Close Kin Mark Recapture for School Shark in the SESSF

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Contents

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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
GGP	Grandparent grandchild 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); sufficient for close kin modelling. Our model estimates a School Shark stock in the region of 50,000 mature individuals during 2000. Although the coefficient of variation (CV) for our abundance estimate ranges from 0.23 to 0.28 over 2000 to 2011 (most precise in 2002-2003, at 0.23) 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, a downward trend 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 close kin model correctly interpreted the close kin data. Our estimate of abundance is three to four times lower than that of the most recent 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 stock, and that the population that we measured is likely to be 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 not 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 provides 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 the 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 (i.e. added to) 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 example, seasonal movement details, unless the latter effect 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 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, Section 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 CPUE certainly is for School Shark, as already explained. Length frequency data is equally problematic because of seasonal and spatial variation in the size of available animals, and the relatively uninformative nature of length data taken by highly selective gillnet gears. 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 (see Methods, Section 3, for further explanation).

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 rely on virgin biomass, 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 (although roughly half were later eliminated from the study, as discussed below).

School Shark have been seen to move from every one of sharkRAG's shark zones to every adjacent 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 (of 2,000 sharks) 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.

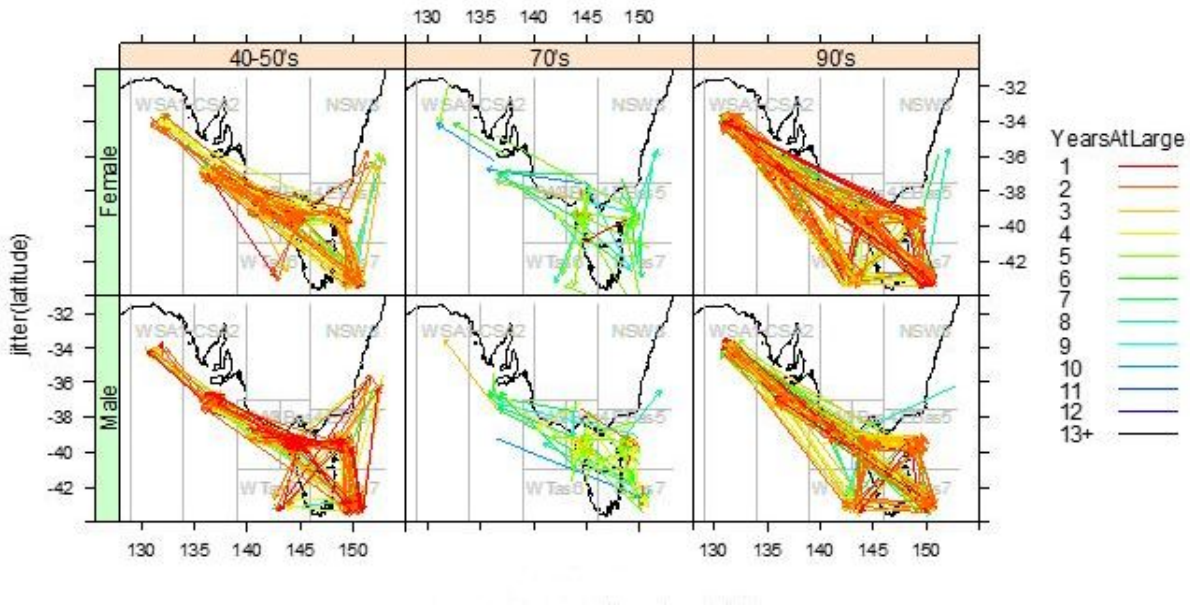

Figure 3.1: Movements of School Shark from conventional tag and recapture data, organised by decade of release. Arrow colour indicates number of years at large. Arrows begin at release point and end at recapture point (arrow head).

In addition to the commercially caught sharks, we sourced tissue samples 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 Section 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

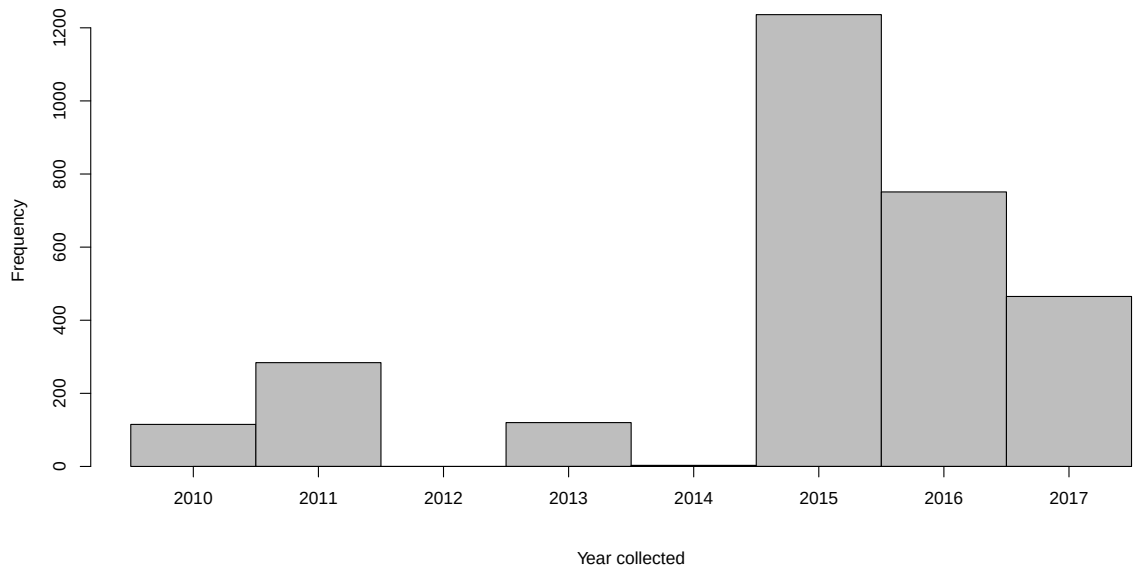

Figure 3.2: Numbers of School Shark samples included in close kin study, by year. Pre-2015 samples were collected by AFMA observers.

this 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 (compared to teleosts): 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 (but still very simple) 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 sections.

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 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 exceeded the collection targets in two locations, the exception being 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).

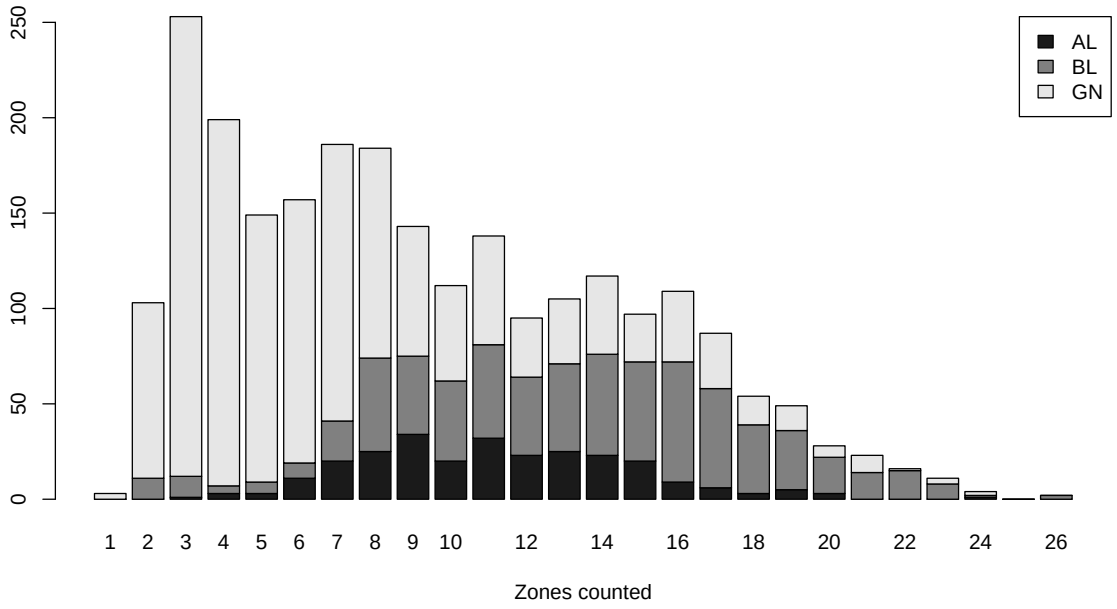

Figure 4.1: Frequency distribution of 'rings' (zones) counted in the vertebrae of the 2,438 animals used for kin finding. Samples were collected from auto-longline (AL), bottom line (BL), or gillnet (GN) fishing vessels.

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 ('Count1') and second ('Count2') counts, as well as between individual readers (using the method of Punt *et al.* (2008)).

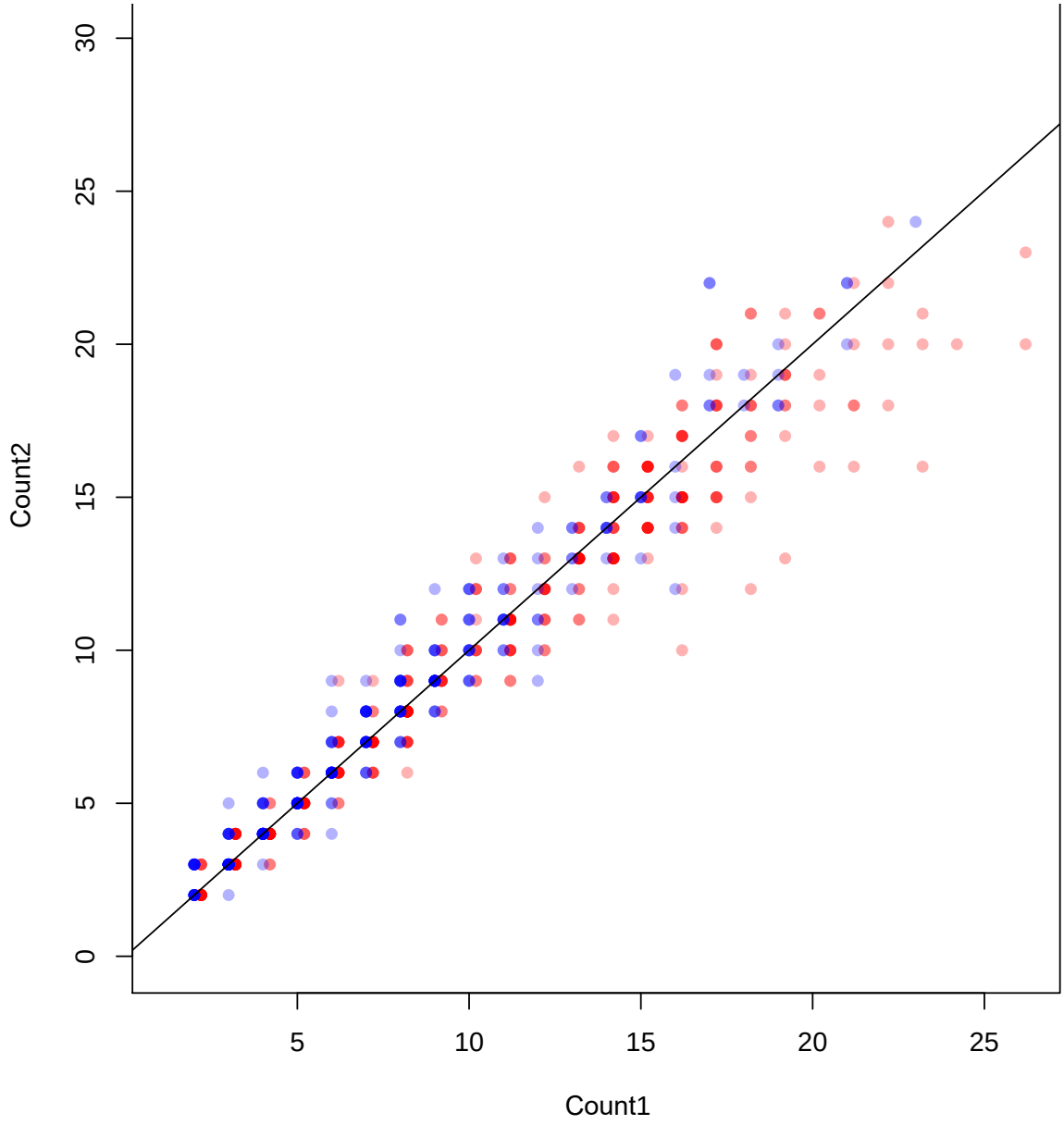

Figure 4.2: Number of vertebral zones ('rings') counted during a first (Count1) and second (Count2) reading of a random selection of School Shark vertebrae. Counts for males sharks (blue dots) are offset slightly so that they do not overlie those for female sharks (red dots). Darker dots indicate more overlaid individuals of the same sex. Note that these are not true ages but deposition zone ('ring') counts.

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

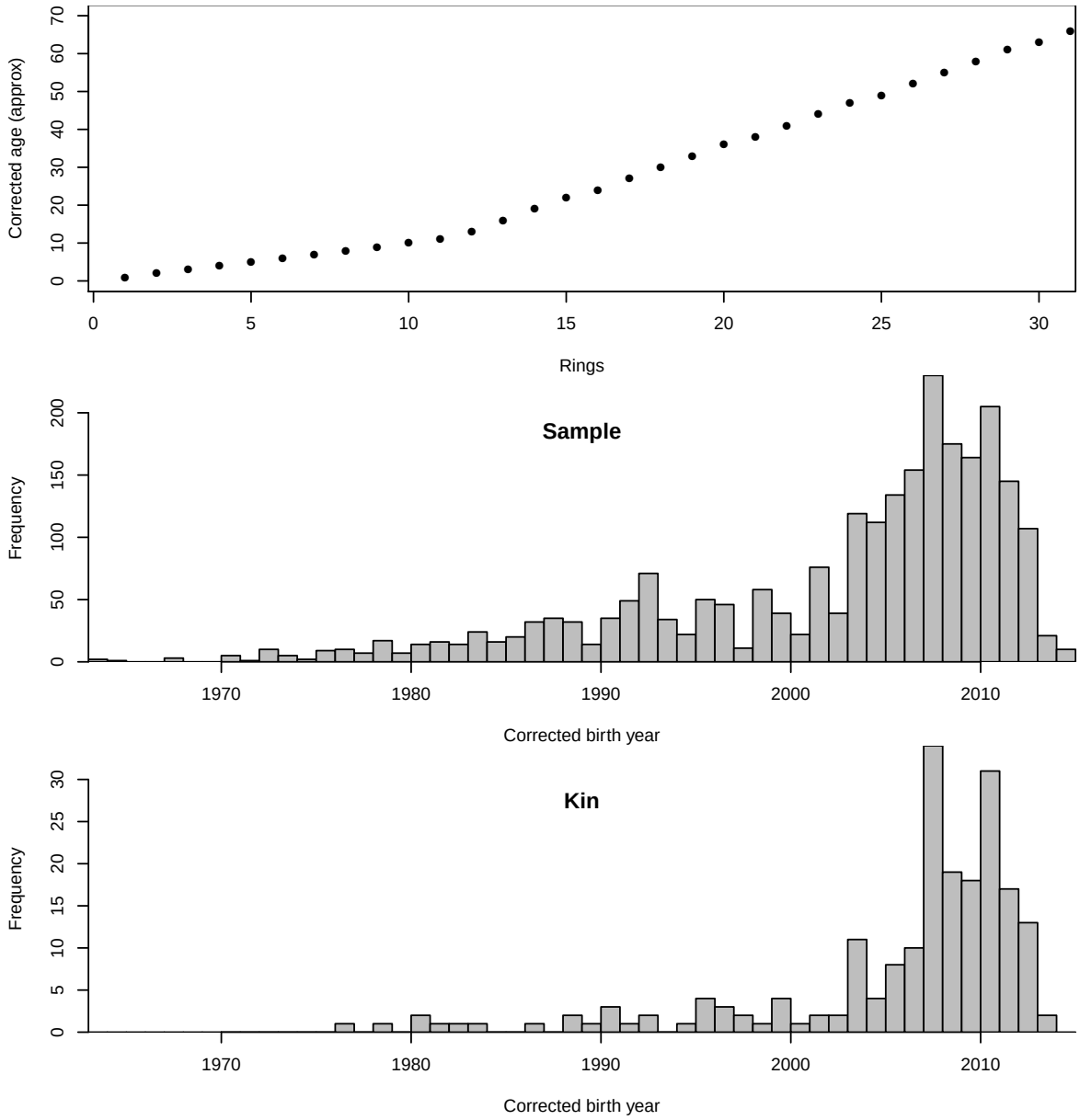

Figure 4.3: Median true age (*Corrected age*) given number of rings counted (upper plot); histogram of inferred birth years for all samples collected (middle plot) and for all animals found in kin pairs (lower plot).

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 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'.

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).

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 to construct an alternative catch time series that did not use 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).

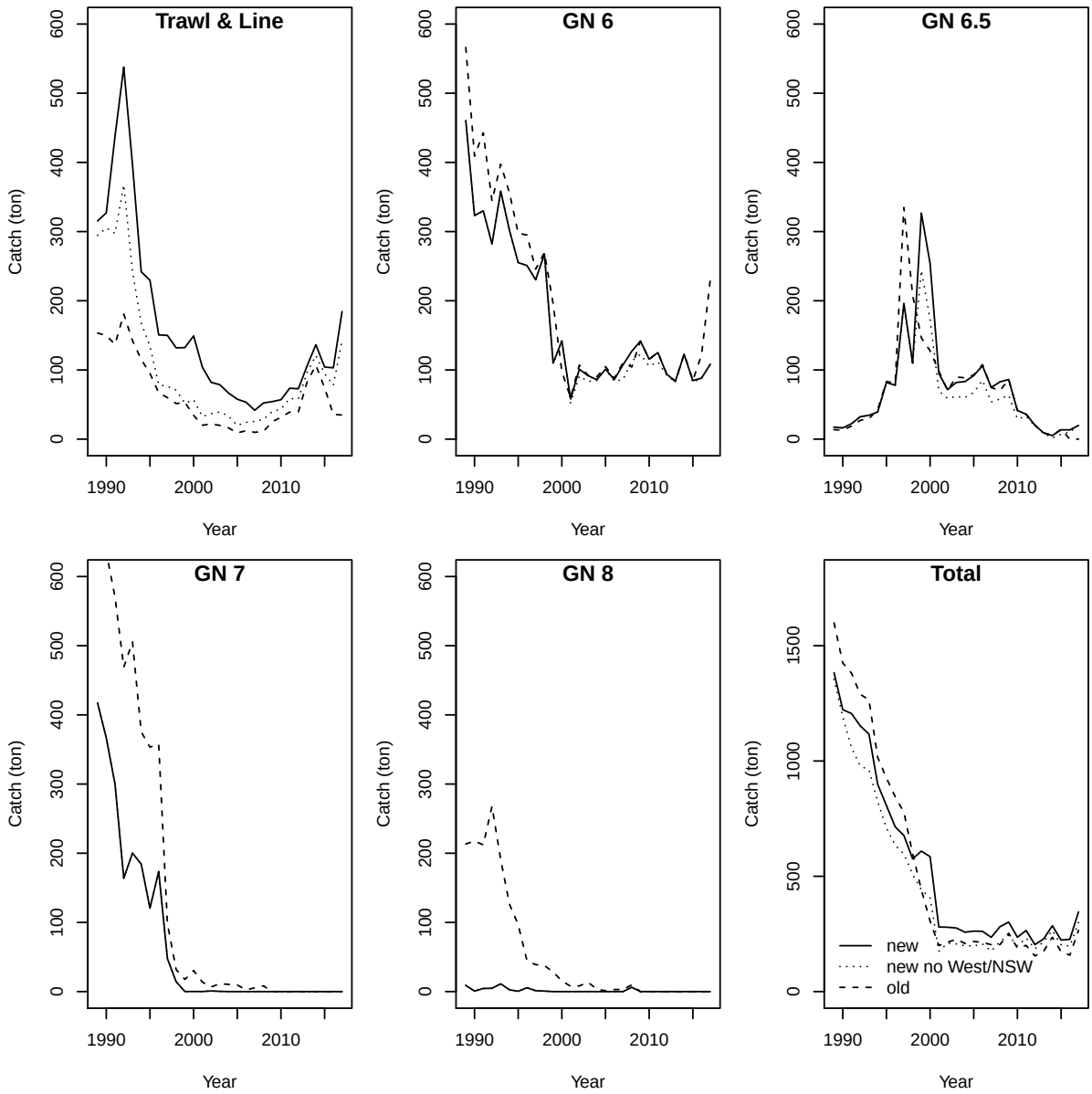

Figure 4.4: Landed catches in tonnes by trawl and line gears, and 6 (GN6), 6.5 (GN6.5), 7 (GN7) and 8 (GN8) inch gillnets as well as total catches for the original time series (*old*) and the new time series using data for all regions (*new*) or excluding WSA, WA and NSW (*new no West/NSW).*

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). All discarded sharks are assumed to be dead.

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 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.

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 use in the stock assessment using the method of Kirk-

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%

wood & 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 then 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 sequencing methodology used in our study might have (but largely did not) show differences. This work has been published (Devloo-Delva *et al.*, 2019) and 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

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)

part, these paired chromosomes (one derived from the father and one from the mother) have near identical sequences, but 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 (Section 3), 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. 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. This was estimated by summing over the product of the proportional haplotype frequencies for the animals in each pair.

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 same assumption was made for SBT, which has similarly high diversity of haplotypes.

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.

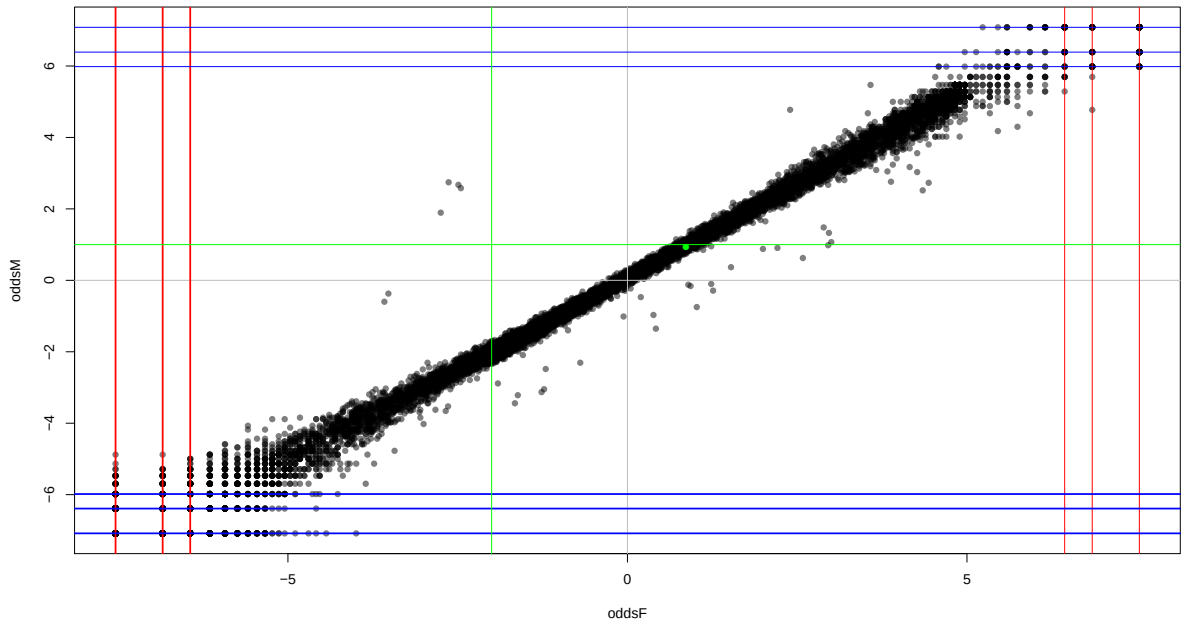

Figure 4.5: The odds ratio of having a particular genetic allele (grey dots) for male vs female School Sharks. The red and blue lines show the odds corresponding with only 1,2, or 3 (thick lines) or all but 1, 2, or 3 (thin lines) animals having a particular allele – these would reveal spurious results 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.

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 individuals have a genetic 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 sampled animals was examined 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. 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, and was able to separate FSPs from POPs for SBT and grey nurse sharks, but has not worked for School Shark; more powerful versions are currently under development. School Shark mature at 9 to 11 years old so separation of FSPs from POPs is easy based on the age gap between individuals in each pair; this is because 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 of little use for 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 are expected) 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 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 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 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 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

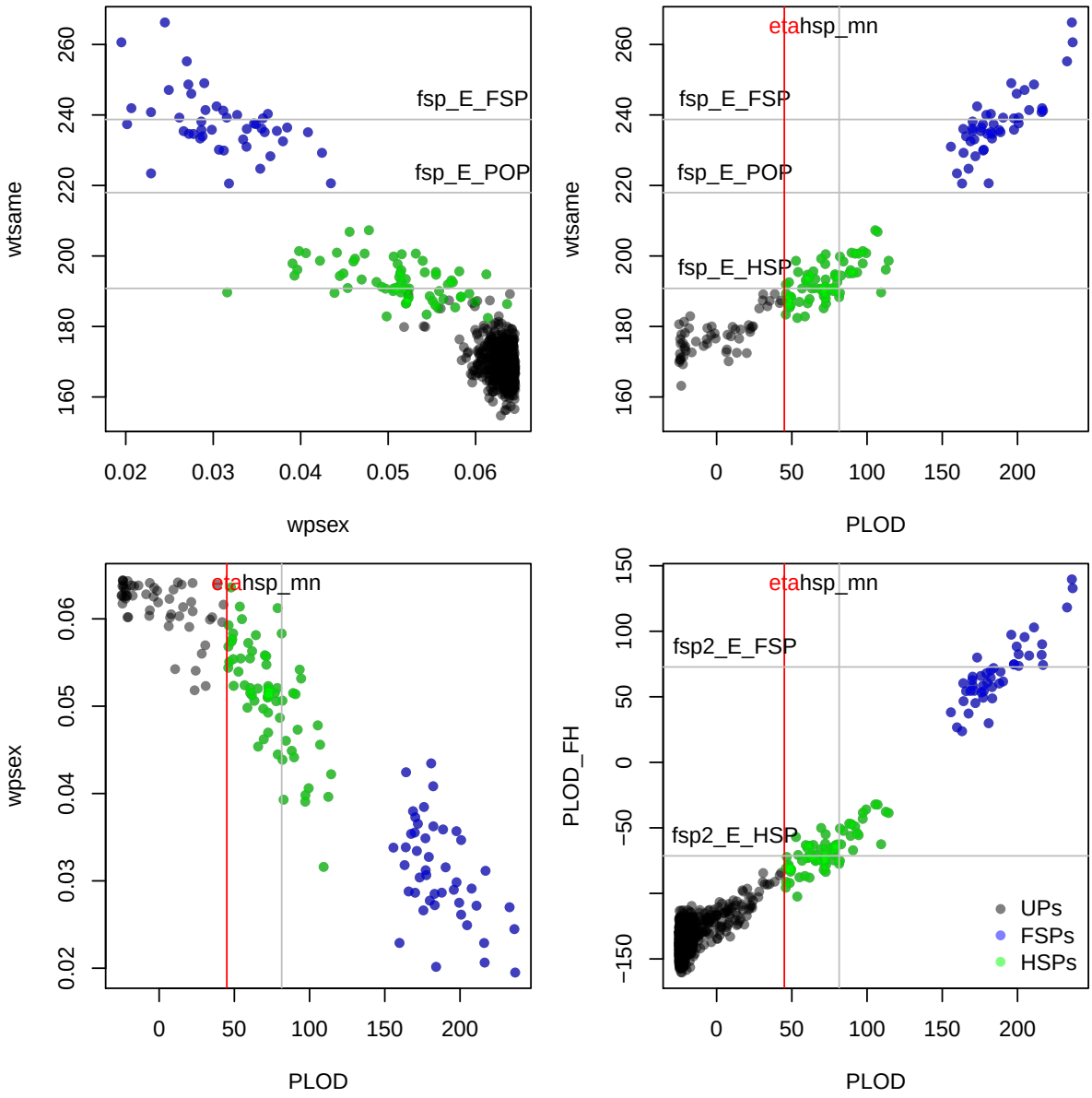

Figure 4.6: Scatter plots showing three statistics for determining relatedness 'wtsame' is optimised for finding FSPs, 'wpsex' for POPs, and 'PLOD' for HSPs. Each dot represents a pair of animals. For clarity, the majority of unrelated (and less related) pairs are not shown. Theoretical mean values for 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).

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

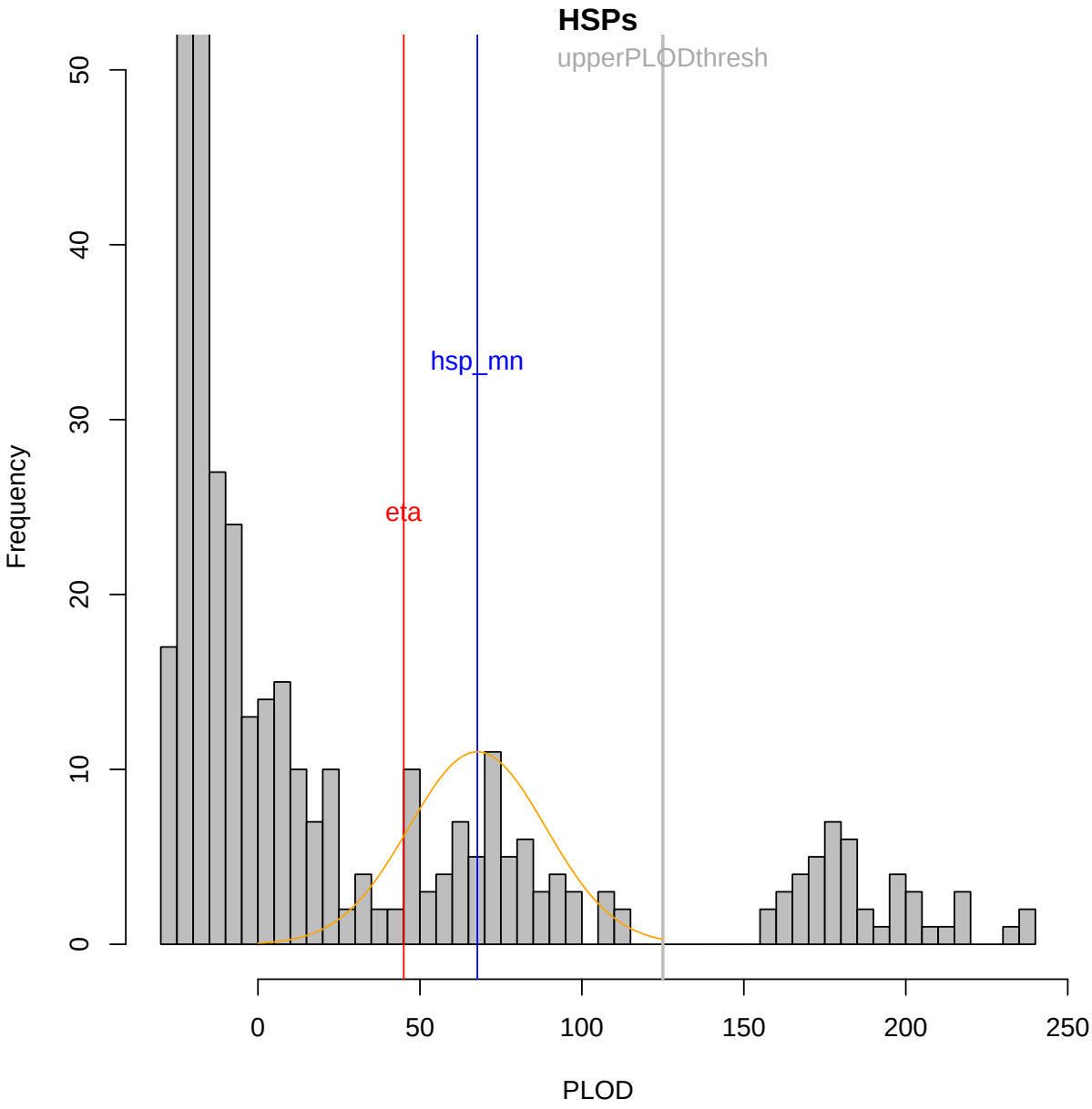

Figure 4.7: Histogram showing the PLOD statistic for more closely related pairs; 'eta' (red line) is the threshold value chosen to separate unambiguous HSPs from the mix of UPs and HSPs (PLOD<eta); 'hsp_mn' (blue line) is the theoretical mean PLOD value for HSPs. An approximation to the theoretical distribution 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.

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.

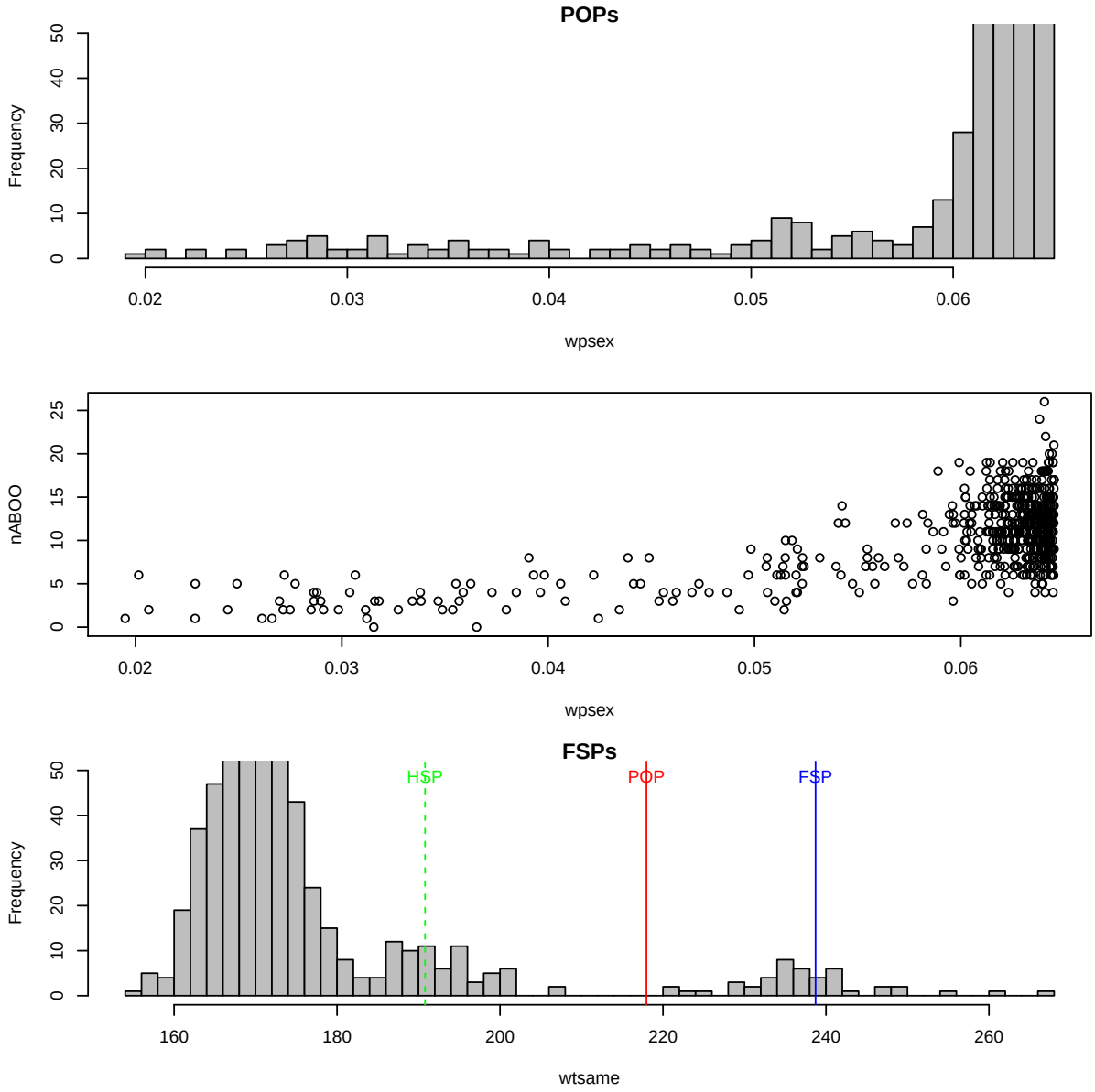

Figure 4.8: The statistic 'wpsex' is shown for the more closely related pairs as a histogram (upper plot) and as a scatterplot (middle plot); 'wpsex' is optimised for finding POPs. The 'wtsame' statistic (optimised for finding FSPs) is shown along with the theoretical values for HSPs (green line), POPs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

Among the younger HSPs, there are more black dots (MHSPs) at a 3-year gap than at 0, 1, or 2 year gaps, consistent with 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

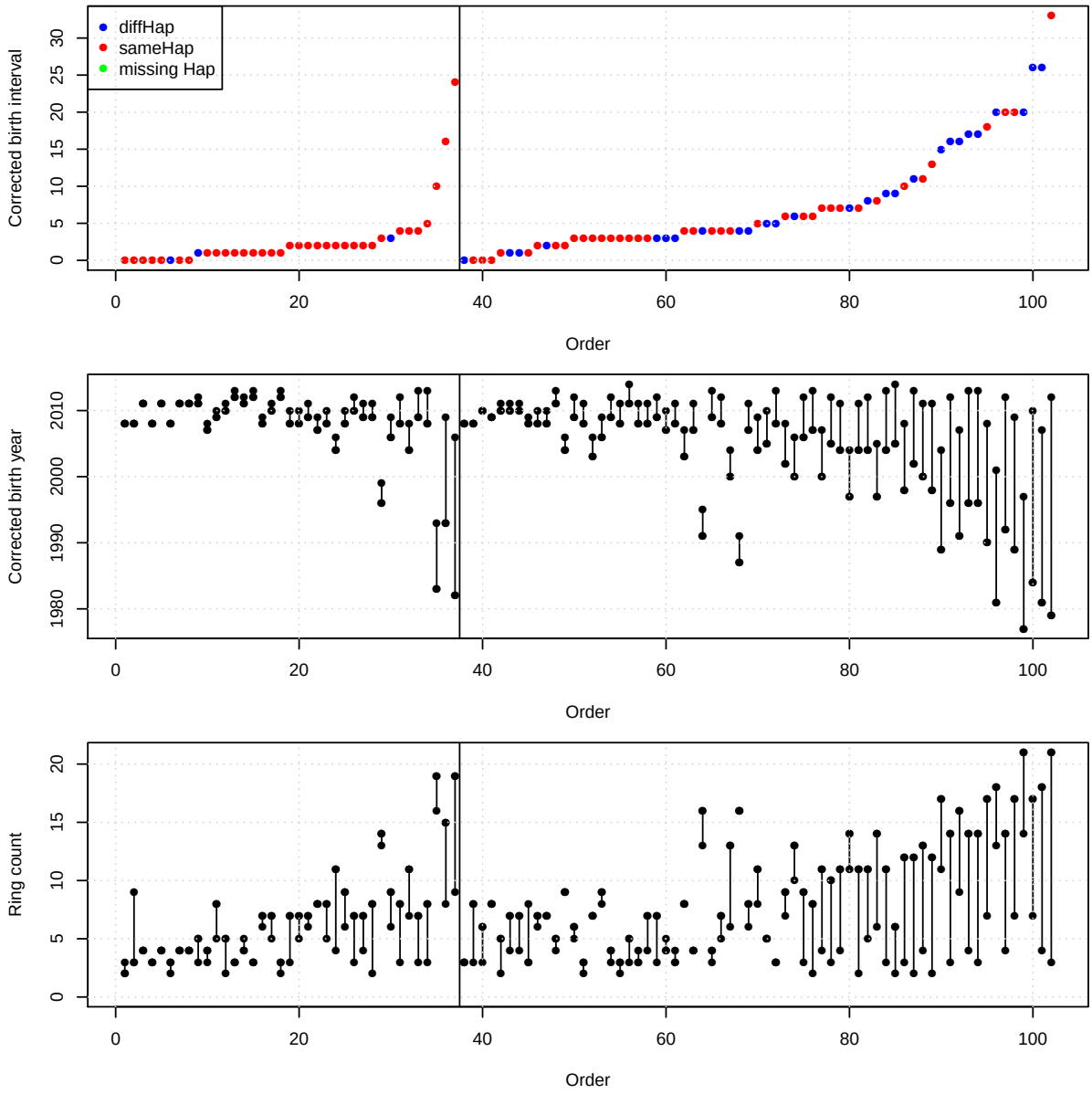

Figure 4.9: Gaps between estimated birth years of each kin pair (corrected for ring deposition rate for those over 11yo), sorted by increasing size (upper plot). FSPs and POPs are shown on the left of the vertical black line; HSPs on the right. Those with the same mtDNA haplotype are shown as red dots,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

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).

The mitochondrial DNA haplotype indicates whether siblings share a mother (matching haplotypes), or a father (non-matching haplotypes). In all of the family groups that we found, the haplotypes and observed kin types were consistent; 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), which 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 almost 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 be addressed in future research.

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

Table 4.5: Eight 'families' of 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. A blank denotes a single animal.

	508	1654
508		**FSP**
29	HSP	HSP

	1609	1738
1609		**HSP**
394	UP	HSP

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

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

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

	1591	2219
1591		**FSP**
472	HSP	HSP

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

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

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

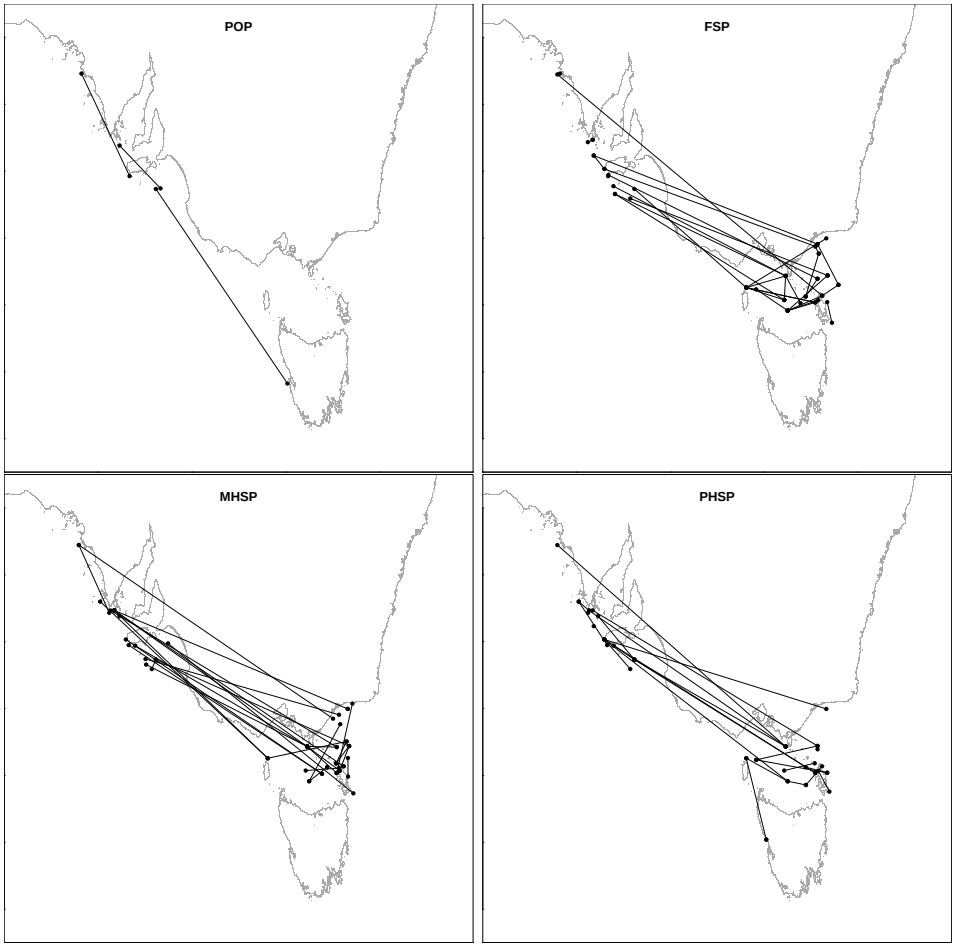

Figure 4.10: Approximate collection locations of the animals found to be parent offspring pairs (POP), full sibling pairs (FSP), maternal half sibling pairs (MHSP), or paternal half sibling pairs (MHSP).

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 4.12) 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
WSA		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**

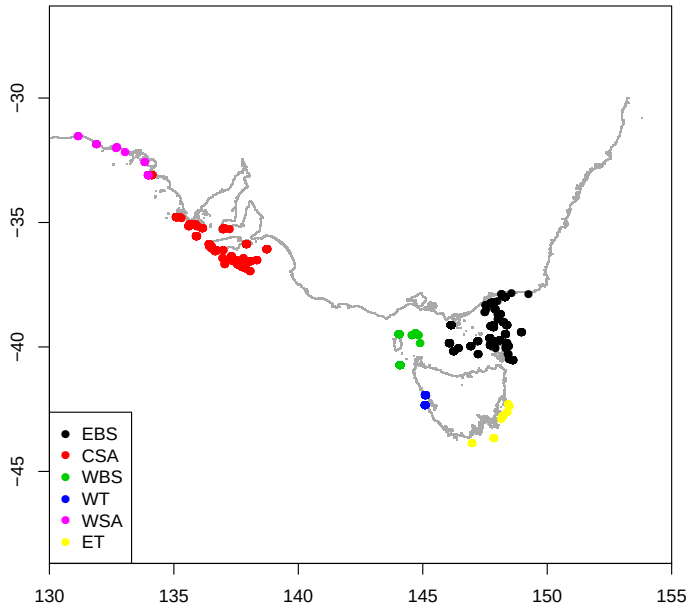

Figure 4.11: Average location of all fishing trips sampled, coloured by shark zone: western South Australia (WSA), central South Australia (CSA), western Bass Strait (WBS), western Tasmania (WT), eastern Tasmania (ET), and eastern Bass Strait (EBS).

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).

2. mtDNA data reinforces the HSP-finding conclusion and reveals substantially more MH-

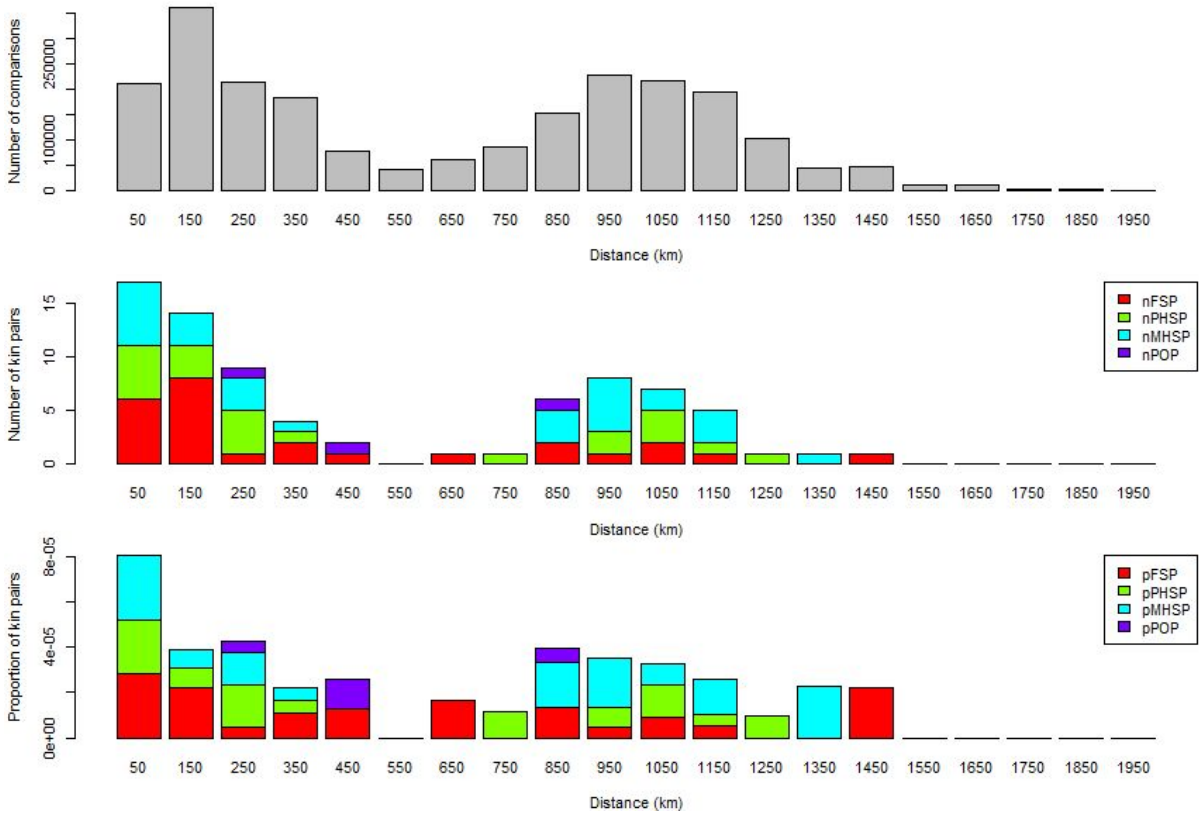

Figure 4.12: Histograms showing the distance (km) between average capture location for (upper plot) every possible pairing of animals sampled; (middle plot) close kin pairs; and (lower plot) the proportion of comparisons that yielded kin pairs.

SPs (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:

1. that all adults of a given sex are equal in terms of fecundity (this is not far from the truth, which is that for female School Shark it changes from roughly 20 to 30 pups per

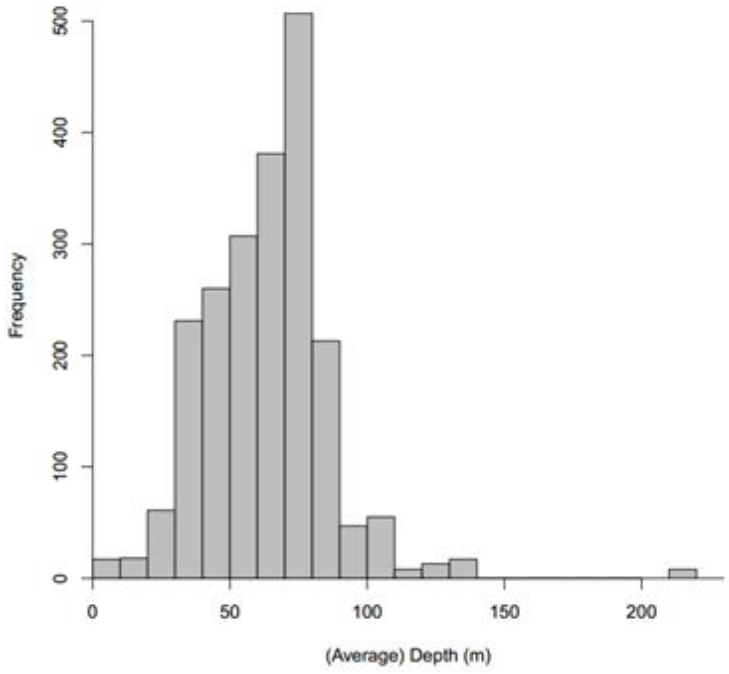

Figure 4.13: Histogram showing the depth of capture for all animals used in the close kin study.

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 (i.e. fishing and natural) 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 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

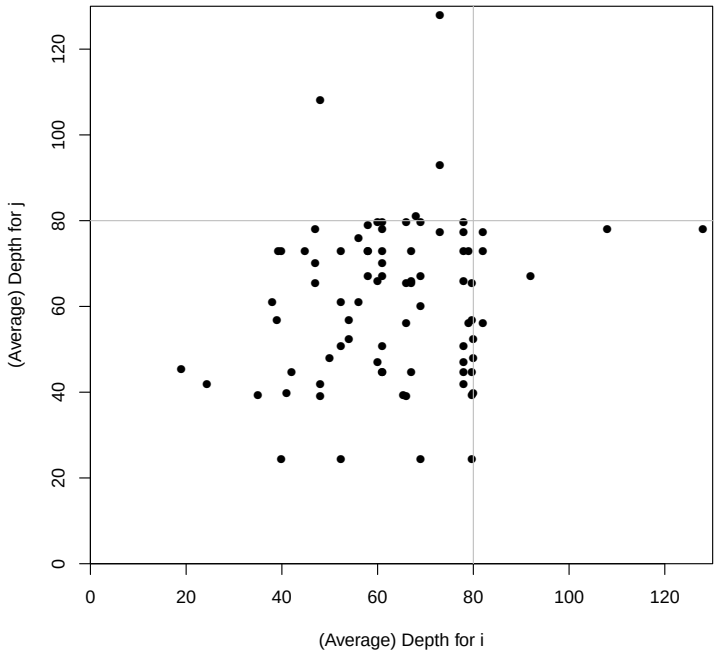

Figure 4.14: Capture depth for animals (named i and j) found to be kin pairs. Each pair is plotted only once, as a single black dot. The average depth for the fishing trip in which the animal was caught is shown (in meters).

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 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

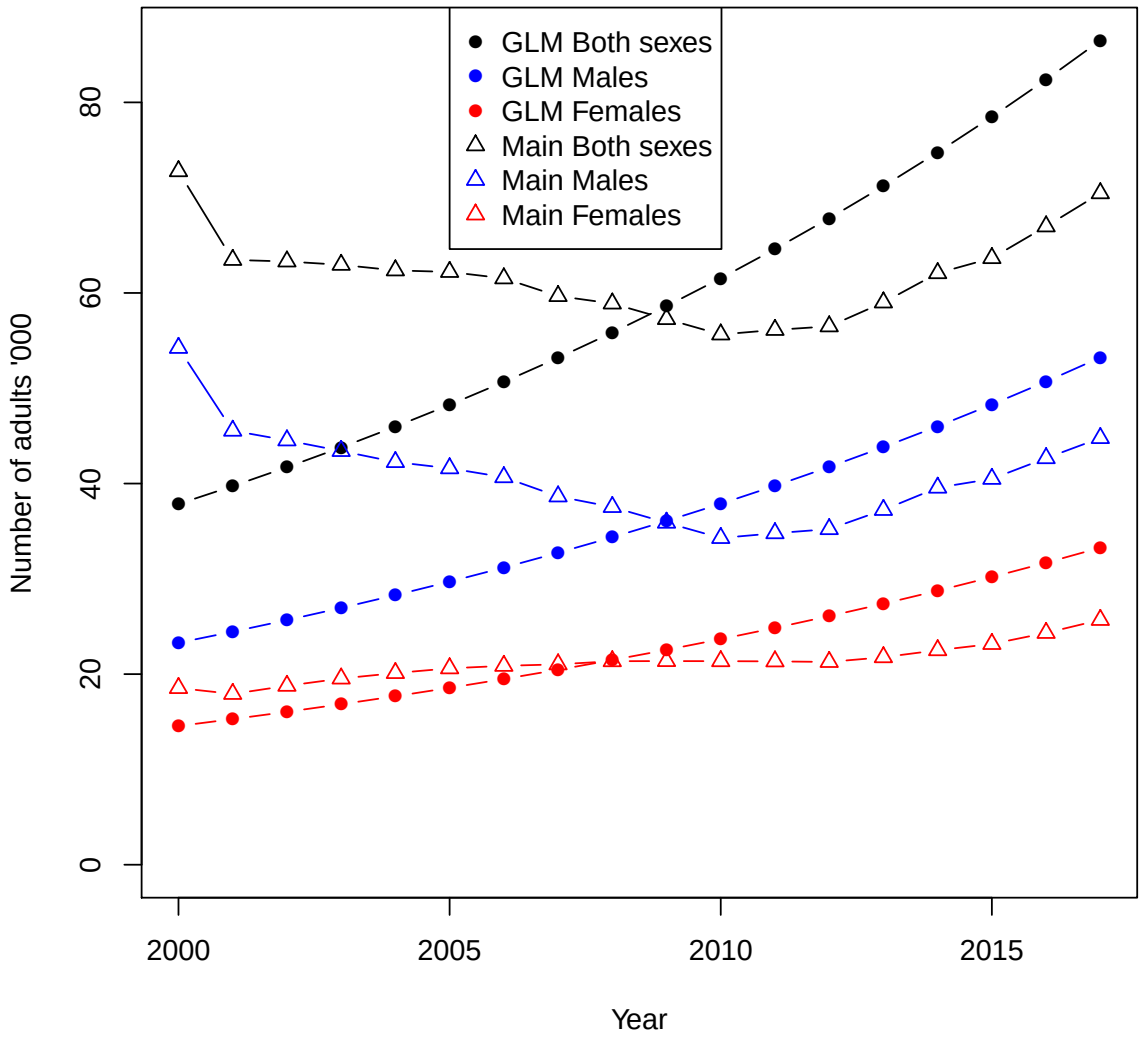

Figure 4.15: Estimated numbers of total (black), female (red) and male (blue) adult School Shark from a simple GLM model (closed circles) and the base case close kin model (open triangles, *Main*).

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 model 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 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 suggest adult abundance of the order of 50,000 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 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 kin 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 dependence 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 ageing was unbiased: 11 rings or less. We placed a "plus group" at age 20, since 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 analysis to ignoring them, and also present a model that uses close kin pairs that involve fathers (i.e. FOPs and PHSPs) to estimate the 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 test 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 (alternatives to this knife-edged form will be explored in future work). 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 L_{inf} 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.

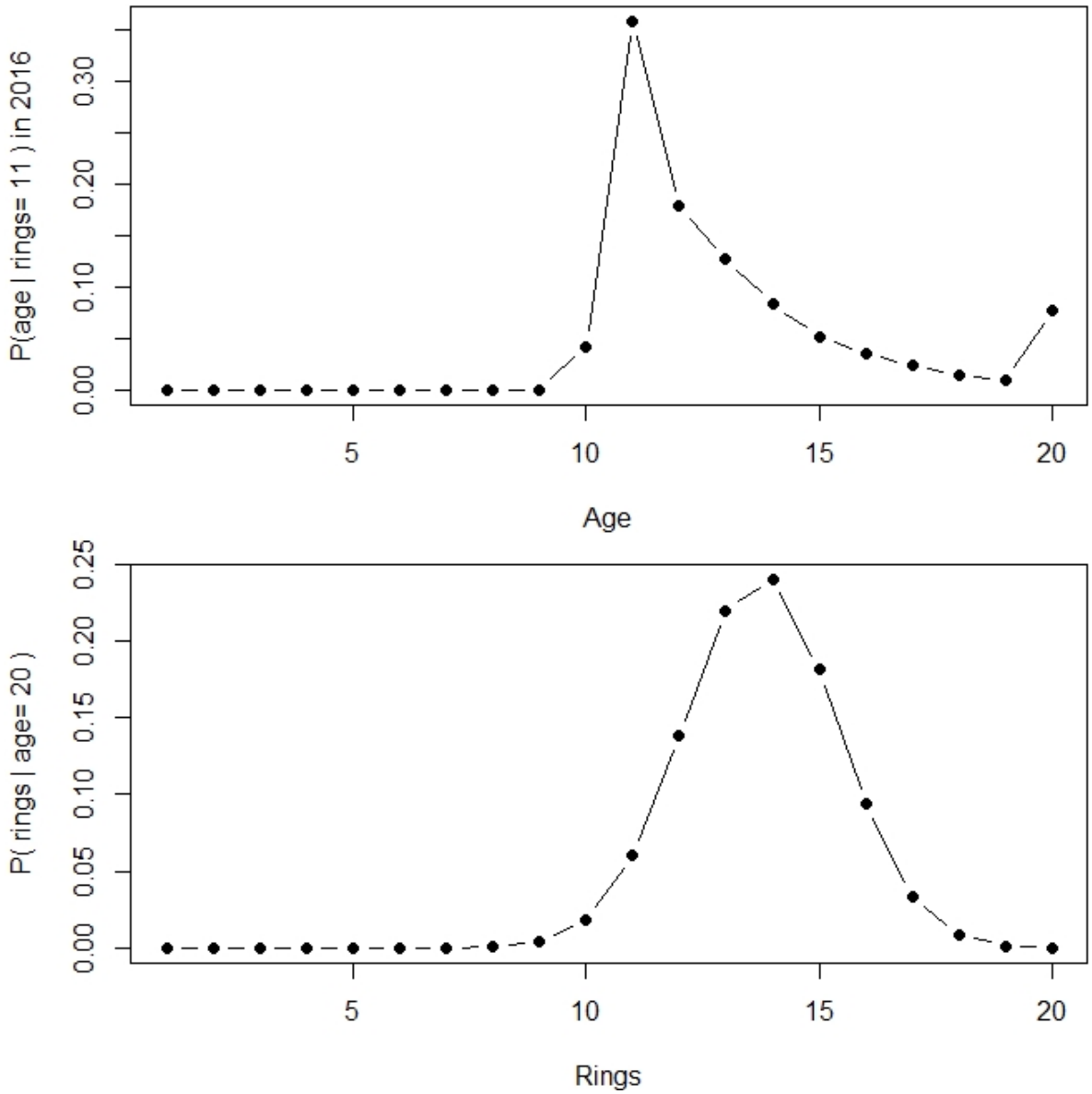

Figure 4.16: The probability distribution of true ages for an animal that has 11 vertebral rings (upper plot) and the probability distribution for the number of rings that an animal aged 20 (the youngest plus group age) will be observed to have in 2016.

Table 4.7: Coefficient of variation (CV) assumed by the School Shark stock assessment model by age group and sex (Punt *et al.*, 2000; Punt, 2001).

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

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 stock might have been relatively small during the 1990s, when catches were high and had been high for decades, so that density dependent juvenile survival might have resulted in a model that could better accommodate those high catches. Catches were steadily reduced from a peak of close to 3,000t during 1985-1987, which could have allowed slow population recovery since that 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.

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 share a father (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)

Two alternative formulations were explored. One uses the model shown here, but with pup 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 in 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). For interest, we have also plotted the results of the simple approach that allowed for natural mortality ("Simple", Section 4.6).

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

Table 4.9: Estimated parameters, negative log likelihood (*-lnL*) and estimated numbers of M/FOPs (*nM/FOP*), FSPs (*nFSP*) and M/PHSPs (*nM/PHSP*) for a range of models. 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

(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 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; 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).

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 (although

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.*, 2018a); 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.*, 2018a) 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 taken.

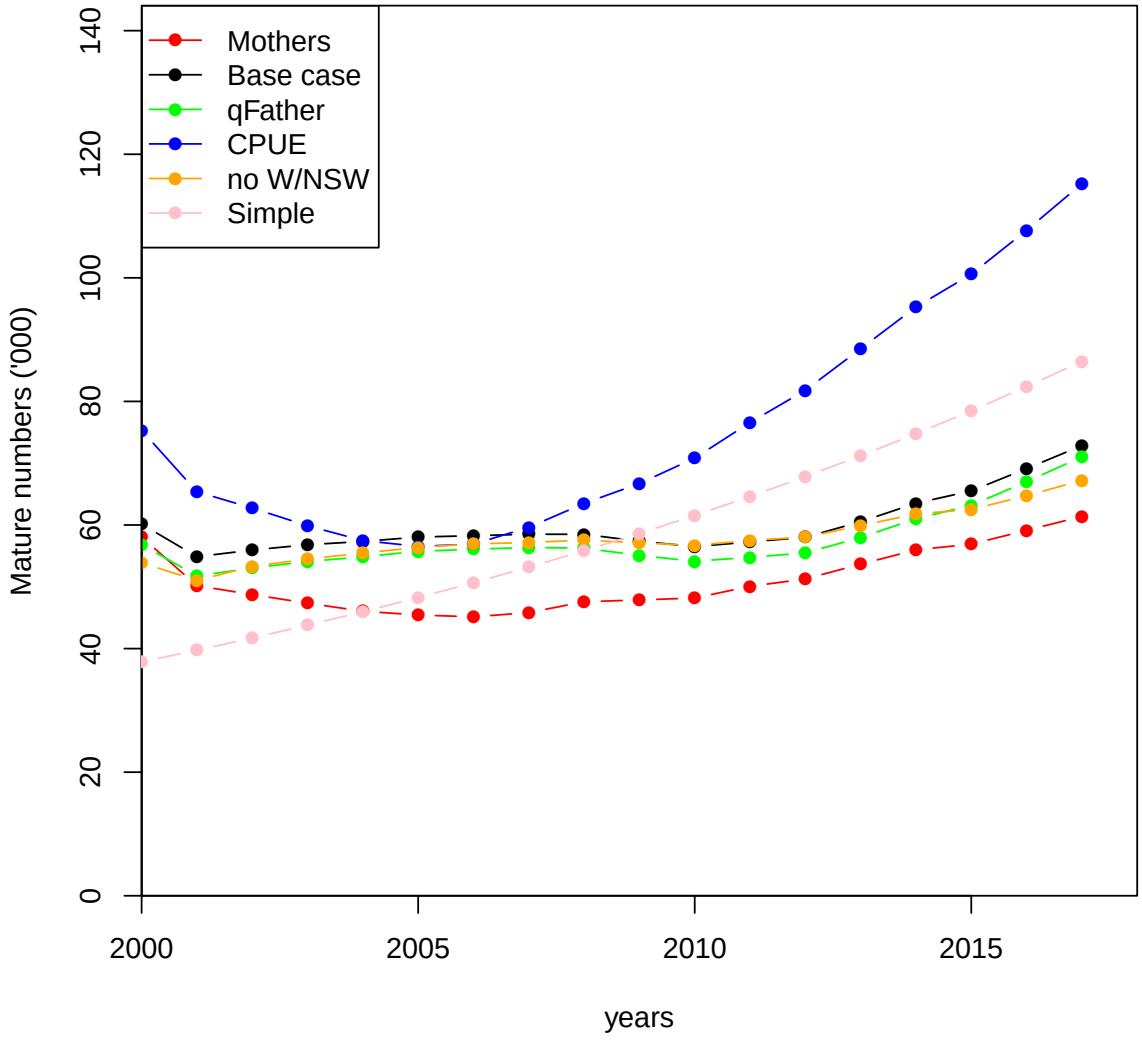

Figure 4.17: Numbers (thousands) of mature School Sharks for the range of models shown in Table 4.9.

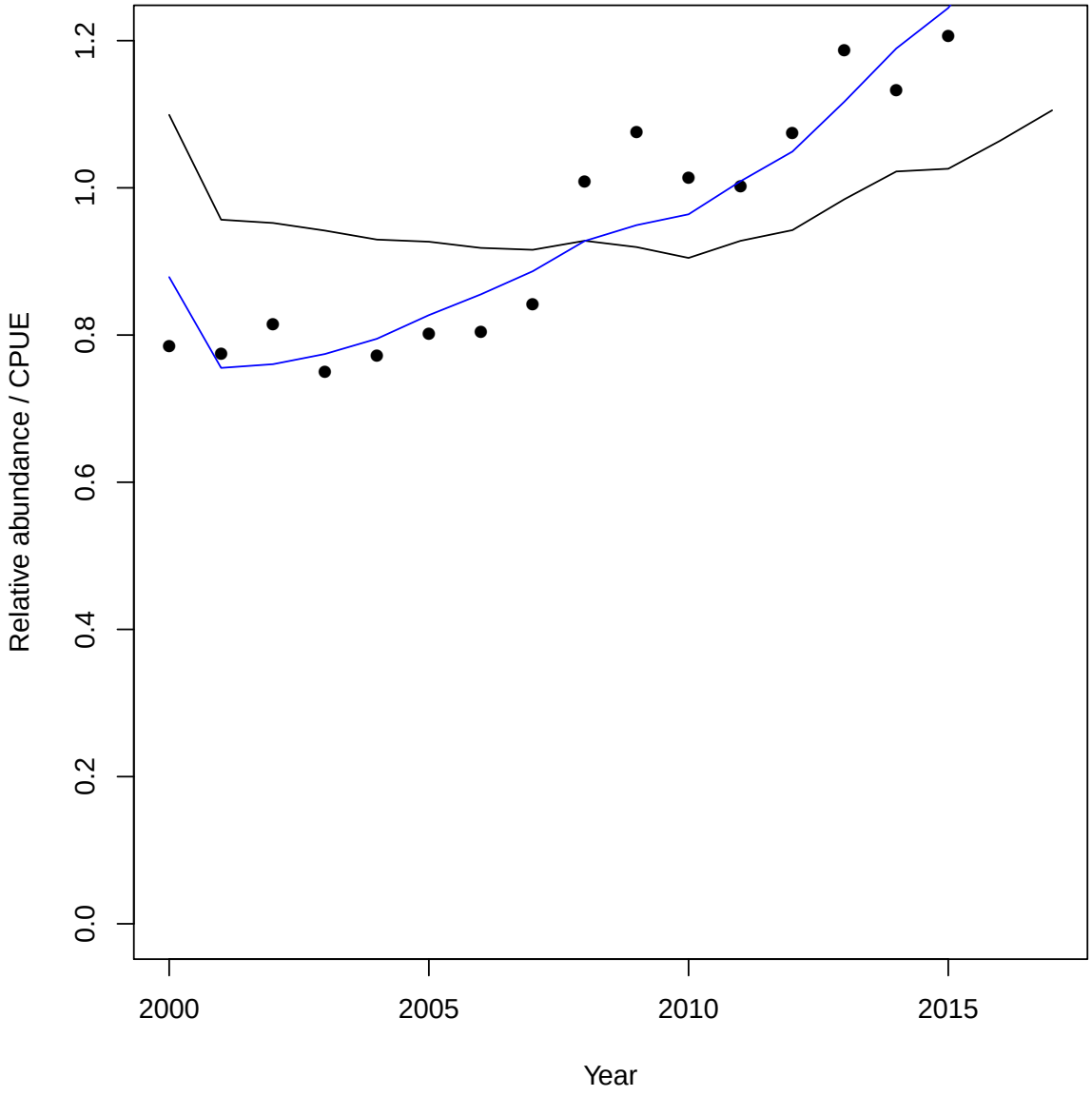

Figure 4.18: The standardized trawl CPUE index (dots) and the model expected values for the base case model (black line) and the sensitivity that is conditioned on the CPUE data (blue line).

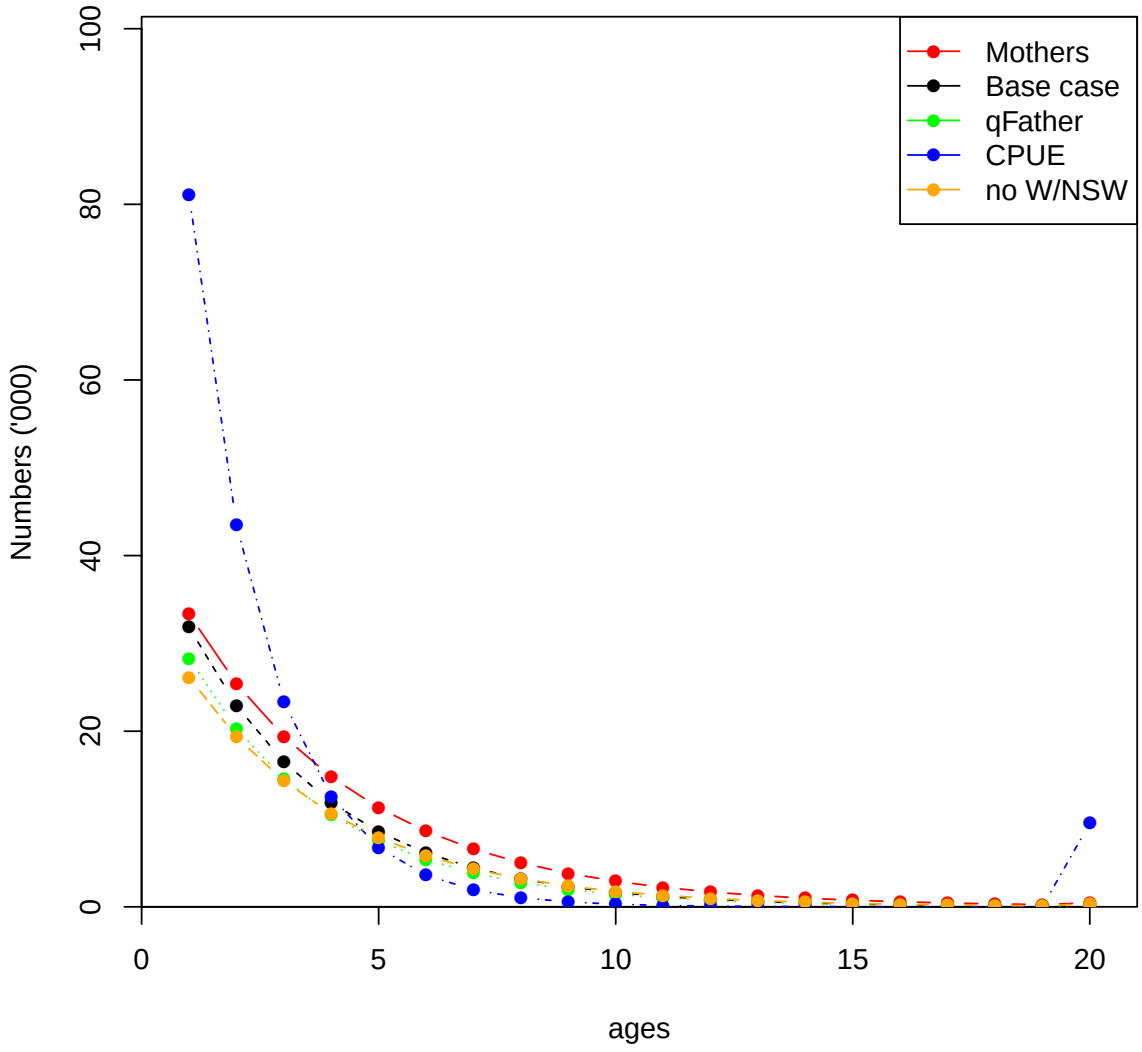

Figure 4.19: Numbers (thousands) at age in 1989 for the range of models shown in Table 4.9.

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 School shark 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)

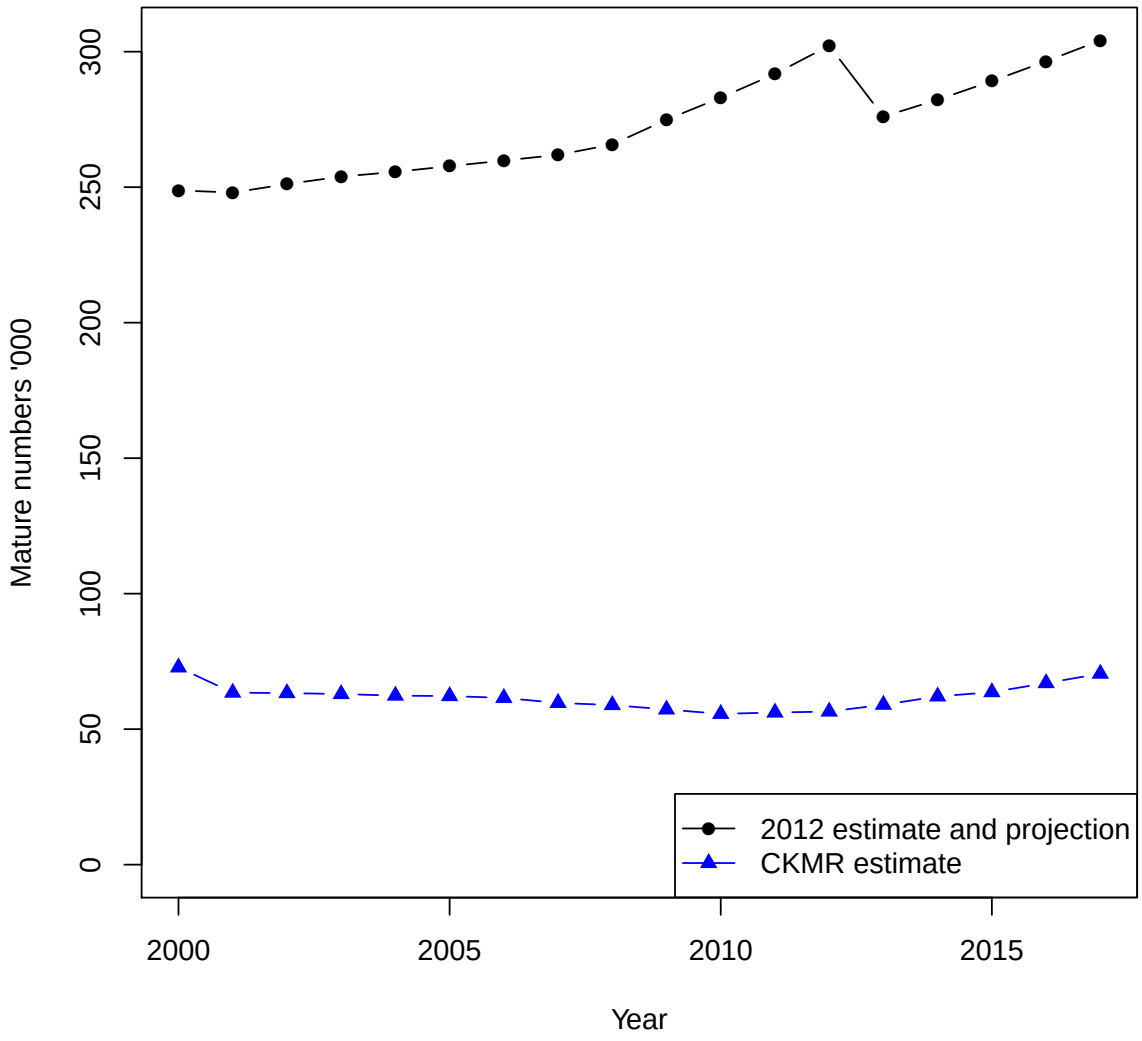

Figure 4.20: Numbers of mature School Shark (females aged 11 and over plus males aged 7 and over) for the full stock assessment model's base case that assumed catches of 225t after 2011 (2012 estimate and projection; black line) and base case close kin model estimate (blue line).

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 should permit 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) and also because of falling School Shark abundance. It is possible for the School Shark stock to be more abundant than Gummy Shark, while also being more depleted (as a function of the much higher starting population size of School 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 (S.E.) 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 S.E. 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 S.E. does greatly reduce over time given consistent sampling (Table 4.13, 700 samples p.a.). Note, however, the S.E. 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

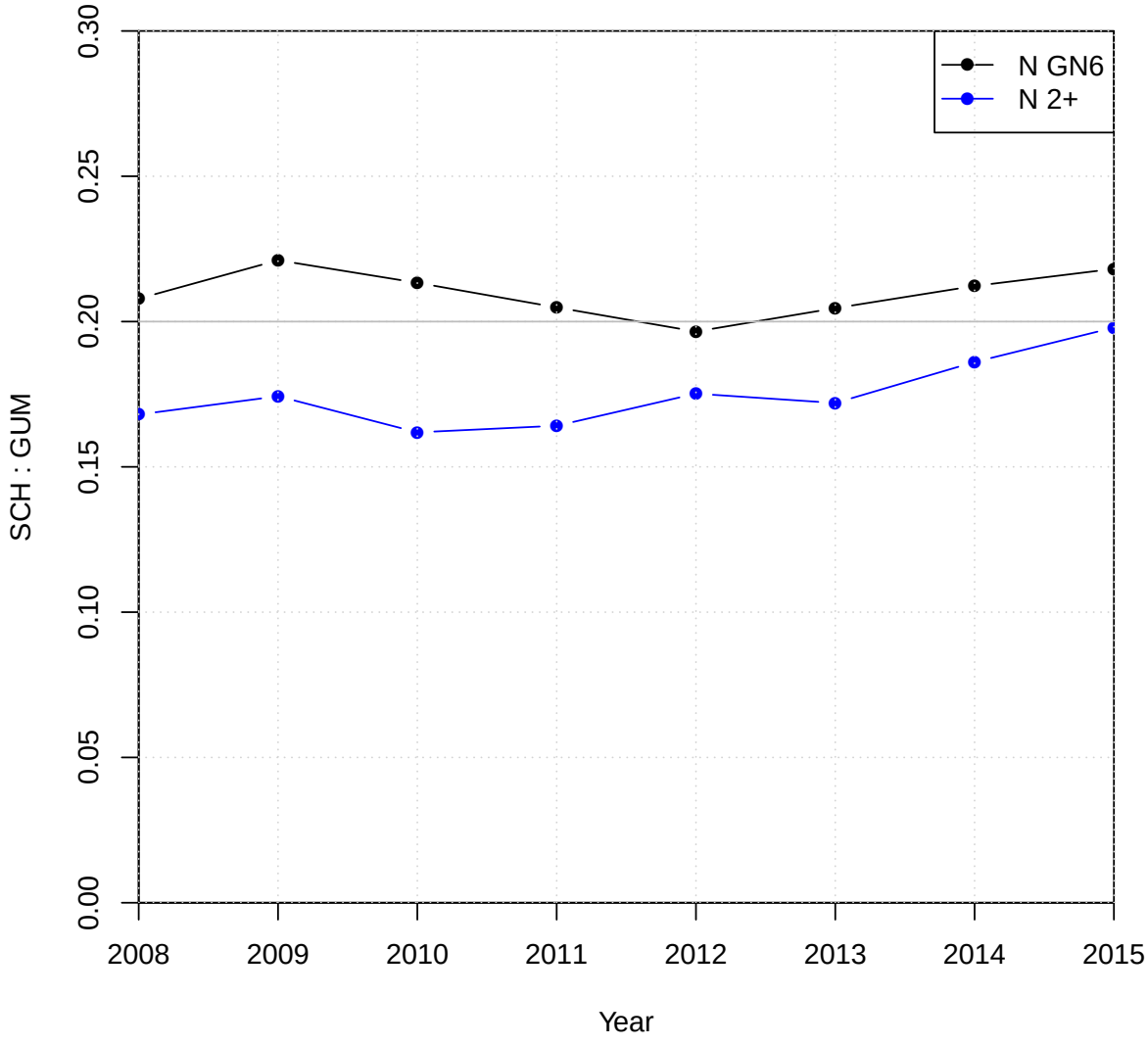

Figure 4.21: Ratio of School Shark to Gummy Shark that are available to 6 inch gillnets *N GN6* and that are aged 2 and over *N 2+* (a proxy for those availably to line and trawl gear).

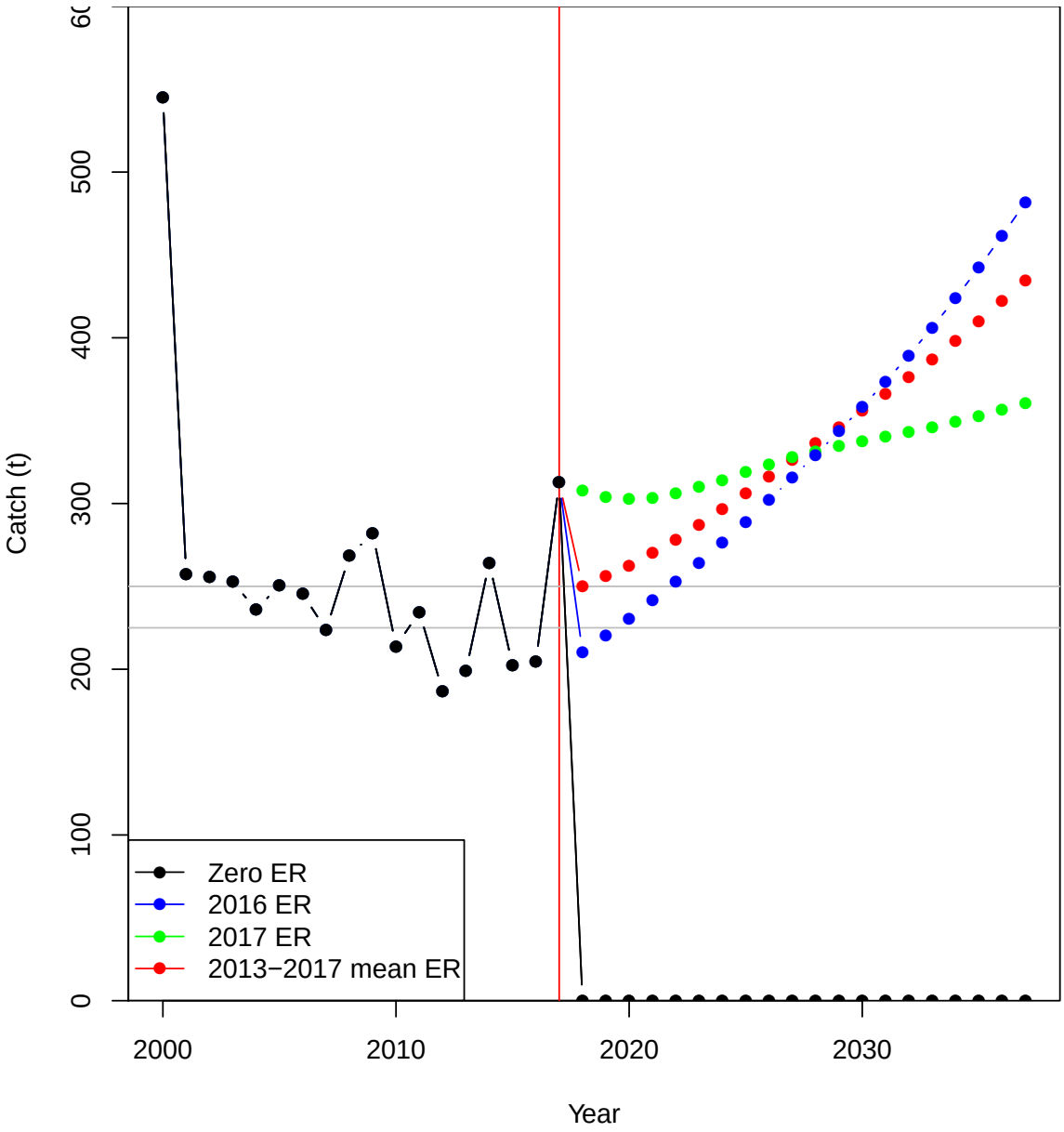

Figure 4.22: Projected catches (median) under zero exploitation (black); the 2016 (blue); the 2017 (green dots); or the average of the 2013-2017 (red) exploitation rates. Past catches are black, and the model is projecting after 2017 (red vertical line)

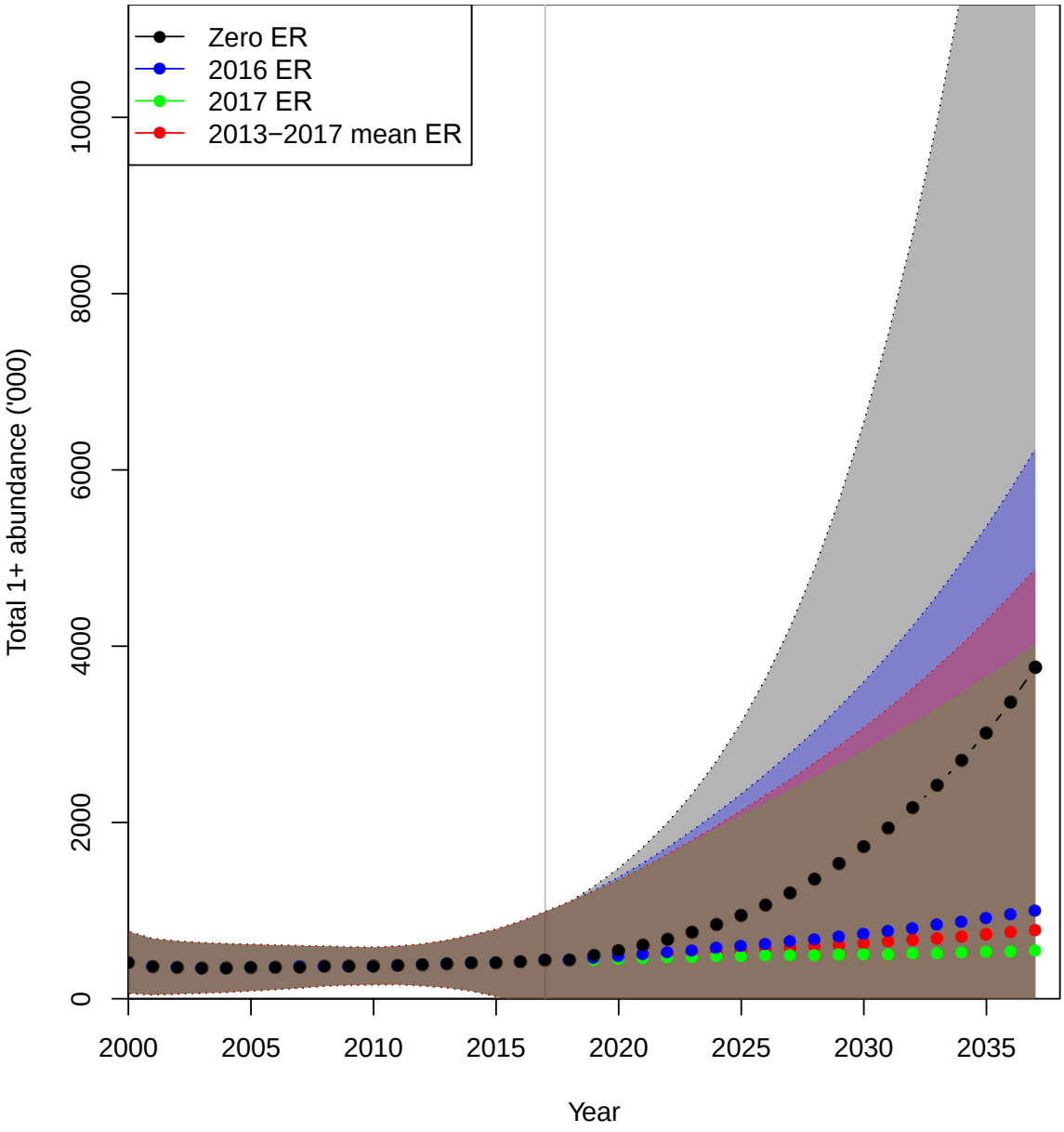

Figure 4.23: Past and projected future 1+ abundance under zero exploitation (black dots, grey shading); the 2016 (blue dots and shading); the 2017 (green dots and brown shading); or the average of the 2013-2017 (red dots and shading) exploitation rates. Shading indicates the 95% confidence interval.

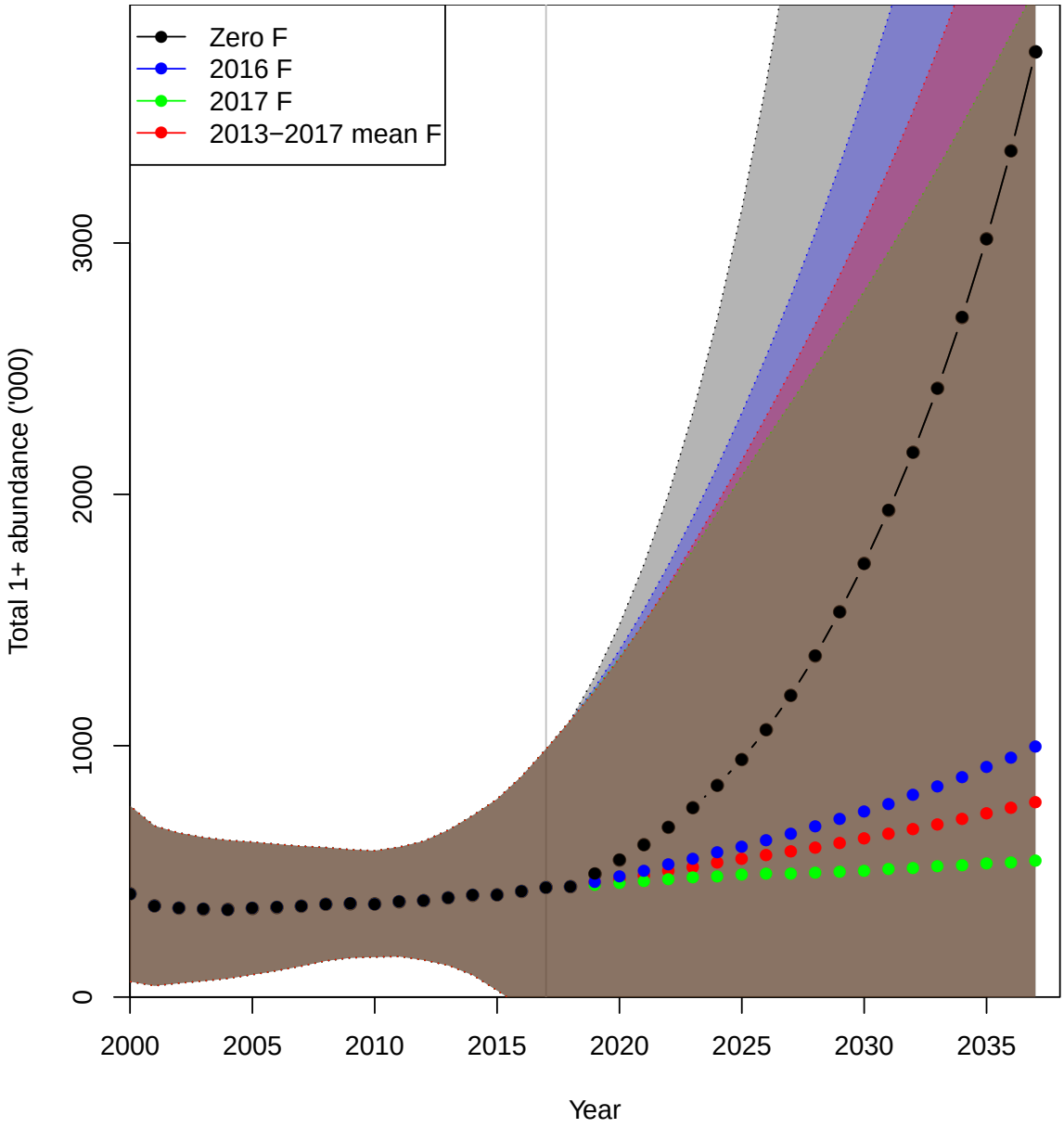

Figure 4.24: An expanded version of Figure 4.23.

therefore relies on only the samples already collected.

These calculations assume a 20% loss of samples during the genetic 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 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 detailed population dynamics model. 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(Thomson & Punt, 2009) and its 225t p.a. catch projection. This is supported by the findings of the simple model approaches which found around 50,000 'typical adults on average' across the 2000s (one-line approach) and roughly 40,000 to 80,000 adults (GLM model). While these estimates 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, 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 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 stocks 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 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 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 turn of the century 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 school shark abundance are too high to allow confidence in the estimate. Further collection of close kin samples will narrow the confidence interval around the trend in population size.

Chapter 7

Recommendations

Ongoing monitoring using close kin
Based on the results presented here, sharkRAG (December 2019 meeting) 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 possible 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 conceivably 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 important pupping grounds (such as 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 underestimated, because pairwise comparisons become non-independent. This variance issue will be addressed in future research.

Population dynamics model
The difficulty that we encountered in modelling the catches takes in the 1990s could no doubt be solved by increasing the complexity of the population dynamics model, specifically by recognising two or more fully intermingled School Shark populations. There would most likely also need to be assumptions made about the relative sizes, productivity, and movements of those populations. Presumably, multiple alternative hypotheses exist that would lead to differing population model formulations, all of which would be consistent with the catch data and the close kin data. However, these would explain the past, not the present and is not clear that such work would be of benefit to School Shark management.

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) and 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.

Understanding stock structure
As stated in Section 5, "...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." Provided we have sampled adequately from the catches, we ought to have calculated the abundance of the adult sharks that gave rise to the juveniles that are available to the fishery. Members of the shark fishing industry have pointed out that we have under-sampled far western South Australia, Western Tasmania, and deeper (trawl fishing) waters. Little School Shark catch comes from those areas, which is why they were not easily sampled, so that if completely separate populations of School Shark exist in those regions, that do not migrate into more often fished waters, then their impact on our estimate of abundance is likely to be slight. Similarly, if the School Shark present in the waters from which our samples came, consist of reproductively discrete populations, then our method ought to provide an accurate estimate of abundance for the sum of these populations. However, if those discrete populations differ in their productivity, then this might impact our result and that phenomenon would be interesting to explore. A future desktop study to better understand the effect of population structure on CKMR estimates of abundance, survival, and other parameters would be desirable.

Chapter 8

Extension and Adoption

The population genetics work (Section 4.4) was presented at an Australian Society for Fish Biology conference (Hobart, 4-8 September 2016) and has been published (Devloo-Delva *et al.*, 2019).

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 sharkRAG meetings (Table 8.1). Verbal updates were also provided to SESSFRAG 'RAG Chairs' meetings, and in March 2019, a brief summary of the project results and implications for management, was prepared at the request of Carolyn Stewardson (FRDC) as an update for COMRAC (see Appendix D). That summary was also widely circulated, by email, amongst relevant industry, science and management stakeholders. There have also been numerous phone calls, emails, and some face-to-face meetings with members of the fishing industry who provided tissue samples for this project. ABC Tasmania's Country Hour program broadcast recorded interviews with one of these industry members, Leigh Castle (15 April 2019, and another with Robin Thomson roughly a week later. These also resulted in an online newspaper article (ABC Rural, 7 May 2019).

Table 8.1: A list of presentations and reports of School Shark close kin work.

Year	Meeting	Presentation	Report or Powerpoint
2016	13 October SharkRAG (Hobart)	Description of genetic work: sequencing quality control genotyping kin finding sex markers Population genetics for AUS vs NZ (Slow) sampling progress	Powerpoint *Close kin for School Shark 2016*
2017	6 December SharkRAG (Hobart)	Sample collection (complete) Location of samples Sex of samples Kin pairs (maps) Proposed models	Powerpoint *Close kin for School Shark 2017*
2018	12 February	CKMR for beginners: conventional Mark Recpature simple CKMR explanation CKMR for a small country town kin types and parameter estimation CKMR for School Sharks Preliminary (incomplete) results (report)	Powerpoint *Close kin for School Shark sharkRAG 2018* Thomson *et al.* (2018b)
2018	February	SESSFRAG (Canberra)	Verbal update
2018	6-7 August SharkRAG (Hobart) *CKMR workshop*	Completed genetics and kin finding results Early mitochondrial DNA results Preliminary model results Punt assessment model Kin finding results	Thomson *et al.* (2018c) PPT Punt model PPT Kin finding
2018	29-30 October SharkRAG (Melbourne)	Exploration of kin pairs: location, distance CKMR model: length frequency data sensitivity tests inclusion of CPUE Estimated trend in abundance	Thomson *et al.* (2018c) Powerpoint
2018	3-4 December SharkRAG (Queenscliffe)	Forward projections (catch scenarios) Plot of gummy with school abundance Kin pairs by depth Scoping future CKMR: cost and benefits sample locations	Powerpoint *Projections and future scoping*
2019	March SESSFRAG (Canberra)	Verbal update	
2019	March ComFRAB	Short written summary	see 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

WILEY Ecology and Evolution Open Access

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

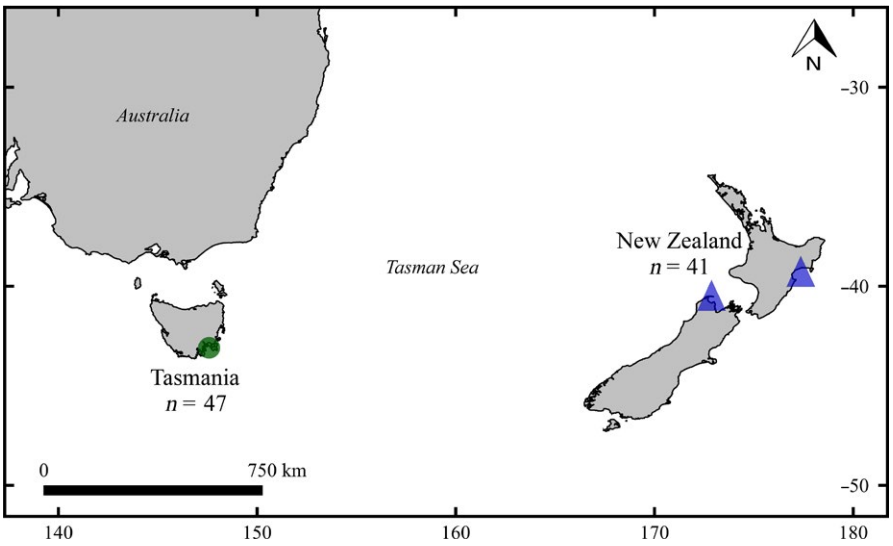

FIGURE 1 Sampling map for neonate school sharks from Tasmania and New Zealand. Green circle represents Pittwater and Norfolk Bay. Blue triangles represent Golden Bay (West, *n* = 33) and Napier (East, *n* = 8)

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,

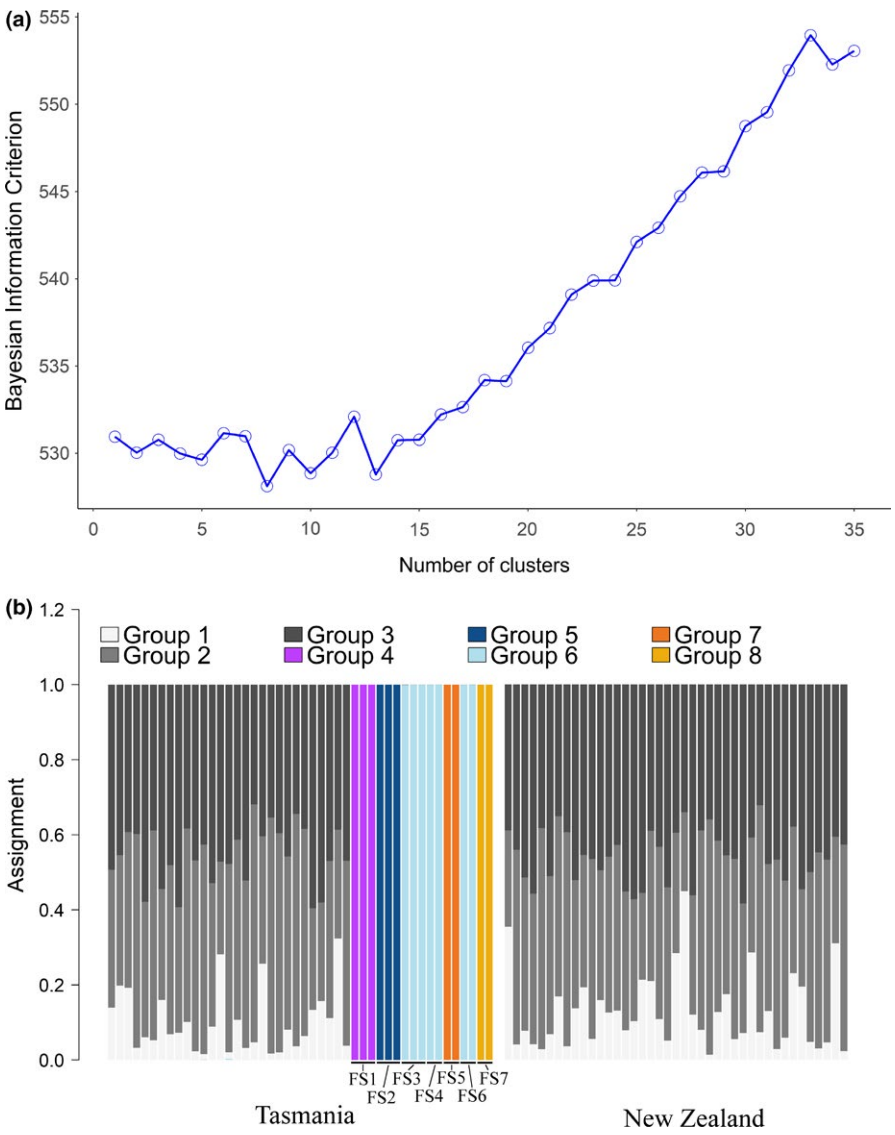

FIGURE 2 (a) Optimal number of cluster selection, based on Bayesian Information Criterion with 29 PCs. (b) DAPC assignment plot between Tasmania and New Zealand (full siblings included), based on seven PCs

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

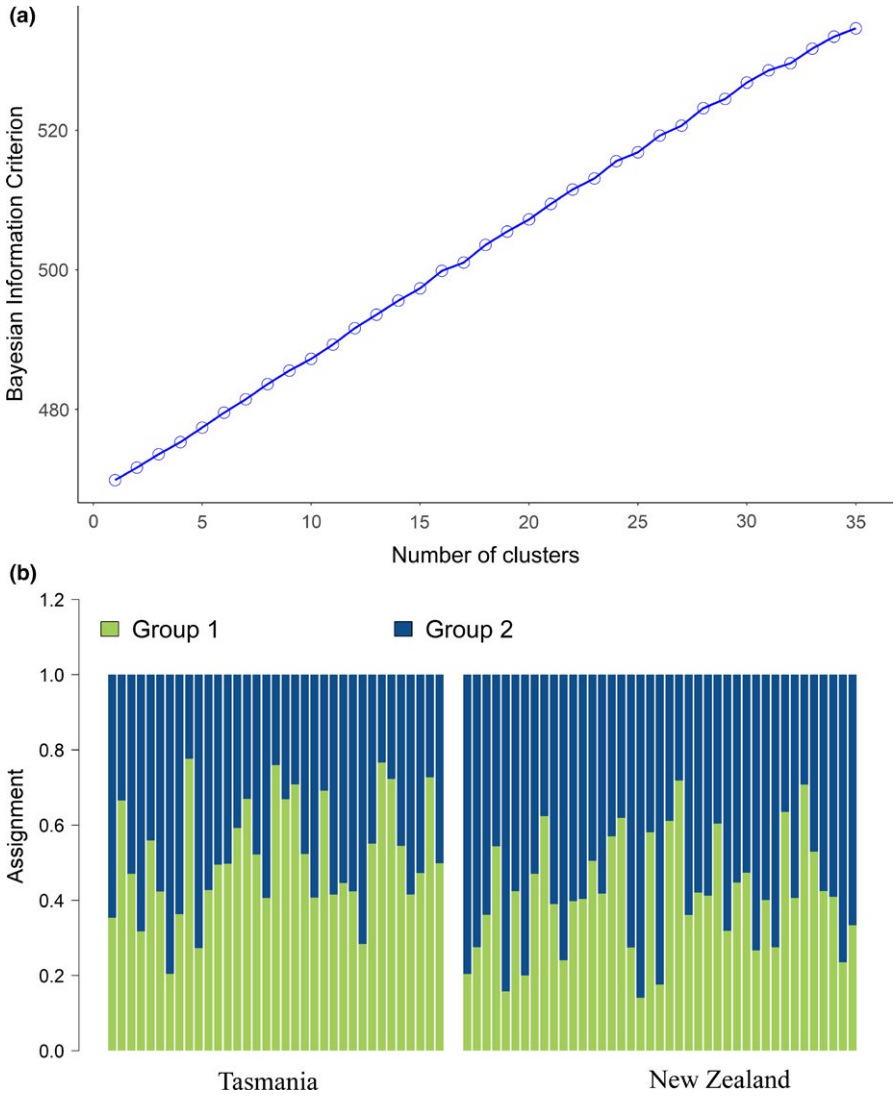

FIGURE 3 (a) Optimal number of cluster selection, based on Bayesian Information Criterion with 25 PC's. (b) DAPC assignment plot between Tasmania and New Zealand (full siblings excluded), based on 35 PCs

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 parameter: 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

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

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. We do not, strictly, need our model to extend back in time to the birth year of the parents (as discussed under 'Inclusion of POPs' in Section 7, we intend to extend our model to incorporate parents born before the start of the model period). The effect of excluding the plus group is neutral because it has been set high enough to have effectively no animals in it. While it might be possible to use the plus group, that would not be a straightforward exercise because we do not know (because we do not track) the age of those animals and

therefore cannot know their relative fecundity in the year of their offspring's birth. It is more straightforward to simply set the plus group at an age high enough to be, effectively, empty of animals, and to then exclude it from our calculations."

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. $b_i = b_j$; same cohort) 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 these same cohort half siblings must have been litter mates (because female 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 because we are modelling just one individual and one point in time (as opposed to the likelihood that the mother of j is the same female as the mother of i, and that she survived from the time of is birth to the time of js). 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.