the resulting abundance in the year 2000, which is a function of all four parameters. The exact interpretation of each of these parameters is not of great interest. Alternative parameterizations that would produce a flexible population distribution in the year 2000, would be equally valid.

The numbers of school shark of sex s and age a present in the population in 1989, $N_{s,1989,a}$ is calculated by assuming constant fishing mortality rate F^{89} (an estimated parameter) prior to that date. To (partly) account for variability in past fishing mortality rates, we estimated an additional parameter τ that influences the size of the plus group (at age A):

$$N_{s,1989,a} = \begin{cases} 0.5\, R^0\, \delta^{89}\, e^{[-(a-1)(M+F^{89})]} & \text{for } a = 1, 2 \ldots (A-1) \\ \tau * 0.5\, R^0\, \delta^{89}\, e^{[-(a-1)(M+F^{89})]} / \left(1 - e^{-(M+F^{89})}\right) & \text{for } a = A \end{cases} \tag{C.1}$$

where M is the natural mortality rate for sharks aged one and over (an estimated parameter),
R^0 is an estimated parameter; broadly interpretable as the number of pups (age 0) in 1989, and
δ^{89} is, broadly, the combined survival rate, and pupping interval, during the first year of life (up to 1989).

Note that the number of animals present in the population in 1989 (N_{89}) is the sum of $N_{s,1989,a}$ over both sexes and all ages. Table 4.9 gives values for N_{89} instead of R^0 because that is a more easily understood figure. The model can be parameterised to estimate either quantity.

We calculate δ^{89} such that the population is in equilibrium during 1989. We trialled models that allowed δ^{89} to be an estimated parameter, but that allow estimated numbers of pups in 1989 that were implausibly larger or smaller than the values in subsequent years (which were calculated based on the number of females in the population and their biologically determined litter sizes). That unusual cohort would move through the population creating an implausible numbers-at-age distribution. Assuming equilibrium, δ^{89} is defined by the sex ratio at birth (50:50) and the reproductive output (i.e. number of pups produced, $R_{f,1989}$) of female sharks of all ages

$$\delta^{89} = 2 \setminus R^f_{1989}. \tag{C.2}$$

The total reproductive output of the population for any year y (R_y^f) is the sum of the number of pups of both sexes produced per individual female shark (R_a^f) summed over all ages a (from age at first maturity for females, m_f, to the plus group age, A)

$$R_y^f = \sum_{a=m_f}^{A} N_{f,y,a}\, R_a^f. \tag{C.3}$$

Later, we will discuss the relative numbers of pups per male, R^m. The number of sharks in the population at the start of the year 2000, is given by applying a term that could broadly be interpreted as the estimated fishing mortality rate between 1989 and 2000, F^{90s} for the 6 and 6.5 inch gillnet gears ($g = 2$ and $g = 3$, respectively). The trawl and line, and the larger mesh gillnet gears were assumed not to be in use. The four parameters that govern dynamics during 1989 to 1999 are likely to be correlated so that none have exact interpretations. As such, we did not use actual catches that were taken between 1989 and 1999.

$$N_{s,y+1,a+1} = N_{s,y,a}\, e^{-(M+S_{2,a}\ F^{90s}+S_{3,a}\ F^{90s})} \quad \text{for } y = 1989, 1990 \ldots 1999. \tag{C.4}$$

Recruitment (number of one year olds) is given by the female reproductive output the previous year and the survival rate of pups (i.e. zero year olds, δ_y) in the previous year. We assume a 50:50 sex ratio at birth:

$$N_{s,y+1,1} = 0.5\ R_y^f\ \delta_y. \tag{C.5}$$

Pup survival in 1989, δ^{89}, was an estimated parameter, as was pup survival from 2000 to 2017 (δ^{2000s}), which was assumed to be constant. During 1989 to 2000 we set annual pup survival rates δ_y by linear interpolation between δ^{89} in 1989 and δ^{2000s} in 2000.

Between 2000 and 2017 we deduct annual catches (in weight) by gear and sex, $C_{s,y,g}$ by estimating the fishing mortality rates $F_{s,y,g}$ that would give the observed catches. We assume a 50:50 split of the catch between sexes, see justification for this in Section 4.2.1. Our calculation required that we convert the numbers of sharks in our model to weights using the size-weight relationship (see Table 4.4). First we deducted half the natural mortality for the year, then we estimated, and deducted, the fishing mortality rate that gave the observed catch $C_{s,y,g}$ for each gear type in turn, and then we deducted the remaining half of the natural mortality. If we name the intermediate numbers-at-age arrays N^a, N^b up to N^f then

$$N_{s,y,a}^a = N_{s,y,a}\, e^{-0.5\ M} \tag{C.6}$$

$$N_{s,y,a}^b = N_{s,y,a}^a\, e^{S_{1,a}\ F_{s,1,y}} \tag{C.7}$$

where $F_{s,y,1}$ was calculated using Newton's method such that the observed catch for gear $g = 1$, $C_{s,1,y}$ in year y is equal to the estimated catch $\hat{C}_{s,y,1}$

$$C_{s,y,1} = \hat{C}_{s,y,1} = \sum_{a=1}^{A} N^{a}_{s,y,a}\ w_{s,a}\ \left(1 - e^{-F_{s,y,1}\ S_{1,a}}\right) \tag{C.8}$$

where $w_{s,a}$ is the weight of a shark of sex s and age a. Note that the catch $C_{s,y,g}$ and the weight-at-age ($w_{s,a}$) must be expressed in the same units (e.g. kg) so that the units of N are individual sharks. Stock assessment models often express catch in tonnes and weight-at-age in kilograms so that the unit of N is thousands of individuals, but in a close kin model where kin pairs are in units of individuals, the N array must also be expressed as single individuals.

Similarly, we derived N^c from N^b using the observed catches from gear 2, and looped over all gears until we had accounted for the catch from all five gears (trawl and line, 6 inch, 6.5 inch, 7 inch and 8 inch gillnets), giving N^f. Note that the catches for 7 and 8 inch gillnets are close to zero and these fleets are only present in the model because we had initially hoped to model the population further back in time, when those gears were in use. The numbers at age in the population at the start of the following year $N_{s,y+1,a+1}$ is then given by taking the remaining half of the natural mortality

$$N_{s,y+1,a+1} = N^{f}_{s,y,a}\, e^{-0.5\ M} \tag{C.9}$$

Age-specific survival rates for each cohort are needed to calculate the probability that the parent of one animal survived until the birth year of their sibling. It is given by

$$Z_{s,y,a} = -\ln\left[\frac{N_{s,y+1,a+1}}{N_{s,y,a}}\right] \quad \text{for } a = 1, 2 \ldots (A-2) \tag{C.10}$$

For ages $A-1$ and A, we assumed the same survival rate as for age $A-2$ in the same year.

C.2 Reproductive output and biologicals

The reproductive output (number of pups per individual, R^f) of female sharks is given by the observed number of pups per female Walker (2005) the parameters of which are given in Table 4.4 of this report. Relative reproductive output for males was based on maturity, as given by testis condition (Walker, 2005). Note that the mean number of pups fathered by males ($\tilde{R}^m$) is not required by the model, instead we use a function that has a maximum value of 1 to scale the probability of being a father relative to sharks of other ages (and lengths).

We specified reproductive output in terms of 1 cm length bins, l (R^f_l, $\tilde{R}^m_l$), as given by (Walker, 2005) and Table 4.4 of this report, and then converted those to functions of age. This was done by multiplying by the probability of being in length class l, given age a, for sex s, $s \in (m, f)$, for length class l, $P(l|a, s)$, and summing over all length classes

$$R^{f}_{a} = \sum_{l=l_1}^{l_2} R^{f}_{l}\ P\left(l|a, f\right) \tag{C.11}$$

and similarly for $\tilde{R}^m_a$.

Similarly, selectivity and weight-at-age were described as functions of length and then converted to age. We calculated $P(l|a,s)$ by assuming a normal distribution for length-at-age, with mean given by the growth curves for each sex, and sex-specific variance taken from unpublished work by Andre Punt (CSIRO and University of Washington, pers commn) that is used in the school shark stock assessment model, see Section 4.7.2 for discussion of those variances.

C.3 Close kin probabilities

The probabilities that any two sampled animals have a particular kin relationship, where that can be parent-offspring PO, half-sibling HS, full-sibling FS, or unrelated UP, are given below. First we present the probabilities in terms of true age, as if that were known. In the following section we account for our imperfect knowledge of age by expressing the kin probabilities in terms of observed ring counts, given probable rings-at-age.

C.3.1 Parent-Offspring pairs

First, we address the probability of two individuals, i and j, having a parent - offspring (PO) kin relationship ($K_{i,j}$), $P[K_{i,j} = PO]$. This probability will depend on measured co-variates for each individual, z_i and z_j. These co-variates are sex, year-of-capture, and we assume, for this section of the report, that z also includes an accurate measure of the true age-at-capture of each animal. Section C.4 below extends our equations to the case where we have only an inaccurate measure of age (as indeed, we do). For school shark, we need to know the sex of the possible parent, s_i as well as its age when j was born, (given by its age at capture a_i, year of capture y_i and the age and year of capture of j, a_j and y_j). The year of j's birth is given by its age at capture a_j and year of capture y_j, $b_j = y_j - a_j$ and we can calculate the birth year of i, b_i similarly. The age of i in the birth year of j, b_j is $a_i^{b_j} = b_j - b_i$. The probability that i and j are a POP is given by the reproductive output of an animal of sex s_i and age $a_i^{b_j}$, divided by the total reproductive output of all mature individuals of the same sex that were alive in the year b_j

$$P[K_{i,j} = PO|z_i, z_j] = \begin{cases} R^{s_i}_{a_i^{b_j}} \Big/ \sum_{a=a_{s_i}}^{A} N_{s_i,b_j,a}\ R^{s_i}_a & \text{for } y_i > b_j, \text{ and } a_i^{b_j} \geq a_{s_i} \\ 0 & \text{otherwise} \end{cases} \tag{C.12}$$

where a_{s_i} is the age at first maturity for sex s_i, and
A is the age of the plus group.

Note that the absolute number of offspring produced by males is not important because a multiplier that converts relative to absolute male maturity would appear in both the numerator and denominator of equation C.12 and would therefore cancel out. It does matter for females, but only through equation C.3.

The school sharks sampled for this study were lethally sampled, so we must also ensure that the year of capture for the parent was after the birth year of the offspring, i.e. $y_i > b_j$. Also, the parent must be mature in year b_j, i.e. $a_i^{b_j} \geq a_{s_i}$.

C.3.2 Cross cohort half sibling pairs

Now we consider the probability that i and j are half siblings, sharing a parent whose sex is denoted by s_p, and given ages and years of capture for i and j, $P[K_{i,j} = HS|z_i, z_j]$. First we consider individuals born in different years ($b_i \neq b_j$). We never observe the shared parent so we have no way of knowing its age and must sum the probabilities for all possible ages of the shared parent. We do know the unseen parent's sex, s_p, from mitochondrial data for the half siblings. The parent must have been mature in the year the older individual was born (b_i) and it must have survived until the year the younger individual was born (b_j). (We will assume, later, when we express these probabilities in terms of ring counts, that we do know which sibling was born first). We describe the annual survival rates for cohorts in equation C.10. The cumulative survival ϕ_{s,a,b_i,b_j} of an individual of sex s_p that has age a in year b_i and age $a+t$, t years later in year b_j (i.e. $b_j - b_i = t$) is given by the product of the $t-1$ quantities

$$\phi_{s_p,a,b_i,b_j} = e^{-Z_{s_p,a,b_i}}\ e^{-Z_{s_p,a+1,b_i+1}} \ldots e^{-Z_{s_p,a+t-1,b_j}}. \tag{C.13}$$

For notational clarity, we define the relative reproductive output of an individual of sex s and age a in year y as $RRO_{s,y,a}$, given by

$$RRO_{s,y,a} = \frac{R^s_{y,a}}{\sum_{a'=a_s}^{A} N_{s,y,a'}\ R^s_{y,a'}} \tag{C.14}$$

The half-sibling probability for an unseen parent of sex s_p that had age a in b_i is the product of (1) the probability that such an individual was the parent of i; (2) the probability that that unseen parent survived from year b_i until year b_j (t years later); (3) that that individual was also the parent of j, and (4) we must allow for underestimation of the number of half sibling pairs due to false negatives when identifying HSPs from their genetic sequences (λ), as described in Section 4.5.3. Because we don't know the age of the unseen parent, we must sum over all possible ages a

$$\begin{aligned} P[K_{i,j} = HS|z_i, z_j] = \sum_{a=a_{sp}}^{A-1} \big[& N_{s_p,b_i,a}\ RRO_{s_p,b_i,a} * \\ & \phi_{s_p,a,b_i,b_j}\ RRO_{s_p,b_j,a+t}\ \lambda \big]. \end{aligned} \tag{C.15}$$

Note that the summation does not include the plus group A because we do not know the birth year of individuals that belong to the plus group. We chose A (and the upper limit on ring counts allowed in the study) such there are negligible numbers of individuals in that group.

For the case where we do not allow fathers to influence the estimate of absolute abundance, we further multiply equation C.15 by the estimable parameter, q_{father}, when we are calculating probabilities for male parents, s_p. This effectively 'decouples' abundance as given by PHSPs from that given by MHSPs.

C.3.3 Same cohort siblings

If we had accurate age data, we would eliminate all FSPs and same cohort HSPs because the 'lucky litter' effect renders same cohort siblings unhelpful for calculating abundance. Because we cannot do that, we must calculate ν_1 the 'lucky litter' effect, and ν_2 the proportion of any litter that are likely to have different fathers (i.e. that are half, rather than full, siblings). The probability that i and j both born in b_i (i.e. $b_i = b_j$) are half siblings that share an unseen parent of observed sex s_p is

$$P[K_{i,j} = HS|z_i, z_j, s_p] = \sum_{a=a_{s_p}}^{A-1} RRO_{s_p,b_i,a}\ \nu_1\ \nu_2. \tag{C.16}$$

If the shared parent is a mother, then the same cohort half siblings must have been litter mates (school sharks do not produce more than one litter per year). We therefore do not need the first row from equation C.15, or the survival probability. Unlike mothers, fathers can contribute to more than one litter each year. We could have estimated an additional parameters, ν_3 to account for the likelihood of paternal half-siblings in a single cohort, but instead we allowed the existing parameters to account for that and checked our choice through estimating the q_{father} parameter. Deviation from 1 would indicate that fathers are giving a different abundance from mothers. This could mean a poor choice for the reproductive output function for males, and / or the need for a ν_3 parameter.

Full siblings, by definition, must be litter-mates because the likelihood (if we assume random mating) of the same two parent animals mating more than once, in different years, is negligible given a population of this size. The probability that i and j are full siblings is expressed in terms of the shared mother $s_p = f$

$$P[K_{i,j} = FS|z_i, z_j] = \sum_{a=a_f}^{A-1} \left[RRO_{f,b_i,a}\ \nu_1\ (1-\nu_2)\right]. \tag{C.17}$$

C.4 Ring counts

The mean number of vertebral 'rings' is assumed to be equal to the true age until age 11, after that rings are deposited at a rate of 0.36 per year. Ageing error was found to be 0.08 and we use this as the CV for younger animals, after age 11 we assume a CV of 0.16 to account for variability in ring deposition rate. The probability that an animal of age a will be observed to have r rings, $P(r|a)$ is given by a Normal distribution

$$P(r|a) \sim \begin{cases} N\left(a, (0.08\ a)^2\right) & \text{if } a \leq 11 \\ N\left(11 + 0.36(a-11), (0.16\ \mu)^2\right) & \text{if } a > 11 \end{cases} \tag{C.18}$$

In the second line of equation C.18, for improved readability, we give the formula for the mean (μ) of the normal distribution and then simply use the symbol μ in the variance formula.

The probability of having true age a given r observed rings is given by Bayes formula

$$P(a|r,s,y) = \frac{P(r|a)\ P(a|s,y)}{\sum_{a'=1}^{A} P(r|a')\ P(a'|s,y)} \tag{C.19}$$

where $P(a|y)$ is given by the numbers at age in the population in year y for sex s. Because this depends on s, the sex of every animal in the close kin sample must be known when calculating kin probabilities, even when the sex is not important as far as pure close kin relationships are concerned. For example, the sex of the offspring in a POP is not, strictly, relevant (see equation C.12); only the sex of the parent matters through its RRO. However, because we do not know the offspring's true age, we must infer that from its ring count, and its sex then becomes relevant through equation C.19.

To convert the close kin probabilities that we expressed as a function of true age, to functions of ring count, we integrate over all possible true ages, given ring counts, for both animals. For example

$$P[K_{i,j} = PO|z_i*, z_j*] = \sum_{a'_i=1}^{A} \sum_{a'_j=1}^{A} P[K_{i,j} = PO|z_i, z_j]\ P(a'_i|r_i, s_i, y_i)\ P(a'_j|r_j, s_j, y_j) \tag{C.20}$$

where z_i* and z_j* are the obsevable covariates for animals i and j (they include ring counts r_i and r_j but *not* true ages a_i and a_j).

Similarly, we derive $P[K_{i,j} = HS|z_i*, z_j*, s_p]$ from $P[K_{i,j} = HS|z_i, z_j, s_p]$ and $P[K_{i,j} = FS|z_i*, z_j*]$ from $P[K_{i,j} = FS|z_i, z_j]$.

C.5 Trawl CPUE

We modelled the CPUE for the trawl fishing fleet by assuming that it would follow the catch rate of the combined trawl and line fleet. We assumed the same knife-edged selectivity used by the stock assessment model (Punt *et al.*, 2000; Punt, 2001). The catch rate of the combined fleet (gear, $g = 1$) U_1, y is proportional to the available biomass

$$U_{1,y} = \sum_{\forall s} \sum_{a=1}^{A} N_{s,y,a}\ w_{s,a}\ S_{1,a}. \tag{C.21}$$

The observed catch rate for the trawl fleet $CPUE_{1,y}$ is approximated by $U_{1,y}$ multiplied by 'catchability', q_1, which we estimated using the least squares method (Polacheck *et al.*, 1993).

$$q_1 = \frac{1}{18} \sum_{y=2000}^{2017} \left[ln(CPUE_{1,y}) - ln\left(U_{1,y}\right) \right]. \tag{C.22}$$

C.6 Likelihood

The likelihood component for the close kin data is a series of Bernoulli trials, one for every possible pairing of individuals, and every type of kin relationship. We observed only three POPs, all of which were eliminated when we restricted the sample to only those with 11 or fewer ring counts. Therefore, the result of every POP trial is a 'failure'. The negative log-likelihood component for POPs, $-lnL_{PO}$, is the sum over all unique combinations of observed covariates (z_i* and z_j*), of the binomial probability of every failure

$$-lnL_{PO} = \sum_{\forall z_i*} \sum_{\forall z_j*} n_{i,j}\ ln(1 - p_{i,j}). \tag{C.23}$$

The model sensitivity that ignores fathers does not include summation over males for individual i.

where $p_{i,j}$ is the probability of success (i.e. observing a POP) given by $P[K_{i,j} = PO|z_i*, z_j*]$, and
$n_{i,j}$ is the number of trials that were done for every unique combination of elements of z_i* and z_j*.

For half siblings we observed successes as well as many failures. For every unique combination of covariates z_i* and z_j*, we observe $c_{i,j}$ successes from of $n_{i,j}$ trials. The joint negative log-likelihood is

$$-lnL_{HS} = \sum_{\forall s_p} \sum_{\forall z_i*} \sum_{\forall z_j*} \left[c_{i,j}\ ln(p_{i,j})\ + (n_{i,j} - c_{i,j})\ ln(1 - p_{i,j}) \right] \tag{C.24}$$

where $p_{i,j}$ is given by $P[K_{i,j} = HS|z_i*, z_j*, s_p]$. The model sensitivity that ignores fathers is limited to $s_p = f$.

The component for FSPs, $-lnL_{FS}$ is derived similarly, with $P[K_{i,j} = FS|z_i*, z_j*]$ giving the probability of a success.

The sensitivity that uses the CPUE for the trawl fleets includes a likelihood component lnL_{CPUE}

$$lnL_{CPUE} = \sum_{y=2000}^{2017} \left(\frac{1}{2\ \sigma^2} \right)\ \left[ln(CPUE_{1,y}) - ln(U_y) \right] \tag{C.25}$$

where the value for σ was taken from the CPUE standardization report (Sporcic & Haddon, 2018).

Appendix D

Brief summary of close kin project

Summary of school shark close kin project FRDC 2014-024

Mark Bravington & Robin Thomson; 8 March 2018

This project has successfully delivered a close-kin-mark-recapture-based (CKMR) abundance estimate for school shark; we found an adequate number of kin-pairs, and there were no insurmountable problems with sampling, genetics, kin-finding, or CKMR modelling. The model has established the current level (i.e. absolute abundance) of the stock; the trend estimate is still imprecise, for reasons discussed below, but will tighten up over the next few years if there is continued sampling and genotyping of sufficient numbers of school sharks.

Sample collection was extremely low cost due to good industry co-operation. A key industry partner left the shark fishery, which caused a delay to the project, but we were ultimately successful in achieving our aspirational target of 3,000 samples. The new industry-led SIDaC initiative will ease future sample collection.

We identified 102 kin pairs. Our results show that the population is smaller, but consequently more productive, than previously thought. The model estimates are consistent with simple back-of-the-envelope approximations based on the idea of "everyone has one mother and one father". There was no need to rely on ambiguous fishery-dependent CPUE, nor to make assumptions about selectivity in order to use length composition data. The small population size indicates that mixing with the New Zealand school shark stock can be discounted.

One unexpected complication was the relatively high proportion of the sample that were over 11 years old, after which age-estimation based on vertebral rings becomes highly uncertain. Older school sharks are thought to lay down, on average, only one vertebral band every three years. Although we were able to build a CKMR model that took account of this uncertainty, it reduced, in some sense, the "effective sample size" and the amount of demographic information that could be extracted quickly.

Despite the substantial sample size and number of kin-pairs, our estimate of recent trend is currently imprecise: the point estimate is positive (indicating recent population increase) but the confidence interval is wide enough that decline cannot be ruled out yet. This is mainly because of the imprecise age data for older sharks, combined with the shift in population dynamics, which have restricted the range of juvenile cohorts usable for trend estimation. However, sampling and genotyping are likely to continue and the trend estimate will become more precise; simply put, if there are more potential parents for future cohorts, then there is a lower chance that two randomly-sampled juveniles will have the same parent, so the proportion of half-siblings will visibly drop. Since the overall process is clearly able to deliver useful numbers of half-siblings, it is only a matter of time before the true trend reveals itself.

There seems to be no way to reconcile pre-2000 catch data with current population dynamics, except by some major shift such as the extirpation of historical breeding sites or greatly reduced productivity. This was already suspected based on CPUE-based assessments, but is confirmed by our work. This mis-match (which results from population biology, not from the use of the CKMR method) precludes the estimation of an historical B_0 for school shark without introducing a much more complex and assumption-driven model; a situation that sharkRAG chose to avoid. The CKMR data (which pertain to post-2000) do make sense internally, but cannot be reconciled with high historical catches indicating an apparent long-term shift in population dynamics and bringing the interpretation of B_0 into question. The implication that this has for appropriate management targets applies, also, to other SESSF species that have showed progressive productivity change (such as silver warehou and jackass morwong). Even without a B_0, CKMR can be used to estimate replacement yield and conservative RBCs could be set below that level.

Future projections using the median results from the CKMR model, along with fixed future levels of fishing mortality, were used to calculate time series of increasing catches that recognise the likely increase in unavoidable bycatch as the stock rebuilds. SharkRAG recommended using catches that relate to the average fishing mortality rate over 2013-2017.

The genotyping technology ("DartCap" from Diversity Arrays Technology), which was new and being tested for the first time, simultaneously, on school shark and southern bluefin tuna, was very successful and economical per sample cost, reliably distinguishing half-sibling pairs; which is a tough challenge for a genotyping method. Future CKMR projects on other species that use the same technology will have much lower development overhead and low unit costs.

We have also gained experience in how to design future CKMR projects, in particular "power calculations" for choosing sample sizes that should achieve specific assessment goals (e.g. some target precision of trend); the approach we have developed requires a significant amount of preliminary work, but helps considerably in the longer term with planning.

Appendix E. Assessment review by Patrick Cordue

A review of the 2019 close kin DNA stock assessment of school shark (Southern and Eastern scalefish and shark fishery)

P.L. Cordue
Innovative Solutions Limited (ISL)
15 October 2019

ISL Client Report
for
The Southern Shark Industry Alliance

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Executive summary

The Southern Shark Industry Alliance (SSIA) requested that Innovative Solutions Ltd (ISL) perform a review of the current school shark assessment in the southern and eastern scalefish and shark fishery. They were concerned that the current assessment may not be adequately representing the true status of the resource.

In the current assessment an age-structured model is fitted to "close kin" data from DNA sampling. It assumes a single stock within a single area and starts in 2000. SSIA is concerned that the stock being assessed through "close kin" may be a different and smaller stock than that which was historically fished as the current model results are inconsistent with catches taken before 2000.

A draft review report was prepared in September 2019 and sent to CSIRO with a request for comments. CSIRO declined to provide any substantive comments.

The principle behind the use of close kin data in the school shark stock assessment is that the number of close kin pairs found from a sample of sharks is inversely related to the population size. The more close kin pairs that are found, for a given sample size, the smaller the population.

Approximately 3000 sharks were sampled from 2010 to 2018 for DNA, sex, and length. The samples were spread over three main areas: south Australia, Bass Strait, and Tasmania. Samples were collected by fishers, fish processors, and scientific observers. Mainly juveniles were sampled but mature sharks were also sampled. DNA was sequenced to sample 15 sex markers and 2000 SNPs (single nucleotide polymorphisms). The raw DNA data were analysed by CSIRO to produce a number of statistics to separate the different close kin relationships (parent of progeny [POP], full sibling pair [FSP], maternal half sibling pair [MHSP], paternal half sibling pair [PHSP] or unrelated pair [URP]). For the sharks in the close-kin pairs, ages were estimated by ring counts.

The assessment was found to be deficient in many regards:

1. According to the DNA results there were a large number of duplicate samples (85) which points to poor laboratory procedures OR genuine duplicates (i.e., identical twins/triplets and/or parthenogenesis which has implications for the analysis)
2. An inability to distinguish between FSPs and POPs using just DNA which points to poor SNP selection OR no genuine POPs (3 POPs were assumed on the basis of age difference)
3. Numerous FSPs with age gaps that are inconsistent with the variance of repeat age readings (which implies huge ageing error which appears to have been ignored in the base model OR the occurrence of repeat matings which has implications for the analysis)
4. The deliberate absence of survey design given a plan to use conditional probabilities. This leads to unnecessary complexity and the need to account for ageing error
5. The assumption that mating is at random which appears to be contradicted by the data
6. **The assumption that the sharks are spatially fully mixed and therefore that the probability of close kin pairs is independent of the distance between capture locations (which appears to be contradicted by the data)**
7. **Neglecting to include nibling pairs (uncle-nephew, uncle-niece, aunt-nephew, aunt-niece) in the set of close kin relationships (which need to be included unless the age range is severely restricted)**

8. **Possibly incorrect calculation of the probabilities of MHSPs and PHSPs (perhaps forgetting to subtract the probability of a FSP from the probabilities of having the same mother/father)**
9. **Incorrect calculation of the likelihood by using independent Bernoulli trials for each close kin relationship for a given pair of sharks (instead of a single multinomial trial)**
10. **Inability to account for known catch history before 2000.**

The current stock assessment contains numerous errors which make it unusable for management purposes. The use of close kin genetic data in the school shark assessment is advantageous. It was the implementation of the approach which was at fault not the use of close kin genetics. The current assessment should not be resurrected with just a few modifications. It uses complex conditional probability equations which are unnecessary if a subset of the data is used to approximate random sampling from the bycatch fisheries.

To get a rough idea of the stock size, an age-structured, individual-based, equilibrium model was fitted to the close kin data. The model results suggest that stock size from 2000 to 2017 could be of the order of 120,000 to 240,000 **mature** sharks (i.e., a factor of 2-4 times higher than the base model results). This is just an educated guess and should not be used for management purposes. A new assessment is needed.

Introduction

The Southern Shark Industry Alliance (SSIA) requested that Innovative Solutions Ltd (ISL) perform a review of the current school shark assessment in the southern and eastern scalefish and shark fishery (SESSF). They were concerned that the current assessment may not be adequately representing the true status of the resource.

In the current assessment an age-structured model is fitted to "close kin" data from DNA sampling (Thomson et al. draft). It assumes a single stock within a single area and starts in 2000. SSIA was concerned that the stock being assessed through "close kin" may be a different and smaller stock than that which was historically fished as the current model results are inconsistent with catches taken before 2000.

ISL undertook a review of the assessment based on the draft report (Thomson et al. draft) and some limited email exchanges with the authors (primarily Robin Thomson). The terms of reference (TOR) for the review were:

1. Evaluation of the ability of the stock assessment for school shark, with the available data, to provide parameter estimates to assess the current status of school shark.
2. Evaluation of the strengths and weaknesses of the stock assessment for school shark.
3. Recommendations for improvements to the assessment of school shark.

Each of the TOR are covered in the report. In addition, a simple stock assessment model was used to investigate and illustrate the effect of various factors and assumptions on estimates of mature stock numbers (when the model is fitted to close kin data). The development and use of a model is unusual when reviewing an existing stock assessment. However, it was found that the existing assessment contains several major errors. Rather than just pointing out the errors it was felt best to explore the likely stock size given the available close kin data.

A draft review report was prepared in September 2019 and was sent to CSIRO with a request for comments so that the review report could be corrected for possible misinterpretations and otherwise improved. CSIRO declined to provide any comments.

Summary of the close kin school shark stock assessment

The principle behind the use of close kin data in the school shark stock assessment is that the number of close kin pairs found from a sample of sharks is inversely related to the population size. The more close kin pairs that are found, for a given sample size, the smaller the population.

Approximately 3000 sharks were sampled from 2010 to 2018 for DNA, sex, and length (Thomson et al. draft). The samples were spread over three main areas: south Australia, Bass Strait, and Tasmania. Samples were collected by fishers, fish processors, and scientific observers. Mainly juveniles were sampled but mature sharks were also sampled. DNA was sequenced to sample 15 sex markers and 2000 SNPs (single nucleotide polymorphisms). The raw DNA data were analysed by CSIRO to produce a number of statistics to separate the different close kin relationships (parent of progeny [POP], full sibling pair [FSP], maternal half sibling pair [MHSP], paternal half sibling pair [PHSP] or unrelated pair [URP]). For the sharks in the close-kin pairs, ages were estimated by ring counts which have been thought to be fairly reliable up to 11 years (Thomson et al. draft).

The DNA analysis was unsuccessful in separating POPs from FSPs and instead this was done on the basis of the difference in ages assuming that repeat matings do not occur (Thomson et al. draft). There were three pairs which had DNA consistent with FSPs but had an age difference that suggested they were parent and pup (assuming no repeat matings between the same pair of sharks). In all, 102 close kin relationships were found.

Two simple models were used with the DNA data to obtain estimates of mature numbers but the actual stock assessment model is an age structured model which minimises a likelihood function based on a series of Bernoulli trials (one for each pair and each close kin relationship). The basic principle followed by Thomson et al. (draft) is to use the observed sex and age of each fish (in each pair) to calculate the probability that each observed pair will have each close kin relationship, and to form the likelihood from those conditional probabilities.

The estimate from the base model is about 60,000 mature sharks in the stock in each year from 2000 to 2017 (Figure 4.20 in Thomson et al. draft). This is much lower than the previous stock assessment estimates of over 250,000 sharks through 2000 to 2017 (Figure 4.20 in Thomson et al. draft). The close kin estimates are inconsistent with the catch history before 2000. This is speculatively explained by Thomson et al. (draft) through a major shift in stock productivity: "There seems to be no way to reconcile pre-2000 catch data with current population dynamics, except by some major shift such as the extirpation of historical breeding sites or greatly reduced productivity."

Despite this major inconsistency the assessment authors claim that the number of mature fish is well determined: "This project has successfully delivered a close-kin-mark-recapture-based (CKMR) abundance estimate for school shark; we found an adequate number of kin-pairs, and there were no insurmountable problems with sampling, genetics, kin-finding, or CKMR modelling. The model has established the current level (i.e. absolute abundance) of the stock…".

Methods

The methods of the review include the reading of the draft stock assessment report, consulting some relevant references, and some email exchanges with the first author (primarily). However, they also include the development of an equilibrium, age structured, individual based model to explore the effect on stock assessment estimates of various factors and assumptions. It was felt insufficient to just point out the technical errors in the existing assessment without providing some indication of how wrong the current estimates could be.

The equilibrium model was fitted to the observed number of close kin pairs used in the base stock assessment model: 0 POPs, 33 FSP, 29 MHSPs, 13 PHSPs. These were obtained from 1627 sharks with 11 rings or fewer (to avoid the worst ageing error) and the number of pair comparisons was reduced by 8% (as sharks caught on the same trip were not compared) (see Thomson et al. draft). As it is an equilibrium model, the samples were all assumed to occur in the same year. A "one-line" calculation (see Thompson et al. draft) was also done fitting to 16 MHSPs and 10 PHSPs.

The model was started in an approximate equilibrium with a specified number of mature sharks (it would been an exact equilibrium except for the integer effects because it is an individual based model). Sex ratio was assumed 50/50 at birth with knife edge maturity for males at 9 years and for females at 11 years. Females were assumed to produce 20 pups each from up to two different fathers. There was a primary male who was the father of approximately 90% of the pups (in a given litter) with a secondary male who was the father for approximately 10% of the pups. Females were not allowed to pup in consecutive years. Mothers and fathers were chosen at random from the

mature sharks in the population. A constant total mortality was assumed. Natural mortality was fixed at 0.1 for sharks 1 year or older and at 1.0 for the first year of life. A "lucky litter" effect was modelled with a proportion of the litters not suffering the high natural mortality in the first year of life. The fishing mortality was specified (0.03, 0.05, or 0.07) and fishing mortality was applied to sharks 4 years and older. A maximum age of 50 years was assumed for establishing the near equilibrium.

The model started in the near equilibrium and was run for 100 years with parents assigned at random to the required number of annual litters (to maintain equilibrium). As the litters were created the names of the parents were recorded. In this way an equilibrium population was established where the genealogical history of the living sharks was available up to at least the grandparents.

The model was fitted by least squares using predicted numbers of close kin pairs given the overall sample size (1627 fish sampled but the number of pairs reduced by 8%). Only two parameters were estimated: the total number of mature sharks and the lucky litter effect. The probability of each type of close kin relationship was calculated from the equilibrium population (it contains all the relevant information). For example, if p is the probability of two sharks sampled at random being a POP then the expected number of POPs is $p \times 0.92 \times 1627 \times 1626 / 2$.

The age range for sampling was restricted to 2 to 13 years inclusive (not 1 to 11 years). This was to allow for some ageing error (e.g., as sharks with 11 rings could be 11 years or a bit older or a bit younger) and because 1 year old sharks did not appear to be sampled. Also, the close kin relationships used in the fitting were: POPs, FSPs, and Others. The relationship "Other" included PHSPs, MHSPs, and nibling pairs (NiPs). A nibling pair is one of uncle-nephew, uncle-niece, aunt-nephew, or aunt-niece. The niblings are not mentioned by Thomson et al. (draft) but genetically they cannot be distinguished from HSPs (e.g., see ISOGG 2019). I did not consider grandchildren-grandparents pairs as these are eliminated by the age restriction.

The individual-based model was extended to include area effects and dominant male/female effects. This was to allow for pups staying in their pupping area for some time and therefore being more likely to yield FSPs and HSPs when sampling was clustered within areas (as it was). The notion of dominant male sharks which father a disproportionate number of pups is a very plausible hypothesis; there may also be dominant females. Shark mating is complex and obviously the sharks do not mate at random.

Results

The equilibrium model has some stochastic elements as the parents of the required number of litters each year are selected at random as are the individuals that die each year due to natural or fishing mortality. So, each population that is generated will deliver slightly different estimates when fitted to close kin observations.

The probabilities of each type of close kin relationship depend primarily on the age range sampled (rather than selectivity within an age range). For example, when the whole population is sampled at random the probabilities of POPs and NiPs are much higher than if sampling is restricted to ages 2-13 years (compare Figures 1 & 2).

Probabilities conditioned on age can vary substantially across models and ages (e.g., Figures 3-5 which show conditional probabilities for MHSPs). There is zero probability of a MHSP for sharks with

an age difference of one year as females cannot pup in consecutive years in this model (or very rarely in reality) (Figures 3-5).

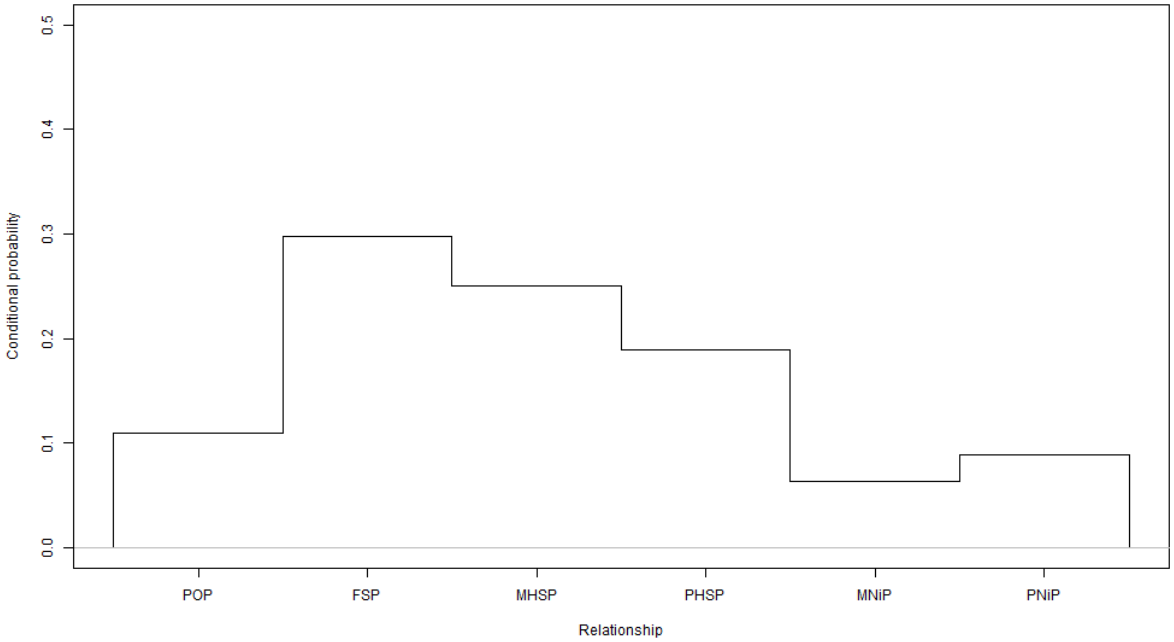

Figure 1: For a model with lucky litter effect 0.12 and fishing mortality 0.05, the conditional probability of the six close kin relationships considered in this document given that one of the relationships has occurred and the population is sampled at random (ages 1-100 years inclusive).

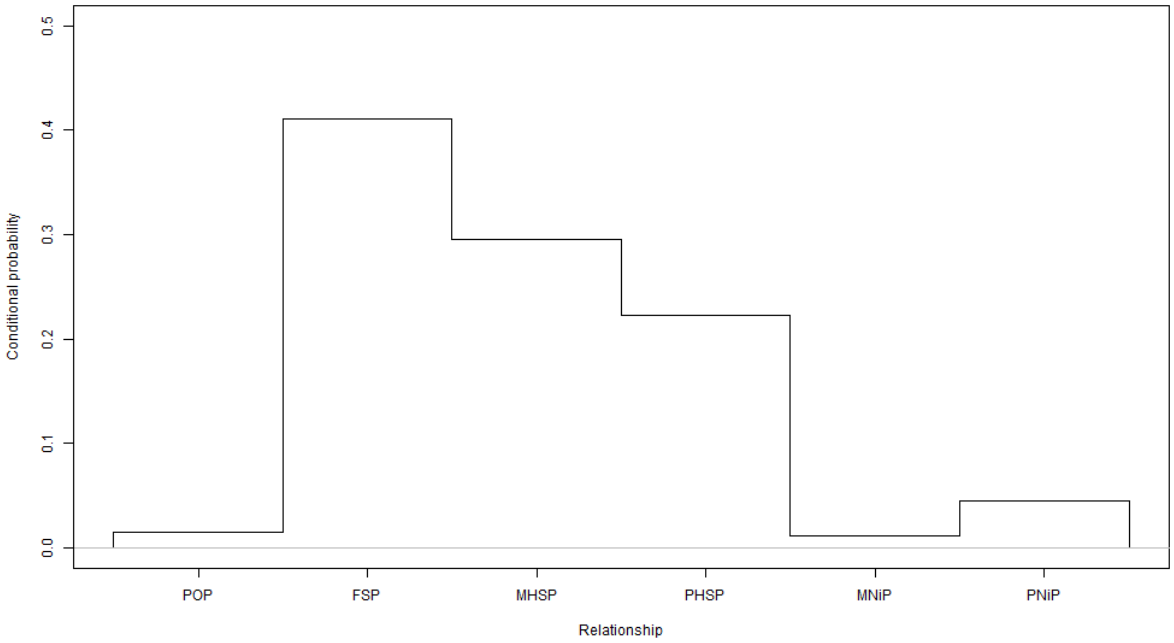

Figure 2: For a model with lucky litter effect 0.12 and fishing mortality 0.05, the conditional probability of the six close kin relationships considered in this document given that one of the relationships has occurred and the population is sampled at random (ages 2-13 years inclusive).

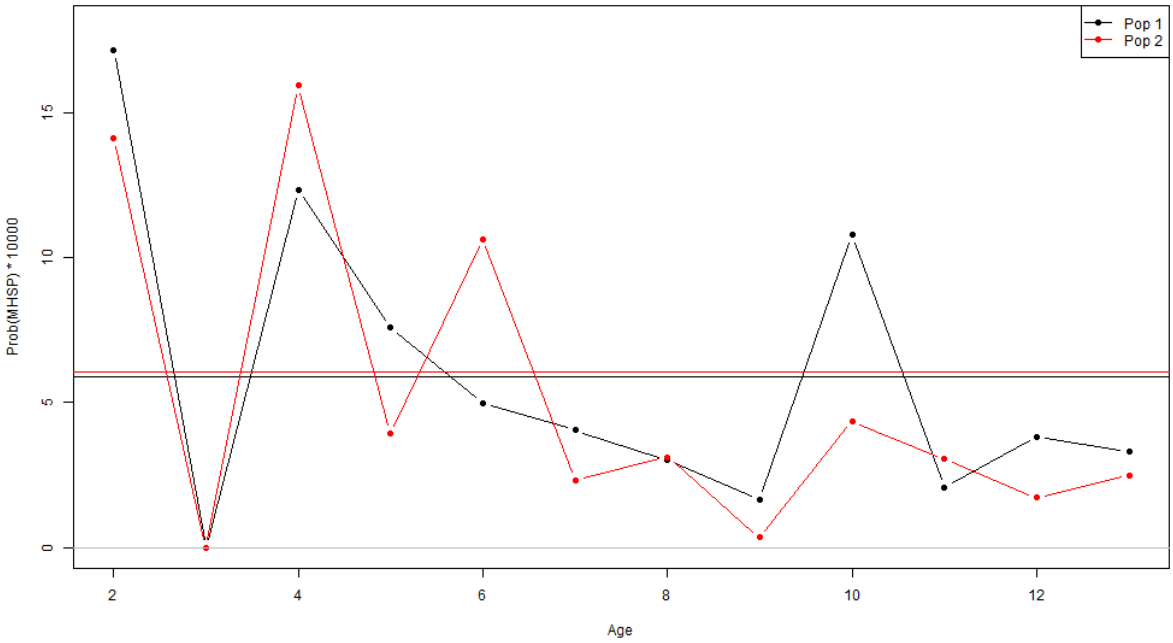

Figure 3: For two populations with lucky litter effect 0.12, fishing mortality 0.05, and 2000 mature sharks, the conditional probabilities of a MHSP when one the sharks in the pair is aged 2 years and the population is sampled at random (ages 2 to 13 years inclusive). The probability is shown for pairs of sharks aged (2,2), (2,3), …, (2,13). The horizontal lines are plotted at the unconditional probabilities.

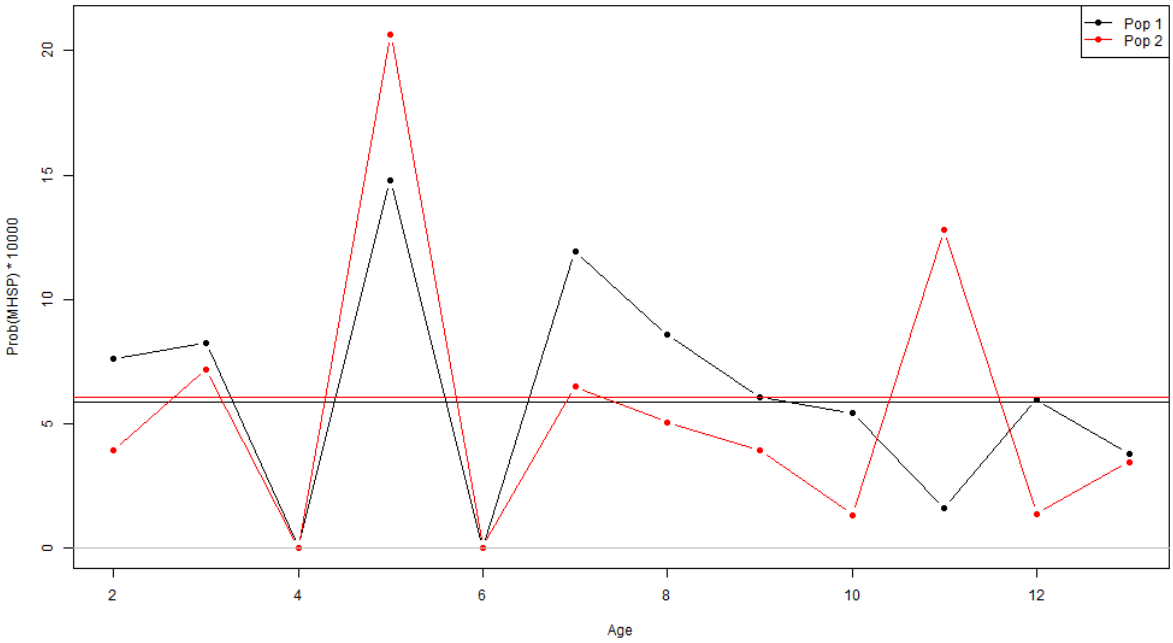

Figure 4: For two populations with lucky litter effect 0.12, fishing mortality 0.05, and 2000 mature sharks, the conditional probabilities of a MHSP when one the sharks in the pair is aged 5 years and the population is sampled at random (ages 2 to 13 years inclusive). The probability is shown for pairs of sharks aged (5,2), (5,3), …, (5,13). The horizontal lines are plotted at the unconditional probabilities.

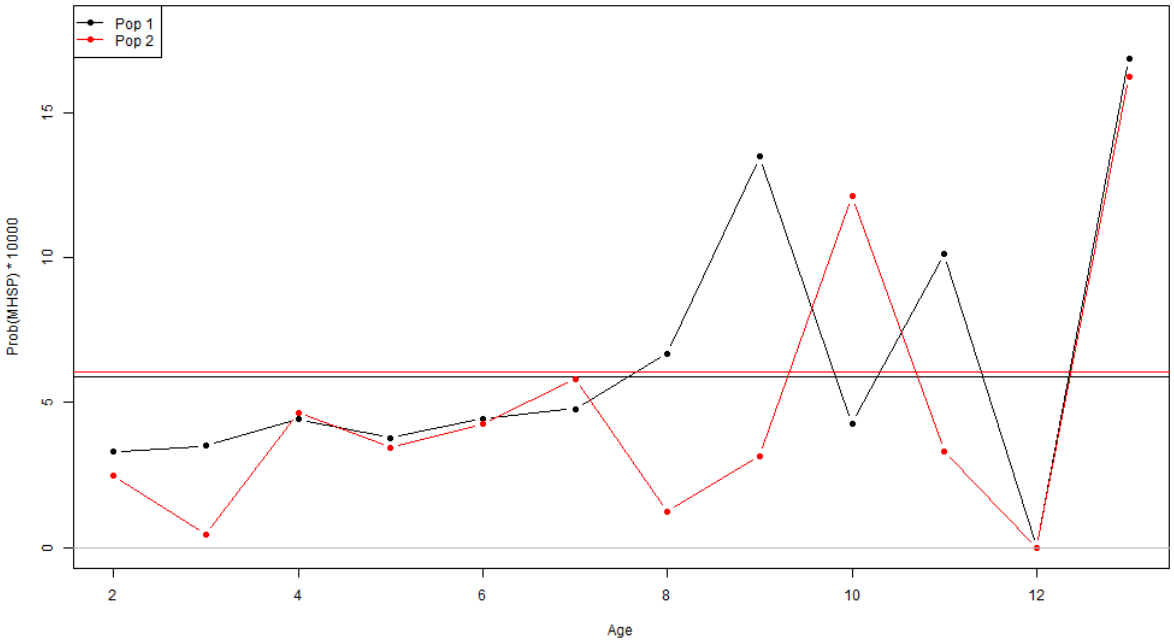

Figure 5: For two populations with lucky litter effect 0.12, fishing mortality 0.05, and 2000 mature sharks, the conditional probabilities of a MHSP when one the sharks in the pair is aged 13 years and the population is sampled at random (ages 2 to 13 years inclusive). The probability is shown for pairs of sharks aged (13,2), (13,3), …, (13,13). The horizontal lines are plotted at the unconditional probabilities.

For the "single-line calculation", a lucky litter effect of 0.12 and a fishing mortality of 0.05 were assumed and just a couple of populations were used which gave the following estimates:

	Number of mature males	**Number of mature females**	**Number of mature sharks**
Population 1	68 200	56 600	124 800
Population 2	75 200	58 400	133 600

For the least squares model, the lucky litter effect and total mature numbers were estimated. This was done for 10 populations for three different assumptions on fishing mortality (0.03, 0.05, and 0.07). The estimates for the lucky litter effect ranged from 0.06 to 0.16, with mature numbers ranging from 102 000 to 152 000 (Table 1, Figure 6).

Table 1: The range of estimates, across 10 populations, for the lucky litter effect (the proportion of litters that do not suffer high mortality in the first year of life) and the total number of mature sharks. The estimates are given for three different assumed levels of fishing mortality.

Fishing mortality	Lucky litter effect	Number of mature sharks (thousands)
0.03	0.06-0.12	126-152
0.05	0.09-0.15	117-126
0.07	0.14-0.16	102-112

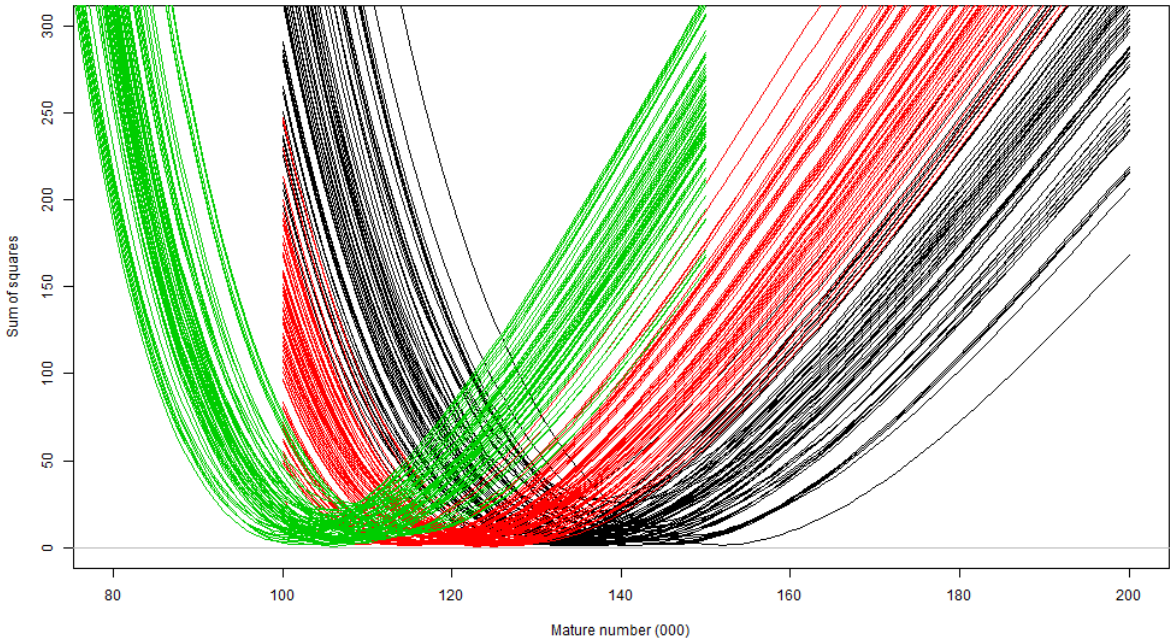

Figure 6: The sum of squares for the least squares models over a grid of lucky litter effects and total mature numbers. The sum of squares surface is shown for 10 models for each assumed fishing mortality (black 0.03, red 0.05, green 0.07). The best fits are achieved when the sum of squares is close to zero (the best fits for total mature number range from 102 000 to 152 000).

The least squares model assumes that mating occurs at random which is not supported by the data (ring counts for FSPs suggest that mating between the same pair of animals quite often occurs in different years which would not happen if mating was random). It also assumes that there is full spatial mixing of the population. Again, this is assumption is not supported by the data as close kin pairs were at least twice as likely to be found when location of capture was within 50 km than if it was more distant (Thomson et al. draft).

A six-area model was run with the areas spatially in "series" (1-2-3-4-5-6) and an annual probability of 10% that sharks would migrate to an adjacent area. A lucky litter effect of 0.12 and a fishing mortality of 0.05 were assumed with a population of 2000 mature sharks. The significant features of the multi-area model are that sharks can only mate with sharks in the same area and the pups have to be born in the area where the mother pups. As movement between areas is relatively limited there will be different probabilities of close kin relationships when sampling within an area as opposed to assuming that sampling is random across all areas (Figures 7 & 8). For some relationships

in some areas there is four times the probability of obtaining the relationship when sampling within the area as opposed to sampling across all areas (Figure 8).

It is crucial to design a sampling scheme and an analysis method that accounts for different probabilities of obtaining close kin relationships for sampling within schools, within areas, and across areas. Ignoring this will potentially lead to gross underestimation of the population size.

I did not fully explore the effect of dominant males/females. It will lead to more FSPs than expected but this may not matter as it will be aliased by estimating a lucky litter effect. Also, I did not fully explore the assumptions of 20 pups per litter and the level of natural mortality in the first year of life. These values were based on reported pupping frequency and first year survival rates (Thomson et al. draft). A higher mean number of pups per litter with the same first year survival rate would give higher probabilities for FSP and HSPs. Also, variability in litter size is important as the probabilities do not just depend on the mean (see Appendix 1).

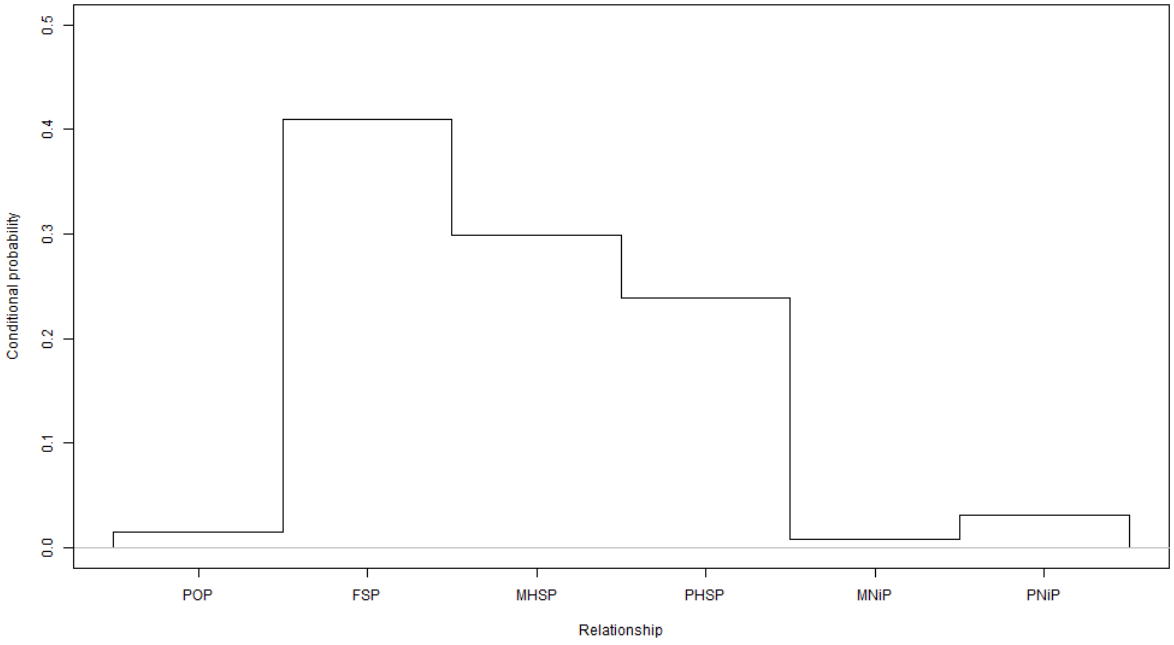

Figure 7: For a six-area model with lucky litter effect 0.12 and fishing mortality 0.05, the conditional probability of the six close kin relationships considered in this document given that one of the relationships has occurred and the population is sampled at random (ages 2-13 years inclusive).

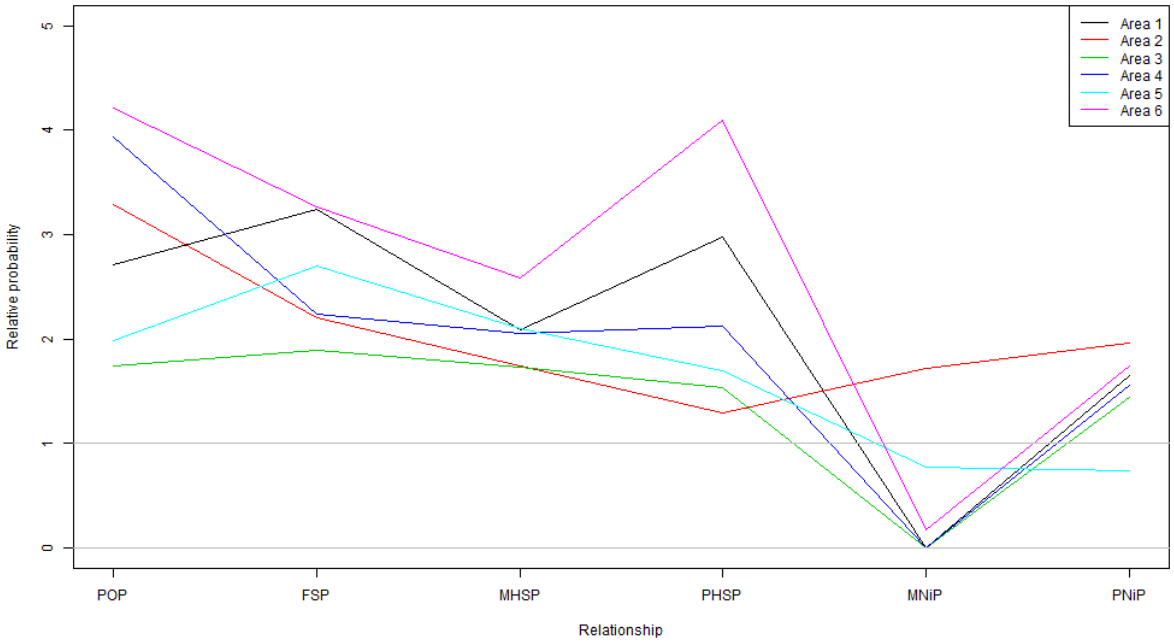

Figure 8: For a six-area model with lucky litter effect 0.12 and fishing mortality 0.05, the ratio of the probability of obtaining a close kin relationship when sampling in a particular area and the probability of the same relationship when sampling the population at random (ages 2 to 13 years inclusive).

Evaluation of the terms of reference

Evaluation of the ability of the stock assessment for school shark, with the available data, to provide parameter estimates to assess the current status of school shark

The assessment methodology is incorrectly implemented. There is inadequate survey design, a poor choice in using conditional probabilities, and several major errors. The current stock assessment should not be used to provide parameter estimates or to assess the current status of the stock. The major issues that need to be considered and the errors that exist in the current assessment are described below.

Survey design

The three most important things to consider when collecting data are: design, design, and design. In the case of the close kin DNA data there was very little design (although this appears to be deliberate). There were three main areas that were targeted for collection of sharks but other than that there was no attempt to stratify or randomise the sampling.

In general, the design should have been based on random sampling from the fisheries but thought should also have been given to obtaining additional samples to help with the estimation of various parameters. School shark presumably got their name from the fact that they school (unlike many shark species). An important parameter is the probability of close kin schooling together – if possible, samples should have deliberately been taken from the same school. Sharks in the same

area may also be much more likely to be close kin than sharks that are distant from each other (especially for juveniles). During mating season sharks may return to traditional mating grounds, and the probability of close kin being found together may be much higher than usual at such places and times. The same applies to pupping grounds. If possible, samples should have been directed to known schooling areas, mating grounds, and pupping grounds.

Sampling at random would also have allowed age frequencies to be produced for the bycatch fisheries which would have enabled the estimation of year class strengths in the model.

The draft stock assessment report (Thomson et al., draft) is generally well written but the description of the reasons for the lack of survey design is largely absent. The likelihood is based entirely on conditional probabilities (for given age and sex of the sampled sharks). If it is assumed that the sharks were sampled at random from the population (for a given selectivity) then the analysis is straightforward. Thomson et al. (draft) have assumed that they sampled at random (with regard to close kin relationships) for the **given** ages and sexes of the sharks – this leads to complex equations and makes a straightforward problem difficult. To me it is a poor choice. Good design simplifies the analysis, but the absence of design (deliberate or not) makes the analysis more complex.

Also, in the document there is poor wording that suggests a misunderstanding of probability theory. From the discussion of the simple model: “Now sample two fish, which for simplicity we will name Peter and Simon, born within a few years of one other (Peter is the elder). What is the probability that Peter and Simon have the same mother, i.e. are a maternal HSP (MHSP)?”. The answer to this question is either 0 or 1 as Peter and Simon either are maternal half siblings or they are not. There is confusion over conditioning on given ages and conditioning on given fish (which leads to a probability of 0 or 1). This confusion is confirmed in the “one line calculation” where a conditional probability is incorrectly used (see below).

Stock structure

The close kin data are not suggestive of multiple stocks although they do not preclude more than one stock. There are close kin relationships found across many combinations of the six shark zones (Figure 4.10, Table 4.6 in Thomson et al. draft). The exception is that none of the sharks from eastern Tasmania were involved in any close kin pairs, but this may be due to the relatively low sample size (only 339 sharks from Tasmania – it is not clear how many were from eastern Tasmania).

The only evidence for two stocks is that the previous stock assessment struggled to fit the data if only a single stock was assumed (Thomson et al. draft). There may be more than one stock but that it is not the main problem with the current assessment.

Duplicate samples

On the basis of the DNA results, a remarkable 85 duplicate samples were reported by Thomson et al. (draft). They attribute these duplicates to the sampling of the same animal more than once and “mix ups in the laboratory”. Only 8 of the duplicates were attributed to sampling of the same animal (although how somebody could do this without noticing that a section of the vertebrae just below the head had already been removed is a bit of a mystery). That leaves 77 laboratory mix ups. If the laboratory procedures were so bad that there were 77 mix ups then all of the laboratory results are suspect.

There is an alternative explanation. It may be that many of the "duplicate" samples were distinct animals that were genetically identical. The occurrence of identical twins or triplets should not be discounted without further investigation. Also, for female duplicates, parthenogenesis is a possible explanation (i.e., virgin birth – see, for example, Chapman et al. 2007). **The duplicates need to be investigated in much more detail.**

Inability to distinguish parent-pup pairs from full sibling pairs

Shark autosomal DNA is found in pairs of chromosomes where one of each pair comes from the mother and one from the father (just like human DNA). Therefore, it should be an easy matter to distinguish parent-pup pairs (POPs) from full sibling pairs (FSP). A pup should be a half match[1] on every SNP with each of their parents whereas this will not be the case between full siblings. The complication is the error rate as not all SNPs will be read accurately. But, nevertheless, a POP should have a much higher half match than a FSP.

It was claimed in Thomson et al. (draft) that three POPs were found. But this was on the basis of age difference with no separation of the "POPs" from FSPs in terms of DNA statistics. The allocation of these shark pairs as POPs relies on the assumption that mating between the same pair of animals in different years does not occur or very rarely occurs. The assumption that mating occurs at random is very dubious for sharks that may have relatively complex mating systems/rituals with some sharks potentially returning to the same mating site regularly (e.g., Pratt & Carrier 2001). The presence of dominant males sharks has been noted which would increase the chances of repeat matings (e.g., Pratt & Carrier 2001). In addition, the age data from the FSPs in this study very strongly suggests that repeat matings do occur (see below).

It is unclear whether any genuine POPs were found. If no genuine POPs were present it would explain the inability to separate the POPs from FSPs by DNA. **It should be a priority to sample a pregnant school shark and her pups for DNA to see what known POPs look like genetically with the current SNPs.**

Age gaps for full sibling pairs

The age data for the FSPs in Thomson et al. (draft) show gaps of up to 7 years. For example, there is a pair with ages 4 years and 11 years (Figure 4.9 in Thomson et al. draft). If this is not the result of a repeat mating then the ageing error is substantially higher than that observed from repeat readings (Figure 4.2 in Thomson et al. draft). For them to be the same age, say 7 years, would require a 7 year old to be read as a 4 year old and another 7 year old to be read as an 11 year old. I counted 10 out of 32 FSPs where the age difference stretches the credibility that repeat matings do not occur. There are also the 3 "POPs" which may just be FSPs.

I think that the conclusion of Thomson et al. (draft) that ageing error is extreme and repeat matings are very rare is dubious. An alternative explanation is that ageing error is not too bad up 11 ring counts (as reported before the conclusions of Thomson et al. draft) and repeat matings do occur (i.e., mating is not at random within the population).

[1] A half match is where at least one allele is shared between two animals for a given location. E.g. "AA" is a half match to "AB" or "AA" but is not a half match to "BB".

Neglecting uncle/aunt – niece/nephew relationships

In several documents that used CKMR methods I searched electronically and found no mention of "aunt" or "uncle" unless they were referring to "half aunts" or "half uncles". Genetically, nibling pairs (uncle-nephew, uncle-niece, aunt-nephew, or aunt-niece) are genetically indistinguishable from HSPs (e.g., ISOGG 2019). So, if the age range is large enough, then a reported HSP could really be a nibling pair (NiP) and these should be accounted for when fitting close kin data in a model. In the base model, the ring counts were restricted to 11 and under. As school shark mature at about 10 years (males earlier) there shouldn't be many NiPs in the school shark pairs used in the base model. But, to not even mention their existence is a strange omission. Perhaps they were ignored because the conditional probabilities are very difficult to calculate. However, as the use of the conditional probabilities is a poor choice there is no need to ignore NiPs in future stock assessments (as the conditional probabilities do not need to be calculated).

Having the same mother is not the same as being maternal half siblings

It is important to note that if two sharks have the same mother then they are not necessarily maternal half siblings. Equally, if two sharks have the same father then they are not necessarily paternal half siblings. Full siblings have the same mother and the same father. To calculate, for example, the probability that two sharks sampled at random are a maternal HSP (MHSP), one must calculate the probability that they have the same mother and subtract the probability that they are a FSP. Similarly, for a paternal HSP (PHSP). The same applies if probabilities conditional on age (or ring count) are calculated. I didn't find any mention of this issue in Thomson et al. (draft). It may be that they have dealt with this in the way they have calculated the conditional probabilities but it is not clear from the documentation.

Maternal half siblings one year different in age

As female school sharks are very unlikely to pup in consecutive years (since gestation is about 12 months) the probability of finding a maternal HSP with an age gap of 1 year is essentially 0. However, the model of Thomson et al. (draft) does not explicitly model a pupping interval for females and so this detail is missed. It is hard to know how much difference it would make, but it is better to include a pupping interval of some sort and test the sensitivity of assumptions rather than to exclude it entirely.

Probability of close kin relationships by distance

The probability of finding a close kin pair given the distance between the capture locations of the two sharks appears to be much larger when the capture locations are less than 50 km (Figure 4.12 in Thomson et al. draft). This is not acknowledged by Thomson et al. (draft) who write; "The distance between the capture locations of close relatives largely follows the same pattern as that shown by calculating the distances between every possible pairing of animals sampled (Figure 4.12) although there seems to be a slight tendency for pairs to be within less than 50 km relative to the overall sample". However, the proportion of close kin pairs within less than 50 km is at least twice that for any other distance bin. This is not a "slight tendency".

It is to be expected that sharks that are found closer together (especially juveniles) are more likely to have a close kin relationship. This needs to be acknowledged in the survey design and the analysis.

A dubious "one-line calculation"

The "one-line calculation" is based on a conditional probability with the "typical" pair of sharks being born four years apart and the probability of the younger shark having the same mother as the older shark (given the mother survived) set equal to $1/N_{fem}$ where N_{fem} is the number of mature females alive in the year of birth of the younger shark. The observed number of maternal HSPs is then equated with the expected number of pairs with the same mother (given the total number of pairs). A similar calculation is done for paternal HSPs.

This is a dubious calculation for two reasons. First, the probability of two sharks having the same mother is not the same as the probability of two sharks being a maternal HSP. The probability that two sharks are a FSP has to be subtracted from the probability of two sharks having the same mother (although if it is assumed that mating is at random and the sharks are four years different in age then the probability that they are full siblings is essentially zero). Second, the conditional probability based on being "given" the older shark should not be used anyway. For this calculation the probability of two sharks selected at random being a maternal HSP should be used.

Possible sensitivity to mean litter size and associated variability

The probabilities of FSPs and HSPs depend on the square of the surviving litter sizes (see Appendix 1). Therefore, it is best to explicitly model average litter size **and** the associated variability. Certainly, there should at least be some sensitivity runs done with different mean litter sizes and variability. Thomson et al. (draft) appear to ignore variability in fecundity and mortality (all animals are identical for given sex and age). Although, for school shark, there is limited scope for high variability in litter size and estimating a lucky litter effect may deal with this to some extent (this would not be true for highly fecund species).

Incorrect likelihood function

The likelihood function used by Thomson et al. (draft) includes a fundamental error (in addition to the poor choice of using conditional probabilities). They create a likelihood which is the product of independent Bernoulli trials: one for each pair of sharks **and** one for each close kin relationship (see Appendix C.6 in Thomson et al. (draft): "The likelihood component for the close kin data is a series of Bernoulli trials, one for every possible pairing of individuals, and every type of kin relationship").

Consider the random sampling of two sharks when we are just looking at POP, FSP, HSP, and URP. This constitutes a single **multinomial** trial with four possible outcomes and not two possible outcomes as for a Bernoulli trial. Instead of using the multinomial probability for a single trial they have split it into four independent Bernoulli trials – but these are not independent trials. If a pair of fish are a FSP then they cannot be a POP or a HSP or a URP. Similarly, if they are a URP that is their relationship, and the probability of any of the other three relationships is 0. The two different approaches yield very different equations; one that is correct and one that is not (Appendix 1).

It should be noted that there is a difference between sampling say 100 sharks and checking each of the 4950 unique pairs for a close kin relationship and, alternatively, sampling 4950 pairs of sharks and checking each independent pair for a close kin relationship. The latter has been assumed by Thomson et al. (draft) in the formation of the likelihood. This is technically incorrect because two pairs of sharks that have one shark in common do not form independent trials. However, it is a

perfectly adequate approximation given the relatively large population size (so that although not strictly independent, such pairs are “almost” independent).

Stock size is underestimated

There are four main errors which have a bearing on the estimates of stock size and three of these will lead to underestimation. Without having the raw data and calculating the conditional probabilities it is hard to judge the effect of each factor but in general terms there should be substantial underestimation - which is confirmed by the equilibrium model results.

The probabilities of MHSPs and PHSPs (for given age and sex) have been overestimated which leads to higher stock size estimates. However, the exclusion of NiPs will lead to some underestimation.

The incorrect likelihood will deliver very much lower probabilities of success for each close kin relationship and lead to underestimation of stock size. This will dominate the two effects already mentioned. The assumption that the sharks were fully spatially mixed could have an additional and substantial effect on the estimates (and lead to further underestimation of stock size).

From the equilibrium model results it looks like there is underestimation by a factor of about 2 for the first three errors combined with the assumption of spatial mixing perhaps contributing as much as another factor of 2. Therefore, a rough estimate of **mature** numbers is from 120,000 to 240,000 **mature** sharks (which is 2-4 times the estimate of about 60,000 **mature** sharks from the base model in Thomson et al. draft).

Projections

Twenty year projections were done by Thomson et al. (draft) at different constant exploitation rates calculated from catches in recent years and their model results. As the model badly underestimates stock size, the estimated exploitation rates are too high, and the projected trend is strongly negatively biased. The mean trend is increasing even for this model so in a corrected assessment a much higher mean rate of increase would be expected for exploitation rates associated with the current level of catches.

Evaluation of the strengths and weaknesses of the stock assessment for school shark

Strengths

The use of close kin genetics in the stock assessment is advantageous. Avoiding the use of CPUE is a strength as the use of CPUE as a biomass index is almost always questionable (for any stock).

The use of close kin genetic data in a stock assessment requires that the genetics for the given species be sufficiently developed that the different close kin relationships can be determined. Appropriate SNPs need to be targeted, laboratory procedures have to ensure excellent quality control, and appropriate statistics (to separate the close kin relationships) have to be developed. I suspect, that despite appearances, this probably has been done in this case. The failure to separate POPs and FSPs on the basis of DNA is quite possibly because there were no POPs. The huge number of “laboratory mix ups” is also possibly due to the occurrence of identical twins/triplets or

parthenogenesis. If this is not the case, then there are serious problems with the DNA and the laboratory (and this paragraph should be under “Weaknesses”).

The authors recognized that there may be a tendency for close kin (e.g., FSPs) to school together and therefore excluded comparisons between sharks caught on the same trip. This was a reasonable approach for dealing with this possible association at the school level.

The draft report is in the most part well written and the attempt to try to verify the close kin model results by using two simple models was sensible.

Weaknesses

Most of the problems have already been described but they are briefly summarised here together with some further issues.

The absence of proper survey design is a weakness as it makes it less defensible to assume that the sampling was random in respect of the fishery. The absence of design was apparently deliberate as the analysis was to be based on the use of conditional probabilities. However, this leads to great complexity which is unnecessary and would not have needed to be considered if proper design had been used. There is no need to bring in the problems associated with ageing error or to do mitochondrial DNA testing to separate maternal and paternal HSPs (they have to be grouped with the NiPs – although through mitochondrial testing the group could be separated into maternal HSPs/NiPs and paternal HSPs/NiPs, but this is not necessary).

Ageing error becomes an issue because of the use of conditional probabilities. It is assumed in the base model that the repeat readings are indicative of the level of ageing error. However, in the analysis of age gaps for FSPs, Thomson et al. (draft) conclude that ageing error is much more substantial than that shown by the repeat readings. To be consistent, the increased ageing error would need to be included in the base model.

The ageing error conclusion is based on the assumption that repeat matings do not occur or are extremely rare – which is based on the assumption that mating is at random. It is difficult to believe that mating is at random and rather than assume that it is, and that ageing error is extreme, it would have been better to explore alternatives.

There are numerous problems with the assessment:

- A large number of duplicate samples (85) according to the DNA which points to poor laboratory procedures or genuine duplicates (i.e., identical twins/triplets or parthenogenesis)
- An inability to distinguish between FSPs and POPs using DNA which points to poor SNP selection or no genuine POPs
- Numerous FSPs with age gaps that are inconsistent with the variance of repeat age readings (which implies huge ageing error which appears to have been ignored in the base model or the occurrence of repeat matings which has implications for the analysis)
- The use of conditional probabilities which leads to unnecessary complexity and the need to account for ageing error
- The assumption that mating is at random which appears to be contradicted by the data

- The assumption that the sharks are spatially fully mixed and therefore that the probability of close kin pairs is independent of the distance between capture locations (which appears to be contradicted by the data)
- Neglecting to include nibling pairs in the set of close kin relationships (which need to be included unless the age range is severely restricted)
- Possibly incorrect calculation of the probabilities of MHSPs and PHSPs (perhaps forgetting to subtract the probability of a FSP from the probabilities of having the same mother/father)
- Not explicitly modelling a pupping interval and therefore over-estimating the probability of maternal HSPs when ages are one year different
- Ignoring variability in surviving litters sizes (except for estimating a lucky litter effect)
- Incorrect calculation of the likelihood by using independent Bernoulli trials for each close kin relationship for a given pair of sharks (instead of a single multinomial trial)
- Inability to account for known catch history before 2000.

Recommendations for improvements to the assessment of school shark

The existing assessment contains several major errors and the use of conditional probabilities is not necessary. Rather than improvements to the existing assessment there should be a reworking of the data and a new model based on unconditional probabilities. Some issues with the DNA need to be sorted out first.

Genetics

There needs to be investigation into the duplicate samples to determine if distinct sharks are involved or not. If there have been 70 laboratory mix ups then laboratory procedures need to be tightened or a new laboratory used.

It needs to be determined whether there are any genuine POPs or not. A pregnant school shark and her pups need to be sampled for DNA to find out what a genuine POP looks like genetically with the existing SNPs. Either there are no genuine POPs or the current SNPs are not suitable for close kin genetics.

Sampling

The existing data can be analysed to get a subset which is roughly consistent with random sampling of the bycatch fisheries. This can then be used in a stock assessment model without resorting to the complex conditional probability equations and trying to deal with the ageing error issue.

Future sampling needs to be properly designed. It should be based on stratified random sampling from the bycatch fisheries. Age can also be sampled to allow the production of age frequencies and the estimation of year class strengths.

Stock assessment model

A purpose written model is required but it can use standard population dynamics with a few extra features. There should be options for multiple areas and non-random mating (e.g., dominant males/females; mating according to age; mating only with suitable sharks in the same area). Mean

litter size and associated variability should be explicitly modelled. There should be the option to estimate a “lucky litter effect”.

The model should extend over the full history of the fishery. There is no reason to ignore the catches which were taken before 2000.

Conclusion

The current stock assessment contains numerous errors which make it unusable for management purposes. The use of close kin genetic data in the school shark assessment is advantageous. It was the implementation of the approach which was at fault not the use of close kin genetics. The current assessment should not be resurrected with just a few modifications. It uses complex conditional probability equations which are unnecessary if a subset of the data is used to approximate random sampling from the bycatch fisheries.

To get a rough idea of the stock size, an age-structured, individual-based, equilibrium model was fitted to the close kin data. The model results suggest that stock size in recent years could be of the order of 120,000 to 240,000 **mature** sharks (i.e., a factor of 2-4 times higher than the base model results). This is just an educated guess and should not be used for management purposes. A new assessment is needed.

Acknowledgements

This work was funded by SSIA. Thanks to Robin Thomson of CSIRO for clarifying some issues that were raised during our email exchanges.

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Appendix 1: Example formulae for close kin probabilities

Whether the close kin data are fitted to a model by maximising the likelihood function, or by least squares, the fundamental building blocks are the probabilities of obtaining a close kin pair when two sharks are sampled at random from the population (possibly filtered through a selectivity).

Take an idealised experiment where we sample two sharks at random from a given population. We need the probability for each of the close kin relationships that we are interested in. Suppose, we are just looking at POP, FSP, HSP, and URP. These are the four possible outcomes for our experiment (we must get one of these relationships for the two sharks we sample). Denote the probabilities of each relationship occurring for our random pair as p_{pop}, p_{fsp}, p_{hsp}, and p_{urp}. These probabilities sum to 1. Also, they are **constant**, being determined by our sample design and the given population. It is straightforward to derive a formula for each of the probabilities by annotating and partitioning the population appropriately.

For example, the formula for a FSP is:

$$p_{fsp} = \frac{N_p\overline{p^2} - n}{n(n-1)}$$

where n is the number of sharks in the population, N_p is the number of pairs of parents (of the sharks in the population), and parent pair i has p_i children alive in the population.

One possible derivation is using counting techniques. The total number of pairs of sharks that can be selected from the population is $n(n-1)/2$. The total number of pairs which have the same parents is:

$$\sum_{i=1}^{N_p} \frac{p_i(p_i-1)}{2} = \frac{1}{2}\sum_{i=1}^{N_p}\left(p_i^2 - p_i\right) = \frac{1}{2}\left(N_p\overline{p^2} - n\right)$$

The probability is the number of pairs of sharks that have the same parents divided by the total number of pairs of sharks (the "2s" cancel and we get the formula already given). Note that this probability is "scalable" (e.g., if we double the number of sharks in the population then the probability is halved, provided $n >> 1$). This means that we don't need to form a population with say 100 000 animals to get the probabilities for when there are 100 000 animals. Instead we can work with a population of 2000 animals and scale the probabilities.

The probabilities for the other relationships can be similarly derived (and are also scalable)

Note, that the testing of a pair of randomly selected sharks is a single multinomial trial, with four possible outcomes in our example. If the outcome of our trial is "relationship" then the contribution to the likelihood function is $p_{relationship}$. This is very different from the product of independent Bernoulli trials as used by Thomson et al. (draft). For example, if the trial delivers a POP then the correct contribution to the likelihood is p_{pop} but they will have $p_{pop}(1-p_{fsp})(1-p_{hsp})(1-p_{urp})$.

Appendix F. CSIRO response to Cordue review

Response to *"A review of the 2019 close kin DNA stock assessment of school shark (Southern and Eastern scalefish and shark fishery) by Patrick Cordue, Innovative Solutions Limited"*

Mark Bravington and Robin Thomson (CSIRO, Castray Esplanade, Battery Point, Hobart)

09 December, 2019

Contents

1 Summary

This report is our response to a review by Dr Cordue of our Close-Kin Mark-Recapture (CKMR) project on school sharks (Thomson *et al* in prep). CSIRO considered that the review should be completed before providing a response. Dr Cordue makes a number of criticisms of our methods and analysis, and reaches the view that our methods and conclusions are unsound, and therefore that our analysis should be re-done. Following our detailed responses below, we conclude that all but one of his comments are either unfounded or irrelevant to our results. The sole exception is the allowance we made for ageing error based on vertebral ring counts: on examination, we agree that the apparent ages of full-sibling pairs (FSPs) in the dataset are not consistent with the amount of ageing error that we assumed in our draft report. There are two possible explanations that would fit the full-sibling data better, but we argue that neither would have much impact on abundance or trend estimates. With respect to his other comments, we do not see any reason to revise our analysis or conclusions. In particular, we strongly disagree with Dr Cordue's suggestion about not using conditional probabilities in CKMR models for complex datasets like school shark. Conditional probabilities that specifically take account of the characteristics of each sample (such as its capture year and estimated age) are the fundamental building-blocks of unbiased CKMR, and their use is based on rock-solid statistical principles; these have been published since 2016 and also scrutinized carefully in a high-profile international stock assessment forum, but Dr Cordue has evidently not read the relevant literature. In future, if more school shark samples become available (presumably with the aim of getting more precise estimates of trend), then we would of course expect to make some adjustments to our model (e.g. in the light of more information on ageing error, and to account for other types of kin that might manifest in longer studies) but we would not change the underlying approach to CKMR model building.

No.	Criticism	Substance?	Implication?	See page
1	Duplicate samples	No	None	6
2	POPs vs FSPs	No	None	8
3	FSP age gaps	Partly	Minor	8
4	Conditional probabilities	No	None	11
5	Random mating	No	None	11
6	Distance	No	None	12
7	Full thiatic pairs	Not yet	None	14
8	HSP probabilities	No	None	14
9	Bernoulli trials	No	None	14
10	Catches pre-2000	No	None	15
11	Pupping interval	No	None	15
12	Litter size	No	None	16
13	One-line calculation	No	None	16

Table 1: A numbered list of Dr Cordue's criticisms, briefly stated, and our response with regard to whether the criticism has any substance, and whether there it has an implication for our estimate of population size.

2 Introduction

Background information regarding the need for a CKMR investigation of school shark is given in Appendix A.

In late 2019, the Southern Shark Industry Alliance (SSIA) commissioned Patrick Cordue (Innovative Solutions Limited, ISL) to review CSIRO's CKMR work on school shark. Subsequently, a lengthy email correspondence occurred between Dr Cordue and Dr Thomson who provided genetic sequence data, background CCSBT reports that provide additional information on the kin-finding algorithms used, a recent non-CSIRO CKMR publication that explains the underlying conditional probability calculations in a very simplified form (Ruzzante *et al* 2019), and basic explanations of how the CKMR method works. Dr Cordue's final report does not incorporate some parts of that correspondence, in particular on differences between kin-finding methods for humans versus those for school sharks, and regarding conditional probabilities. Following that correspondence, CSIRO declined to provide further comments on Dr Cordue's findings in draft form, preferring to comment

once more only on his final report.

The structure of this document is as follows. In the next few sections of the introduction, we provide some general comments on the development and review of CKMR in other applications. After a summary of the logic behind our school shark CKMR project, we present our responses to Dr Cordue's numbered list of criticisms (his pages 2-3), as well as to some additional criticisms from the main body of his report. For each, we lead up to two questions: (i) is there any substance to the criticism; and (ii) if so, then how much impact could it have on the population estimate from our study? In Appendix A we give an expanded version of our responses, providing a more technical treatment for interested readers. Particularly technical expansions to some of our points are given in Appendix B.

2.1 Development and review of the CKMR method

School shark is only the second large-scale application of CKMR to a commercially exploited species. CKMR is still a fairly new technique that demands a wide range of specialist expertise to implement successfully. In CKMR projects at CSIRO we separately use geneticists, specialist laboratory technicians, stock assessment experts, and statisticians, both for kin-finding and for the fundamentals of statistical inference from data. Papers are still sparse, but there are two complete applications (Bravington *et al* 2016a; Hillary *et al* 2018; both have since been updated substantially with more data), and one comprehensive description of the statistical principles (Bravington *et al* 2016b) as well as an alternative derivation of the same key kinship equations (Skaug 2017). We are also aware of two published CKMR applications outside CSIRO, although both are on salmonid fish in rivers where very simple models are possible (Forland 2014; Ruzzante *et al* 2019). Marine fish (and sharks) generally have more complicated life-histories and sampling arrangements, which require more nuanced models to avoid bias.

It is unrealistic to expect any single reviewer to have adequate expertise across all the above areas to be able, on their own and from a "cold start", to review a complex CKMR study like school shark. Dr Cordue does not cite, and does not draw on the contents of the key papers above especially Bravington *et al* (2016b) which explains how CKMR can be applied across a wide range of situations so as to lead to unbiased estimates of abundance and other demographic parameters.

2.2 Advice for SSIA

When the first CKMR project was developed at CSIRO (on Southern Bluefin Tuna, SBT; Bravington *et al* 2016a), we were very aware of the issues of breadth-of-expertise and unfamiliarity. We knew that the final statistical analysis would be unfamiliar to stock assessment scientists at CCSBT (the international commission that manages SBT), and that the underlying genetics in particular would simply seem like magic— and also that the moment of presenting results is exactly the wrong time to be aiming for agreement about methods. Therefore, we deliberately engaged an expert steering committee right from the start of the SBT project. To find the right expertise, the committee had to be drawn internationally as well as domestically: Waples, Anderson (USA: genetics and parentage); Ovenden (Australia: genetics); McGarvey (Australia: mark-recapture); Parma (Argentina and CCSBT: advanced stock assessment). Every year, we gave the Steering Committee a detailed report, and held a webinar. This ongoing expert committee process was essential in ensuring smooth uptake of our results when it came to the end of the five-to-six-year initial project, in 2012. There was still a vigorous interrogation process at CCSBT itself from the absolute top tier of international stock assessment ((Butterworth, Hilborn, Parma, Ianelli, Pope), but the findings of the CKMR investigation were accepted without difficulty, and ongoing CKMR is now firmly integrated into the CCSBT management process.

Given there had been a rigorous review process during development of CKMR for SBT, there did not seem to be a need for a formal expert committee for the school shark application; periodic review through sharkRAG seemed adequate. Perhaps this was a mistake - it might be worth returning to the external steering committee model for future school shark work.

2.3 The basic ideas behind our project

Before tackling the details, it is worth re-emphasizing the basic logic of our study from the viewpoint of school shark assessment. Our approach to CKMR for school shark is to collect a large sample of juvenile sharks of more-or-less known age, genotype each sample, and conduct pairwise comparisons of every sample against every other sample to see which pairs turn out to be close-kin pairs. Our estimates of adult abundance and trend are based on the numbers of cross-cohort half-sibling pairs, i.e. born in different years and sharing either a mother or a father. Complications that arise from same-cohort half-siblings and full-siblings are dealt with by estimating additional 'nuisance' parameters.

1. The proportion of pairwise comparisons between samples that turn out to be cross-cohort siblings, determines the adult population size: the chance that two animals born just a few years apart have the same mother, is basically 1/number-of-adult-females.
2. The changing proportion of siblings in newer versus older cohorts determines the adult population trend. If the adult population abundance is increasing, then a recent offspring has more potential mothers to "choose" its real mother from, and so recent cohorts will yield a lower proportion of siblings than older cohorts.
3. While some simple back-of-the-envelope calculations along the above lines can be useful, the abundance and trend aspects are in fact deeply linked so that the points above are somewhat over-simplified. To avoid biases in estimates of abundance and trend, it is necessary to produce a more complicated analysis which also takes account of mortality rate (which is of interest in its own right) as well as handling several important details to do with, for example, litter-size variation, multiple paternity, ageing error, and uncertain detection of siblings.
4. The more complicated analysis (mentioned in 3 above) requires the use of conditional probabilities so that it can calculate trend (which relies on knowing the birth years of the offspring), mortality (for which the gaps between birth years of half-siblings is informative), and ageing error (which adds uncertainty to those birth years); conditional probabilities must also account for the sex of the parents (in POPs, and shared by HSPs) because fecundity-at-age (size) must be accounted for to avoid bias.

In the long run, if the adult population really is increasing, then CKMR inevitably must reveal it, via a drop in the proportion of half-sibling pairs (see point 2 above): more parents to "choose from" means that fewer juveniles will share the same parent.

3 Summarised response to criticisms

We have focused on responding to Dr Cordue's numbered list of ten criticisms. We have re-ordered some of them for clarity, but have retained the original numbering system. We have also addressed three further criticisms raised in Dr Cordue's main text but not in his summary, numbered 11–13 below. For brevity, our references to "section X.X in our draft report" always pertain to Thomson *et al* (in prep) - the draft report on our school shark CKMR work, which was provided to the FRDC in June 2019. An expanded version of our responses below can be found in Appendix A.

For consistency with the close-kin literature, we use the following notation:

CKMR: Close-Kin Mark-Recapture (Dr Cordue refers to *close kin DNA* and to *close kin genetics*)

POP: Parent Offspring Pair (Dr Cordue mentions *Parent of Progeny* and *Parent of Pup*)

FSP: Full-Sibling Pair (two animals that share both parents)

HSP: Half-Sibling Pair (two animals that share either a mother MHSP, or a father PHSP, but not both)

FTP: Full-Thiatic Pair (aunt/uncle and niece/nephew pair i.e. an individual and the offspring of their full-sibling); (Dr Cordue uses the term "nibling", which is found in some human genetics literature)

HTP: Half-Thiatic Pair (an individual and the offspring of their half-sibling)

Below, we repeat (in italics) each of Dr Cordue's numbered criticisms, and then give our response (note the expanded version in Appendix A). The key points to consider for each of Dr Cordue's comments are (i) is there any substance to it, and only if yes (ii) how much impact could it have on our estimate of population abundance? We answer those at the end of each of our responses.

3.1 Duplicates

1. According to the DNA results there were a large number of duplicate samples (85) which points to poor laboratory procedures OR genuine duplicates (i.e., identical twins/triplets and/or parthenogenesis which has implications for the analysis)

There were no such large-scale duplicate samples. The samples in question were clearly not school shark, based on the genetics; presumably, they were gummy shark sampled in error (see section 4.1.1 of our draft report). Unfortunately, section 4.5.3 of our draft report contradicts Section 4.1.1 in that it refers to the removal of "85 accidental duplicates" but this is a reporting error which we will correct when our report is finalised - in Section 4.5.3 we conflated the relatively small number of actual duplicate tissue samples (27, representing 13 individuals that were sampled on, or close to, the same day) taken by samplers in the processing factories with the 58 suspected gummy shark samples. Section 4.1.1 provides an accurate discussion of these samples.

Is there any substance to this? No

Implications for assessment? None

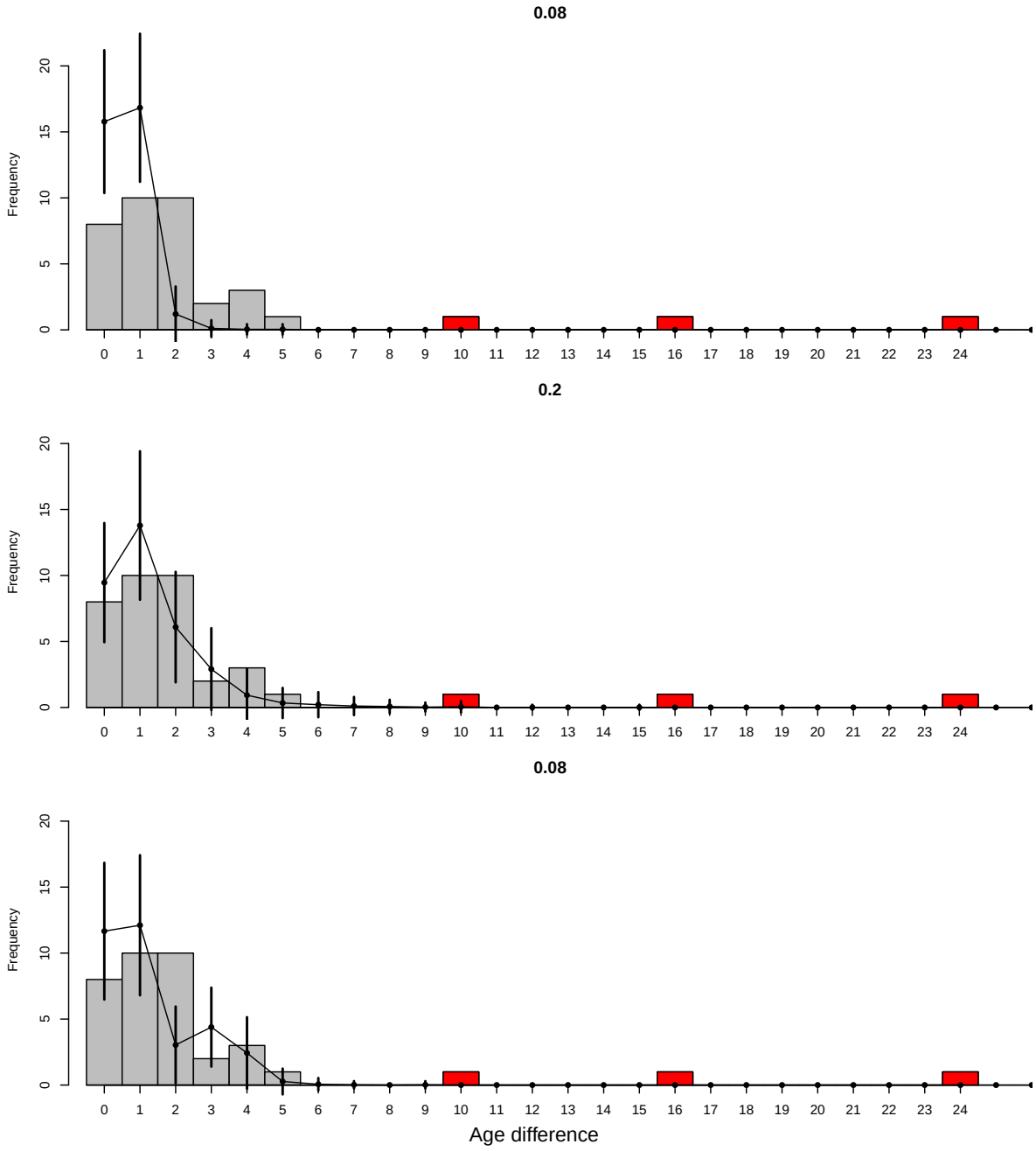

Figure 1: Frequency of apparent age gaps between pairs of animals found to be either FSPs (grey bars) or POPs (red bars). The black line (with error bars of 2 standard deviations) is based on aging error with a CV of 0.08 (upper and lower plot) or 0.20 (middle plot) and same cohort FSPs (upper two plots) or allows gaps of 3 years for ten pairs (lower plot).

3.2 FSPs vs POPs

2. An inability to distinguish between FSPs and POPs using just DNA which points to poor SNP selection OR no genuine POPs (3 POPs were assumed on the basis of age difference)

Our loci (SNPs) are fine for their core purpose, which is to identify HSPs from unrelated/less-related pairs—quite a challenging task. As to FSPs and POPs: it is quite clear, based on the estimated ages of the samples in these FSP/POPs, which ones were POPs and which ones were FSPs, so that is the criterion we used. Figure 1 (upper plot) shows the distributions of estimated birth-gap for FSPs/POPs. The gaps are clustered close to 0 with a small number out to a 5-year gap; then there is a big gap to three pairs separated by 10 years or more. FSPs should be close together in age (see also response to next point); POPs, of course, have to be separated in birth-date by at least the age-of-maturity (9 years or more). The large birth year gaps of the three POPs are definitely much larger than those of the cluster of FSPs. Further, the existence of a few POPs in the dataset is to be expected *a priori*, given our estimates of abundance from HSPs and the age distribution of the samples.

We did try a simple sub-optimal purely-genetic algorithm for distinguishing FSPs from POPs, but it did not give clear-cut results. Since we could do that job without genetics, we did not spend the effort to improve the FSP-vs-POP algorithm, although we could have done so (see Appendix A for more detail); in contrast, we carefully optimized our HSP-finding algorithm because it was necessary to do so. One key difference between our genetic dataset and those commonly used in human genealogy (with which Dr Cordue is familiar), is that our set of loci contains many "heritable null alleles", whereas such loci are generally avoided in human genetics. Kin-finding algorithms that can cope with null alleles need to be more complex; see expanded response in Appendix A.

Is there any substance to this? No

Implications for assessment? None

3.3 FSP age gaps

3. Numerous FSPs with age gaps that are inconsistent with the variance of repeat age readings (which implies huge ageing error which appears to have been ignored in the base model OR the occurrence of repeat matings which has implications for the analysis)

While we did allow for some ageing error in the analysis (CV=0.08 based on repeat readings of vertebrae), we agree that the FSP data point to there being more ageing error in reality, and/or some sperm-storage across one pupping interval; both are biologically plausible. However, repeat matings are inconsistent with the data (see criticism 5 below).

Figure 1 (upper plot) shows the distribution of observed and expected FSP age gaps, if all FSPs are truly same-cohort and the ageing error has CV = 0.08 (same CV assumed for all ages). The expected distribution clearly is too narrow; there should not be any apparent gaps of 3–5 years, but in fact we found six (as noted above, the three very long gaps of 10 or more years, are POPs).

Figure 1 (middle plot) shows what is expected if the CV is actually 0.20; then the match would be quite good. Is it possible that the true juvenile ageing CV is much higher than 0.08? In fact it is: the figure of 0.08 accounts just for repeat-reader error, not for genuine biological variation in observable rings. There is data on ring-deposition variability in Walker *et al* (2001); we felt that it is too sparse to be relied on for a quantitative estimate in the model but, as shown in Appendix A, the additional variation could be substantial.

Sperm storage is the other possible explanation for FSPs with apparent age-gaps of say 3–5 years (i.e. a true gap of 3 years across one pupping interval, possibly plus some ageing error). Storage of at least 4 years has been verified for another shark species in captivity (Bernal *et al* 2015). This is discussed in Section 4.5.4 of our draft report:

"The remainder of the leftmost pairs must be FSPs; the maximum apparent gap is 5 years. Most of the FSPs have a gap of approximately 2 years, which is entirely explicable in terms of ageing error on animals from the same cohort. The six FSPs with gaps of approximately 5 years are either due to ageing error (certainly

plausible), or (conceivably) to sperm storage, whereby a female uses sperm from one mating to fertilize not just one litter but also the next (two or more likely three years later). Sperm storage is known to occur in several shark species, including in school shark, at least for a few months immediately following the mating season (Walker, 2005)."

Figure 1 (lower plot) shows the expected FSP age distribution if there were truly 10 cross cohort FSPs (i.e. the six that have an apparent gap of 3-5 years as well as some of those that have a gap of 2 years) but keeping the original ageing-error CV of 0.08. The fit is again reasonably good. It is clear that sperm storage, or perhaps a combination of sperm storage with a somewhat higher CV on ageing error, could explain the observed FSP age gaps quite satisfactorily.

It is important to distinguish between sperm-storage and repeat-mating (the interpretation suggested by Dr Cordue), since they have different implications for demography and for how to properly build the CKMR model. The FSP age-gap data specifically show that repeat-mating with the same partner is at best rare (as would be expected in a very large population, unless there is pair-bonding). If it was common, then there would be a longer tail of FSP age gaps, reflecting same-parents at intervals of 6 and 9 etc years— more like the HSP distribution (see Figure 2). But there are no observed FSP gaps in the range 6–9 years (and the few gaps after that are consistent with being POPs).

While we do assume— with good empirical support— that repeat mating is rare, we do not assume that mating (i.e. partner choice) is completely at random among the whole population. Our response to criticism 5 addresses the wider issue of random mating.

3.3.1 Implications

There are two reasonable biological mechanisms that could explain the FSP age gaps: more ageing error, and/or sperm storage. If we were to include them in the model, what might the effects be? Note that we cannot check the impact of sperm storage using our current code. To do that, we would need to rewrite the code to specifically allow for 3-year pupping intervals, which would be a substantial job. (See response to criticism 13 for why we did not consider the explicit-pupping-interval version necessary under a purely-ageing-error model.) However, there are some straightforward arguments as to why the effects are likely to be small.

First, the effect of under-estimating the CV for ageing uncertainty would be minimal through FSPs *per se*, because nuisance parameters ensure that they have little impact on abundance estimation. The impact via HSPs, though, would be to somewhat overstate the true range of birth-years among the samples. The total number of HSPs would not change, so the impact is unlikely to be large, but would likely cause the current trend and natural mortality estimates to be biased downwards. However, even if the assumed CV really is too low, the uncertainty in the estimated trend is currently so large (point estimate 0.11 increase over 5 years; standard error 0.40; Table 4.11 of our draft report) that fixing a slight bias is clearly not going to turn a statistically insignificant trend into a significant one.

Second, the main implication of having ignored genuine cross-cohort FSPs from sperm storage would be that we had somewhat underestimated the "effective" number of maternal HSPs (MHSPs); this would imply that we somewhat over-estimated the adult abundance, although there are also more subtle effects related to multiple-paternity and same-cohort HSP parameters so it is hard to predict precisely. The number of FSPs involved is small relative to the number of cross-cohort HSPs in the model (i.e. $< 20\%$), so the effect should not be large. We discussed this point in section 4.5.4 of our draft report:

"If school shark are storing sperm for use in litters that are two or three years apart, then apparent cross-cohort FSPs (from successive matings) should be treated demographically as if they were MHSPs; if not, they can be basically ignored in the CKMR model. We have chosen to assume that all the FSPs are same-cohort, and hence that the longer (3 to 5) year gaps in apparent birth cohort are due to ageing error. If we had chosen to assume that sperm storage occurs, we would somewhat lower the estimated abundance."

Is there any substance to this? Partly— the age-gap distribution CV we assumed for ageing error is either somewhat too low and/or there is a modest incidence of sperm storage. There is no evidence of repeat mating, though.

Implications for assessment? Sperm storage would mean our current abundance estimates are somewhat too high. Higher CV on ageing error would have little effect on results, but might cause a minor downward bias on the estimates of current trend and natural mortality.

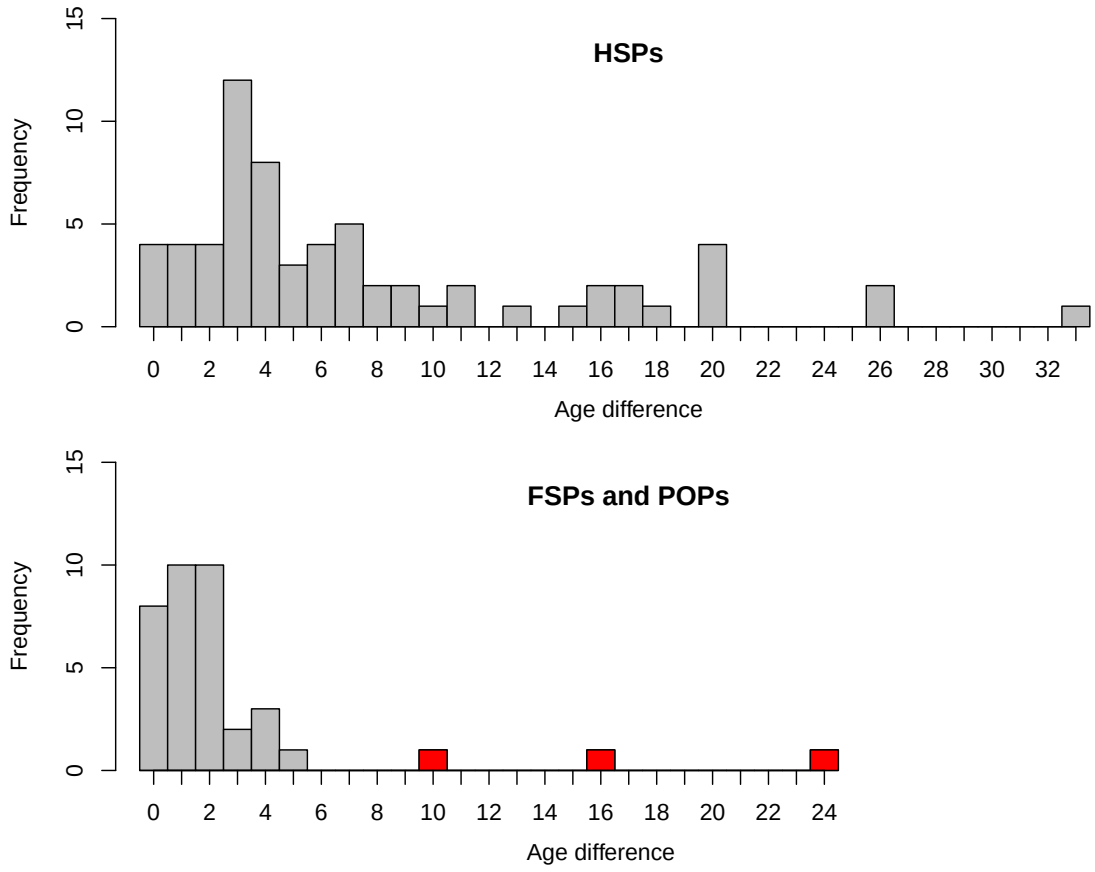

Figure 2: Frequency of apparent age gaps between pairs of animals found to be (upper plot) HSPs, or (lower plot) FSPs (grey bars) and POPs (red bars). HSPs have a wider spread than FSPs.

3.4 Random mating

5. The assumption that mating is at random which appears to be contradicted by the data

If the overall school shark population was mating non-randomly i.e. being somehow split into tiny persistent breeding groups such that the chance of finding the same partner again is quite high (the extreme being pair-bonding) then the FSP age-gap distribution would, just like the HSP distribution, have a long right-hand tail; but, as shown in Figure 2, it does not.

To unpick this a little further, Dr Cordue states (p18 of his report):

"The ageing error conclusion is based on the assumption that repeat matings do not occur or are extremely rare – which is based on the assumption that mating is at random. It is difficult to believe that mating is at random ..."

The assumption that repeat matings are extremely rare (which we do make), does not imply that matings are completely at random (i.e. that an individual's future mate choice is completely independent of their past mate choice). All that is required is for the pool of possible mates to be large for most individuals on most mating occasions. Given total adult abundances of the order of a hundred thousand, this seems entirely plausible. School shark are reported by industry members of sharkRAG to mate in large aggregations. We do not expect "pod structure" (e.g. sperm whales) or pair-bonding (e.g. albatrosses). Even if the population were split into a large number of separate sub-populations, each of which mates only within itself, the size of these groups is evidently big enough that repeat mating is rare. This is evidenced by Figure 2; if repeat-mating were occurring, FSPs would should show the same broad distributions of age-gaps that is shown by HSPs, which they clearly do not. Appendix A gives more details, and a further consideration of the implications of possible sub-population structure can be found in Appendix B (see also response to criticism 10).

Is there any substance to this? No, as shown by the data.

Implications for management advice? None.

3.5 Conditional probabilities

4. The deliberate absence of survey design given a plan to use conditional probabilities. This leads to unnecessary complexity and the need to account for ageing error

There are a number of serious problems in Dr Cordue's argument. To explain further, we turn first to a related comment on p19 of Dr Cordue's report:

"the use of conditional probabilities is not necessary. Rather than improvements to the existing assessment there should be a reworking of the data and a new model based on unconditional probabilities."

To be clear: the phrase "conditional probabilities" means that, for each pairwise comparison between two samples, we take account of (i.e. condition the probabilities on) the specific covariates of that pair: years of capture, ring counts, and sexes— this is how we have built our model, exactly along the lines of Bravington *et al* (2016b). That paper proves mathematically that a conditional analysis will give unbiased estimates, provided the structure of the probabilities is set up to correctly match the biology and sampling. It also gives a systematic recipe to construct the conditional probability equations, based on the notion of Expected Relative Reproductive Output, and sets out a maximum likelihood framework for estimating all the unknown demographic parameters.

Dr Cordue's claim about "unconditional probabilities" appears to be that samples should be taken at random (see below) in such a way that the conditional probabilities for pairwise comparisons across the sample can be averaged out (see below), to give a single probability value for each kinship type (MHSP, PHSP, FSP, POP) that presumably depends on only a small number of unknown parameters, including average abundance; the observed kinship rates are then calculated from the pairwise comparison results; and finally a simple model is used to estimate the parameters. This might sound appealingly simple— but it can't work for the following reasons.

1. Close-kin models fundamentally cannot separate average abundance from abundance trend without using conditional probabilities. An observed number of kin-pairs overall can be explained equally well by a smaller stable population, or by a currently larger population that is increasing (Skaug 2001). Conditional probabilities do not always average out in the way Dr Cordue might hope, and an "unconditional" model is fundamentally biased in the presence of trend. The only solution is to (correctly) use conditional probabilities.
2. Without conditional probabilities no trend can be estimated, which would defeat the point of the exercise for school shark, where CKMR is being used to show evidence of recovery.
3. "Random sampling", by which Dr Cordue presumably means sampling in such a way that there is an equal probability of sampling all living sharks, is not possible in a commercial fishery because of selectivity (resulting both from gear selectivity and differential availability of fish resulting from age- or size-based behavioural patterns) and it is rare to have really good estimates of selectivity. Our conditional model bypasses that problem entirely; it simply requires knowing the facts about each sample (e.g. capture date, ring count, sex).
4. As a general statistical principle, stratified sampling is more efficient than "random sampling" e.g. fish are not usually sampled randomly with respect to size for the purpose of constructing an age-length key.
5. Ageing error is inescapable and simply must be accounted for, especially when accounting for the lucky-litter effect and multiple paternity that influence same-cohort but not cross-cohort sibling pairs. These parameters cannot be estimated if their influence is somehow "averaged out" through an unconditional model, without leading to bias.
6. With regard to "unnecessary complexity": the conditional probabilities in CKMR (which are scarcely more complex than expressions that feature in typical age-structured stock assessment models) merely reflect correctly the important and inescapable features of sampling and biology.

It is also incorrect to claim that we did not have a survey design. Specifically, we took some care to plan the number of samples that would be required to get a reasonable number of kin-pairs — based on the spread of sizes collected by the fishery and given the stock assessment model available at the time. We assumed that samples would be collected in proportion to their occurrence in the fishery. Note that industry co-operation in collecting samples for this project has been essential, and that meant keeping the burden from field instructions low. We planned from the start to use a “conditional” analysis, as we have in every CKMR project undertaken at CSIRO, and concluded that the sample spread and sample size we were likely to get should give a good chance of building a usefully precise model. Our use of conditional probability allowed us to make use of our data even though a change in the fishery lead to our receiving many more samples from longline gear than we had anticipated.

Is there any substance to this? No

Implications for assessment? None

3.6 Distance

6. The assumption that the sharks are spatially fully mixed and therefore that the probability of close kin pairs is independent of the distance between capture locations (which appears to be contradicted by the data)

We found no evidence of higher rates of sibship at shorter distances. Dr Cordue is incorrect in suggesting that movement rates for school sharks are “relatively limited”, as shown by conventional as well as satellite tagging studies.

Figure 4.12 of our draft report shows the number of comparisons, and the number of kin pairs found, against binned distances apart for the two captured animals. Each bar represents 100km. We replotted the data using 25km instead of 100km bins (Figure 3). This shows no indication of a tendency for kin pairs to come from samples collected closer together, rather, the height of the 0-100km bar in our draft report results from one higher bar at 75-100km distance, which is easily explained as the result of chance.

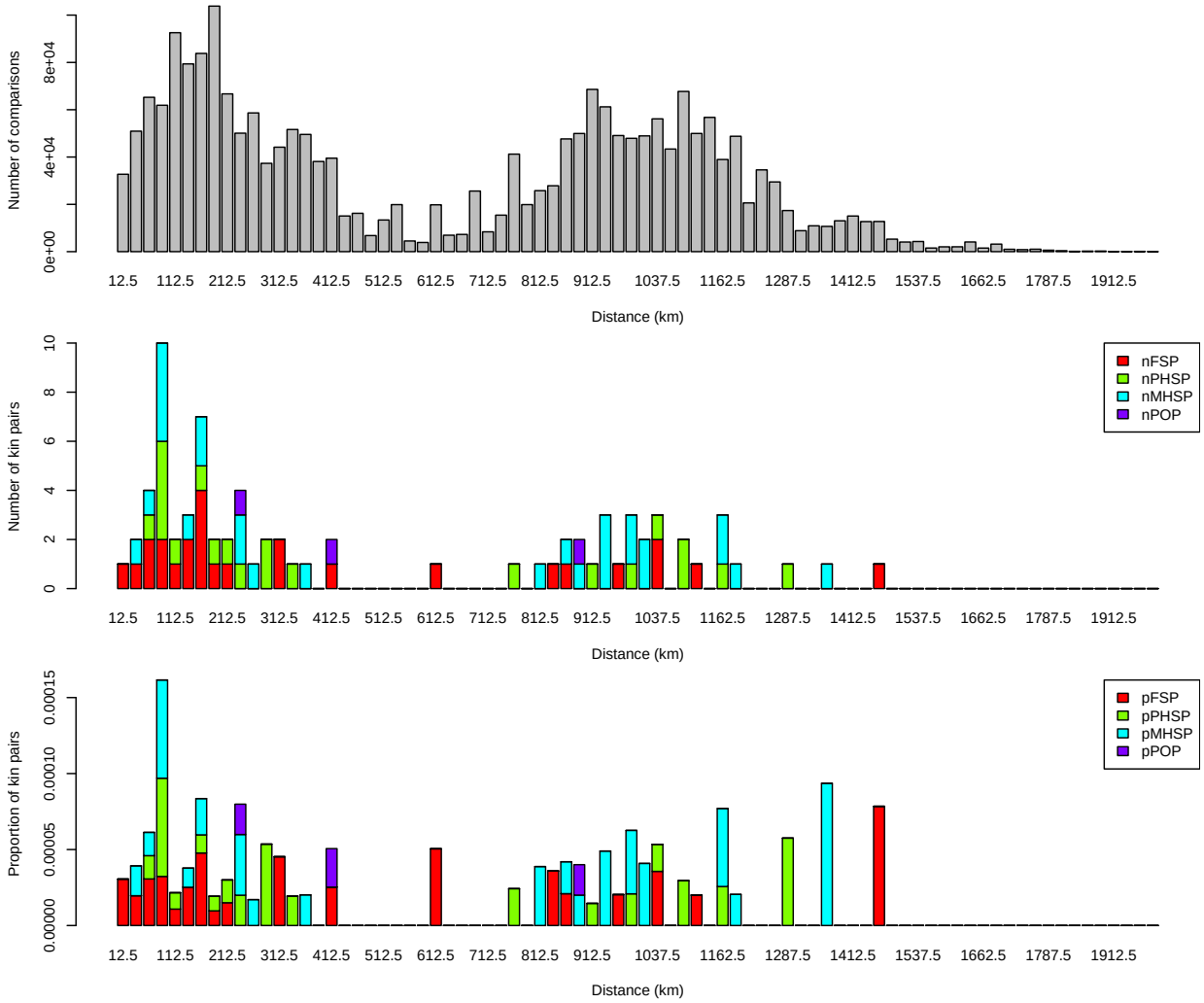

Figure 3: Histograms showing the distance (km) between average capture location for (upper plot) every possible pairing of animals sampled; (middle plot) close kin pairs; and (lower plot) the proportion of comparisons that yielded kin pairs. Bins are 25km wide and the value on the x-axis shows the central distance for each bin.

In his modelling scenarios, Dr Cordue considered a scenario in which "movement between areas is relatively limited" (bottom of p10) but school sharks do not show limited movement; indeed they have a reputation for impressive movement. School shark pups have been shown to travel from south-eastern Tasmania (Pittwater) past Flinders island and back again before they are a year old (McAllister *et al* 2015; p64 of our draft report). Conventional tagging has shown prodigious movement (Figure 3.1, draft report) from every sharkRAG zone to every other zone, and movement between New Zealand and Australia is not uncommon (p64, draft report). Satellite tracking of pregnant females has shown large-scale, rapid, movement (Ian Knuckey, Fishwell Consulting, pers commn) so it is in no way unreasonable for us to have developed a model that does not confine school shark to "limited movement" out of the regions in which they were born. The locations of the kin pairs found in our study tested, and supported, our assumption (Figure 4.10 and Table 4.6 of the draft report as well as the lower plot of Figure 3 below).

Is there any substance to this? No

Implications for assessment? None

3.7 Full Thiatic Pairs (FTPs)

7. Neglecting to include nibling pairs (uncle-nephew, uncle-niece, aunt-nephew, aunt-niece) in the set of close kin relationships (which need to be included unless the age range is severely restricted)

This is not relevant, because we did severely restrict the age range in our "base model". On p15 of Dr Cordue's report, he notes: *"As school shark mature at about 10 years (males earlier) there shouldn't be many NiPs in the school shark pairs used in the base model."* Dr Cordue's draft review report did not include this acknowledgement, so it is likely to have been a more recent realisation on his part that may not have percolated through to his summary. The demographic reasoning is elucidated in Appendix A.

Is there any substance to this? No, not yet. If the study expands in future to cover more years, we would adjust the CKMR model to allow for the possibility.

Implications for management advice? None.

3.8 HSP probabilities

8. Possibly incorrect calculation of the probabilities of MHSPs and PHSPs (perhaps forgetting to subtract the probability of an FSP from the probabilities of having the same mother/father)

No subtraction is necessary. The probability of a sibling being a half- versus a full-sibling is modelled using a "nuisance parameter" ν_2 which dictates the expected proportion of every litter that consists of maternal half-siblings (different fathers) as opposed to full-siblings (same father). Note that the parameter does not appear in equations for cross-cohort half-siblings because repeat mating is improbable (see above). However, if we need to allow for sperm-storage in a future version of our model, there will need to be some minor modifications here— probably another nuisance parameter.

On p15 of his report Dr Cordue writes *"It may be that they have dealt with this in the way they have calculated the conditional probabilities but it is not clear from the documentation"*. We certainly try to explain it, several times: in the parameter list of Table 4.8 on p49; on p90, where we wrote "ν_2 *Proportion of the litter that are likely to have different fathers (i.e. that are half, rather than full, siblings)*". Equation C.17 on the same page is even more explicit: "*The probability that i and j are full-siblings is expressed in terms of the shared mother*"; and this is followed by the probability equation for full-siblings, which involves the term $1 - \nu_2$ in contrast with equation C.16, the probability calculation for same-cohort half-siblings, which involves just ν_2.

Is there any substance to this? No

Implications for assessment? None

3.9 Bernoulli trials

9. Incorrect calculation of the likelihood by using independent Bernoulli trials for each close kin relationship for a given pair of sharks (instead of a single multinomial trial)

As Dr Cordue notes on p16: *"This [treating the comparisons as independent Bernoulli trials] is technically incorrect because two pairs of sharks that have one shark in common do not form independent trials. However, it is a perfectly adequate approximation given the relatively large population size (so that although not strictly independent, such pairs are almost independent)."* In that, he is correct. Like point (8) above, this second realization is absent from Dr Cordue's draft report so that he seems to have corrected himself but not fully modified his final report.

The full technical explanation can be found in Bravington *et al* (2016b) (section 4.3) on "sparse sampling" of large populations. The point is really that, with a high enough adult abundance (and school shark certainly qualifies), only a small proportion of the population needs to be sampled to find enough kin-pairs to fit a statistical model; and then only a small proportion of the sample will actually be involved in any kin-pairs at all. We used approximately 3000 samples and found roughly 100 kin pairs i.e. 200 animals; so only 7% of our sample are involved in a pair. Therefore, we might expect of the order of 7% of our pairs to overlap with each other; and indeed, we discovered eight triads (Section 4.5.5 of the draft report). Comparisons within those

triads are clearly not statistically independent, but make up only a small proportion of the total statistical (Fisher) information. This justifies both the use of independent Bernoulli trials that repeatedly use the same animals (last paragraph of p16 of Dr Cordue's report) as well as our decision to ignore the fact that a pair found to be a POP cannot also be an HSP (penultimate paragraph). By treating all the pairwise comparisons as independent, we get a computationally tractable framework, a very good approximation to variance and, importantly, we do not incur any bias (section 4.1 of Bravington *et al* 2016b).

Is there any substance to this? No

Implications for assessment? None

3.10 Catches pre-2000

10. Inability to account for known catch history before 2000.

This phenomenon has been evident for many years with CPUE-based analyses of school shark; it is not restricted to CKMR, as we noted in the Discussion (chapter 5) of our draft report. Punt *et al* (2001) encountered the same problem in marrying CPUE with catch data for school shark in southern Australia. Like Punt, we hypothesised that there were two or more stocks of school sharks, at least one of which is no longer present but that was present in the past when catches were high; and we referenced DEWR (2008), describing the degradation of Victorian pupping grounds.

The direct information from our CKMR analysis is on the absolute abundance and trend of those adults that gave birth to the juveniles in the fishery (i.e. the samples), over the years when those juveniles were born— basically 2005–2015. Our final report, and this response, discusses a number of potential issues around our abundance estimates, and we do not see any major reasons to expect bias.(See also Appendix B of this report, which discusses whether our current estimates could be susceptible to any issues from stock structure within the remaining population; in all, serious bias from any current stock structure seems unlikely.) Our abundance estimates are consistent across the simplest approximate "one-line" calculation, through a more sophisticated but still approximate GLM, right to a full-strength (and less approximate) stock assessment model— so coding errors are unlikely. Overall it really does seem as though something fundamental has changed about the biology/distribution/population structure of school shark in southern Australia since the 1990s— and the extirpation of former pupping grounds seems a plausible explanation.

Is there any substance to this? No. The tension between pre-2000s catches and current abundance estimates is not a shortcoming of the CKMR method; it is the reality of school shark history and stock structure.

Implications for assessment? None. As we noted in our draft report, there are substantial implications for management; see further discussion in Appendix A.

3.11 Pupping interval

(11.) Not explicitly modelling a pupping interval and therefore over-estimating the probability of maternal HSPs when ages are one year different

Our main argument for not needing to explicitly model a pupping interval, is that enough cohorts are involved for the "skip-spawning" effect on probabilities to more-or-less cancel out overall. Criticism (11) omits the counterpart: our model also under-estimates by a factor of 3 the true MHSP probability in comparisons whenever the true birth-interval is 3 or 6 or 9 etc years, because only 1/3 of the female population is pupping each year. Making the same assumption with regard to both the kin pair probability *and* the pairwise comparisons has the affect of, basically, cancelling the over-estimation in probability that occurs when the birth-interval is not a multiple of 3 years.

In addition, the substantial ageing errors in school shark mean that the true gap in birth-years between any pair of samples is quite uncertain, so that even if we were to calculate "exact" probabilities for each possible gap under a 3-year pupping cycle, the spikes and troughs are substantially averaged for any particular pair because of uncertainty about the pair's true gap. Since CKMR models with explicit pupping/spawning

intervals are somewhat harder to code, we opted to keep the model simple. We have made a similar decision for other species where skip-spawning occurs, including SBT (where ageing error is small, but there are many cohorts).

Is there any substance to this? No

Implications for assessment? None

3.12 Litter size

(12.) Ignoring variability in surviving litters sizes (except for estimating a lucky litter effect)

This is wrong on two counts. Firstly we don't ignore variability, and secondly only certain aspects of variability actually matter in properly-constructed CKMR estimates of abundance. Regarding the first: systematic effects at the level of individual adults due to body-size / fecundity / growth are explicit in our probability calculations. Regarding the second: random variability in litter size does matter for within-cohort comparisons (the lucky litter effect), but not for between-cohort comparisons, which are the ones used to estimate abundance in the CKMR model.

Litter size variability certainly does matter in CKMR, especially in HSP-based studies like ours. The key points are explained clearly in Bravington *et al* (2016b), section 3.2, equations (3.8) and (3.9). We recap the points here, but the published equations are unambiguous and may actually be easier to follow (and are outlined in Appendix A). There are two components to variability: systematic variation, whereby one adult is likely to have more or fewer offspring than another because of individual factors (e.g. big female teleosts make more eggs); and random variation, whereby some litters just happen to be larger than others and/or have higher survival rates, by chance. When calculating CKMR probabilities, we deal explicitly with the former, by incorporating the known size / fecundity and growth relationships for female school sharks.

The remaining issue concerns random variation. Appendix A gives a technical explanation that demonstrates that the random variability of litter size tends to systematically increase the number of siblings within cohorts, but not between cohorts, which is why the lucky-litter parameter that we estimate is required for the former but not the latter.

The Appendix of Dr Cordue's report fails to break down the probabilities into within- and between-cohort cases, which disguises the true nature of the term $\bar{p}^2$ on p21 of his report, and that presumably is what led to his comment. Mean litter size *per se* does not play a significant role in our model, although relative mean litter size as a function of female body size is important, and we account for that.

Is there any substance to this? No

Implications for assessment? None

3.13 "One-line" calculation

(13. from p16 of review report) *First, the probability of two sharks having the same mother is not the same as the probability of two sharks being a maternal HSP. Second, the conditional probability based on being "given" the older shark should not be used anyway.*

The first comment is irrelevant, and the second (although hard to follow) is either a misunderstanding or incorrect. For our "one-line calculation" of a ballpark abundance estimate, we only used cross-cohort comparisons, where full-siblings are rare at best, so that HSPs are the only relevant kinship type. We did that to avoid the genuine complications of lucky-litter effects and multiple-paternity, and our selection criterion was to look only at pairs where the apparent birth-years are at least 4 years apart.

As to the second comment: neglecting pupping intervals and other minor complications, if two animals are born about Y years apart from a steady-state non-pair-bonding population, then for the pair to be an MHSP, the first-born's mother has to survive the Y-year interval until the second animal is born, and if so, she then has an equal probability with every other living female adult of being the mother of the second-born. This conditional probability is equation (3.10) in Bravington *et al* (2016b).

In our one-liner, we are making many comparisons of this basic type, but the intervals Y differ between comparisons. Rather than taking the exact approach of dealing with them individually— which requires a lot more than one line— we make the first-order approximation of using the average interval $\bar{Y}$ to allow for adult mortality (see Appendix A for more detail). This is crude, but it is definitely the correct type of calculation; it is based on an exact conditional probability that is then averaged, albeit approximately, across the conditions in our sample. Dr Cordue's final comment on the one-liner, on p16, says: *"For this calculation the probability of two sharks selected at random being a maternal HSP should be used."* He does not define exactly what *"at random"* means; but in fact that is (up to approximation) exactly what we have calculated, assuming "at random" means "at random from the samples that were collected". Note that the adult mortality adjustment is very important in all calculations of HSP probabilities; we are not sure whether or not Dr Cordue has correctly applied it in his own calculations (e.g. it does not feature in his Appendix).

Our one-line calculation (section 4.6.1 of our draft report), and the simple GLM that follows it (section 4.6.2), are not meant to be exact— they are meant to be as simple as possible, at the inevitable cost of being quite approximate. There are two motives for including them. The first is to illustrate "basically" where the signal is coming from in CKMR estimation, in order to help a reader build some confidence in the principles with as few technical complications as possible. The second is as a rough check on our own code, to provide reassurance that there aren't big mistakes within the unavoidably-complicated machinery of our full CKMR-based stock assessment. The estimates from the three different models should at least be reasonably similar, which they are. Given that the rough check is passed, we strongly recommend the estimates from the full-complexity CKMR-based assessment, rather than from the simpler models.

Is there any substance to this? No

Implications for assessment? None

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A Appendix: More detailed response to review critiques

This Appendix contains a more detailed response to Dr Cordue's criticisms than that given above in the "Summarised response to review points" section. The material in that section is repeated here, along with additional material, so that this section can be read instead of the one above, by interested readers willing to tackle more technical details.

First, we introduce the reason for a CKMR investigation of school shark. School shark in southern Australia used to be the mainstay of the flake and chips market. It was been heavily fished during the 1900s, and by the end of the 1990s was reduced to very low levels (estimated to be at between 12 and 18% of B_0 in 1997, Punt *et al* 2000). Management during the 1990s and 2000s was based on an age-structured stock assessment model (Punt *et al* 2000; Punt 2001; Thomson & Punt 2009; Thomson 2012). That model relied on commercial gillnet CPUE time series as an index of abundance. However, increasingly stringent management measures that were introduced to protect what was by then a bycatch species in a fishery mainly catching gummy shark, caused school shark CPUE to break down as an index of abundance, perhaps as early as the mid-1990s.

Without reliable CPUE, a conundrum eventually develops: if the management measures are actually working and the stock is recovering, but there is no way to prove it and therefore no basis for adjusting the effectively-fixed-TAC policy (set at 215t since 2013), then there would be an increasingly and unnecessarily severe choke on fishing effort directed mostly at gummy shark. As a way to resolve this impasse, managers then turned in 2014 to close-kin mark-recapture (CKMR) to provide a school shark stock assessment that is not susceptible to past (or future) changes in fishing behaviour. Between 2014 and 2019, we presented a series of progress reports at meetings of AFMA's shark resource assessment group (sharkRAG), leading up to a first CKMR estimate of school shark abundance, trend, and other demographic parameters in our draft report to the FRDC (Thomson *et al* in prep). Our analysis suggests that the remaining population is smaller but more productive than previously thought. While our point estimate is that some rebuilding has occurred, the statistical uncertainty around trend is still very high, and even some decrease cannot yet be ruled out. Forward projections from our model indicated that catch levels very similar to those currently taken would (when looking at the median but ignoring the large confidence interval) allow rebuilding. The quota available to the Commonwealth shark fishery has nevertheless been reduced, primarily to account for increased levels of discarding in that fishery, as well as catches by the States.

Our responses to Dr Cordue's criticisms follow.

A.1 Duplicates

1. According to the DNA results there were a large number of duplicate samples (85) which points to poor laboratory procedures OR genuine duplicates (i.e., identical twins/triplets and/or parthenogenesis which has implications for the analysis)

There were no such large-scale duplicate samples. The samples in question were clearly not school shark, based on the genetics; presumably, they were gummy shark sampled in error (see section 4.1.1 of our draft report). Unfortunately, section 4.5.3 of our draft report contradicts Section 4.1.1 in that it refers to the removal of "85 accidental duplicates" but this is a reporting error which we will correct when our report is finalised - in Section 4.5.3 we conflated the relatively small number of actual duplicate tissue samples (27, representing 13 individuals that were sampled on, or close to, the same day) taken by samplers in the processing factories with the 58 suspected gummy shark samples. Section 4.1.1 provides an accurate discussion of these samples.

Is there any substance to this? No

Implications for assessment? None

A.2 FSPs vs POPs

2. An inability to distinguish between FSPs and POPs using just DNA which points to poor SNP selection OR no genuine POPs (3 POPs were assumed on the basis of age difference)

It is clear, based on the estimated ages of the samples found to be FSP/POPs, which ones were POPs and which ones were FSPs, so that is the criterion we used. Figure 1 (upper plot) shows the distributions of estimated birth-gap for FSPs/POPs. The gaps are clustered close to 0 with a small number out to a 5-year gap; then there is a big gap to three pairs separated by 10 years or more. FSPs should be close together in age (see also response to next point); POPs, of course, have to be separated in birth-date by at least the age-of-maturity (9 years or more). The large birth year gaps of the three POPs are definitely much larger than those of the cluster of FSPs. Further, the existence of a few POPs in the dataset is to be expected *a priori*, given our estimates of abundance from HSPs and the age distribution of the samples. Thus, the kin pair data do seem to tell an internally consistent story.

Given the clarity of the signal from age— and the fact that neither POPs nor FSPs make much difference in the final model, which is HSP-based— there was no need to look at the genetics for FSP/POPs. Nevertheless, for interest we did in addition try out a simple-but-inefficient statistical algorithm for FSPs-vs-POPs, which proved not particularly effective (section 4.5.3 of the draft report). We did not bother trying to improve that algorithm in this project, although it remains on our future to-do list for CKMR tools in general. Our SNP selection was designed for the core important task— finding HSPs— for which it worked well; and our algorithm for HSP-finding was carefully optimized to give maximum statistical power.

A.2.1 Null alleles

Although the details of kin-finding algorithms are really beside the point here, it is worth commenting on one related aspect in the body of Dr Cordue's report, because it does indicate a lack of understanding over one technical aspect of the genetics. On p14, Dr Cordue describes identification of POPs in humans or other species that are genetically well-studied. But he has not reflected an email conversation between himself and Dr Thomson, where the role of "null alleles" was discussed. Loci with null alleles i.e. where some allelic variant is physically present in the genome, but that particular variant systematically (and heritably) fails to reveal itself under genotyping— do complicate analyses, and therefore are generally excluded from human studies, where cheap genotyping methods have been specifically tweaked and alternative loci are plentifully available. Awareness of nulls tends to be low among many practising geneticists— loci with nulls are just seen as a nuisance to be avoided. However, with the ddRAD-based techniques that DArT uses, "nulls" have turned out to be surprisingly common in fish/sharks, and it can be hard to find enough null-free loci. In order to minimize genotyping costs, we in fact deliberately target some loci with moderate frequencies of nulls, because the presence of a null can to some extent be detected (based on the read-counts of non-null variants) and the null then acts as a "3rd allele", which improves kin-finding power per unit cost. The downside is that we have had to develop special-purpose "null-friendly" algorithms, which takes work. The FSP/POP discrimination is one example; our simple algorithm allows for the presence of nulls (and it has to, because nulls are common in our data and the calculations would be wrong otherwise— existing methods from the human genetics literature won't work in the presence of nulls) but the presence of the nulls reduces its statistical power. We could— and eventually will— circumvent that by adapting the algorithm to use detected nulls, but it is not a simple task. For HSPs, where it really matters, we use a full-strength algorithm that incorporates partial null-detection.

Is there any substance to this? No

Implications for assessment? None

A.3 FSP age gaps

3. Numerous FSPs with age gaps that are inconsistent with the variance of repeat age readings (which implies huge ageing error which appears to have been ignored in the base model OR the occurrence of repeat matings which has implications for the analysis)

While we did allow for some ageing error in the analysis (CV=0.08 based on repeat readings of vertebrae), we agree that the FSP data point to there being more ageing error in reality, and/or some sperm-storage across one pupping interval; both are biologically plausible. However, repeat matings are inconsistent with the data.

Figure 1 (upper plot) shows the distribution of observed and expected FSP age gaps, if all FSPs are truly

same-cohort and the ageing error has CV = 0.08 (same CV assumed for all ages). The expected distribution clearly is too narrow; there should not be any apparent gaps of 3–5 years, but in fact we found six (as noted above, the three very long gaps of 10 or more years, are POPs).

Figure 1 (middle plot) shows what is expected if the CV is actually 0.20; then the match would be quite good. Is it actually possible that the true juvenile ageing CV is much higher than 0.08. The figure of 0.08 accounts for repeat-reader error, not for genuine biological variation in observable rings. The only data available on variation in ring deposition in school sharks is based on just 14 female and 4 male sharks (Table 2 of Walker *et al* 2001) and the data on the number of rings laid down each year is not broken down by shark age (size). We therefore could not use these data to calculate ageing error for juvenile sharks, but we note that the standard deviation in ring deposition rate (from the more accurate of the two methods used - alizarin staining) is 1.78, which is considerable. Age reading error would further inflate the overall variation in observed rings-at-age. A CV of 0.20 therefore seems quite reasonable.

Sperm storage is the other possible explanation for FSPs with apparent age-gaps of say 3–5 years (a true gap of 3 years across one pupping interval, possibly plus some ageing error). Storage of at least 4 years has been verified for another shark species in captivity (Bernal *et al* 2015). This is discussed in section 4.5.4 of our draft report:

"The remainder of the leftmost pairs must be FSPs; the maximum apparent gap is 5 years. Most of the FSPs have a gap of 0–2 years, which is entirely explicable in terms of ageing error on animals from the same cohort. The six FSPs with gaps of 3–5 years are either due to ageing error (certainly plausible), or (conceivably) to sperm storage, whereby a female uses sperm from one mating to fertilize not just one litter but also the next (two or more likely three years later). Sperm storage is known to occur in several shark species, including in school shark, at least for a few months immediately following the mating season (Walker, 2005)."

Figure 1 (lower plot) shows the expected FSP age distribution if there were truly 10 cross cohort FSPs (i.e. the six that have an apparent gap of 3-5 years as well as four of those that have a gap of 2 years) but keeping the original ageing-error CV of 0.08. The fit is again reasonably good. It is clear that sperm storage, or perhaps a combination of sperm storage with a somewhat higher CV on ageing error, could explain the observed FSP age gaps quite satisfactorily. However, in order to inflate the expectation for a 3-5 gap we have under-estimated the observed numbers at 2 years. This shows the influence of assuming that only sperm-storage is occurring, without invoking additional ageing error (even though it seems reasonable from Walker *et al* 2001 that additional ageing error is present).

Although length measurements are generally not reliable in our samples (section 4.1.3 of our draft report), it is interesting to compare recorded lengths within those four FSPs where the apparent age-gap is 4 or 5 years. In three of the four pairs, one animal is recorded as under 90cm (with a ring count of 3) and the other animal as over 130cm (with a ring count of 7 or 8). In the fourth pair, a shark with ring-count 7 and length 109cm is a full-sibling of another with ring-count 11 and length 164cm. The members of each pair were either captured in the same year as each other, or one year apart. While the ring-count differences alone might be consistent with a high CV in ring-counts, the length differences (notwithstanding possible measurement inconsistencies) do seem too big for animals of the same true age (i.e. from the same cohort). This does suggest that these large-apparent-age-gap FSPs might indeed be drawn from two successive litters with sperm storage.

A.3.1 Age uncertainty in adults

Dr Cordue seems to misunderstand the reason for restricting the sample to animals found to have 11 or fewer age 'rings'. Dr Cordue's report states *ages were estimated by ring counts which have been thought to be fairly reliable up to 11 years* (p4) and *11 rings or fewer (to avoid the worst ageing error)* (p5) but ageing error alone can be (and was) accounted for in the model. The reason for excluding sharks older than 11 was that ageing is thought to be biased for older sharks (Walker *et al* 2001). Such bias can also be accounted for (and is, for ages over 11) but only if the extent of the bias is known.

A.3.2 Implications

Our main CKMR analysis assumes that all the FSPs are same-cohort (i.e. that there is no appreciable sperm storage), and that the CV on age estimates is 0.08 for juveniles. However, Figure 1 (upper plot) shows that the data are inconsistent with at least one of these assumptions. The implications turn out to be different depending on which assumption is at fault, but in either case there is no reason to expect a major impact on our conclusions, as explained next.

First, if the no-sperm-storage assumption is correct, then the CV of juvenile ageing error must be higher than 0.08. This probably does not matter for the FSPs themselves, since they are largely peripheral to the main CKMR model and mainly impact on estimates of nuisance parameters (if they are from the same cohort). However, the choice of CV does have some effect on the model outputs via HSPs. Using too low a CV would tend to somewhat overstate the true range of birth-years among the samples— although most of that range is driven by the genuinely wide span of juvenile cohorts in our samples, so the impact of an incorrect CV value would be limited. Presumably, by analogy with "errors-in-variables" problems in statistics (Carroll *et al* 2006), this would mean that the current trend estimate was somewhat attenuated (i.e. biased towards zero). The current mortality rate estimate would also be biased downwards for basically the same reason, but since the overall number of HSPs is unaffected, presumably there would be little bias in the overall abundance estimate. However, even if the assumed CV really is too low, the uncertainty in the estimated trend is currently so large (point estimate 0.11 increase over 5 years; standard error 0.40; Table 4.11 of our draft report) that fixing an "attenuation bias" is clearly not going to turn a statistically insignificant trend into a significant one.

Second, what if sperm storage is the explanation for all or most of the FSPs with bigger apparent age-gaps, and the CV of 0.08 on juvenile age is about right? The issue then would be that the cross-cohort FSPs should actually be treated as if they were maternal HSPs. The mother is alive when two separate litters are born, but the father is only involved in one mating event. There are not that many likely cross-cohort FSPs (assuming sperm storage, and removing the animals older than 11) — perhaps 5 compared to about 42 HSPs— so the effect of including them on the abundance estimate would to be reduce it appreciably, though not massively. It is not obvious that there should be a systematic effect on trend estimates. The estimated mortality rate might increase (since the new "MHSPs" are only one pupping-interval apart, and so would have lower age-gaps than typical MHSPs) and there would be minor impacts on the other nuisance parameters, such as multiple-paternity-rate. We made this point in section 4.5.4 of our draft report:

"If school shark are storing sperm for use in litters that are two or three years apart, then apparent cross-cohort FSPs (from successive matings) should be treated demographically as if they were MHSPs; if not, they can be basically ignored in the CKMR model. We have chosen to assume that all the FSPs are same-cohort, and hence that the longer (3 to 5) year gaps in apparent birth cohort are due to ageing error. If we had chosen to assume that sperm storage occurs, we would somewhat lower the estimated abundance.""

A.3.3 Ageing error in general

It is fair to say that the extent of ageing error/uncertainty in school sharks did surprise us during analysis, and a lot of our effort went into dealing with it— in particular for adults, where the uncertainty is relatively much worse (section 4.1.2 of our draft report), and we explored several different approaches before reluctantly deciding it was best not to use adults at all in the final model. When we designed this project back in 2013, we were expecting to have good age measurements (e.g. CV of 0.08) from the vertebrae, as well as length measurements; and we were not expecting to have many adults, because at that point the fishery mostly used gillnets designed not to catch adults. In principle, for HSP-based CKMR, a good age measurement is much more informative than a good length measurement. For POP-based CKMR on teleosts, the situation is more complicated; age is still best for juveniles, but for adults both age and length are useful, and if only one is available, then length might actually be preferable, which is why we emphasized the need for vertebrae. At the time of design, we therefore expected that we would be able in our final analysis to treat age estimates as basically exact, which would have kept the model simple. However, the fishery changed during the project so that our samples eventually included rather more older juveniles and many adults, with their much higher age uncertainty; the length measurements were unfortunately made inconsistently and are not reliable enough

for general use (section 4.1.3); and, based on the FSP age-gaps, either the CV of vertebral age estimates for juveniles is higher than 0.08 and/or sperm storage is reasonably common.

For several of our other shark CKMR projects, the samples mostly come from live biopsies and only length measurements are available, so we had to build uncertainty about age-given-length into those models. That seems to have worked well when the length measurements were reasonably accurate (e.g. Hillary *et al* 2018, for white sharks). With school sharks, we are in the situation of having reasonable though imprecise age measurements for juveniles only, but currently no useful length data. If the length measurements had been consistent, we could have used them in conjunction with vertebral age estimates to reduce the overall uncertainty about the true age of each sample. Unfortunately that is not the case at present, but hopefully will be resolved in future sampling. In particular, having more FSPs with reliable length measurements might resolve uncertainty about the ageing CVs and the extent of sperm-storage, if any. When enough suitable data become available, there should be no problem in modifying our existing model accordingly (by changing the CV and/or allowing a proportion of sperm-storage).

Is there any substance to this? Partly— the age-gap distribution CV we assumed for ageing error is either somewhat too low and/or there is a modest incidence of sperm storage. There is no evidence of repeat mating, though.

Implications for assessment? Sperm storage would mean our current abundance estimates are somewhat too high. Higher CV on ageing error would have little effect on our resuls.

A.4 Random mating

5. The assumption that mating is at random which appears to be contradicted by the data

If the overall school shark population was mating non-randomly, i.e. being somehow split into tiny persistent breeding groups (that show site-attachment) such that the chance of finding the same partner again is quite high (the extreme being pair-bonding) then the FSP age-gap distribution would, just like the HSP distribution, have a long right-hand tail; but, as shown in Figure 2, it does not.

Although our main argument is that repeat-breeding is likely to be very rare, and moreover that the observed age gaps can be explained better by a combination of ageing error and sperm storage and to-be-expected POPs, it is worth noting that the number of potential distant FSP gaps is very low relative to the number of HSPs— treating those FSPs as MHSPs could not change the estimates much.

To unpick this a little further, Dr Cordue states (p18 of his report):

"The ageing error conclusion is based on the assumption that repeat matings do not occur or are extremely rare – which is based on the assumption that mating is at random. It is difficult to believe that mating is at random ..."

The assumption that repeat matings are extremely rare (which we do make), does not imply that matings are completely at random (i.e. that an individual's future mate choice is completely independent of their past mate choice). All that is required is for the pool of possible mates to be large for most individuals on most occasions. Given total adult abundances of the order of a hundred thousand, this seems entirely plausible except in species with "pod structure" (e.g. sperm whales) or pair-bonding (e.g. albatrosses). Even if the population were split into a large number of separate sub-populations, each of which mates within itself, the size of the mating group would be large enough for consistency with the assumptions of our CKMR model.

The affect of mating group structure on CKMR is elaborated in Appendix B, where it is concluded that, if two juveniles are sampled entirely at random from the fishery, the chance they share a mother is the same (i.e. $1/N$) whether or not separate mating groups exist. This conclusion does not require group membership to be heritable— it only requires that the composition of the juvenile sample reflects the proportions of the adult populations.

Sampling from the same "school" or pod would, of course, result in bias. (It violates the fundamental condition of CKMR, which is that the fact of one animal happening to be sampled should not, given all covariates, affect the chance of the other animal being sampled). So we didn't do that.

Is there any substance to this? No, as shown by the data. See the previous point about possible sperm-storage, as opposed to repeat-mating. As to the more general point about "random mating", see Appendix B.

Implications for management advice? None.

A.5 Conditional probabilities

4. The deliberate absence of survey design given a plan to use conditional probabilities. This leads to unnecessary complexity and the need to account for ageing error

There are a number of serious problems in Dr Cordue's argument. In unpicking the issues, we turn first to a related comment on p19 of Dr Cordue's report:

"the use of conditional probabilities is not necessary. Rather than improvements to the existing assessment there should be a reworking of the data and a new model based on unconditional probabilities."

To be clear: the phrase "conditional probabilities" means that, for each pairwise comparison between two samples, we take account of (i.e. condition the probabilities on) the specific covariates of that pair: years of capture, ring counts, possibly lengths and sexes— this is how we have built our model, exactly along the lines of Bravington *et al* (2016b), and in that paper it is proved mathematically that a conditional analysis will give unbiased estimates, provided the structure of the probabilities is set up to correctly to match the biology and sampling. Dr Cordue's claim about "unconditional probabilities" appears to be that: samples should be taken at random (see below) in such a way that the conditional probabilities for pairwise comparisons across the sample can be averaged out (see below), to give a single number for each kinship type (MHSP, PHSP, etc) that presumably depends on only a small number of unknown parameters, including average abundance; the observed kinship rates are then calculated from the pairwise comparison results; and finally a simple model is used to estimate the parameters. It might sound appealingly simple— but it can't work for the following reasons:

1. Close-kin models fundamentally cannot separate average abundance from abundance trend using only a single point (e.g. an empirical estimate of one probability, say of mother-offspring pairs, where all covariates such as age and date have been collapsed). Consider two populations with similar demographics and the same current adult abundance, except that one is rising and the other is falling; there will be more kin-pairs in the rising population. In other words, you can "explain" some observed overall rate of kin-pairs equally well with a larger rising population, or a smaller static or declining population. An average abundance estimate can only be obtained by assuming there has been no trend (which would rather defeat the point of the exercise, for school shark); and if there has in fact been a trend, then the average estimate will be biased. This level-vs-trend trade-off, which is unusual in statistics and perhaps specific to CKMR, has been known since the very first paper proposing a single-sample version of CKMR (Skaug 2001)— with random sampling— which ran into exactly this problem.
    - A CKMR analysis using conditional probabilities looks at many different probabilities at once, depending on dates, ages, etc. This is precisely where the power of CKMR comes from, to simultaneously estimate abundance level, trend, mortality rate, and other demographic parameters— exactly what a stock assessment should do.
2. "Random sampling" (presumably meaning equiprobable sampling, i.e. an equal probability of being sampled for all living sharks— but, within what age range? over what time period?) is not possible in a commercial fishery; just about all fishing gear is selective, and it is rare to have really good estimates of selectivity.
    - Our conditional model bypasses that problem entirely; it simply requires knowing the facts about each sample (date, ring count, etc).
    - As a general statistical point: even when equiprobable sampling is possible, it is in general not a statistically efficient way to design an experiment. When trying to estimate the parameters of a linear regression of y on x, it is not efficient to collect x randomly across its range; the best

precision (lowest CVs) for a given sample size is obtained by taking half the samples near one end of x's range, and the other half near the other end. And in fisheries, construction of age-length keys is much more efficient if sampling is stratified by length, rather than drawing randomly (somehow) from the entire population, which will be dominated by the youngest age classes. Efficiency (i.e. getting a low variance on parameter estimates, for a given total cost or sample size) does matter for CKMR; our ability to decide whether there really has been an upward trend in school shark abundance is restricted by the variance on our estimates.

3. Complexity: the conditional probabilities in CKMR (which are scarcely more complex than expressions that feature in typical age-structured stock assessments) merely reflect correctly the important and inescapable features of sampling and biology. To paraphrase Einstein: a model should be as simple as possible, but no simpler.

    - Ageing error is unavoidably important— much as we might wish otherwise. For example, when birth-year is uncertain, it becomes impossible to bypass the complications of same-cohort comparisons— lucky-litter effects and multiple-paternity— just by excluding certain comparisons. Instead it is necessary to introduce and estimate extra parameters. The overall proportions of siblings in the sample are substantially inflated by lucky-litter effects, so there is really no hope of somehow "averaging out" these phenomena through an unconditional model without leading to bias. (It is notable that Dr Cordue's Appendix does not distinguish same-cohort from cross-cohort comparisons.)

It is incorrect to claim that we did not have a survey design. Specifically, we took some care to plan the number of samples that would be required to get a reasonable number of kin-pairs — based on the assessment at the time, and fully aware that that assessment might be off the mark. We assumed that samples would be collected in proportion to their occurrence in the fishery. Note that industry co-operation in collecting samples for this project has been essential, and that meant keeping the burden from field instructions low. We planned from the start to use a "conditional" analysis— as we have in every CKMR project undertaken at CSIRO— and concluded that the sample spread and sample size we were likely to get should give a good chance of building a usefully precise model— which by-and-large has turned out to be the case. In fact, just as the project began, the fishery switched some gillnets to longlines, with quite different selectivity, so any attempt at more precise control over samples would have been futile. Certainly, at the time we designed this project, we only had crude tools for CKMR design, e.g. just to predict the number of kin-pairs that might be found. Over several projects and several years since, we have developed sophisticated tools. We could, in theory, now do a more nuanced job of design for school shark, e.g. to concentrate on smaller or bigger animals. In practice, though, the practicalities of sample collection in the field mean that we will always have to be prepared to analyse whatever we get, regardless of what the plan might have been— and that means: conditionally.

Is there any substance to this? No

Implications for assessment? None

A.6 Distance

6. The assumption that the sharks are spatially fully mixed and therefore that the probability of close kin pairs is independent of the distance between capture locations (which appears to be contradicted by the data)

We found no evidence of higher rates of sibship at shorter distances (which would indicate limited mixing), neither in our draft report nor when re-examining the data for this response.

Figure 4.12 of the draft report shows the number of comparisons, and the number of kin pairs found, against binned distances apart for the two captured animals. Each bar represents 100km. We replotted the data using 25km instead of 100km bins (Figure 3). This shows no indication of a tendency towards kin pairs to come from samples collected closer together, rather, the height of the 0-100km bar results from a higher bar at 75-100km distance, which is easily explained as the result of chance.

In his modelling scenarios, Dr Cordue considered a scenario in which "movement between areas is relatively

limited" (bottom of p10) but school sharks do not show limited movement. They have a reputation for impressive movement. Young of the year school shark have been shown to travel from south-eastern Tasmania (Pittwater) past Flinders island and back again before they are a year old (McAllister *et al* 2015; p64 of the draft report). Conventional tagging has shown prodigious movement (Fig 3.1, draft report) from every sharkRAG zone to every other sharkRAG zone, and movement between New Zealand and Australia is not uncommon (p64, draft report). Satellite tracking of pregnant females has shown large-scale, rapid, movement (Ian Knuckey, Fishwell Consulting, pers commn) so it is in no way unreasonable for us to have developed a model that does not confine school shark to 'limited movement' out of the regions in which they were born. The kin pairs found in our study tested, and supported, our assumption (Fig 4.10 and Table 4.6 of the draft report).

A.7 Full Thiatic Pairs (FTPs)

7. Neglecting to include nibling pairs (uncle-nephew, uncle-niece, aunt-nephew, aunt-niece) in the set of close kin relationships (which need to be included unless the age range is severely restricted)

This is not relevant, because we did severely restrict the age range in our "base model". On p15 of Dr Cordue's report, he notes: *"As school shark mature at about 10 years (males earlier) there shouldn't be many NiPs in the school shark pairs used in the base model."* Dr Cordue's draft review report did not include this acknowledgement, so it is likely to have been a more recent realisation on his part that may not have percolated through to his summary.

We ignored FTPs because there are good reasons to expect them to be negligibly rare in the data we used for modelling. It is worth spelling out the logic; there are in fact two reasons.

The first, already alluded to, concerns age ranges and study duration; we have capitalized the important terms, for clarity. An FTP occurs when the sample contains both an Offspring, and a Full-Sibling of the Offspring's Mother (or Father; the same argument would apply). Since the Mother must have been mature when the Offspring was born, and since Full-siblings are mostly or exclusively from the same cohort, the Full-sibling must also be mature; FTPs must be born about 10 years or more apart. Not many of the comparisons we actually use involve animals born that far apart, in the first place. Also, our ring-count restriction excludes the great majority of adults from our comparisons; and only a small proportion of Offspring will come from Mothers in the youngest age-classes of adults; so FTPs must be pretty rare.

There is a minor leak in that argument, because it is occasionally possible for an immature Full-sibling to be sampled (lethally) and yet for the Mother to reach maturity and be able to give birth to a sampled Offspring. This could happen through sperm-storage (if it occurs), whereby there will be a few immature Full-siblings who are one pupping-interval younger than a Mother who is just mature. Also, if the sample collection covered enough years, then an immature Full-sibling could be sampled early on while the Mother from the same cohort goes on to survive and produce an Offspring that gets sampled late in the study. Our samples were collected across just a couple of years, and the great majority are age 3 or greater, so this is not an issue yet. However, there would be some opportunity for FTPs to occur in a longer study, e.g. if more samples are collected in future.

That first argument is entirely adequate to deal with school sharks (at least with the samples we have now), but as a matter of general interest for CKMR, there is also a second argument based on general demographics for species that do not pair-bond or repeat-mate-with-same-partner. It is not easy to put this succinctly into words— pictures or equations might be clearer— but the gist is as follows. In a long-term equilibrium population, and making unconditional comparisons across many years, there are twice as many thiatic pairs (TPs) as sibling pairs (SPs) (either full or maternal; the point is that they share a mother): each Offspring has two grandmothers that each might be the mother of some other animal (yielding a TP), but only one mother that could also be someone else's mother (yielding an SP). Separately, the proportion of TPs that are FTP rather than HTP is driven by the proportion of breeding events that are same-cohort, because FTPs stem originally from full-siblings which come almost entirely from the same cohort, whereas HTPs stem originally from half-siblings which are mostly cross-cohort. If there are many breeding events during the lifespan of an average adult, FSPs will be uncommon compared to HSPs, and thus FTPs will be uncommon relative to HTPs. Overall, the ratio of FTPs to HSPs is

$$\text{(fraction of SPs that are FSP)} \times \text{(ratio of TPs to SPs)} \times \text{(fraction of SPs that are HSP)} = \text{(uncommon)} \times 2 \times \text{(close to 1)}$$

which is still "uncommon". This argument is diminished if the lucky-litter effect is large and/or if the number of expected breeding events per lifetime is small, since both lead to an increase in the overall ratio of FSPs to HSPs.

While FTPs certainly would be a non-ignorable complication for some species— e.g. pair-bonding birds, and octopuses with just one large litter per lifetime— they are not yet of importance to school shark CKMR. If we do get more school shark samples in future, we could adjust our CKMR model accordingly: the true FTP probability can be calculated demographically using similar expected relative reproductive output (ERRO) principles to our other kinship probability calculations, and added to the true HSP probability, to form the probability of the genetically-detectable kinship category "HSP-or-FTP". We have done a similar thing in other CKMR projects to allow for Grandparent/Grandchild Pairs (GGPs), which are also indistinguishable from HSPs (note that GGPs are not an issue for our school shark analysis, both because of restricting the age-range to juveniles, and because sampling a juvenile prevents it from going to breed). It is certainly possible to do that, but it also involves a lot of extra effort and will not affect analysis of the current dataset, so there is no point doing so until it becomes necessary.

Is there any substance to this? No, not yet. If the study expands in future to cover more years, we would adjust the CKMR model to allow for the possibility.

Implications for management advice? None

A.8 HSP probabilities

8. Possibly incorrect calculation of the probabilities of MHSPs and PHSPs (perhaps forgetting to subtract the probability of an FSP from the probabilities of having the same mother/father)

No subtraction is necessary. The probability of a sibling being a half- versus a full-sibling is modelled using a (nuisance) parameter (ν_2) which dictates expected proportion of every litter that consists of (maternal) half-siblings (different fathers) as opposed to full-siblings (same father). Note that the parameter does not appear in equations for cross-cohort half-siblings because repeat mating is improbable (see above). However, if we need to allow for sperm-storage in a future version of our model, there will need to be some minor modifications here— probably another nuisance parameter. On p15 of his report Dr Cordue writes *"It may be that they have dealt with this in the way they have calculated the conditional probabilities but it is not clear from the documentation"*. We certainly try to explain it, several times: in the parameter list of Table 4.8 on p49; on p90, where we wrote "ν_2 *Proportion of the litter that are likely to have different fathers (i.e. that are half, rather than full, siblings)*". Equation C.17 on the same page is even more explicit: *"The probability that i and j are full-siblings is expressed in terms of the shared mother"*; and this is followed by the probability equation for full-siblings, which involves the term $1 - \nu_2$ in contrast with equation C.16, the probability calculation for same-cohort half-siblings, which involves just ν_2.

Is there any substance to this? No

Implications for assessment? None

A.9 Bernoulli trials

9. Incorrect calculation of the likelihood by using independent Bernoulli trials for each close kin relationship for a given pair of sharks (instead of a single multinomial trial)

As Dr Cordue notes on p16: *"This [treating the comparisons as independent Bernoulli trials] is technically incorrect because two pairs of sharks that have one shark in common do not form independent trials. However, it is a perfectly adequate approximation given the relatively large population size (so that although not strictly independent, such pairs are almost independent)."* In that, he is correct. Again, the explanation can be found

in Bravington *et al* (2016b) (section 4.3) on "sparse sampling" of large populations, where n refers to the sample size and N to the adult population size:

As to approximate independence: the set of pairwise comparisons cannot be fully independent, because one outcome can sometimes predict another. If j's mother is already found, she cannot be found again. However, a heuristic justification can be made provided sampling is sparse enough for expected kin-triads (within which the pairwise comparisons are clearly not independent) to be rare compared to kin-pairs. If $n \propto O(N^{1/2})$*, as per the previous paragraph, then the ratio of triads to pairs is* $O_p(N^{-1/2})$*, so sparse sampling [amounts to N being large enough. (In longer-term studies with substantial turnover of adults, total n can presumably be larger, because triads will remain rare.) The key point is that only a small proportion of samples will be involved in any kin-pair at all. For most comparisons between one sample i and another j, there will be little predictive power even from knowing the outcomes of all other comparisons involving i and j, since all those outcomes will usually just be Unrelated, and thus largely uninformative about i's relationship to j. In other words, under sparse sampling the expected marginal information from a comparison is similar to its expected conditional information given all other comparisons, so approximate independence is reasonable.*

The point is really that, with a high enough adult abundance (and school shark certainly qualifies), only a small proportion of the population needs to be sampled to find enough kin-pairs to fit a statistical model; and then only a small proportion of the sample will actually be involved in any kin-pairs at all. Our main model uses about 3000 samples and finds about 100 pairs, i.e. 200 animals; so only 7% of our sample are involved in a pair. Therefore, we might expect of the order of 7% of our pairs to overlap with each other; and indeed, we discovered eight triads (Section 4.5.5 of the draft report). Comparisons within those triads are clearly not statistically independent, but make up only a small proportion of the total statistical (Fisher) information. This justifies both the use of independent Bernoulli trials that repeatedly use the same animals (last paragraph of p16 of Dr Cordue's report) as well as our decision to ignore the fact that a pair found to be a POP cannot also be an HSP (penultimate paragraph). By treating all the pairwise comparisons as independent, we get a computationally tractable framework, a very good approximation to variance— and, importantly, we do not incur any bias (section 4.1 of Bravington *et al* 2016b).

Is there any substance to this? No

Implications for assessment? None

A.10 Catches pre-2000

10. Inability to account for known catch history before 2000.

This phenomenon has been evident for many years with CPUE-based analyses of school shark; it is not restricted to CKMR, as we noted in our draft report (Chapter 5, Discussion). Punt *et al* (2001) encountered the same problem in marrying CPUE with catch data for school shark in southern Australia. Like him, we hypothesised that there were two or more stocks of school sharks, at least one of which is no longer present but that was present in the past when catches were high; and we referenced DEWR (2008), describing the degradation of Victorian pupping grounds.

The direct information from our CKMR analyses is on the absolute abundance and trend of those adults that gave birth to the juveniles in the fishery (i.e. the samples), over the years when those juveniles were born— basically 2005–2015. It is hard to see how the estimates could give a tremendously biased picture of the currently important adult population— see our discussion of potential issues in our draft report, and in this response (including Appendix B, which discusses whether our current estimates could be susceptible to any issues from stock structure within the remaining population; in all, serious bias seems unlikely. Our abundance estimates are consistent across the simplest approximate "one-line" calculation, through a more sophisticated but still approximate off-the-shelf statistical model (a GLM), right to a full-strength (and less approximate) stock assessment model— so coding errors are unlikely. Overall it really does seem as though something fundamental has changed about the biology/distribution/population structure of school shark in southern Australia since the 1990s— and the extirpation of former pupping grounds seems a plausible explanation.

Trend: Irrespective of CKMR, there is no question that, even if there were other abundant stocks of school shark in the past, the current population/stock was severely depleted by fishing prior to the year 2000. While the CKMR data suggest that there may have been some increase since, the trend is still far from statistically significant. However, if more data is collected in future and a significant upward trend is confirmed (so that management is demonstrably working), then it would presumably become appropriate to increase TACs somehow. But deciding how much of an increase will surely beg another question: what would constitute "recovery"?

Level: i.e. biomass reference point. If some of the historical stocks have been severely depleted, it may never be possible within any reasonable human timeframe for the entire southern Australian population of school shark to recover to AFMA's usual target proportion of the original mature stock biomass (B_0), even in the complete absence of fishing. Recovery to some fraction of current carrying capacity might on the other hand be possible (and even perhaps demonstrable), but would not satisfy current management targets.

Is there any substance to this? No. This isn't a problem with CKMR— it's an issue for school shark in general, and our results simply confirm something that was already suspected from previous CPUE-based assessments, and for which there is a plausible biological mechanism.

Implications for assessment? None. As we noted in our draft report, there are substantial implications for management; see above.

A.11 Pupping interval

(11.) Not explicitly modelling a pupping interval and therefore over-estimating the probability of maternal HSPs when ages are one year different

The large ageing error, which is accounted for in the model, obviates the need to specify a three yearly cycle when probability calculations are estimated. Individual sharks are not tracked, so that it would be computationally onerous to split the female population into three components, that each pup in separate three yearly cycles. Such a split is unlikely to alter the result of the model, even if ageing were perfect, and with ageing as imperfect as it is, is clearly not warranted.

There is no need to explicitly model a pupping interval, mainly because enough cohorts are involved that the "skip-spawning" effect cancels out. Comment (11) omits the counterpart: our model also under-estimates by a factor of 3 the true MHSP probability in comparisons whenever the true birth-interval is 3 or 6 or 9 etc years, because only 1/3 of the female population is pupping each year. This basically cancels the over-estimation in comparisons where the birth-interval is not a multiple of 3 years. The cancellation is not completely exact in a short study because, across all pairwise comparisons, the number that are truly a multiple of three-years apart is unlikely to be exactly one-third of the total.

What's more, ageing error for school sharks means that the true gap is unknown for any given pair. Thus the appropriate probability to use for any given pair must be averaged out across the possible true gaps for that pair, covering a range either side of the gap estimated from the pair's ring-counts. This further smears out any difference in exact probabilities between the ideal skip-spawning version and the non-skip-spawning approximation.

We met a similar situation with white sharks and even with SBT, where the CKMR data itself shows that young adults tend to pup only every other year (using POPs not HSPs in that case). For the main abundance-estimation model, we did not bother moving to an explicit skip-spawning formulation because the overall numbers of odd- and even-gap comparisons were fairly similar (juvenile age is known almost exactly, and adult year-of-capture is certain, so the estimated gaps are accurate for SBT).

In our school shark model, we do allow for the general effect of fecundity-at-age, and for uncertainty about age, and for "lucky litter" effects, but for the reasons above we do not explicitly allow for skip-spawning. While it is possible to develop CKMR models that do explicitly model "pupping intervals", the code and the details become more complicated, and there is little point unless dealing with a situation where the number of cohorts sampled is small compared to the average gap; an extreme example would be a short-term study

of leatherback turtles, where the inter-birth interval is about 8 years. CKMR studies should if possible be designed to avoid that necessity, and so we did with this one.

That said, if we expand our school shark model in future to allow for possible sperm-storage, it will become necessary to allow explicitly for pupping intervals, because we will need to estimate extra parameter(s) to deal specifically with sperm-storage across a gap of exactly 3 years.

Is there any substance to this? No

Implications for assessment? None

A.12 Litter size

(12.) Ignoring variability in surviving litters sizes (except for estimating a lucky litter effect)

This is wrong on two counts— we don't ignore variability, and/but only certain aspects of variability actually matter. Systematic effects at the level of individual adults due to body-size/fecundity/growth are explicit in our probability calculations; and random variability in litter size does matter for within-cohort comparisons (the lucky litter effect), but not for between-cohort comparisons, which are where all the CKMR information really comes from.

The key points are explained clearly in the underlying paper: Bravington *et al* (2016b), section 3.2, equations (3.8) and (3.9). We recap the points here, but the published equations are unambiguous and may actually be easier to follow.

Litter size variability certainly does matter in CKMR, especially in HSP-based studies like ours. There are two components to variability: systematic variation, whereby one adult is likely to have more or fewer offspring than another because of individual factors (e.g. big female teleosts make more eggs); and random variation, whereby some litters just happen to be larger than others and/or have higher survival rates, by chance. When calculating CKMR probabilities, we deal explicitly with the former, by incorporating the known size/fecundity and growth relationships for female school sharks, using whatever adult age distribution arises from the population dynamics inside the model.

The remaining issue concerns random variation. There are two quite different situations to consider: between- and within-cohort comparisons. For simplicity, this explanation assumes there are no systematic individual effects, no adult mortality between cohorts, and merges FSPs with MHSPs (our actual model of course deals with all of those). Suppose there are N adult females, the expected fecundity (surviving litter size) of each mother in a single cohort of juveniles is r. Consider a single adult female (we'll call her Lucy) - the actual number of offspring produced by Lucy in the ith cohort is $R^{[i]}_{\text{Lucy}}$, for i=1,2,3.

1. Comparisons across cohorts. Lucy actually produces $R^{[1]}_{\text{Lucy}} \times R^{[2]}_{\text{Lucy}}$ distinct sibling pairs. The expected number of "her" pairs, i.e. $\mathbb{E}\left[R^{[1]}_{\text{Lucy}} R^{[2]}_{\text{Lucy}}\right]$, is exactly r^2, because the litter sizes are by definition statistically independent, so "the expectation of the product is the product of the expectations", statistically. Non-independence would violate the "no systematic Lucy effect" assumption; any such effects need to be accounted for separately. Technically, a 3-year pupping interval would also violate non-independence, with positive and negative correlations between the R's according as the interval is a multiple of 3. However, in fact the average number of all Lucy's sibling pairs across a reasonable span of years covering several pupping occasions still comes out correctly.

2. Comparisons within a cohort. This time Lucy actually produces $\left(R^{[1]}_{\text{Lucy}}\right)^2$ sibling pairs. The expected number of her pairs is now $r^2 + \mathbb{V}[R]$, which is bigger than in the cross-cohort case unless litter size is constant (aside from adult mortality effects etc). (This skates over the slight complication of double-counting in same-cohort comparisons, but that is just a book-keeping detail.)

Thus the random variability of litter size has a *systematic* effect on the number of siblings *within* cohorts, but not between cohorts, which is why a lucky-litter effect is required for the former but not the latter. The litter sizes for school shark are high enough for lucky-litter effects to be possible, so the *"limited scope for high*

variability" (p16 of Dr Cordue's report) is not right; for example, Hillary *et al* (2018) found an appreciable lucky-litter effect even for white sharks (average litter size around 10).

The Appendix of Dr Cordue's report fails to break down the probabilities into within- and between-cohort cases, which disguises the true nature of the term $\bar{p^2}$ on p21, and that presumably is what led to this comment. Mean litter size *per se* does not play a significant role in our model, although relative mean litter size as a function of female body size is important, and we account for that.

While on this general point, it is worth clarifying that we do make an important assumption about litter size variation: that, except via body size in females, there is no substantial persistent individual effect on surviving litter size. This assumption cannot be tested directly from the kin-pairs, so it is necessary to think about whether there are any biologically likely ways that it could be violated. One way would be if a substantial proportion of adults are biologically infertile— but we do not think that is likely for school shark. (And if it was, the infertility would somehow need to affect males as well as females at a similar rate, since we found the male and female adult estimates to be consistent, see Section 4.7.3 of our draft report, $q_father = 0.82$.) Infertility aside, there certainly are species where this assumption would be inappropriate— for example, terrestrial mammals that hold breeding territories, where a mother holding a "good" territory will persistently produce more surviving offspring than the norm. Again, that particular scenario is in our view implausible for school sharks, but could there be other mechanisms? There is only one that seems even remotely plausible: stock structure (within the remaining post-2000 population) where the stocks are differentially vulnerable to the fishery, presumably due to different spatial distributions. Appendix B deals with the stock-structure issue at some length. In summary, if the effect was real and strong, we would expect to see it via spatial patterns in the HSPs—which we do not see (Figure 3 and associated text); and if the effect is weak, there is unlikely to be much bias.

Is there any substance to this? No

Implications for assessment? None

A.13 "One-line" calculation

(13. from p16 of review report) *First, the probability of two sharks having the same mother is not the same as the probability of two sharks being a maternal HSP. Second, the conditional probability based on being "given" the older shark should not be used anyway.*

The first comment is irrelevant, and the second (although hard to follow) is either a misunderstanding or incorrect. For our "one-line calculation", we only used (in terms of calculating abundance) cross-cohort comparisons, where full-siblings are rare at best, so that HSPs are the only relevant kinship type. We did that to avoid the genuine complications of lucky-litter effects and multiple-paternity, and our selection criterion was to look only at pairs where the apparent birth-years are at least 4 years apart.

As to the second comment: neglecting pupping intervals and other minor complications, if two animals are born about Y years apart from a steady-state non-pair-bonding population, then the probability that the second-born shares a mother with the first-born is

$$\frac{\exp(-zY)}{N_{\text{♀}}}$$

where z is the annual adult mortality rate and $N_{\text{♀}}$ the abundance of adult females. Simply put: for the pair to be an MHSP, the first-born's mother has to survive the interval until the second animal is born, and if so she then has an equal probability with every other living female adult of being the mother of the second-born. (This equation is derived formally in Bravington *et al* (2016b), where it appears as equation (3.10) for a special case of half-sibling probabilities.) In our one-liner, we are making many comparisons of this basic type, but the intervals Y differ between comparisons. Rather than deal with them individually and properly, we make the first-order approximation that the average of the $\exp(-zY)$ terms is about equal to $\exp\left(-z\bar{Y}\right)$ where $\bar{Y}$ is the average interval. This is crude, but it is definitely the correct type of calculation, based on an exact conditional probability that is then averaged, albeit approximately, across the conditions in our

sample. Dr Cordue's final comment on the one-liner, on p16, says: *"For this calculation the probability of two sharks selected at random being a maternal HSP should be used."* He does not define exactly what "at random" means; but in fact that is (up to approximation) exactly what we have calculated, assuming *"at random"* means "at random from the samples that were collected". Note that the adult mortality adjustment is very important in all calculations of HSP probabilities; we are not sure whether or not Dr Cordue has correctly applied it in his own calculations (e.g. it does not feature in his Appendix).

Our one-line calculation (section 4.6.1 of our draft report), and the simple GLM that follows it (section 4.6.2), are not meant to be exact— they are meant to be as simple as possible, at the inevitable cost of being quite approximate. There are two motives for including them. The first is to illustrate "basically" where the signal is coming from in CKMR estimation, in order to help a reader build some confidence in the principles with as few technical complications as possible. The second is as a rough check on our own code, to provide reassurance that there are no serious mistakes within the unavoidably complicated black box machinery of our full CKMR-based stock assessment i.e. as a "laugh test", the estimates from the three different models should at least be reasonably similar, which they are. Given that the laugh test is passed, we definitely recommend the estimates from the full-complexity CKMR-based assessment, rather than from the simpler models.

Is there any substance to this? No

Implications for assessment? None

B "Population structure", random mating, and HSP-based CKMR

The "random mating" comment comes up several times in Dr Cordue's report. Partly it is related to the incidence of repeat-breeding and ageing error, but there may also be some suspicions about the general implications for CKMR of possible "mating groups" or cryptic sub-populations. This rejoinder is a good opportunity to clarify that "cryptic population structure"— i.e. persistent mating groups— is not on its own a source of bias in a school-shark-like CKMR setting, even if such groups do exist. The reasoning is most clearly explained using some algebra.

Suppose there is an overall adult female population of size N which is actually split into two separate mating groups of size N_1 and N_2, where $N_1 + N_2 = N$. For simplicity, we omit all the real-life complications about multiple cohorts and lucky litters and selectivity and mortality and size/fecundity relationship and full-siblings, so as to concentrate on the group-structure aspect. Juveniles from both groups are mixed randomly in the fishery. If two juveniles are sampled entirely at random from the fishery, what is the chance they share a mother, i.e. that they are an MHSP?

Each of the juveniles can come from (i.e. have its mother in) either group 1 with probability $\frac{N_1}{N_1+N_2}$, or group 2 with probability $\frac{N_2}{N_1+N_2}$. If they both come from group 1, the probability of MSHP is $1/N_1$; if they both come from group 2, the probability is $1/N_2$; if they come from different groups, the probability is 0. Since they are sampled independently of each other (and thus their groups are independent), the overall probability is

$$\begin{aligned}
&\mathbb{P}\left[\text{MHSP}\right] = \\
&\left(\frac{N_1}{N_1+N_2}\right)^2 \times \frac{1}{N_1} + 2\frac{N_1}{N_1+N_2}\frac{N_2}{N_1+N_2} \times 0 + \left(\frac{N_2}{N_1+N_2}\right)^2 \times \frac{1}{N_2} \\
&= \frac{N_1+N_2}{\left(N_1+N_2\right)^2} = \frac{1}{N_1+N_2} \\
&= \frac{1}{N} \qquad (1)
\end{aligned}$$

In other words, the true probability takes the same value as if it were calculated ignoring the possibility of mating groups (as we have done). This means that persistent mating groups do not intrinsically lead to bias.

With some effort, though, it would still be possible to get a biased abundance estimate: specifically, if animals from the same mating group tend to stay together *and* then get sampled together. (This is different to the "lucky litter" effect.) Then the overall number of HSPs becomes inflated, because comparisons within one sampling occasion are not independent with respect to group membership, so that equation 1 no longer applies. This risk can be avoided simply by not doing the pairwise comparisons between animals that may have been sampled together, as we did for school shark (section 4.1.4 of our draft report).

B.1 More complicated situations

For the record, CKMR is not immune to "population structure in general"; applying a simple "no-structure" model can in certain circumstances lead to bias, although the circumstances need to be more complicated than a cryptic-sub-population/persistent-mating-group scenario, as just explained. There are so many possible "structure" cases that it is hard to give general principles but, in general, CKMR needs more care when there are multiple spawning sites *and* parents and/or very young juveniles are mostly sampled near those sites. Spatial variants of CKMR models can be devised in principle (section 3.1.5 of Bravington *et al* 2016b), but if possible it is better to avoid such modelling complexities by sampling differently. We investigated one such situation in a scoping study for the eastern stock of Atlantic Bluefin Tuna (Davies *et al* 2015). That particular situation of course does not apply to school shark anyway, which are highly mobile in the age range that is sampled, and where fishing (and sampling) is deliberately not allowed in nursery areas.

To illustrate the issues that *might* occur with a different type of "population structure", consider an extension of the previous two-mating-group case where juveniles from the two groups have different vulnerabilities to the fishery (and to sampling). This could only really arise if the two groups have different but partly-overlapping spatial ranges as juveniles *and* if the fishery does not cover both ranges. For definiteness, suppose that group 1 juveniles spend all their time in the fishery zone and are fully vulnerable to the fishery, but that juveniles from group 2 spend only a proportion α of their time there. (There are no other fisheries on group 1 animals, but group 2 animals may or may not also be caught somewhere else.) The probability that a sampled juvenile is from group 1 now changes, to become

$$\frac{N_1}{N_1 + \alpha N_2}$$

and there is a similar change for group 2. However, the probabilities of sibship *conditional* on group membership stay at $1/N_1$ and $1/N_2$ respectively, so that the overall probability is

$$\begin{aligned} &\mathbb{P}\left[\text{MHSP}\right] = \\ &\left(\frac{N_1}{N_1 + \alpha N_2}\right)^2 \times \frac{1}{N_1} + \left(\frac{\alpha N_2}{N_1 + \alpha N_2}\right)^2 \times \frac{1}{N_2} \\ &= \frac{N_1 + \alpha^2 N_2}{\left(N_1 + \alpha N_2\right)^2} \end{aligned} \tag{2}$$

which is not the same as eqn1 except in the extreme cases $\alpha = 1$ (equal vulnerability) and $\alpha = 0$ (no overlap at all).

The expected adult abundance estimate from "naive no-structure" CKMR is, in this *highly* simplified scenario, just $1/\mathbb{P}\left[\text{MHSP}\right]$, in other words:

$$\hat{N}_{\text{naive}} = \frac{\left(N_1 + \alpha N_2\right)^2}{N_1 + \alpha^2 N_2} \tag{3}$$

This is a complicated formula involving α; how does it relate to the "right" composite abundance? Actually, it is by no means clear what the "right" abundance really would be in this situation— it depends considerably on management goals and practices. However, there are a couple of obvious possibilities:

1. The total number of adults that *could* contribute juveniles to the fishery is $N_1 + N_2$. However, it is decidedly questionable whether group 2 adults are really "worth" as much to management as group 1 adults, especially if their proportional contribution of juveniles is small (i.e. if $\alpha N_2 \ll N_1$). The claim that "there is an additional invisible huge stock out there which contributes only a small proportion of itself but yet sustains the fishery" has been fairly widespread in the history of fisheries— but it has not stood the test of time very well!

2. Given perfect CPUE or abundance-index data, what would a "classical" stock assessment come up with? Essentially, this turns out to be the abundance of group 1, plus a fraction of the abundance in group 2. This seems a reasonable measure— the second group is not "worth" as much as the first, because it does not contribute as many juveniles per capita. It is certainly open to question whether it is appropriate to manage this kind of mixed fishery with a single naive assessment, but nevertheless it is probably frequent practice. As shown in Box B.1, depending on what other fisheries affect the second group, possible answers could include $N_1 + \alpha N_2$ or $\frac{(N_1+\alpha N_2)^2}{N_1+\alpha^2 N_2}$. The former is perhaps the most appealing; remarkably, the latter is exactly the same as the expected "naive no-structure" CKMR estimate, eqn 3.

Mixed-group stock assessments

If a standard age-structured stock assessment was applied naively (i.e. ignoring groups) to "perfect" CPUE or survey-index data from a mixed-group fishery where the two groups have different vulnerabilities, as per the main text: what would the expected abundance estimate be, in terms of the abundances of the two groups? To make things tractable, consider the naive assessment as a simple depletion analysis on a group of cohorts (whether juvenile or adult). There is then an index U (CPUE or survey index) which is proportional to abundance (of the mixed groups) with unknown catchability q. We measure $U^{[1]} = qN^{[1]}$ at one moment, remove a known catch C from $N^{[1]}$ to leave $N^{[2]}$ animals, then measure $U^{[2]}$. The change $\Delta U = U^{[1]} - U^{[2]}$ should satisfy $\Delta U = qN - q(N - C) = qC$, so we estimate $\hat{q} = \Delta U/C$, and then estimate $N^{[1]}$ via $\hat{N} = U^{[1]}/\hat{q}$. The mixed-group reality, however, is that the index depends on the fishable abundance $N_1 + \alpha N_2$, i.e. $U^{[i]} = q\left(N_1^{[i]} + \alpha N_2^{[i]}\right)$; so the task is to express $\hat{N}$ in terms of the group-specific N's and α, the relative vulnerability of group 2 compared to group 1.
The calculation depends on how much fishing mortality is imposed on group 2 elsewhere (from fisheries which do not catch group 1 animals).

B.1.1 Group 2 experiences similar fishing mortality in the other part of its range

The ratio N_1/N_2 does not change, and so removing C causes a proportional change in $N_1 + \alpha N_2$; thus the naive standard assessment will lead to an abundance estimate of $\hat{N}_{\text{ass1}} = N_1 + \alpha N_2$.

B.1.2 Group 2 unfished elsewhere

The catch C removes $C\frac{N_1}{N_1+\alpha N_2}$ animals from group 1, and $C\frac{\alpha N_2}{N_1+\alpha N_2}$ from group 2. The index will be

$$\begin{aligned} U^{[2]} &= q\left(N_1 - C\frac{N_1}{N_1 + \alpha N_2} + \alpha\left(N_2 - C\frac{\alpha N_2}{N_1 + \alpha N_2}\right)\right) \\ &= q\left(N_1 + \alpha N_2\right) - qC\frac{N_1 + \alpha^2 N_2}{N_1 + \alpha N_2} \end{aligned}$$

Thus the naive standard assessment will lead to $\hat{q} = q \times \frac{N_1+\alpha^2 N_2}{N_1+\alpha N_2}$, and

$$\begin{aligned} \hat{N}_{\text{ass2}} &= \frac{U^{[1]}}{\hat{q}} \\ &= \frac{\left(N_1 + \alpha N_2\right)^2}{N_1 + \alpha^2 N_2} \end{aligned}$$

It is of course also possible that group 2 experiences *higher* fishing mortality in its "other" fishery. In that case, though, it becomes very difficult to see the naive standard assessment as relevant to management.

So how would a no-structure CKMR estimate compare to these three? It is easy to prove mathematically (see Box) that the expected naive CKMR estimate is always lower than the first (total), higher than the "standard stock assessment" estimate if group 2 is fished equally hard elsewhere, and exactly equal to "standard stock assessment" if group 2 is unfished elsewhere. In other words, although the naive CKMR estimate tends to underestimate absolute total abundance of the combined stock, it tends to *overestimate* compared to arguably the most useful measure of "adult abundance". Table 2 shows percentage bias of the naive CKMR estimate, relative to those three possible definitions, assuming 50% vulnerability for group 2 juveniles. Biases are modest compared to the total $N_1 + N_2$; for the weighted combination, bias is modest until group 2 is making up 50% or more of the juveniles (rows 4 and 5).

N_2/N_1	Ppn. of group 2 in catch	N_1+N_2	$\hat{N}_{\text{ass1}}$	$\hat{N}_{\text{ass2}}$
0.1	5%	-2	2	0
0.5	20%	-7	11	0
1	33%	-10	20	0
2	50%	-11	33	0
10	83%	-6	71	0

Table 2: Percent bias in abundance estimate from "naive no-structure" CKMR, compared to three possible definitions of "adult abundance". A value of 0 means no bias. N_1+N_2 is the total abundance of both populations; $\hat{N}_{\text{ass}}$ are two different possibilities for what a standard stock assessment might give, as per Box B.1. Rows are relative stock size of the second stock compared to the first. Vulnerability of stock 2 is 50% of stock 1's throughout.

Directions of bias when applying naive no-structure CKMR to groups with unequal vulnerability

Notation follows the main text. Vulnerability is by convention lower in group 2, so $\alpha < 1$. Let ρ be the ratio N_2/N_1, so that $\rho > 1$ means that the less-vulnerable group is also less abundant, and for simplicity set $N_1 = 1$ (we are only interested in the direction of bias here). From eqn 3, the expected value of the naive CKMR estimate is

$$\bar{N}_{\text{naive}} = \frac{(1+\alpha\rho)^2}{1+\alpha^2\rho}$$

First, the absolute total abundance is $N_{1+2} \triangleq 1+\rho$. We have

$$\begin{aligned}
&(1-\alpha)^2 > 0 \\
\implies &1-2\alpha+\alpha^2 > 0 \\
\implies &1+\alpha^2 > 2\alpha \\
\implies &\left(1+\alpha^2\right)\rho > 2\alpha\rho \\
\implies &1+\left(1+\alpha^2\right)\rho+\alpha^2\rho^2 > 1+2\alpha\rho+\alpha^2\rho^2 \\
\implies &(1+\rho)\left(1+\alpha^2\rho\right) > (1+\alpha\rho)^2 \\
\implies &1+\rho > \frac{(1+\alpha\rho)^2}{1+\alpha^2\rho} \\
\implies &N_{1+2} > \bar{N}_{\text{naive}}
\end{aligned}$$

Second, the "ideal weighted" abundance (i.e. what a conventional stock assessment with perfect data might give, *if* group 2 is fished equally hard in the rest of its range) is $N_{\text{ass1}} \triangleq 1+\alpha\rho$. This time we have

$$\begin{aligned}
&\alpha < 1 \\
\implies &1+\alpha^2\rho < 1+\alpha\rho \\
\implies &(1+\alpha\rho)\left(1+\alpha^2\rho\right) < (1+\alpha\rho)^2 \\
\implies &1+\alpha\rho < \frac{(1+\alpha\rho)^2}{1+\alpha^2\rho} \\
\implies &N_{\text{ass1}} < \bar{N}_{\text{naive}}
\end{aligned}$$

Third, as already noted in eqn 3 and Box B.1, the naive CKMR abundance estimate has the same expectation as a naive conventional stock assessment might have.

The implications for CKMR-based trend estimates are less clear, since the two groups might have different rates of change over time— but the basic intuition remains solid: more contributing adults over time means lower proportions of HSPs in the sample.

As a final general comment, it is worth explaining the reason for "bias" in terms of the fundamental principles of CKMR as presented in BSA16 ("bias" in the sense that the most desirable abundance estimate, whatever it might be, is probably not eqn 3). The point is that "group membership" of an *adult* is a source of unmodelled and persistent (through her lifespan) difference in Expected Reproductive Output, as measured among "samplable" juveniles. Female adults belonging to group 2 are likely to generate systematically fewer *sampled* juveniles per capita than females in group 1, because the group 2 juveniles are per capita less likely to be caught. The CKMR formulation is only guaranteed to give unbiased estimates when there are no such important unmodelled persistent effects.

The above discussion is quite general, so it is time to bring it back school shark. The main points are as follows:

1. If a population consists of several distinct "persistent separate mating groups" which are fished equally, e.g. cryptic sub-populations that mix on the fishing grounds, then that does not lead to bias in a "naive" CKMR assessment which ignores stocks.

2. If the fishery exploits the main "persistent mating group" more heavily than another (e.g. if the second group spends some of its time outside the fished zone), then bias could arise from applying CKMR naively. It is not clear what the "right" answer is in such settings, but one reasonable reference might be a weighted sum which reflects the lower "input" from the second group. Under certain circumstances but not all, that is also the expected output from a standard single-stock assessment applied to good abundance-index data (which of course does not exist for school shark), though the standard assessment can also be biased upwards sometimes. Anyway, using that reference, the CKMR estimate is if anything likely to be biased somewhat *upwards,* though the bias does not seem particularly large provided the contribution of the second group to the fishable juveniles is not dominant.

3. How far might any of this apply to school shark? Certainly, there is historical evidence that (at least in the past) there were several distinct stocks along the southern Australian coast, with distinct pupping grounds. There may still be more than one stock contributing to the currently-fished "population" of juveniles, although it also seems likely that some of the stocks have collapsed completely (see response to Dr Cordue's point 10). Juvenile school shark move *a lot*, though, so even if they are pupped in different places, their spatial distributions over their growing years (and therefore their vulnerability to being sampled) could overlap entirely— in which case there is no bias. If there is substantial systematic mismatch in spatial distribution between offspring from different groups, then there could be some bias, probably upwards— but then we would expect to see HSPs clustered spatially, which we don't (see Figure 3 and associated text). If there is another large and almost-completely-separate stock that occasionally contributes a few juveniles to the fishery, then CKMR will certainly underestimate the *combined* total adult abundance from both stocks— but that would presumably not be sensible for management purposes, in any case.

4. Overall, the potential for problematic bias from "persistent mating group structure" seems fairly low for this species— which is not to say that stock structure can *always* be ignored in CKMR settings.