A method for estimating the absolute spawning stock size of SBT, using close-kin genetics

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Abstract

We describe a method for estimating the absolute spawning stock size of SBT, based on genetic identification of parent-offspring matches in samples from Indonesia and the GAB. The method is related to mark-recapture, and provides an estimate of true adult numbers (not the unrelated genetic concept of "effective population size"). No catch or CPUE data is used, so the estimate is not subject to the biasses and interpretational problems associated with recent SBT assessments. We explain the statistical basis of the method, comment on its robustness, and describe progress with data collection and future plans.

1 Introduction

The CCSBT and its Scientific Committee cannot currently do a stock assessment of SBT. The recent discovery of serious flaws in the historical catch and CPUE data have undermined the main data sources for the assessment, and estimates of absolute SSB— which were always much less precise than relative depletion estimates— are now completely uncertain. Even if a new version of the historical CPUE index can be reconstructed, it seems unlikely (given the inherent difficulties of partial spatial coverage) that uncertainty in the series will genuinely be low enough to allow a precise assessment. It may be possible to develop a new assessment based on different future data sources (e.g. a different CPUE-style relative abundance index), but there will not be enough new data anytime soon to allow this. As to other existing data sources, tagging gives very little information about spawning-age fish, and even for sub-adults it is handicapped by various reporting rate issues in all the major fleets. The juvenile aerial survey in the GAB is of limited value on its own, as it is only a relative index and covers only the GAB-visiting part of the juvenile population, and anyway can say nothing direct about adult abundance[1].

This paper presents a completely different way to estimate, within a couple of years and using fishery-independent data, one key management quantity: the absolute abundance of adult SBT. The basis is counting parent-offspring pairs in samples of juveniles and adults (i.e. fish old enough to spawn). Intuitively, for a sample of fixed size, there will be fewer parent-offspring pairs if the population size is higher. This idea can be developed into a formal statistical estimate of absolute adult abundance (but not juvenile abundance), using ideas from mark-recapture. DNA fingerprinting can be used to actually identify the parent-offspring pairs. The approach was pioneered by Skaug, 2001, who applied the method to a small sample of North Atlantic minke whales, and by Nielsen et al., 2001, who estimated the abundance of male humpback whales in the West Indies. Skaug's dataset exhibits a number of difficult features which don't apply to SBT (see **Discussion**), most notably the inability to distinguish adults and juveniles; these features forced Skaug to make extra assumptions during analysis, which are not required for SBT. Nielsen *et al.* were able to directly compare genetic samples from males with calves, given also a genetic sample from the calf's mother, and were able to use a simpler analysis with fewer assumptions. Although their sample size was very limited, their results were consistent with independently-obtained estimates of male abundance.

[1]However, a significant trend over time in a juvenile abundance estimate is an important *indirect* indicator of spawner abundance.

We are embarking on a project to estimate the absolute abundance of SBT adults, based on identification of parent-offspring pairs between adults in the spawning grounds off Indonesia and juveniles in the GAB. The proposal was already being developed before overcatch was reported, but the urgency of the work is now much greater. Our method is more similar to Nielsen *et al.*'s than Skaug's, in that we are able to distinguish adults from juveniles, but there are a number of important differences, described in this paper. The project includes four years' sampling from the Indonesian spawning-ground fishery (2005/6-2008/9), and at least three years' sampling from the GAB juvenile fishery (2006-2008), with results expected by CCSBT 2009. Our target is to have at least 7000 fish genotyped (about 50/50 adults/juveniles); we have based this on approximate sample-size calculations, aiming for ∼70 parent-offspring pairs to get an overall[2] CV of ∼12%. We have already done some careful preliminary genetics to develop suitable loci, so that we will be able to establish parent-offspring relationships with high confidence in a cost-effective way.

Although underpinned by genetics, our approach has nothing in common with "effective population size"; it is a direct estimate of recent[3] adult abundance, and is based on mark/recapture principles rather than population genetics theory. Genetics is only used as a "mark" (in the juvenile) which can be "recaptured" (in the parent), and the only theory required is that of biparental inheritance.

For clarity, we will first describe the principles as they would apply to a single-year study where all adults have an equal probability of being sampled: how to estimate abundance, and how to estimate the CV. We then describe the current status of our SBT work and our future plans. The setup is a little more complicated than the basic case described in **Basic methods**, so we go on to outline how the basic idea can be modified (or whether it needs to be) to deal with a number of potential wrinkles for the case of SBT:

- sex-specific effects
- multi-year sampling
- age- or size-dependent catchability and fecundity
- additional reproductive variability
- population substructure

The final section includes a summary, some comments on how the immediate results (which pertain only to adults) might be used in management (which pertains to a much wider entire age range), and some thoughts about how the approach might be extended in future as part of a long-term monitoring and assessment framework.

[2]The real CV will likely be different, for several reasons explained later. Mainly, the sample size calculation depends on the abundance, which is of course the thing that is very uncertain before doing the project.

[3]I.e. with a 3-year lag.

2 Basic methods

DNA tests are commonly used to test parenthood. Colloquially, for a typical "gene" with several variants in the population and two copies of the gene in each animal, a parent and its offspring must have at least one identical variant, whereas unrelated individuals might have totally different variants. Formally, a parent and its offspring must have at least one matching allele at every diploid locus. If a locus has a large number of different alleles, there is a low probability that two unrelated animals will have a matching allele at that locus just by chance. If we examine a large number of loci on each animal, the probability that two unrelated animals will have a matching allele at *every* locus is therefore extremely low. Hence, we can in principle completely rule out "false positives", i.e. apparent parent/offspring pairs that are really unrelated. False negatives are almost impossible if scoring is reliable, so from now on we assume that the genetic evidence is an exact indicator of a parental relationship.

Now suppose you have a sample of m_A randomly-selected adults[4] and that, one year later, you collect a sample of m_J one-year-old juveniles. Pick one of the juveniles and one of the adults, and genotype both of them at enough loci to rule out any possibility of false-positives. What is the probability of a "hit"— i.e. that the chosen adult is actually a parent of the juvenile? Since the juvenile must have had two parents, the probability that the chosen adult is one of those two is $2/N_A$, where N_A (or just N) is the number of adults alive when the juveniles were spawned. Now repeat the comparison for the same juvenile and all the other adults. The expected number of hits between that juvenile and the entire set of m_A adults is $2m_A/N$. Now repeat this for all the juveniles: the expected total number of hits, $\mathbb{E}[H]$, is $2m_J m_A/N$. Thus, if h is the actual number of hits, we can form an approximately unbiassed estimate[5] of N in the obvious way (formally, by using the "method of moments") via:

$$\hat{N} = 2m_J m_A/h$$

Note that the method cannot tell us anything about the total abundance of juveniles. The logic doesn't work in reverse: although we know that each juvenile must have had two parents, we don't know how many juveniles on average each parent would have had. In mark-recapture terms, each juvenile "marks" exactly two adults which might subsequently be recaptured, allowing us to estimate the number of adults. Looked at the other way round, though, each adult "marks" an unknown number of juveniles— which makes it impossible to use mark-recapture analysis directly to estimate the abundance of juveniles[6].

There are two crucial points to emphasize. First, the derivation of $\hat{N}$ does require that the adults are randomly sampled, but does *not* require that the juveniles are randomly sampled; in particular, the juvenile samples do not have to be mutually independent. Of course, the juveniles must be selected

[4] Collected just after the spawning season, to avoid removing the very parents that we seek.

[5] As with most maximum-likelihood estimates, the estimate is only *asymptotically* unbiassed, i.e. the bias disappears if the expected number of recaptures is large enough. For $h > \tilde{}10$, the relative bias is about $1/h$, i.e. about 1.5% for the SBT project given the "target" of 70 for h.

[6] Skaug's method estimates adult and juvenile abundance together, and uses the number of half-sibling etc. matches as well as parent-offspring pairs. However, the method is less direct and requires extra assumptions which would not make sense for SBT.

independently of the adults— the method breaks down if applied to mother-calf pairs, for example.

Second, the derivation of $\hat{N}$ does *not* require that all "adults" make an equal reproductive contribution. The key point is actually the random selection of adults. In fact, the "adult" population might be defined as "that set of animals which have equal probability of appearing in our m_A-sample". The trickiest part of applying the method to SBT, is correcting for unequal sampling probabilities among the "adults"; see **Wrinkles**.

2.1 Basic CV & sample size calculations

To get an idea of the uncertainty in $\hat{N}$, one further assumption is needed: that the numbers of hits from different juveniles are independent (see **Wrinkles**). Then some algebra (see **Appendix**) shows that

$$CV\left(\hat{N}\right) \approx \frac{\sqrt{2}}{m}\sqrt{N} \tag{1}$$

where m is the combined sample size (for optimality, split equally between adults and juveniles). Given some *a priori* notion of N, we can use (1) to set the sample size; e.g. a 10% CV requires about $15\sqrt{N}$ samples. For SBT, using a guesstimate from a now-obsolete assessment of $N \approx 350,000$ (the number of fish $\geqslant 160cm$, the lower limit of maturity), a target CV of 12% implies a sample size of 7000, with about 70 hits being expected and about 1% of the adults being sampled. We stress that this is only a sample-size calculation, and the achieved CV will be different for a number of reasons; see **Discussion**.

The remarkable thing about (1) is that it is (inversely) *linear* in sample size. By contrast, in the great majority of statistical settings, CV depends (inversely) on the *square root* of the sample size, meaning that diminishing returns usually set in as more data are collected. With close-kin abundance estimation, though, there is a quadratic gain in efficiency[7], basically because each new (juvenile) sample is compared against *all* existing (adult) samples, hence generating far more than one "data point".

3 Status of SBT work

3.1 Sampling

Our adult sampling program uses the infrastructure of the existing Indonesian catch sampling programme in Benoa, Bali. Samples for genotyping are taken throughout the fishing (spawning) season from all possible SBT 165cm and up. This size limit was chosen based on maturity data, to safely

[7] Unless the sampling fraction becomes "large", or the period of sampling becomes so long that a high proportion of parents of "early" juveniles have died.

encompass all fish big enough to have been parents two years previously, when the youngest juveniles in the corresponding sample were spawned. Mouth tissue is collected by a trained sampler and deep-frozen for shipment to Australia. All genetically-sampled fish are lengthed and sexed (by checking for residual female gonads; see Farley et al., 2007) as part of the regular catch sampling programme, and a portion of the genotyped fish form part of the otolith-collection set and so will be of known age. To make sure we are sampling only spawners, and in the absence of precise information on fishing location, we have excluded all SBT from trips with a high proportion of sub-adult fish, since in the last couple of years, boats from some fishing companies have been fishing further south, outside the SBT spawning ground. Coverage of the spawning grounds and spawning season is good; the fleet that lands into Benoa covers the main part of the SBT spawning grounds, although a much smaller unsampled catch of SBT is taken further west from the Cilicap fleet, in an area of apparently lower SBT spawning density (Proctor et al., 2003; Far Seas Fisheries Research Laboratory, 1985).

To date we have collected two adult samples from Indonesia ($m = 220$ from 2005/2006, and $m_A = 1200$ from 2006/2007), and we plan two more years with similar sample sizes to this year. That number of adults is about the maximum possible given the logistics of sampling.

For juveniles, we collected a sample of 4000 juveniles from Port Lincoln in 2006, and are arranging a similar number for this year (about 800 collected so far) and 2008. The 2006 sample contained mostly 3-year-old fish ($\sim$90%), with about 4% 2-year-olds and 6% 4-year-olds. Only a subset of fish will be genotyped to begin with, in case that gives enough precision overall. Genotyping of juveniles will be restricted to fish within length bands that allow an unambiguous determination of age, to ensure that we can accurately track the birth-year (actually this does not exclude very many fish).

None of the samples have been genotyped yet. Although our current plans are to genotype a total of around 7000 fish, if it does turn out that we get many fewer hits than expected (i.e. much bigger N) then we will have a large number of spare juvenile samples than can be genotyped to improve precision. This is quite possible if the true spawning stock size turns out to be much higher than we assumed in our sample size calculations.

3.2 Genetics

Genotyping costs money, and given that the sample size will be quite large, it is important to minimize the number of loci that need to be tested. The key is to do some careful preparatory work to pick the best set of loci, and to use a two-pass approach: first genotype every sample at some set L_1 of loci and then, whenever a possible matching pair is found, check the pair by genotyping a further set L_2 of loci. L_1 needs careful design; it must include enough highly-informative loci to rule out a high proportion of samples on the first pass, but not so many that expense is pushed needlessly high. An outline of the calculations required is given in the Appendix; the number of loci required depends only on the sample size, not on N. Because only a small proportion of animals will be tested in the second pass, design of the second pass is less critical.

The costs of genotyping go up stair-wise with number of loci (e.g. 6 loci might be almost as cheap as 5, but 7 might cost almost twice as much), so it is worth spending considerable efforts to develop really informative loci and to organize them efficiently for mass genotyping. The ideal loci for close-

kin studies are more variable than is useful for traditional population genetics, for which an excessive number of alleles actually reduces statistical power.

With SBT, we have done a considerable amount of preliminary work to identify new, powerful, and reliable loci (microsatellite library enrichment, locus discovery, primer design, amplification optimization, trialling on a sample of 16 fish, allele frequency calculations). A basic set of 20 tetra-nucleotide[8] loci have been selected and, based on their allele frequencies in the sample, using any 12 of the more powerful of these as L_1 will eliminate well over 90% of unrelated fish on the first pass. In other words, even if some of the best loci fail for some unexpected reason such as strongly preferential amplification of short alleles, we still have plenty of other candidates. A subset of the remaining loci can be used L_2.

4 Wrinkles

The base case described above is very simple, but does not apply directly to our SBT project. There are a number of wrinkles which require attention. To keep the descriptions as clear as possible, each wrinkle is discussd independently of the other wrinkles, usually in terms of adjustments to estimators. In practice, though, the wrinkles interact, and it will be necessary to move to a fully parametric likelihood-based framework for estimation. That will complicate the statistical programming, but the comments below about estimability and precision made below will not change.

4.1 Sex

There is a sex-bias in spawning-ground samples of SBT, and a different bias in the whole adult population (Farley et al., 2007). Nevertheless, each juvenile must have had one male and one female parent. Since the sex of the adult SBT sample is known, the simplest way to deal with any sex bias is just to make independent estimates $\hat{N}_m$ and $\hat{N}_f$ of the adult male and adult female abundances, using the male and female adult samples respectively. This separation-by-adult-sex should be assumed throughout the rest of this paper, but is not mentioned explicitly. There is a small effect on the CV— see Appendix.

4.2 Sampling delays and multi-year sampling

The abundance estimate described above is retrospective: N is the number of adults that were alive in the year when the juveniles were spawned, rather than when the juveniles or adults were sampled. This remains true for the multi-year SBT project, but there are extra complications of modelling, sampling, and interpretation that arise from the multi-year nature of the project. There are really three aspects. The first is that, for a given cohort of juveniles, the potential parents will be sampled

[8]Loci with tetra-nucleotide repeat sequences are easier to score reliably than the di-nucleotide loci which (being easier to find) are more commonly encountered in population genetics.

across several years, rather than in one year. Linked to this is the second aspect: there will generally be a delay of several years before the potential parents of a given juvenile cohort are sampled, during which some of the parents will die. The third aspect, which is quite separate, is that there are multiple cohorts of juveniles.

With respect to delayed adult sampling, it is obvious that a given juvenile could only have been spawned by fish that were big enough to be adult in its birth-year. Therefore, depending on the birth-year of the juvenile and the sampling-year of the adults, it is necessary to restrict the set of potential parents that are checked for hits, to make sure they were all mature in the birth-year; this can be done by using an age or length cut-off projected forwards to cover the delay. In other words, it is important to do all checks against the *same* population of potential parents of a given juvenile across the years of adult sampling; in mark-recapture terminology, the population must remain "closed". This ensures that the abundance estimate pertains to the original size of the adult population in the juvenile's birth-year.

With respect to possible mortality between juvenile "marking" and parental "recapture", it turns out that the date of adult "recapture" does not affect the probability of that adult being the parent of a particular juvenile (under reasonable assumptions, and after addressing some of the other wrinkles, in particular **Multi-year-breeding cycles**; see Appendix for justification). Hence sampling delays do not lead to bias. For a given cohort of juveniles, the basic model could in fact be extended to the multi-year adult-sampling case by simply aggregating the potential-parent samples across years, using the year-dependent size cut-off.

With respect to the multiple cohorts of juveniles, it is necessary to allow for possible changes in adult abundance over the different birth-years of the juvenile cohorts. In the context of the basic model, this could be done easily by constructing independent estimates of adult abundance in each birth-year[9], and then averaging (to reduce noise). In practice, the interaction with age- or size-dependent catchability will necessitate a more complicated likelihood-based multi-year model for SBT.

Overall, the multi-year and delayed-sampling issues entail a small number of extra parameters, but should have little impact on CV. It is worth noting that, because the number of hits is proportional to the square of the sample size, and because each new year of samples gets cross-matched to earlier years as well as to itself, most of the hits will not be found until the final year of this 3-year study.

4.3 Multi–year breeding cycles

It is theoretically possible that SBT— even big ones— have a multi-year breeding cycle and do not turn up to spawn every year. Suppose there was a two-year cycle: then a one-year project that sampled only one cohort of juveniles would either coincide with an "off" year or an "on" year for the parents of that cohort, and there would either be no matches or twice as many as the total abundance suggests— and there would be no way to detect either phenomenon. However, in a two-year program, or a one-year program with two age-classes of juveniles, the overall number of matches

[9]The estimates for different birth-years are effectively independent because there is a negligible probability of any adult matching multiple juveniles from different cohorts.

comes out right and the bias disappears (see Appendix). In practice, the effect of a regular breeding cycle would, if important enough to matter, be obvious in the samples, by comparing sampling-year of identified parents against birth-year of corresponding offspring. If this revealed a 2-year cycle, say, then the method would need an adjustment to differentiate between

$$\mathbb{P}\,[\text{random adult on spawning ground is my parent}|\text{odd number of years since my birth}]$$

and the even-year equivalent. This only adds one extra parameter (or $p-1$ in the case of a p-year cycle), and so would have limited effect on precision. In the SBT project, adult samples will be collected over at least 3 years and will cover at least 4 cohorts of juveniles, so the project should be able to cope with breeding cycles of 4 years or less.

Irregular breeding cycles (for example, breeding on average only one year in two, but at random rather than alternately) don't affect the basic method— if adults are present at random on the spawning grounds, then the chance of any one being your parent is still $2/\!\!/N$. However, if there is an age-related effect on the probability, some adjustments are required, as described next.

4.4 Age-dependent sampling probability

In our design for SBT, adults are sampled on the spawning grounds. Sampling probabilities will therefore *not* be equal across ages. For example, suppose there is a gradual maturity ogive rather than knife-edge maturity; then, in any given year, the proportion of fish at "age of 50% maturity" that are available for sampling in the spawning grounds will be lower than the proportion available within the fully-mature age classes. This necessitates an adjustment to the basic method, using information on the spawning biology of SBT. The description below is mostly in terms of age, for simplicity, but in fact the main driver is length (or, equivalently, body-weight), and the actual statistical models used on the data will probably need to be length-based.

SBT are multiple spawners, remaining on the spawning ground for days or weeks, with a daily spawning cycle possibly punctuated by rest periods (*Farley and Davis, 1998*); also, some mature fish may simply not visit the spawning grounds in some years. The more time a fish spends on the spawning ground, the more eggs it will produce, *and* the more likely it is to be caught. Bigger/older fish of a given sex seem to spend more time on the grounds (Davis et al., 2003; and consistent with the apparent over-representation of older fish in spawning ground samples found in Farley et al., 2007), and certainly produce more eggs per day[10] (Farley and Davis, 1998).

Although the histological studies above have shown how daily egg production relates to size (and age), there is no independent data on residence time[11] as a function of age. Nevertheless, the quantities required to provide an unbiassed estimate of N can be estimated from three sources: the age profile

[10] Males don't produce eggs. The method used for unbiassed estimation of male abundance is statistically similar to that proposed for females, but differs in biological detail. Some extra collection of male gonads will be necessary, as fewer males have been studied than females.

[11] Residence time has two components: the probability of coming to the spawning grounds in a particular year, times the average residence when actually on the grounds. There is no way to separate these two components, but it is only their product that is important.

of sampled adults on the spawning grounds, the age profile of identified parents, and histological data on daily egg (or sperm) production. In the hypothetical example considered in the Appendix (with single-year sampling and no sampling delays), it is assumed that residence time, daily egg production, and abundance are exponential functions of age with coefficients r, g, and z respectively and only g known *a priori*. The Appendix shows that r and z can then be estimated, and that the basic abundance estimate needs to be adjusted by a factor $\frac{(r+z)(g+r+z)}{z(g+2r+z)}$. Note that if $r = 0$, i.e. that residence times and therefore sampling probabilities are equal for all adults, then the adjustment is 1 whatever the value of g: that is, variations in adult fecundity do not bias the estimate unless correlated with sampling probability.

In reality, residence time and daily egg production will be asymptotic rather than exponential functions of age, so some non-linear estimation will be necessary and more than one parameter will be involved. Also, a length- rather than age-based model will probably be required. A joint likelihood model for all the data will be necessary, and the estimation of extra parameters (i.e. z and r in the hypothetical example) will have some impact on the CV. However, a greater impact on the CV will come from the fact that the expected number of hits depends on g and r. Because of r, our project will sample more heavily from the more fecund fish, so the number of hits (and thus the precision) might actually be better than the "target" even if our guesstimate of N happens to be about right. Further, it might be useful or even preferable to construct an age-weighted version of N, e.g. for direct comparison with spawning ground catches; such an $\hat{N}$ would have yet again a different precision. All these aspects can only be quantified once the data is available.

In principle, we could take advantage of age-specific fecundity and catchability by changing the adult sampling design to concentrate even further on older/bigger fish, which would increase the number of hits per genotype. In practice, though, there are too few adult fish available to make this worthwhile.

4.5 Random reproductive variability

As shown in previous section, systematic reproductive variability between adults does not bias the basic $\hat{N}$ unless correlated with adult sampling probability. Nevertheless, in some fish populations, a small number of mating events can, by chance, contribute a very high proportion of the surviving juveniles. Would this random reproductive variability have implications for a close-kin abundance estimate? The short answer is that there is almost[12] no bias. As noted at the end of **Basic methods**, there is no requirement for adults to make equal reproductive contributions, as long as they are sampled with equal probability (or that any unequal sampling is allowed for, as just described). If you are a juvenile, then the chance that a randomly-chosen adult is your parent is still $2/N$ whether you have no siblings or 1,000,000 siblings.

However, random reproductive variability could affect the precision of $\hat{N}$. If two juveniles happen to be sibs or half-sibs, then the results of comparing the second juvenile against the adult sample are not independent of the results of comparing the first one. Hence, if there are many sibs or half-sibs

[12]Actually there is a small amount of bias because $\hat{N}$ itself is slightly biassed for finite-sized samples, as noted in **Basic methods**. Reproductive variability decreases the effective sample size, so worsens the sub-asymptotic bias—but this should be very small for SBT with 70 hits expected.

in the juvenile sample, then the effective sample size will be substantially reduced. The good news is that this kind of event can easily be detected, by examining the amount of allele-sharing within each juvenile cohort. It is less easy to be sure about individual half-sib identifications than about individual parent-offspring identifications, because the genetic overlap is much less, but nevertheless an overall statistical excess is easy to check. And unless the reproductive variability is actually manifest in the juvenile *sample* (rather than the cohort as a whole), there is no impact on precision.

Seriously high reproductive variability is mostly documented for landlocked species with small populations, such as bass and salmon. With SBT, the large population, prolonged spawning season, pelagic spawning, and multiple mating behaviour all make the phenomenon *a priori* less likely. Further, the juvenile sample is a tiny fraction of the juvenile population. To the extent that the sample is "random" (although this is not a requirement of the method), the incidence of siblings in the juvenile sample should therefore be far lower than the incidence in the cohort (following the same argument used to derive the CV of $\hat{N}$, with $\mathbb{E}[H_{JJ}] \propto m_J^2/N_J$) . Nevertheless, the sample of ~1500 juveniles per year is actually drawn from a much smaller number of schools, and samples from the same school could contain siblings or half-siblings from the same spawnings. Only time, and data, will tell; but the point is that any effect strong enough to reduce precision should be apparent and estimable from the data.

4.6 Closely-related individuals

Accidental hits between related individuals that are not parent-offspring pairs will not be a problem. First, it is only non-parental relationships between juveniles and adults that would matter; within-group comparisons are not used. The number of full-siblings between juveniles and adults will be miniscule, because any such pair would have to result from two matings between exactly the same individuals at least 8 years apart. The number of half-sibs and grandparent-grandchild pairs could be of the same order of magnitude as the number of parent-offspring pair, but such distant relatives only share 1/4 of their alleles, and need not share any alleles at any given locus; hence the chance of say a pair of half-sibs having at least one allele in common in all 18 hypervariable loci is very low. Since there should not be vastly more close-relative pairs than parent-offspring pairs in the adult-juvenile comparisons, and the probability of the former mimicking the latter is very low, false-positives from close relatives are most unlikely to be a problem.

4.7 Population structure

So far, it has been assumed that SBT form a single population with complete interbreeding. Although no previous study has found evidence of population structure, conventional population genetics applied to large populations is a notoriously blunt tool for that task. It turns out (see **Appendix**) that the basic method is unbiassed even when there is population sub-structure, providing that sampling is proportional to abundance across either the sub-populations of adults, or the sub-populations of juveniles. In our SBT project, juvenile samples come only from the GAB, so if there are substantial numbers of non-GAB juveniles out there somewhere, then juvenile sampling will obviously not be proportional. However, adult samples should cover the spawning season and spawning area (, al-

though not necessarily in strict proportion to adult SBT density. Hence, the basic estimator would exhibit population-structure bias if and only if three conditions all apply:

1. adults exhibit fidelity across years to particular parts of the spawning season and/or spwaning grounds;
2. the timing or location of spawning affects a juvenile's chances of going to the GAB (rather than going elsewhere or dying young);
3. sampling coverage of the spawning grounds (in time and space) is substantially uneven, and correlated with the fidelity patterns in (1). (In other words, if adults showed timing-fidelity but not spatial-fidelity, whereas coverage was even across the spawning season but not across the spawning grounds, then the uneven spatial coverage would not matter.)

There is no direct information on condition 1. With respect to condition 2, much the greatest part of SBT spawning occurs within the North Australian Basin (Far Seas Fisheries Research Laboratory, 1985), and particularly towards the east and south of the basin beyond the Australian shelf, where the Indonesian through-flows in summer would tend to push the larvae together into the Leeuwin current. These conditions seem unlikely to induce a strong location-of-spawning effect on most juvenile's subsequent propensity to go to the GAB[13], although a timing-of-spawning effect is possible. With respect to condition 3, the Benoa-based operations that we are sampling coincide well with this main spawning area (Proctor et al., 2003, Figure 4.3.1; note that the fishing range has expanded southwards since then, as per Proctor et al., 2006). Approximate timing-of-effort information could be probably be obtained from the sampling program; spatial information has proved harder to get, but the data obviously do exist somewhere at the company level, and some insights may be obtainable through, for example, the observer program (Sadiyah et al., 2007) or the Fishery High School program (Basson et al., 2007).

Fortunately, there is enough information in the project data to check the first two conditions. If the seasonal/spatial distribution of identified parents of GAB juveniles is substantially different to the seasonal/spatial distribution of all adult samples, then that is a clear signal that the first two conditions do apply. Such evidence of population structure[14] would be of major qualitative importance to management, regardless of its impact on quantitative results.

If and only if the first two conditions do apply, then the third could be checked using timing (and perhaps location) information on Indonesian samples. And if all three conditions do apply, then it should be possible to adjust for the uneven adult sampling probabilities, again using sampling coverage information. That is very much a bridge to be crossed only if we come to it; but because the sampling coverage is at least fairly complete[15] even if not necessarily balanced, we would in principle be able to develop a correction if required.

[13] A small proportion of larvae are found to the north of the NAB and west of it. Different oceanographic conditions apply there, and those larvae could well end up somewhere different as juveniles. However, at least until 1981, this proportion was small.

[14] "Population structure" is probably the wrong phrase, because the behaviour does not have to be heritable; adult spawning preference need not be related to earlier juvenile GABness, even if offspring's GABness is driven by adult spawning preference.

[15] Again: over the great majority of the spawning area.

5 Discussion

5.1 Comparisons with previous close-kin work

It is worth taking a moment to compare our project with Skaug, 2001 and Nielsen et al., 2001. Both studies had very limited sample sizes, since the data were collected for other purposes, and consequently low expected number of hits and low precision. Both studies also had use very limited set of loci, originally developed for different purposes and at a time when genotyping was much more expensive than nowadays. Consequently, the "false-positive" probabilities were so high that both studies had to rely on probabilistic evidence of a match, complicating the statistics. In Skaug's case, it was both necessary (in order to get a bigger number of hits) and unavoidable (because of the equivocal genetic evidence from using a small number of loci) to allow for other close relationships, in particular half-sibs and grandparent-grandchild. This entails further assumptions about reproductive variability and equilibrium age distributions and abundance, which (as Skaug notes) was a major problem for the close-kin approach in that particular example. Nielsen *et al.* had an easier time— and required far fewer assumptions— because the only relationship of interest was parent-offspring, and because knowledge of the mother's genotype makes it much easier to exclude an unrelated father, even with limited loci. They did note that there could be a complication arising from the adult male sampling probability being correlated with reproductive output— noisy males that make a conspicuous display are easier to find, both for females and for biopsy crews. Nielsen *et al.* lacked the data to address that issue, which is essentially the same one that we face with size-specific catchability on the spawning grounds; in our case, though, the histological information about daily egg production should be enough to compensate for unequal sampling probabilities. Overall, in our study as compared to the earlier studies, the much larger expected number of hits (and hence potential precision) should mitigate the need to estimate a few extra parameters.

Close-kin abundance estimation does not seem to have been much used since those two papers. In a marine context, most fish species are simply too abundant to have made the method cost-effective, although this may change as genotyping costs continue to drop. For many fish, the possibility of undetected population substructure would also be a deterrent, as this can bias estimates if not allowed for (see earlier).For cetaceans, where the method was first developed, there are usually alternative methods of estimating abundance, either through line-transect surveys (as with Skaug's minke whales) or through mark-recapture using photo-ID and/or "genetic tagging" from biopsies. SBT is in many ways the ideal species for a close-kin abundance estimate: the spawning population is not that large, there is not thought to be serious population substructure, the species is valuable enough to make mass genotyping affordable, and sampling can be arranged fairly easily on top of existing programmes. Most importantly, though, there is a *need*, because there is not (and never really was) a reliable alternative estimate of absolute spawner abundance.

5.2 Comparisons with conventional mark-recapture

In principle, a conventional mark-recapture program might be a competitive way to quickly estimate adult abundance (although tagging large numbers of huge spawning-age SBT is not that easy). The

potential downside of genetic mark-recapture (either of individuals, or of closely-related animals such as in our study) is that only a limited set of animals are checked to see whether they are recaptures, so the sample size can be greatly reduced in theory compared to an ideal tag-recapture program in which every tag recovered was reported. However, genetic mark-recapture does have one great advantage over conventional mark-recapture, in that there is no confounding between reporting rate and recapture rate. With conventional tags, a non-recovery could be either due to non-reporting or non-recapture, but with genetic tags, non-reporting (i.e. forgetting to send in a sample— it is impossible to tell whether the sample is a recapture or not) simply reduces the sample size without leading to bias. This is an important point which underlines the "fishery-independent" nature of the data.

5.3 Implications of the results for management

The most immediate result for management will of course the adult abundance estimate; given the great uncertainty about the current status of SBT, the obvious first thing to do with it is to compare it against current catches of adults, as a bottom-line check on adult mortality rates. In addition, based on the discussion of age-specific factors, there should be a direct estimate of recent Z among the adults. (This is a Z in the same sense that the slope of a catch-curve is; it combines total mortality with any trend in recruitment.) Combining the N and Z estimates, and using current catch information on older sub-adults (assumed correct in future, but not necessarily in the past), it should be possible to make some inference about likely current mortality rates on older sub-adults, too. There are a variety of ways that such *ad hoc* calculations might be done, and might be extended back to younger fish. Obviously, such calculations do not constitute a full assessment (see next section), but they do allow a sanity check in an environment where there is both great uncertainty and great concern about the status of SBT.

In the slightly longer term, estimates of N (and recent Z) can play a key role in conditioning whatever Operating Model gets developed for testing Management Procedures. (This is how we had originally envisaged the results being used, before the issue of overcatch was raised.) When there are as many dimensions of uncertainty as SBT now has, it is an exceedingly hard task to capture the range of plausible scenarios; there are very many "parameter" combinations are consistent with very uninformative data. Cutting down the "scenario space" is essential part of making MP-testing feasible.

The precision of $\hat{N}$ obviously has implications for how the results feed into management. As per **Wrinkles**, there are a number of model-related reasons why the CV will differ from the basic-case sample size calculations, but probably the dominant factor is that the real abundance might be considerably higher or lower than we have assumed. If the abundance is lower, then the CV will be improved. But even if the actual abundance turned out to be $10\times$ higher than in our sample size calculation, the basic-method CV would still be around 40%, and by tripling the juvenile sample using the "reserve pool", this could be brought down under 25%. This is pretty good precision compared to many fisheries measures, and by virtue of the quadratic efficiency of the method, adding extra years of data will bring down the CV rapidly. This leads on to the question of whether a continuation of close-kin sampling, and an adaptation of the method, could play a larger role in assessment and

management of SBT.

5.4 Scope for future close-kin work

Given the early stage of our close-kin project, it is well beyond the scope of this paper to speculate too far into the future, but a little reflection suggests that close-kin abundance estimation for SBT might be even more useful as an ongoing assessment tool than as a one-off exercise. For one thing, the quadratic gain in efficiency with sample size means that CVs should drop rapidly with the accumulation of more years of data[16]. Second, with far more hits, it may become possible to track individual cohorts through the adult population in terms of their changing proportional contribution to annual juvenile production. Most importantly, though, there becomes a possibility of extending the model to cover earlier age classes. There are several ways this might be done, but conceptually at least, one easy way might be to use the close-kin estimates of absolute adult abundance by cohort (by looking at changes in $\hat{N}$ over time and against adult catches) to set the end-points of a VPA-style back-calculation using catches from pre-adult ages. In turn, this might allow assessment of age-specific selectivity without depending on a relative abundance index. As with the various wrinkles discussed in this paper, the best way to do all this in practice would probably be through an "integrated" likelihood-based assessment model. In that context, it is important to note that the the close-kin estimate provides not just an absolute estimate of abundance, but also an absolute estimate of precision (unlike, say, a CPUE index).

More speculatively, a sampling programme for close-kin genetics could also open up some quite different opportunities. For example, the CCSBT's program of conventional tagging of juveniles could be supplemented by a juvenile biopsy program as part of genetic mark-recapture. The several thousand juvenile samples that could be genotyped annually from Port Lincoln as part of a close-kin adult-abundance estimation, would be a no-cost source of recaptures; one would test the samples against the original biopsy "tags" for individual matches (not close-kin). Additional expense would be incurred in genotyping the "tagged" fish, and obviously the recapture rate would be much lower than is *potentially* achievable with conventional tags, because only a small fraction of the penned fish are genotyped in the close-kin project. On the plus side, though, there would be no problem with unknown/variable reporting rates, nor any need for tag-seeding. And if sample collection from the longline fleets could also be arranged, this would open up even more possibilities for genetic mark-recapture.

5.5 Conclusions

In this paper, we have described how a close-kin abundance estimate for adult SBT can be obtained, and outlined our progress to date. There are a number of important details of sampling and analysis that need to be taken care of, in order to avoid issues that could lead to biassed or hopelessly imprecise estimates. As far as we can foresee, though, just about all these potential issues can be addressed

[16] Eventually this levels out, of course, when there are too few of the original adults left alive to score hits against. By then, though, the focus is no longer on a single estimate of adult abundance, but rather on a time series.

using data that will be available either now or during the project. The sample sizes required seem unlikely to be exorbitant, and the genetic feasibility has been established.

Any proposed new method will, of course, have caveats attached to it until the results are in. However, it is important to bear in mind the problems of all the other data sources on SBT. In comparison, the close-kin estimate (albeit of a limited part of the age range) rests on rather few assumptions. As well as providing fairly swiftly a one-off estimate to calibrate operating models and serve as a bottom-line comparison for catch rates, a close-kin sampling program might even constitute an important part of future management procedures for SBT.

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6 Appendix: mathematical justifications

6.0.1 CV calculations

"Baseline" case The expected number of hits is $2m_J m_A/N$ and the maximum possible is $2m_J$ if both parents of every juvenile occur in the sample of adults. The true distribution of H is hypergeometric, assuming all comparisons are independent, but since presumably $N \gg m_A$ (i.e. only a small fraction of the adults are sampled) a Poisson approximation will be fine. Hence the CV of H will be $\sqrt{N/(2m_J m_A)}$, and since $CV(X) \approx SE(\log X)$ and $\log \hat{N} = const - \log H$, this will also apply to $\hat{N}$. To minimize this CV for a fixed total sample size $m = m_J + m_A$, it is most efficient to set $m_J = m_A$, giving

$$CV\left(\hat{N}\right) = \frac{\sqrt{2N}}{m}$$

When there is an unequal sex ratio in the adults, but sampling is in proportion to the sex ratio, then the CV is unaffected. When the sex ratio in the sample is different to the sex ratio in the adult population (which then has to be estimated), the CV worsens slightly. For SBT, spawning ground catches are about 2:1 female:male, and assuming a true 1:1 sex ratio, the CV would increase by a factor of about 1.05. Calculations for these are as follows:

Population proportion known and equal to sample proportion Let p be the proportion of females in the adult population, so that the abundance of adult females is Np and the sample size of adult females is $m_A p$. Then H_f, the number of hits to adult females, is approximately Poisson distributed with mean $m_J(m_A p)/(Np) = m_J m_A/N$. The variance of $\hat{N}|h_f$ is approximately $N^3/(m_J m_A)$, by the Delta-method. An independent estimate can be constructed from the males; note that H_m has exactly the same expectation, so $\mathbb{V}\left[\hat{N}|h_m\right] = \mathbb{V}\left[\hat{N}|h_f\right]$. Since we have two independent estimates with the same variance, we can average them to obtain an overall estimate with

$$\mathbb{V}\left[\hat{N}|h_f, q, p = q\right] \approx \frac{N^3}{m_J m_A}\frac{1}{2} \tag{2}$$

The corresponding CV is the same as for the baseline case.

Population proportion known but not equal to sample proportion Suppose $q \neq p$ is the proportion of females in the adult sample. Similar reasoning shows that

$$\begin{aligned} \mathbb{V}\left[\hat{N}|h_f, p, q\right] &\approx \frac{N^3}{m_J m_A}\frac{p}{q} \\ \mathbb{V}\left[\hat{N}|h_m, p, q\right] &\approx \frac{N^3}{m_J m_A}\frac{1-p}{1-q} \end{aligned}$$

and the inverse-variance-weighted combination has

$$\mathbb{V}\left[\hat{N}|h_m, h_f, p, q\right] \approx \frac{N^3}{m_J m_A}\frac{p\,(1-p)}{q\,(1-p)+p\,(1-q)} \tag{3}$$

For the cases $p = q$ and $p = 0.5$ (any q), the right-hand fraction is still 0.5.

Population proportion unknown but not equal to sample proportion We have $\mathbb{E}\left[H_f\right] = m_J m_A q/\left(Np\right)$. If p is unknown, the best we can do is estimate the product Np from the females, and $N\left(1-p\right)$ from the males, and then add the two to estimate N. Using the Poisson approximation together with the Delta-method, we get

$$\mathbb{V}\left[\hat{N}|h_m, h_f, q\right] \approx \frac{N^3}{m_J m_A}\left(\frac{p^3}{q} + \frac{\left(1-p\right)^3}{1-q}\right) \tag{4}$$

The extra uncertainty compared to equation (3) arises from having to estimate p. For SBT, $q \approx 2/3$, and for $p = 0.5$ (from catch data on adults outside the spawning season) q, the term in brackets is $\sim$0.56, compared with 0.5 used in our sample size calculations. The sex bias will therefore inflate the real CV by a factor of $\sqrt{0.56/0.5} \approx 1.05$.

6.0.2 Sampling delays and probability of being a parent

Suppose we have one juvenile born in year 0 and a sample of adults taken in year y, all of whom were mature when the juvenile was born. What is the probability that one particular adult from the sample will be a parent of the juvenile? Let ℓ be the length of this adult in year 0 (probably inferred from its length in year y), let P be the event that this adult was a parent of that juvenile in year 0, and let Y denote the event that this adult occurred in the adult sample y years after that juvenile's birth. Note that y implies that the adult first survived for y years, and was then captured in the adult sample.

$$\begin{aligned}\mathbb{P}[P|\ell,Y] &= \frac{\mathbb{P}[Y|\ell,P]\,\mathbb{P}[P|\ell]}{\mathbb{P}[Y|\ell]} \\ &= \mathbb{P}[P|\ell]\,\frac{\mathbb{P}[\text{survived to } y|\ell,P]\,\mathbb{P}[\text{sampled in } y|\text{survived to } y,\ell,P]}{\mathbb{P}[\text{survived to } y|\ell]\,\mathbb{P}[\text{sampled in } y|\text{survived to } y,\ell]}\end{aligned}$$

Assume that:

1. survival probability (conditional on length) is independent of whether the adult was a parent of anything in year 0, and
2. sampling probability (conditional on length) in year y is independent of parental status in year 0.

Then the P-conditionals on the top of the fraction are irrelevant, and we have

$$\mathbb{P}[P|\ell,y] = \mathbb{P}[P|\ell]$$

so that year-of-sampling is irrelevant (conditional on length)[17].

The first assumption is probably reasonable for adult SBT, since their annual survival is pretty high— the differential mortality (fishing+natural) associated with spawning or not spawning in a particular year is likely low. The second assumption could be violated if, for example, there was a 2-year breeding cycle; the probability of being in the sample would alternate between low and high according as y was odd or even. This possibility is addressed next.

6.0.3 Multi-year breeding cycles

Clearly, if adults have a two-year breeding cycle, bias will occur if we sample in only one year on the spawning ground and the juvenile ground; either there will be too many matches compared to a no-cycle population of the same adult size, or too few.

To show that bias disappears if we sample a population with a two-year breeding cycle over two years, suppose we sample m_J juveniles and m_A adults overall, split evenly over each of the two years, with a single age-class of juveniles sampled in each year. Crucially, we must also assume that only adults who are going to spawn in a given year will turn up in the adult sample for that year— this is true for SBT. where all fish that have been checked histologically on the spawning grounds have been in spawning condition. When both years' data are analysed, the Year 1 juveniles will match only against Year 1 adults, and the Year 2 juveniles will match only against Year 2 adults. Suppose there are N_1 adults in the "odd-year spawning group" and N_2 adults in the "even-year spawning group"; then

[17] If we did not condition on length, then the fact that the fish was a parent in year 0 provides some information on the size of the fish, and thus on its subsequent survival probability, so the conclusion would no longer be valid.

	Number sampled				Expected number of hits	
Year	Jc1	Jc2	Ac1	Ac2	Same cycle	Other cycle
1	$m_J/2$	0	$m_A/2$	0	$2\left(m_J/2\right)\left(m_A/2\right)/N_1 = m_A m_J/\left(2N_1\right)$	0
2	0	$m_J/2$	0	$m_A/2$	$2\left(m_J/2\right)\left(m_A/2\right)/N_2 = m_A m_J/\left(2N_2\right)$	0
Total	m_J		m_A		$m_A m_J\left(1/N_1 + 1/N_2\right)/2$	

Table 1: Wthin- and between-year hits, given a two-year breeding cycle

For simplicity, suppose that $N_1 \approx N_2$; this is reasonable for a long-lived species where the odd & even breeding groups are made up of multiple cohorts, as with SBT. The total expected number of matches becomes $m_A m_J/N_1 = 2m_A m_J/N$ where the total adult population is $N = 2N_1$. Compare this with sampling m_J & m_A from a freely-interbreeding population of adult size N in a single year; the expected number of hits is again $2m_A m_J/N$. Hence there is little bias as long as we sample both years.

Actually, there is a slight bias arising from the difference between arithmetic means and harmonic means for the odd- and even-year spawning groups. This should not be large for SBT, where so many age classes contribute to spawning. In any case, if the breeding pattern is so clear, it will be possible to detect it, by following cohorts of juveniles and seeing whether they match predominantly against adults sampled in particular years, and then to fit two separate models to remove the arithmetic-harmonic effect.

With a three-year study and a two-year cycle, bias would reappear. However, if the pattern is clear enough to cause bias, it will also be clear enough to detect; matches will only ever occur between samples collected across a gap of a fixed number of years. A more complicated cyclic model could then be constructed.

6.0.4 Number of loci required in two-phase testing

The need to eliminate a high proportion of samples as "definitely non-relatives" in the first pass, sets a stringent limit on p_1, the probability of an accidental hit on the first pass. To eliminate say 90% of samples, we need a 90% probability that a given juvenile will not match *any* of the m_A adults by chance, so that $(1-p_1)^{m_A} = 0.9$. In general $p_1 \approx (1-X)/m_A$ where X is the proportion to be ruled out on the first pass; for m_A of a few thousand and X of around 0.9, this means choosing L_1 to achieve a p_1 on the order of 10^{-5}. Given a set of loci and their allele frequencies, computation of p_1 (the probability that two unrelated individuals will share at least one allele at every locus) is a straightforward exercise in genetic probabilities. Some consideration should really be given to scoring error, which makes the computation more tedious.

6.0.5 Age-specific catchability

The relative fecundity of an SBT aged a is determined by four factors:

$$\text{rel fec}_a \propto \text{average residence time}_a \times \text{spawning frequency}_a \times \text{batch fecundity}_a \times \text{viability}_a$$

Note that average residence time itself has two components: the probability of actually turning up in any given year, times the average residence time given that the fish turns up. There is no need to separate the two for abundance estimation purposes, so we just deal with average residence time.

Of these four factors, we neglect any age-effects on viability[18]. The other three factors determine the number of eggs produced. Previous histological work can be used to estimate the relative spawning frequency while present on the grounds, and the batch fecundity[19]. Therefore we can write

$$\text{rel fec}_a \propto \text{ave res time}_a \times \text{rel eggs per day}_a$$

where relative-eggs-per-day$_a$ is estimated externally. The probability of capture on the spawning grounds is also proportional to average residence time[20]. Until and unless enough archival tag data is found, we do not have any external estimates of average-residence-time$_a$, but these turn out not to be necessary for estimating N. To show this in principle, we will assume (purely for simplicity of presentation) that numbers-at-age, average residence time, and relative eggs per day are all negative exponential functions of age, with coefficients z, r, and g respectively. Then we can perform two "catch curve" analyses, as follows:

1. Use the log-slope of the age profile of adults in the spawning ground to estimate $z + r$.
2. Use the log-slope of age for *identified parents* to estimate $(z + r) + (r + g)$. The first term arises because older fish are more likely to be sampled, and the second because they generate more eggs. Note that there is at least a 2-year gap between spawning and being identified as a parent, since juveniles are not being sampled until they are age 2; hence there is no need to worry about adult-sampling removing potential spawners.
3. The difference between the two log-slopes is therefore an estimate of $r + g$. Since we have an external estimate of g, we can also estimate r.
4. Subtract the estimate of r in (3) from the estimate of $z + r$ in (1) to get an estimate of z.
5. The expected number of hits is $m_J m_A \mathbb{P}[\text{hit}]$. For notational simplicity, define the age-of-maturity as 0, with N_0 animals at that age and N adults in total. Then we have

[18] Whether or not this is correct, it is standard practice e.g. for calculating SSB. In fact, SSB calculations typically just assume that juvenile production is proportional to bodyweight, whereas we "go one better" by estimating the relationship empirically.

[19] For females, batch fecundity is proportional to $W^{2.4}$ where W is body weight. This is based on the change in gonad weight before and after a spawning event. For males, a slightly different approach is needed, based on absolute gonad weight, but the general idea is similar.

[20] A small further source of variability arises from fish of different sizes having different depth frequency distributions while on the spawning grounds, and thus potentially having different catchabilities per unit time. This is tied in with the estimation of spawning frequency earlier on. However, the effect (examined in Davis et al., 2003) is not large.

$$
\begin{aligned}
\mathbb{P}\left[\text{hit}\right] &= \sum\nolimits_{a\geqslant 0}\left(\text{propn}-\text{sampled}-\text{adults}-\text{aged}{-}a\right)\times\mathbb{P}\left[\text{adult}-\text{aged}{-}a{-}\text{is}-\text{my}-\text{parent}\right] \\
&\quad \sum\nolimits_{a\geqslant 0}\left(\text{propn}-\text{sampled}-\text{adults}-\text{aged}{-}a\right)\times 2\times\frac{\#\text{eggs}-\text{from}-\text{an}-\text{age}{-}a}{\text{total}-\#\text{eggs}-\text{released}} \\
&= 2\sum\nolimits_{a\geqslant 0}\frac{e^{(r+z)a}}{\sum_{a'\geqslant 0}e^{(r+z)a}}\times\frac{e^{(g+r)a}}{N_0\sum_{a'\geqslant 0}e^{(g+r+z)a}} \\
&= \frac{2}{N_0}\frac{\sum_{a\geqslant 0}e^{(g+2r+z)a}}{\left(\sum_{a\geqslant 0}e^{(r+z)a}\right)\left(\sum_{a\geqslant 0}e^{(g+r+z)a}\right)}
\end{aligned}
$$

For ease of exposition, replace the sums by integrals, and note that

$$
N=\#\text{adults}=N_0\sum\nolimits_{a\geqslant 0}e^{za}\approx N_0\int_0^\infty e^{az}da=\frac{N_0}{z}
$$

We then have

$$
\begin{aligned}
\mathbb{P}\left[\text{hit}\right] &\approx \frac{2}{N_0}\frac{(r+z)(g+r+z)}{g+2r+z} \\
&= \frac{2}{(N_0/z)}\frac{(r+z)(g+r+z)}{z(g+2r+z)} \\
&= \frac{2}{N}\frac{(r+z)(g+r+z)}{z(g+2r+z)}
\end{aligned}
$$

Note that if $r=0$ (i.e. no age-dependent catchability) then the right-hand fraction cancels to 1 whatever the value of g, and we retrieve the base-case formula. However, if $r\neq 0$, we do need to know g.

6.0.6 Population substructure and sampling bias

Suppose the entire adult population of N is made up of two sub-populations with proportions π and $1-\pi$, and that adults are sampled proportionally from their respective sub-population, so that the overall adult sample contains $m_A\pi$ fish from the first sub-population and $m_A(1-\pi)$ from the second. Juveniles, though, are not necessarily sampled in proportion to sub-population abundance; let m_{J1} and m_{J2} be the numbers sampled from each sub-population.

If the entire dataset is analysed without regard to sub-populations, then the expected number of hits can be calculated by considering samples from each sub-population separately (since there will be no cross-hits between juveniles from one sub-population and adults from the other):

$$\begin{aligned}
\mathbb{E}[H] &= \frac{2m_{J1}(\pi m_A)}{\pi N} + \frac{2m_{J2}(1-\pi)m_A}{(1-\pi)N} \\
&= \frac{2m_{J1}m_A}{N} + \frac{2m_{J2}m_A}{N} \\
&= \frac{2m_J m_A}{N}
\end{aligned}$$

just as in the case without sub-populations. In other words, the basic estimate is unbiassed provided at least one life-stage is sampled in proportion to sub-population abundance. If both are sampled disproportionately, though, there will be bias.