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Mark Bravington and Campbell Davies

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Mark Bravington, Campbell Davies

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Abstract

ORIGINAL: Close-Kin data are now playing a key role in SBT assessment, partly as a fishery-independent abundance index but also providing direct information on reproductive-output-at-size and on survival. The expensive part was getting this far: developing suitable genetic markers, and genotyping enough samples to get a useful number of Parent-Offspring Pairs. A continued program would have much lower running costs, because fewer samples are now needed to yield each POP (thanks to the existing genotype pool and the "quadratic magic" of CK). It could provide a fishery-independent time series of of SSB (the focus of the rebuilding plan), also helping to narrow down the range of plausible scenarios considered in the OM. It is therefore worth thinking about the detailed options for a continuation CK program, in terms of:

#1: what CVs on what quantities would result from different designs

#2: the pros and cons of changing the suite of genetic markers (i.e. away from microsats to one of the newer, less labour-intensive, and hopefully cheaper platforms based on SNPs).

REVISED: The SBT Close-Kin project has successfully delivered fishery-independent estimates of adult abundance, reproductive output as a function of size, and adult survival. The CCSBT Scientific Committee has agreed the close-kin data should be incorporated into the OM. There are efficient procedures for obtaining samples, considerable know-how on the genetics processing and quality control and, crucially, a substantial catalogue of genotyped (2006-2010) and unprocessed samples (2007-2013). It would be possible to extend the program to provide a time series that directly tracks SSB, the goal of CCSBT's interim rebuilding plan. Thanks to the existing genotypes, and the "quadratic magic" of Close-Kin, future sampling and genotyping levels (and cost) could be considerably lower than in the past while still yielding precise abundance estimates; the precision of other important quantities, such as the effect of body size on reproductive output, would also improve with an extended time series of data. Turnover of adults due to mortality means that after some years the existing bank of samples will become irrelevant to estimating the then-current adult abundance; while this is not an immediate issue, it does suggest that an ongoing low-level program might be more sensible than restarting a Close-Kin program from scratch after a long gap. This paper discusses some of the options and issues in more detail, showing in broad terms how the information content of samples varies with date of collection and length of study. There appears to be merit in detailed investigation of cost-effective options for an ongoing close-kin program to monitor the spawning stock directly and to provide valuable abundance indices and other parameters for the OM. Such a study should consider: the interaction between the close-kin and other data sources in the OM, what precision will accrue to which parts of the OM, the balance of sample sizes between adults and juveniles, the value of genotyping existing archived samples (2006-2012), the cost-effectiveness of alternative genetic markers, and potential cost-savings if sample collection and genotyping were shared with a gene-tagging program.

1 Introduction

The CSIRO-FRDC SBT Close-Kin (CK) project has successfully delivered fishery-independent[1] estimates of adult abundance, reproductive output as a function of size, and survival. The Scientific Committee has agreed that the CK data should be incorporated into the OM (Anon. [2012]), and this process is well under way with promising results thus far (Hillary RM and M [2012], Hillary RM and C [2013], Anon. [2013]). There are existing frameworks for sampling in Indonesia and Australia[2], considerable know-how on the genetics (which is far from a push-button exercise) and, crucially, a substantial "bank" of genotyped samples. Given the success of SBT CK so far, the point of this paper is to stimulate discussion about its possible roles in future monitoring and assessment of SBT.

Since CK has already delivered a one-off estimate that is fairly precise, plus important biological information (Relative Reproductive Contribution by Body Size - RRCBS) that changes the interpretation of key quantities in the OM, it would be possible to just stop. But it would be remiss to do so without first considering what could be gained from continuing the program at some level to generate a time-series of abundance estimates[3]— particularly since the goal of CCSBT's interim rebuilding plan is adult stock size, for which CK is the only SBT data stream that can provide direct information.

[1] I.E. without using total catch or CPUE data. However, it does use Indonesian length and age frequency data, which require fewer assumptions to interpret.

[2] However, these frameworks are not currently supported with ongoing funding for continued collection of tissue samples.

[3] In practice, CK data would presumably be used within the OM for this, rather than on its own; however, the adult abundance estimates would be mainly driven by the CK data.

If CK does continue, it will need more genotyped samples. There are two ways: use existing archived samples, and/or collect new ones. As to the former: although sample collection as part of the CSIRO-FRDC SBT CK project ended in 2010, CSIRO and the Australian SBT Industry Association have continued from 2011 through 2013 to fund collection of tissue samples from adults (via the Benoa monitoring program in Indonesia) and juveniles (at harvest in Port Lincoln). These samples have been collected and archived (the cheaper part) but not genotyped (the more expensive part). Also, there are also several thousand archived samples from 2007-2010 that were collected but not genotyped during the CSIRO-FRDC project[4]. A subset of archived samples could be genotyped to improve precision in the short term. However, in the medium term, some kind of ongoing collection and genotyping of up-to-date samples would be necessary in order to use CK to monitor rebuilding. There are currently no plans to do so.

CK is at heart a mark-recapture exercise, and as such it is able to "re-use" and get extra value from the existing bank of genotyped samples by comparing them with future samples. (This is in contrast to direct or indirect density-estimation exercises, such as line-transect surveys and CPUE, where every year starts as a fresh blank sheet.) For this reason, future sampling levels (and costs) could be considerably reduced compared to those for the CSIRO-FRDC project; it will not require nearly so many new samples to yield one Parent-Offspring Pair (POP) as it did when the project began. Exactly how much sampling would be required is not obvious, though, and this paper sets out some of the points to be considered.

2 Rate of detection of Parent-Offspring-Pairs (POPs)

The key point in in the design of CK is how many POPs are detected, since the main driver of the CV on adult abundance is simply Poisson variability in the POP count. Specifically, if P POPs are found then the CV has to be at least $1/\sqrt{P}$. The study was originally designed with an aim of finding 70 POPs, giving a CV of at least $1/\sqrt{70} \approx 12\%$. In fact, the stock size turned out to be much higher than the 2005 point estimate which had to be used in the original design, meaning that we needed to genotype more samples than planned in order to get a statistically respectable number of POPs. In the end, even though the study took longer than planned because of the extra sampling and genotyping, it actually stopped "early", in the sense that the number of POPs eventually found was 45, well below the original target of 70.

With 45 POPs, the CV cannot be less than $1/\sqrt{45} \approx 15\%$. There are additional sources of uncertainty, but these turned out to be fairly trivial in comparison (at least for abundance estimation), causing the overall CV[5] to rise only to 17%[6]. The total number of POPs arises from summing single comparisons between each adult and each juvenile, each of roughly equal probability, so in principle there is a quadratic increase in the number of POPs found as the sample size increases. This is eventually mitigated by individual growth and population turnover (see Bravington et al., 2013), which affect the "roughly equal probabilities". Given these mitigating effects, it is interesting to see how samples from different years are now contributing to the expected total number of POPs.

Table 1 shows the relative chance of finding a POP when comparing an adult to a a juvenile, as a function of when the two samples were caught[7]. As above, more POPs mean lower CVs, so the numbers in the Table are a reasonable reflection of relative sample informativeness or "power" (on an arbitrary scale). Since every juvenile sample is compared with every adult sample[8], we can sum across columns and/or rows weighted by sample size to get an idea of *relative* power over time, and to show e.g. how the power of a sample collected "now" increases over time as newer samples are collected to compare it with.

The most relevant features of Table 1 are:

- Currently, a new adult sample is slightly more powerful than a new juvenile sample (74 vs 70; i.e. adults are better value, but not much). The most valuable sample right now, in terms of POPs per dollar, would be an adult from 2008 (but all adult samples from that year have already been genotyped). As time progresses, then "the most valuable sample" will change.

- Each new year of sampling is not just useful in its own right, but also empowers all the existing samples because they can be compared against the new ones. Consequently, the overall power in 2010 (as measured e.g. by summing the WSUM row) was about 43% higher than in 2009 (not shown, but computable), despite there only being about 20%

[4] All our adult samples have already been genotyped, because they were scarcer than juvenile samples and therefore had higher "information content".

[5] This is the CV on mid-study adult abundance from the "mini-assessment" first presented in CCSBT-ESC/1208/19, and then adapted intersessionally to allow for (i) random variability in recruitment, (ii) internally-estimated overdispersion in Indonesian length-frequency data, and (iii) uncertainty in the analysis of daily-fecundity-at-size.

[6] These CVs are conditional on a particular structural assumption about adult selectivity in Indonesia: that it is directly proportional to residence time. A stand-alone CK assessment requires such an assumption. However, there may be additional uncertainty related to aspects of adult selectivity beyond residence time, which cannot be reduced just by collecting more samples for CK; a different type of data would be needed. However, early results from the CK-adapted OM indicate that making different assumptions does not actually change the CV that much.

[7] For clarity in a sampling-design context like this, the years shown in Table 1 are sampling-years, rather than juvenile *birth*-years as shown in, for example, Figure 4.3 of CCSBT-ESC/1208/19. Almost all genotyped juveniles were born 3 years prior to sampling.

[8] Subject to genetic quality, and to feasibility of juvenile-birth-date relative to adult-capture-date.

Table 1: Comparison of the informative power of samples from different age-groups and years

		2006	2007	2008	2009	2010	WSUM
	Ju\Ad	214	1457	1526	1394	1164	
2006	1523	13	11	9	8	6	50
2007	1707	15	13	11	9	8	61
2008	1448	18	16	13	11	10	74
2009	1338	0	18	16	14	12	85
2010	1432	0	0	19	17	15	70
WSUM		71	85	102	87	74	

Juvenile capture-years run down; adult capture-years run across. The Ju\Ad row and column show sample sizes. Entries in the central rectangle are proportional to expected POPs-per-comparison; the zeros in the lower left are because an adult cannot breed after death. Entries in the WSUM column are proportional to expected POPs for *all* comparisons between one specific juvenile sample in that year and all the adult samples. Similarly, entries in the WSUM row are proportional to the total expected POPs involving one specific adult sample from that year and *all* juveniles.

extra samples. Evidently, SBT CK is still benefitting from "quadratic magic": the increased number of comparisons arising from new samples is still comfortably outstripping the turnover of adults.

- Thus, if 2010 sampling levels were to continue, CVs would continue to improve rapidly. Stabilization will eventually occur when the top-right corner start filling with 0's, say in around 2017 at 2010 sampling levels. By then, because of adult turnover, a CK study would be providing information about a different set of adults than when it started—i.e. it would be directly tracking changes in the spawning population.

It is a little difficult to be "precise about precision" here since, as the time-series of samples lengthens, so does the number of incoming recruitments, and the question arises of precisely what we want to be precise about. To date, the dominant issue has simply been getting enough POPs to be able to make any kind of statistically reasonable estimate of anything, but eventually the information in those POPs becomes spread out across different parameters, so "the CV" depends on what is being considered. For example, SSB at the start of the study eventually becomes mathematically independent of SSB at the end, because of demographic turnover. Under steady CK sampling levels, CV of SSB at the start of the study will decrease (i.e. improve) for a while but will eventually level out because of turnover, whereas CV of the *trend* in SSB will improve faster and for longer[9], and the same goes for adult survival rate. It is not obvious what would happen to the CV of, say, SSB in last year sampled. Nor is it obvious how turnover and accumulation of POPs would affect the CV of other static parameters such as RRCBS, whose CV has not even been calculated from the data so far: it will certainly get more precise as more samples are added, but how quickly and with what asymptote? In the longer term, uncertainty about RRCBS might turn out to be important for tracking rebuilding, because the "spawning stock" is effectively the adult size composition weighted by the RRCBS, and the age (and thus size) composition of the adult stock is sure to change over the next decade.

The question "CV of what" warrants careful thought, as it naturally has implications for the utility of CK data in the OM, and in considering performance indicators for monitoring rebuilding of the stock.

3 Interaction with gene-tagging

A CK program could run independently of gene-tagging (GT), just as it has to date. However, there is some opportunity for synergy, because GT would require genetic samples from a large number of juveniles (much larger than CK has used or would need in future). This *might* both eliminate the need for separate CK juvenile samples, and reduce the number of adult CK samples required. The key point with CK is to keep the total number of adult-juvenile comparisons high. For a given total sample size, this is most efficiently done by sampling similar numbers of adults and juveniles, but it could also be achieved by sampling a much larger number of juveniles (which GT could provide "for free") together with a reduced number of adults.

Certainly, a GT program might provide CK with juvenile tissue samples and extracted DNA for free. However, it is not certain at this stage to what extent GT would also provide "free genotypes" for CK; it depends on which genetic markers, and how many, are used in GT. Since IDing an individual requires many fewer markers than IDing POPs, and the number of markers needs to be kept low for cost reasons, additional genotyping of a subset of the GT samples might be required before those samples could be used for CK. The details still need to be worked out, but at this stage we can distinguish best-case and worst-case scenarios:

[9]Longer periods are always better for estimating trends, because the cumulative effect of the trend is greater, at least until it becomes necessary to worry about changes the trend itself.

Best case: the GT markers on juveniles are powerful enough to exclude most of the juveniles from being in POPs with any of the (fully-genotyped) CK adults. Only the remaining potential-POP juveniles need extra genotyping to determine whether tey are POPs or not, and since few fish are involved, this would be cheap. Also, because the juvenile sample size would automatically be much larger than a CK study would need, the adult sample size (and cost) in the latter could be proportionally reduced compared to the requirements of a stand-alone CK program. In that case, the marginal running cost of CK, needing only some hundreds of adults per year, would be negligible.

Worst case: the GT markers are insufficient to exclude most of the juveniles as POPs. In that case, it will be most efficient just to select an arbitrary subset of the unexcluded GT juveniles for CK purposes, the same number that would be needed in a stand-alone CK program, and genotype type them at extra loci[10]. The number of adults required would be the same as in a stand-alone program. Nevertheless, by virtue of the GT program having collected and DNA-extracted and partially genotyped the "CK juveniles", the CK program would be spared a good fraction— say one-half— of its juvenile-related costs. This would reduce the overall CK running costs by maybe one-quarter compared to a stand-alone program.

Note that any synergy is largely one-way: CK itself is of no direct benefit to GT, except of course through the genetic know-how and infrastructure already developed.

4 Discussion

SBT CK has worked well, and/but quite a lot of money has been spent to get it to this stage. The total cost so far has been about the same as two GAB aerial surveys, with about half going on development (which would not need to be repeated) and about half on "routine processing" (which would have to continue if further samples were genotyped). The main benefits and issues associated with continuing some kind of CK program are:

- CK offers a direct way to monitor the projected rebuilding in the spawning stock— the goal of CCSBT's interim rebuilding plan. It is the only abundance index currently available to do this.
- Because of the CK back-catalogue, "precision per sample" is still going to improve substantially over the next 5 or so years. Therefore, sampling levels could presumably be reduced over this period while still maintaining precision on current abundance. Also, the longer the time-series, the better the precision on trend and on adult survival, something that is again not available from any other current monitoring series.
- Notwithstanding the value of the back-catalogue, CK samples do have a "sell-by date" because of adult turnover. Waiting, say, 5 years and then restarting a CK study would (aside from possible loss of human expertise in the genetics) waste much of the capital invested in the existing samples.
- Collecting and archiving samples is cheap (about $30K PA at 2010 levels). Maintaining the collection programs seems a sensible interim measure. If you collect and archive a sample, you can always decide later not to genotype it, thus saving most of the expense; but if you don't collect it in the first place, there is no chance to change your mind later.
- To figure out a cost-effective[11] CK strategy (if any) for the future, a design study would be required, addressing *inter alia*:
  - the interaction between CK and other data in the OM
  - what precision will accrue to different parts of the "assessment"
  - sampling levels in Indonesia and Port Lincoln
  - the value of genotyping existing archived samples from 2007-2012
  - choice of genetic markers (there may now be cheaper options than when the study began)
  - possible cost savings if run in conjunction with a gene-tagging program.
- Now that the OM has a natural mechanism for handling CK data, such a study could be run fairly easily by using the OM machinery to simulate future population dynamics and data, and the CK mini-assesssment (which runs much faster than the OM) to estimate resulting CVs.

[10] The already-excluded ones would still "count", but would not need extra genotyping.
[11] I.E. in terms of CV obtained

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