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Report on gene-tagging design study

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Abstract

A gene-tagging project for juvenile SBT has been proposed as part of the CCSBT Scientific Research Program 2014-2018. Gene-tagging can provide estimates of absolute abundance, fishing mortality and natural mortality of the cohort(s) being tagged, depending on the design of the project. Our current focus is on obtaining estimates of juvenile abundance, as a potential replacement for the scientific aerial survey and to reduce reliance on CPUE. The estimates of juvenile abundance can be used in the SBT operating model (OM) and, potentially, future management procedures (MPs). In 2015 all SBT recruitment monitoring ceased, including the aerial survey, which is essential for the adopted MP, and therefore finding new cost-effective methods is now critical.

Gene-tagging is similar to conventional tagging but uses the genetic fingerprint of a fish in place of plastic spaghetti tags. Genetic fingerprints from the catch tissue samples are compared to those from tagging tissue samples, and an exact match is analogous to a conventional recapture. The key advantages of a gene-tagging program are that it overcomes the reporting rate issues that led to the cessation of the CCSBT conventional tagging program in 2006 (Anon. 2007; Davies et al. 2007; Harley et al. 2008; Davies et al. 2008), the tag is invisible and lasts (essentially) forever, there is no tag shedding and no tag reward costs. There is, however, still the need to tag and recapture thousands of individuals, which requires dedicated sea time and careful experimental design.

This report discusses and refines the experimental design most appropriate for a pilot gene-tagging program to estimate abundance of recruits, following the preliminary work in Preece et al. (2013). The aim of the pilot study is to test the feasibility and logistics for the simplest gene-tagging design. We propose that in the pilot gene-tagging experiment, tag samples be taken from age 2 fish in the Great Australian Bight (GAB) during the summer of a given year, and that recapture samples be taken from age 3 fish the following year at farm harvest from fish that were caught in the surface fishery earlier that year. The tag and recapture sample sizes required to achieve a CV of 25% in the age 2 abundance estimate depend on the actual size of the age 2 population, and whether overdispersion is allowed for, and are in the order of 5000 for the tagging sample and 6500-14000 for the recapture sample. Costs for this pilot study have been updated with the most recent information. We also consider potential issues that may need to be addressed to obtain unbiased parameter estimates. Most of these are not specific to gene tagging, but apply to any tagging program for SBT. Extensions to the basic design, such as tagging and resampling fish from regions outside the Great Australian Bight (GAB) and tagging and resampling multiple age classes, should be considered after the initial logistics have been tested and demonstrated to be cost-effective in the pilot tagging study.

Gene-tagging has the potential to provide fishery independent estimates of the juvenile abundance for use in the SBT OM and potentially in future MPs, and would be a highly informative and potentially cost-effective method for recruitment monitoring. Integration of gene tagging abundance estimates into the SBT OM is demonstrated and potential future use in MPs is discussed.

1 Introduction

A gene-tagging program for juvenile SBT has been proposed as part of the CCBST Scientific Research Program 2014-2018 as a potential alternative source of information on recruitment and juvenile mortality. Gene-tagging is similar to conventional tagging but uses natural gene-tags (the genetic fingerprint of a fish from a tissue sample) in place of plastic spaghetti tags. Analogous to conventional tagging, gene-tagging has the potential – depending on the design of the study – to provide estimates of absolute abundance, fishing mortality and natural mortality of the tagged cohort(s) (Polacheck et al. 2010; Davies et al. 2007; Davies et al. 2008; Preece et al. 2013). The key advantages of a gene-tagging program are that it overcomes the reporting rate issues that led to the cessation of the CCSBT conventional tagging program in 2006 (Anon. 2007, Davies et al. 2007; Harley et al. 2008; Davies et al. 2008,the tagging is invisible and lasts (essentially) forever, there is no tag shedding and no tag reward costs. These and other advantages, as well as potential disadvantages, of using gene-tags versus conventional tags are discussed below in the "Background on gene-tagging" section.

Considerable work went into the design and planning of the 2001-2006 CCSBT conventional tagging program, based on over 40 years of tagging experience on SBT, and we have drawn heavily on that collaborative work and expertise (Anon 2001a; Polacheck et al. 2001; Itoh et al. 2007; Davies et al. 2007). The aims of that program remain as the motives for the current proposed gene-tagging program: to improve the information base for assessing the SBT stock and to reduce the dependency upon CPUE (e.g. Anon 2001b; Anon 2007).

The focus of the gene-tagging program design discussed here is recruitment monitoring, namely to provide estimates of juvenile abundance for use in the SBT operating model (OM) and future management procedures (MPs), and to monitor rebuilding of the SBT stock. In 2015 all SBT recruitment monitoring ceased, including the aerial survey, which is essential for the adopted MP, and therefore finding new cost-effective methods is now critical. Without the aerial survey, the operating models and, especially, the MP need another source of information on juvenile abundance. Gene tagging abundance estimates have several advantages over aerial survey estimates; they provide absolute rather than relative abundance estimates, provide an estimate for each tagged age class, rather than assumed proportions of several age classes (2-4), provide estimates in numbers rather than in weight (which needs to be converted to numbers in operating models), and are not affected by environmental conditions and observer ability.

In considering the design of a pilot program, we have restricted most of the discussion to the simplest and least expensive design that will give the minimum information required to monitor recruitment. The simplest design involves tagging and resampling a single cohort of fish (e.g. tagging fish from a given cohort at age 2 and resampling the same cohort a year later at age 3), to provide an estimate of absolute abundance for the tagged cohort at the time of tagging (i.e. age 2). Costs associated with this form of design with various sample sizes and assumptions were presented in Preece et al. (2013). Here we refine the experimental design of a gene-tagging program for an absolute abundance estimate of juvenile SBT and consider in greater depth the issues that may potentially bias the realised parameter estimates. Most of these issues are not specific to gene-tagging, but apply to any tagging program for SBT.

We demonstrate methods for integrating the gene-tagging data and/or derived abundance estimates in the SBT OM, and discuss their potential use in future MPs, possibly as a replacement to the aerial survey index of recruitment. Consideration is given to whether a less frequent than annual abundance index could be used in the OM or MP; for example, tagging in every second year would reduce costs compared to annual recruitment monitoring, but their use in a future MP would need to be evaluated in the context of the overall MP performance in meeting the Commission's rebuilding objectives.

This paper provides a framework for the pilot gene-tagging project where the aim is to test the logistical feasibility of gene-tagging SBT in the GAB. The field work for such a pilot project is currently included in the 2016 and 2017 CCSBT Scientific Research Program work plan. If the pilot study were to commence in January 2016, then results, including an abundance estimate, would be available in late 2017. Importantly, many of the logistic and technical feasibility issues would be better understood in time for discussion at the 2017 ESC and available for consideration in review of the MP (scheduled for 2017).

2 Objectives

The objectives of this design study are to refine the experimental design and approximate costs of a pilot gene-tagging program and, using simulated data, demonstrate methods for integration of the data into the SBT operating models, for consideration by the ESC.

3 Background on gene-tagging

Gene-tagging involves taking a tissue sample from a wild fish that is then released back into the wild. The tissue sample is genotyped at multiple loci to provide a unique DNA fingerprint. It is this unique DNA fingerprint that forms the gene-tag. Subsequently, tissue samples are taken from a subset of the catch and processed to determine their DNA fingerprint. The DNA fingerprints from the catch samples are compared to those of the tagged fish; an exact match is analogous to a conventional recapture.

In the past there has been a significant cost associated with DNA fingerprinting of tissue samples. However, these costs have reduced rapidly over recent years as new techniques and genetic markers have become available and from further optimisation and streamlining of procedures for quality control of the genotyping and identification of "matches".

Gene-tagging has several key advantages over conventional tagging:

- Gene-tags do not require reporting rate estimates, which are difficult and costly to obtain.
- Gene-tags are invisible and thus remove any onus for reporting by the processor/fishers, and therefore are fishery independent in this respect.

- The natural gene-tag does not shed and does not deteriorate during the life of each fish. The tag lasts the whole life of the fish, and the recapture sample can be taken at any time into the future.
- Taking a tissue sample (particularly with a specially designed tool – see below) is less invasive than spaghetti tagging and reduces the chance of tagging mortality.
- A vast amount of additional genetic data can be collected at little extra cost (e.g., there is potential for cost savings through overlap in data collection with the close-kin genetics program designed to estimate spawning stock abundance[1]).

Potential disadvantages of gene-tagging are:

- Cross-contamination of DNA between samples – colleagues at CSIRO have developed and tested a tissue sample tool that collects a clean tissue sample, free of cross-contamination, and suitable for large-scale field applications, which eliminates this issue to the greatest extent possible (Bradford et al. *in press*)
- High quality tissue samples and fingerprinting methods are required to avoid the risks of false negative or false positive matches. Genetic methods for matching an individual to itself with high certainty have been developed for SBT, and require far fewer loci than matching parent-offspring or other more distantly related pairs (as required for the close-kin project) (Bravington and Davies 2013; Bravington et al. 2015). Rigorous protocols for obtaining and preserving good quality DNA samples will be followed, but if the DNA for a particular sample is poor, the sample will simply be omitted from comparisons.
- If catch sampling is done at farm harvest or at market, information about a "recaptured" fish at the time it was caught in the wild (such as location and fish length), and thus about movement and growth, is unlikely to be available. However, there is potential for market samples to be linked to other data collection operations, for example through the CCSBT catch-documentation-scheme (CDS) data.
- If a design that provides estimates of fishing mortality and natural mortality is considered desirable (i.e. Brownie estimator), then catch-at-age (CAA) data are required because the proportion of the catch sampled for "recaptures" needs to be known/estimated. This is not the case for conventional tagging studies as the entire catch is assumed to be examined for recaptures, or for the design of a pilot gene-tagging program considered here (i.e. Petersen estimator).

[1] Gene tagging and close-kin abundance estimation methods are different, despite shared DNA profiling technology. They differ in the methods used for calculating abundance estimates, the type of parameters that can be estimated, and how genetic samples "tag" a fish (see Preece et al. 2013 for further explanation of these differences). The relevant links between the methods are: (i) the "technology infrastructure" (genetic markers and techniques), and (ii) the fact that a gene tagging program could provide tissue samples from juveniles for use in a close-kin estimate of spawning stock abundance for no additional cost.

4 Pilot gene-tagging design

4.1 Petersen design for estimating absolute abundance

As discussed in Preece et al. (2013), the simplest and lowest cost design for a gene-tagging program is for estimating absolute abundance of a tagged cohort of juveniles. In this case, a single cohort of fish is tagged at a point in time (i.e., at a particular age), and after a sufficient amount of time to allow for mixing with the untagged population, the cohort is resampled and the number of tagged individuals in the recapture sample is determined.

An estimate of cohort abundance at the time of tagging (N) is given by:

(1) $N = T * S/R$

where T is the number of fish in the cohort that were tagged, R is the number of tagged fish "recaptured" in the recapture sample, and S is the recapture sample size. Eq. (1) is often referred to as the Petersen (or Lincoln-Petersen) estimator of abundance (e.g. Seber 1982), so we will refer to this tagging design as the Petersen design.[2]

Assuming a Poisson recapture process, the coefficient of variation (CV) of the abundance estimate can be approximated by:

(2) $CV = \text{sqrt}(N/(T*S))$

$= \text{sqrt}(1/R)$

An important observation is that it is not necessary to account for mortality (natural or fishing) of "tagged" individuals between tagging and resampling because the total mortality of the tagged and untagged populations will be equal if the population is well mixed (see Preece et al. 2013 for a more detailed explanation).

In the previous conventional tagging studies for SBT, multiple cohorts were tagged over a number of successive years and resampled over several years – referred to as a Brownie design. This design allows for estimation of natural and fishing mortality (e.g. Anon. 2001a; Polacheck et al. 2006). This design is more expensive (because it involves tagging larger numbers of fish across multiple age classes and years) and, in the case of a gene-tagging study, has the drawback that the proportion of the catch sampled for "recaptures" needs to be known, or estimated (i.e., catch at age data are required). As such, we recommend that, for the pilot gene-tagging program, we use the Petersen design.

4.2 Design considerations

Specifics of a Petersen gene-tagging study that need to be decided are:

[2] Equation (1) is an asymptotically unbiased estimate for N, but can have large biases when sample sizes are small (see Seber 1982). Sample sizes expected under the SBT gene tagging program should be large enough that the biases would be small. However, bias-corrected estimators, such as that proposed by Chapman (1951) (namely, $N = (T+1)*(S+1)/(R+1) - 1$), can be applied and the results compared.

1. where and when to “tag” fish (by taking a tissue sample and releasing the fish alive),
2. where and when to take the “recapture” samples,
3. the age(s) at which to tag and recapture,
4. how many tag and recapture samples to take.

4.2.1 Tagging

Juvenile fish are most easily tagged in large numbers in the GAB and off Western Australia (WA) during the summer months (Jan-Mar), as demonstrated in the previous conventional tagging programs. This is because surface schools can be targeted with pole and line gear, which allows for large numbers to be caught, “tagged” and released with minimal handling time. In this case, tissue samples would be taken, by experienced taggers, on board a chartered pole and line fishing vessel, either after the main fishing season (mid Feb-Mar), or away from the main fishing operations, in order to reduce the number of very short term recaptures. Note that short-term recaptures are not identifiable with gene-tags (as they are with conventional tags), and would bias the abundance estimate if a substantial number of tagged fish were caught before having mixed.

In addition to pole and line releases, tagging could potentially be undertaken in the longline fisheries. This would require appropriately trained individuals on board to take the tissue samples and to ensure a reasonable distribution of releases. The condition of fish caught in the longline fishery is not always suitable for tagging, so only lively and uninjured fish could be tagged and released. If this were to be considered, effort should be targeted at those components of the longline fisheries that catch sufficient numbers of the target age class.

In order to develop a time series of abundance estimates, tagging would need to be carried out on a regular basis. We consider annual and bi-annual tagging in our discussion of including the estimates in the OM and potential future MPs (section 9).

4.2.2 Recapture samples

For recapture sampling, large numbers of juveniles could most easily be resampled from the surface fishery in the GAB. Resampling should not occur until after tagged fish have had time to fully mix with untagged fish from the same cohort. Hence, the earliest this should occur would be the summer following initial tagging (i.e. after approximately 12 months at liberty). Tissue samples would most practically and efficiently be taken at the time fish are harvested from the farms (i.e., around 4-6 months after wild capture – leading to a lag of around 16-18 months between initial tagging and recapture tissue sampling).

Recapture tissue samples could also potentially be taken by observers or trained individuals on longliners or from fish at major markets. As with tagging, this would only be practical if sufficient numbers of the target recapture age class are available to be sampled.

4.2.3 Age class

Age of fish at tagging and resampling needs to be estimated from length, and this is most accurate for the youngest, fastest growing age classes since their length frequency distributions overlap the

least with neighbouring age classes. To minimize incorrect ageing, it is optimal to tag 1-year olds and resample 2-year olds, with the next best being to tag 2-year olds and resample 3-year olds. The expected percent of incorrect ages and consequences for the abundance estimate, as well as methods to correct for this, are considered below.

Tagging more than one age class would provide an abundance estimate for each age class tagged. Resampling more than one age class can be highly informative with regard to mixing assumptions. For example, if we tag fish at age 2 and recapture them at age 3 and again at, say, age 5 from across the fisheries, then we can still only estimate age 2 abundance but we can compare the estimates derived from the age 3 and age 5 recaptures – if they differ, this may be an indication of non-mixing. However, the ageing error for older fish may be too high to get an estimate with enough precision to make a meaningful comparison. Nevertheless, these are additional options that may be worth considering in future.

As noted above, if multiple consecutive age classes are tagged and recaptured over multiple years, these data can be used in a Brownie framework. These data can be highly informative when used in the OM with regard to natural mortality – as was demonstrated when the 1990s conventional tagging data were included in the OM using a Brownie framework (Anon. 2009; Eveson and Davies 2009; Hillary et al. 2014) – and on abundance of year classes when used in conjunction with other data sets, such as close-kin (e.g. Hillary et al. 2013).

4.2.4 Sample sizes

The tag and recapture sample sizes required to achieve a given level of precision, whilst minimizing costs, can be estimated for a particular Petersen design (see Preece et al. 2013). Because the CV depends on the size of the population being estimated (the CV increases as the population size increases; see eq. 2), larger sample sizes are required to achieve the same precision for an estimate of age 1 abundance than an estimate for age 2. Although genotyping a tissue sample costs the same regardless of whether it is a tag or recapture sample, the at-sea and vessel charter costs to tag and release fish are substantially more expensive than taking recapture samples at harvest (or from the market), therefore it is more cost effective to take larger samples at recapture than increasing the tagging sample size.

4.3 Pilot study design recommendations

Based on the above considerations, we propose that for the pilot gene-tagging experiment, tagging samples be taken from age 2 fish in the GAB during the summer of year *y*, and that recapture samples be taken from age 3 fish in year *y*+1 at farm harvest from fish that were caught earlier that year in the GAB fishery. The tag and recapture sample sizes required to achieve a 25% CV in the estimate of abundance of age 2 using this design depend on the actual size of the age 2 population, and also on whether overdispersion is allowed for (see section 5.3) in the estimator. However, the samples sizes are in the order of 5000 for tagging and 6500-14000 for recapture (see section 8).

The focus here is the design for a pilot gene-tagging project. Extensions to the basic design, such as tagging and resampling fish from regions outside the GAB and tagging and resampling multiple

age classes, should be considered after the initial logistics have been demonstrated in the pilot study (see section 6 “Recommended extensions to the pilot tagging design”).

5 Potential sources of bias and uncertainty

5.1 Spatial dynamics and mixing

There are a number of potential issues related to spatial dynamics and mixing of tagged fish within the population that could affect abundance estimation from the proposed gene-tagging experiment. We address these issues and their likely impact on the abundance estimates below.

5.1.1 Uncertainty in the proportion of the target age class present in the GAB at the time of tagging

This issue can be broken down into several different scenarios:

1. Not all age 2 fish are in the GAB at the time of tagging because some have not yet arrived and/or some have already left:
    a. If all fish return to the GAB at age 3, we still get an estimate of abundance for the entire age 2 population at the time of tagging, provided tagged and untagged fish have mixed during the winter.
    b. If only a fraction of the age 2 population return to the GAB at age 3, but it is a random selection (i.e., tagged and untagged fish have mixed during the winter and are equally likely to return), we still get an estimate of abundance for the entire age 2 population at the time of tagging.
2. Not all age 2 fish are in GAB at time of tagging because a fraction of the age 2 population never went to the GAB:
    a. If the whole age 2 population mixes in winter and a given percent returns to the GAB at age 3, then we still get an estimate of abundance for the entire age 2 population at the time of tagging.
    b. If the same fish that weren’t present in the GAB at age 2 don't return the next year at age 3, then we get an estimate of abundance for the age 2 "GAB population” (i.e., only the population that visits the GAB).

Scenario 2b is the only case that would not give an abundance estimate for the whole population, but it would still give an abundance estimate for the component of the stock in the GAB, which could be incorporated into the SBT OM with a variable that estimates that proportion in the GAB (assuming the proportion remains roughly consistent over the years for which the tagging study provides abundance estimates).

Scenario 2b would be consistent with the hypothesis that a percent of the juvenile population resides off South Africa, for example, and does not come to the GAB in summer. This hypothesis was explored in detail as part of a large FRDC project “Spatial Interactions among Juvenile

Southern Bluefin Tuna at the Global Scale: A Large Scale Archival Tag Experiment", which concluded it was unlikely there was a significant percent of the juvenile component of the population residing off South Africa. Total catches of juveniles off South Africa have remained very small. For easy reference, a subsection from the project final report (Basson et al. 2012) which summarises their findings with regard to this issue is included in Appendix A3.

The design of the pilot study being proposed would not be able to further resolve the scientific question of whether or not a percentage of the age 2 population never goes into the GAB. However, as noted in Preece et al. (2013), if a second recapture sample were taken at a later date (e.g. from the longline fishery in New Zealand when fish are age 5 or older), and assuming that the age 2 fish that never visited the GAB have mixed with the rest of the population, there would be an indication of scenario 2b from the second recapture sample estimates of abundance (i.e., the abundance estimate would be larger). This would confirm that there is a bias that needs to be addressed. Otolith micro-chemistry also appears to be a promising method for understanding the spatial-temporal behaviour (Davies et al. 2014; Clear et al. 2014).

5.1.2 WA fish having different spatial dynamics than GAB fish

In the CCSBT Scientific Research Program (SRP) conventional tagging data from the early 2000s, a significantly smaller percent of fish tagged at age 1 off of WA were recaptured at subsequent ages than those tagged at age 1 in the GAB (Polacheck et al. 2007). This difference was not observed in the 1990s tagging data. Possible reasons have been identified as: (1) high tag-related mortality and/or tag shedding for age 1 fish tagged off WA; (2) high natural mortality rates for age 1 WA fish; and (3) incomplete mixing of age 1 WA fish with the entire age 1 population. (Refer to Polacheck et al. 2007 for a detailed discussion of each.)

Since we are proposing to estimate age 2 abundance by tagging age 2 fish, the first two hypotheses of high tag shedding and/or high tag-related or natural mortality of age 1 fish would not affect the estimate. Furthermore, tag shedding is not an issue for gene-tags, and the probability of tag-related mortality should be reduced. The remaining of the three hypotheses is incomplete mixing of age 1 WA fish with the entire age 1 population, and the possibility that a large fraction of age 1 WA fish does not return to the GAB at older ages. This would give rise to scenario 2b described in the previous section, in which a proportion of the age 2 population would not be present in the GAB at the time of tagging and would not return the next year at age 3. As noted above, under this scenario the proposed design would still provide an abundance estimate for the component of the stock that goes to the GAB, and other projects have the potential to answer the question of juvenile spatial dynamics (e.g. otolith micro-chemistry; Davies et al. 2014).

5.1.3 Spatial heterogeneity/incomplete mixing

To obtain an unbiased abundance estimate, the Petersen method assumes complete mixing of tagged and untagged fish from the population of interest (in our case, the tagged cohort) throughout their range. Spatial heterogeneity and incomplete mixing could occur, for example, if all tagging was done late in the season in one location and these fish have different spatial dynamics than fish present earlier in the season and/or in a different location (e.g., they migrate

to the Tasman Sea instead of the Indian Ocean for winter, where they experience different fishing mortality rates).

Analyses of the 1990s tagging data suggested that mixing was reasonably good (Polacheck et al. 2006; Basson et al. 2012); although the data were not sufficient to be definitive, due to the lack of tagging in regions outside WA and the GAB and the lack reporting rate estimates from the longline fisheries. Data from the 2000s experiments suggest that changes may have occurred in the spatial dynamics of juvenile SBT between the 1990s and early 2000s that could indicate non-mixing is a greater concern (as discussed the in previous section 5.1.2 and in Polacheck et al. 2006).

Recaptures from the longline fisheries in winter would enable an evaluation of spatial heterogeneity, and could be considered in an extension to the pilot tagging experiment (see section 6).

5.2 Errors in determining age from length

To estimate abundance of a given cohort requires correctly determining the cohort to which fish belong (i.e., their age) at tagging and recapture. Since age cannot be generally be observed directly, it must be inferred from length measurements. Incorrect ages will bias the abundance estimate.

In the proposed design we aim to tag a cohort at age 2 and resample it the next year at age 3. Table 1 summarizes the otolith age and length data that is available for SBT caught in the Australian surface fishery since the 2002 fishing season (see Farley et al. 2014 for details of the sample collection and ageing procedures). Based on these data, there are two 5-cm length bins for which over 90% of the fish belong to age class 2, namely 70-74 cm and 75-79 cm (noting that fish length is recorded to the nearest cm).

If the “tagging” tissue samples are taken from the GAB during the same months as the fishing season (Jan-Mar), the age-length data in Table 1 should be relevant to those samples (assuming growth has remained relatively stable since 2002). Thus, if we choose fish between the lengths of 70 and 79 cm for sampling, we would expect 91% to be 2-yr olds, 4% to be 1-yr olds, and 5% to be 3-yr olds.

The proposal is for “recapture” tissue samples to be taken the next year from the surface fishery catches at the time of harvest from the farms (generally Jun-Aug). However, for illustrative purposes, assume that it is taken during the months of Jan-Mar so that the age-length data in Table 1 are relevant. We want to sample fish that are age 3. The 5-cm length bin which maximizes the proportion of fish belonging to age class 3 is 95-99 cm, with 77% estimated to be 3-yr olds, 12% to be 2-yr olds, and 10% to be 4-yr olds. There may be logistical difficulties with collecting enough samples (>5000) from such a narrow length range, so the optimal 10-cm length range would be 95-104 cm for which we would expect 74% to be 3-yr olds, 8% to be 2-yr olds and 18% to be 4-yr olds.

To see what affect ageing error could have on the abundance estimate, assume that the true abundance of age 2 fish at the time of tagging is N=2 million. Suppose we tag T=5000 fish of lengths 70-79 cm and assume they are all 2-yr olds, and resample S=15000 fish from the catch of lengths 95-104 cm the next year and assume they are all 3-yr olds (T and S based on the maximum

sample sizes given in Table 2 of section 8 below). The number of "recaptures" (i.e., matches in DNA fingerprints), R, will be the number of tagged 2 year olds resampled at age 3, plus the number of (mistakenly) tagged 3-yr olds resampled (mistakenly) at age 4. In this case, the second component is too small to need to consider further. Thus the expected number of recaptures, or matches, is:

$R = (.74*S)*(.91*T)/N = 25$

This would give an estimated age 2 population size of:

$\widehat{N} = S*T/R = 3$ million

So we would overestimate the age 2 population size by about 1 million.

If we have good estimates of the percent ageing errors, then we can correct for this. This would be best achieved by collecting otoliths from fish within the target length range at the time of tissue sampling (i.e., at the time of harvest from the farms). Such otolith data may be informative for the close-kin project as well, which also requires accurate age estimates from samples taken at the time of harvest.

It is important to note that this bias is not specific to gene-tagging, but would result from conventional tagging as well under the same experimental design (since length is used to determine age regardless of tag type). The difference is that in previous conventional tagging experiments for SBT, the recapture sample was the entire catch, and in that case the bias will not be as large.

Table 1. Age-length data derived from GAB otolith samples collected during fishing seasons 2002-2014.

LENGTH	AGE ESTIMATE										
bin (cm)	1	2	3	4	5	6	7	8	9	10	11
[45,50)	4	0	0	0	0	0	0	0	0	0	0
[50,55)	12	0	0	0	0	0	0	0	0	0	0
[55,60)	25	1	0	0	0	0	0	0	0	0	0
[60,65)	15	9	0	0	0	0	0	0	0	0	0
[65,70)	5	6	0	0	0	0	0	0	0	0	0
[70,75)	2	36	1	0	0	0	0	0	0	0	0
[75,80)	1	39	3	0	0	0	0	0	0	0	0
[80,85)	0	65	8	0	0	0	0	0	0	0	0
[85,90)	0	61	28	1	0	0	0	0	0	0	0
[90,95)	0	38	81	8	0	0	0	0	0	0	0
[95,100)	0	16	103	14	1	0	0	0	0	0	0
[100,105)	0	5	87	32	0	0	0	0	0	0	0
[105,110)	0	2	59	64	4	0	0	0	0	0	0
[110,115)	0	0	44	76	13	0	0	0	0	0	0
[115,120)	0	0	8	89	19	3	0	0	0	0	0
[120,125)	0	0	5	47	36	2	0	0	0	0	0
[125,130)	0	0	0	20	62	16	0	0	0	0	0
[130,135)	0	0	0	8	31	18	4	0	0	0	0
[135,140)	0	0	0	2	11	27	4	1	1	0	0
[140,145)	0	0	0	1	3	16	9	4	0	0	0
[145,150)	0	0	0	0	0	6	7	7	0	0	0
[150,155)	0	0	0	0	0	4	5	3	0	0	0
[155,160)	0	0	0	0	0	0	1	2	0	0	0
[160,165)	0	0	0	0	0	0	1	0	0	0	0
[165,170)	0	0	0	0	0	0	0	0	1	1	1

5.3 Overdispersion

There are a number of reasons why we would expect the CV of the Petersen abundance estimate to be larger than given by eq. 2 (i.e. for there to be greater variability in the observed number of recaptures than the model predicts). Extra variability than predicted by the model is referred to as overdispersion.

One reason for this would be if fish tagged in the same school remained together until they were recaptured, so their recapture probabilities are not independent. For the experimental design being proposed, where fish will be at liberty for a year before recapture and will have migrated from the GAB to the southern ocean and back, it seems unlikely that large numbers will remain in

the same schools for the duration. However, even if some degree of long-term school fidelity exists, this would not bias the abundance estimate, it would only lead to greater variance. Furthermore, one of the tagging protocols would be to spread tagging over as many schools as possible in order to minimize this source of overdispersion (see Appendix A1. Protocols for tagging and recapture).

Another reason would be false negatives or false positives in the DNA fingerprint matching, such that the observed number of recaptures is incorrect. Again, assuming that such genetic errors are equally likely in either direction, this would not bias the abundance estimate but would increase the variance. Given the rigorous genetic methods and statistical quality control protocols that have been developed (Bravington et al. 2015), the error rate for fingerprint matching should be very small, and so is unlikely to be a substantial source of the overdispersion.

We have already discussed how errors in ageing can bias the abundance estimate, and how we can attempt to correct for this bias by estimating the proportion of fish in the recapture sample that are incorrectly aged. This correction factor will of course contain uncertainty, which would translate to greater uncertainty in the final abundance estimate.

6 Recommended extensions to the pilot study design

1. Collect otoliths from an appropriate subsample of the recaptured fish to estimate percent ageing errors and use the correction in the abundance estimate. Note this would only be feasible with the agreement and co-operation of the surface fishery and processors.
2. Consider tagging age 1 fish off WA to resolve uncertainty about spatial dynamics of 1 year olds. Because conventional tagging stopped in 2006, we do not know if the difference in recapture rates of age 1 WA versus GAB fish still remains, or if those were anomalous years. As such, we recommend tagging age 1 fish both off WA and in the GAB in at least one early year of the gene-tagging recruitment monitoring program (i.e. after the pilot gene-tagging study). If the difference in recapture rates of WA versus GAB fish still remains, then it would be important to investigate why (e.g. through recapture sampling in the longline fisheries to see if the WA fish are being recaptured in those fisheries and just not returning to the GAB). If the reason is non-mixing (i.e. a large fraction of age 1 WA fish does not return to the GAB at older ages), then the abundance estimates derived from the GAB releases and recaptures would not represent the entire component of the stock. If the reason is very high natural mortality on age 1 WA fish, this would not affect the GAB-derived age 2 abundance estimates, but would highlight the need to address uncertainties in natural mortality rates. In terms of tagging off WA, the Japanese knowledge and experience in WA in recent years would be very valuable, however, opportunistic tagging as part of the trolling survey at its current scale would not capture enough fish for the sample sizes required.
3. Consider tagging multiple cohorts over multiple years using gene-tagging to estimate fishing and natural mortality rates. The data from the previous tagging programs are highly informative in the SBT operating models for key uncertainties in natural mortality (e.g. Anon.

2009; Eveson and Davies 2009). This tagging program would be similar to previous SRP programs, and would be more expensive than the pilot tagging program. Large scale tagging programs are a key recommendation for tuna RFMOs from the KOBE II workshop of experts on provision of scientific advice (Anon, 2010). The previous CCSBT SRP tagging program was cancelled because of the non-reporting of tags and inability to estimate reliable reporting rates, but gene-tagging is not subject to these forms of biases. The tagging data incorporated into the SBT OM are from the 1990s, now 20+ years old, and we cannot assume natural mortality rates to have remained stable since that time. The cost of such a program is unlikely to be excessive relative to previous CCSBT tagging programs. The relative cost-effectiveness of different sources of monitoring data is something that should be explored in the context of the review of the MP.

7 Genetics methods

The SBT gene-tagging program will use new genetic methods and techniques that have been developed for use in the close-kin project (see Bravington et al. 2015). In the original close-kin work a suite of DNA micro-satellite loci were used to determine the unique DNA profile or genetic fingerprint of a fish and to identify parent-offspring pairs. Matching parents to offspring requires a higher level of genetic resolution (i.e., more loci) than is required for gene-tagging, where the aim is to accurately match an individual fish with itself.

The new DNA profiling methods recently developed at CSIRO are similar to RAD-seq technology. These “genotyping by sequencing” (GBS) technologies are considered highly informative, robust, repeatable and substantially cheaper to develop and run than the former micro-satellite technologies (Bravington et al. 2015). Use of these methods for identifying unique SBT and familial relationships between individuals has been evaluated, and markers applicable for both gene-tagging and future close-kin work have been identified.

The costs for the genetic processing component of the gene-tagging work are based on the costs for the close-kin genetic processing stages. In some cases use of the same tissue sample for both projects may also be possible and desirable. There is potential for the gene-tagging work to be even further simplified and a robotic system used to rapidly process large numbers of samples. This aspect of further optimisation would be investigated as part of the gene-tagging pilot study.

8 Precision and costs

8.1 Precision

In considering the design of the gene-tagging program and the precision of the estimates that could be generated, a hypothetical CV of 25% for the estimates of abundance was set. Using this target, sample sizes can be adjusted and optimised against the cost information to provide combinations of tagging and recapture sample sizes that minimise the overall costs. The 25% CV

was chosen as being relatively more informative than the aerial survey and CPUE (as they are implemented in the OM).

Overdispersion (discussed in section 5.3) is additional variance that is not considered in the Petersen model. The scale of the realised overdispersion won't be known until the data are integrated in the OM. In the cost estimates below, an overdispersion factor has been included in the calculations. The effect of this factor is that larger sample sizes are required to improve the CV of the abundance estimates and therefore costs are higher. This could be managed by taking larger numbers of tissue samples at release or recapture at little additional cost, but only genotyping a subset of these until the overdispersion factor is evaluated. In the case that it is unacceptably high, additional samples could be processed to reduce the CV further. The advantage of taking a large number of samples also means that a larger number of matches will be found, and the target length class could be more finely defined.

8.2 Costs

Costs associated with obtaining a single age 2 abundance estimate were estimated via an optimisation model that minimised costs based on varying tagging and recapture sample sizes (see Preece et al. 2013). The cost estimates use information on current abundance, a target CV of the abundance estimate, and costs of tagging, recapture sampling, genetics processing and the project set-up costs.

The following inputs were used:

1) Abundance estimates based on the range of estimates in the last decade, from the 2014 reference set of operating models. We have used 2 million fish as an estimate of current abundance of 2-year olds and 3 million fish as the maximum. The 2013 and 2014 estimates of numbers of age 2 fish are very high relative to the previous years, and are highly influenced by the 2014 aerial survey data and have very little other data to inform them. See Appendix A2 for more information.
2) Genetics processing costs of $25 per sample, which includes DNA extraction, equipment and labour.
3) Vessel charter and labour costs. Preece et al. (2013) examined a range of options for minimising costs by minimising days at sea. From these calculations, and based on information from the 2000s CCSBT SRP tagging program that aimed to tag 8,000-10,000 fish in 40 days at sea in SA, we have fixed the initial tag sample size at 5000 fish and included costs for 20 days at sea (this is the minimum needed to allow for bad weather and time to find fish). The at-sea tagging costs include vessel charter and costs for two taggers. For the pilot study, at least one tagger should be a highly experienced person with knowledge of the tissue sampling and quality control protocols. Two or three separate trips to sea are anticipated in the 20 days, and therefore several less experienced taggers could be involved or trained.
4) Tissue sampling at recapture, when fish are being harvested, has been included but cost estimates are very preliminary as these would need to go to tender.
5) Administration, project management, at-sea equipment and preparation, analysis and fixed costs have been included at $150,000.

6) Target CV = 0.25 is used in the optimisation model to estimate tagging and recapture sample sizes that minimise costs. This is hypothetical and other target CVs could be considered.
7) Overdispersion. We examined the potential impact on costs and sample sizes of an overdispersion factor, as is used in the OM for the 1990s conventional tagging data (Anon. 2009; Hillary et al. 2014). As noted above, the actual overdispersion factor cannot be estimated until gene-tagging data are integrated into the SBT OM. Results for no overdispersion and for an overdispersion factor of 1.5 (similar to the current value of 1.82 in the 2014 OM) are provided as the two extremes.

It is important to note that costs for chartering a vessel are preliminary estimates based on historical charter costs from the 2000s CCBST SRP tagging program. The costs of various aspects of the gene-tagging project would be clarified and fine-tuned as part of the pilot tagging program, to inform the ESC and Commission of the likely future costs of a recruitment monitoring program using this method. Also, as the population increases, the sample sizes will also need to increase to maintain the same CV of the estimates (but at a slower rate than the population size increases – see eq. 2).

The estimated sample sizes and estimated costs for a pilot gene-tagging project that would provide a single estimate of the absolute abundance of the age 2 cohort are in Table 2. The costs would be spread over approximately 22 months starting with tagging tissue collection and ending with the abundance estimation step.

Table 2. The cost estimates and tagging and recapture tissue samples sizes for the gene-tagging pilot study, for a target CV of the estimates of abundance with and without inclusion of an over dispersion factor of 1.5, and for two assumptions for the numbers of fish in the age 2 cohort: 2 million fish (approximate recent average), and 3 million fish (recent maximum).

TARGET CV OF ABUNDANCE ESTIMATES	ASSUMED COHORT SIZE	TAGGING SAMPLE SIZE	RECAPTURE SAMPLE SIZE	COST ESTIMATES
0.25 + no overdispersion	**2 million**	5000	6500	AUD $705,000
0.25 + no overdispersion	**3 million**	5000	9600	AUD $782,000
0.25 + 1.5 overdispersion	**2 million**	5000	9600	AUD $782,000
0.25 + 1.5 overdispersion	**3 million**	5000	14400	AUD $915,000

An additional cost for otolith collection, ageing and archiving to calculate and correct for ageing errors would be approximately $4000 for 100 otoliths sampled. This information may also be of use in the proposed future close-kin project, and therefore costs only need to be included in one project, not both.

Opportunistic tissue sampling of age 3 fish across the fishery (or sampling the tagged cohort at older ages) would include costs for sample collection, freight and handling costs, genetics and labour for the genetics processing. A rough minimum estimate of these costs is $25/sample, excluding any costs for sampling.

9 Inclusion of gene-tagging data in the SBT operating model and management procedure

As with other data sources, the gene-tagging data could be integrated into the SBT OM regardless of whether or not the aerial survey data continued to be collected and incorporated (Anon. 2014). Overlapping years of the two recruitment monitoring surveys are not required in the OM. Aerial survey abundance estimates, however, are essential and formally required for the operation of the management procedure until a new MP is developed, tested, adopted and implemented.

9.1 Integration in the SBT OM

The proposed tag design would provide a time series of age 2 abundance estimates, along with standard error estimates, for all years of tagging. It is straightforward to include these estimates in the OM using a simple Gaussian likelihood. Because the tagging study provides an absolute, not relative, abundance series, it would not be necessary to scale the estimates (e.g., with a "q" parameter, as for the aerial survey indices), provided that the estimates are for the entire age 2 population. If, however, a proportion of age class 2 never enters the GAB, and we assume the proportion is consistent across years, then a "q" scaling parameter could account for this. We suggest including a "q" parameter in the likelihood to see what the value is estimated to be – if it is close to 1, then the abundance estimates are consistent with the model predictions of age 2 abundance. If it is less than 1, it *may* indicate that not all of the age 2 population goes to the GAB; however, it may simply indicate the Petersen estimates are not consistent with other data sources informing the age 2 abundance estimates.

As discussed in Section 5.3 on overdispersion, we expect the standard errors estimated directly from the tagging data to be under-estimated. Thus, the likelihood can be adjusted to allow for additional process error, as is done currently with the aerial survey data. The adjustment factor for overdispersion could be determined after a few years of abundance estimates were obtained and their fit and compatibility with other data sources in the OM evaluated.

To demonstrate how these data could be used in the OM, we modified the OM ADMB code (sbtmod.tpl) to include a likelihood for gene-tagging abundance estimates. As per the above discussion, we included a "q" scaling parameter, and also allowed for an overdispersion factor to be specified by the user (i.e. the factor by which the variance estimates will be inflated).

The code does not require an annual series; e.g., it is able to accommodate data from gene-tagging every second year. We would not expect the impacts of a bi-annual series to be substantial, although there would be potential for unusual recruitment patterns in the "off" years if there is conflicting information in the data sources integrated in the model. The frequency of the gene-tagging abundance estimates will be a greater issue for their use in a new MP, and relative performance of that MP (see Section 9.2).

We tested the code on simulated data to ensure it was working correctly (e.g., when the estimates were generated to be consistent with the mean age 2 abundance estimates from the 2013 OM base grid runs, "q" was estimated to be close to 1). Furthermore, to demonstrate the derivation of Petersen estimates and their inclusion in the OM using real data, we used the 1990s

conventional tag data. A time series of Petersen abundance estimates can be derived for each age class that was tagged, based on recaptures from any subsequent age class (excluding the same age as tagging to allow for mixing). As noted previously, the recapture sample size, S, for the 1990s tagging experiment is the entire catch, so catch-at-age estimates are needed. Furthermore, to get the appropriate number of recaptures, R, we need to adjust the observed numbers of tag returns by the estimated reporting rates. We used catch-at-age data and reporting rate estimates corresponding to the data used in the current base OM runs (i.e. adjusted for overcatch using LL1 scenario 1 and surface scenario 1).

The numbers of releases and recaptures, the catch numbers, and the derived Petersen estimates of abundance and CVs (calculated using eq. 2) for each age and year of release and recapture are given in Table 3.

Table 3. Tag release and recapture numbers, and catch numbers, and Petersen estimates of abundance and uncertainty (AbundEst and CV) using the 1990s conventional tag data. Age 2 estimates based on age 3 recaptures are shaded as these are the estimates we illustrate using in the OM. Ntag = number of tag releases; Nreturn = observed number of tag returns; RepRate = reporting rate estimate; Nrecap = estimated number of tag recaptures accounting for non-reporting.

RelAge	RecAge	Year	Ntag	Nreturn	RepRate	Nrecap	Ncatch	AbundEst	CV
1	2	1991	3301	41	0.50	81	38,348	1,556,075	0.11
1	2	1992	2147	21	0.58	36	45,444	2,685,452	0.17
1	2	1993	4898	41	0.22	188	23,004	599,106	0.07
1	2	1994	9003	110	0.42	260	28,594	989,941	0.06
1	2	1995	8594	87	0.32	274	19,793	619,779	0.06
1	3	1991	3301	46	0.49	94	150,195	5,248,951	0.10
1	3	1992	2147	56	0.36	156	159,618	2,196,950	0.08
1	3	1993	4898	202	0.51	395	123,108	1,528,353	0.05
1	3	1994	9003	401	0.48	844	137,545	1,466,840	0.03
1	3	1995	8594	640	0.66	970	196,223	1,739,039	0.03
2	3	1992	4715	163	0.49	335	150,195	2,115,821	0.06
2	3	1993	3161	90	0.36	251	159,618	2,012,603	0.06
2	3	1994	3177	169	0.51	330	123,108	1,184,914	0.06
2	3	1995	5968	404	0.48	851	137,545	965,134	0.03
2	3	1996	2524	344	0.66	521	196,223	950,222	0.04
2	4	1992	4715	103	0.37	281	149,800	2,516,652	0.06
2	4	1993	3161	77	0.42	185	117,008	1,998,213	0.07
2	4	1994	3177	78	0.37	211	135,146	2,036,699	0.07
2	4	1995	5968	374	0.55	682	127,256	1,112,798	0.04
3	4	1993	2909	87	0.37	237	149,800	1,838,244	0.07
3	4	1994	3737	156	0.42	375	117,008	1,166,021	0.05
3	4	1995	2728	106	0.37	286	135,146	1,286,894	0.06
3	4	1996	1516	205	0.55	374	127,256	515,709	0.05
3	5	1993	2909	36	0.14	250	70,102	815,705	0.06
3	5	1994	3737	31	0.14	217	67,612	1,165,517	0.07
3	5	1995	2728	76	0.32	238	80,244	921,710	0.07

Although all of the estimates in Table 3 could be included in the OM, we only included the age 2 estimates based on age 3 recaptures, as this is the design being proposed for the gene-tagging pilot study. Figure 1 shows how these estimates compare with the age 2 abundance estimates for years 1992 to 1996 from the 2013 OM base grid runs.

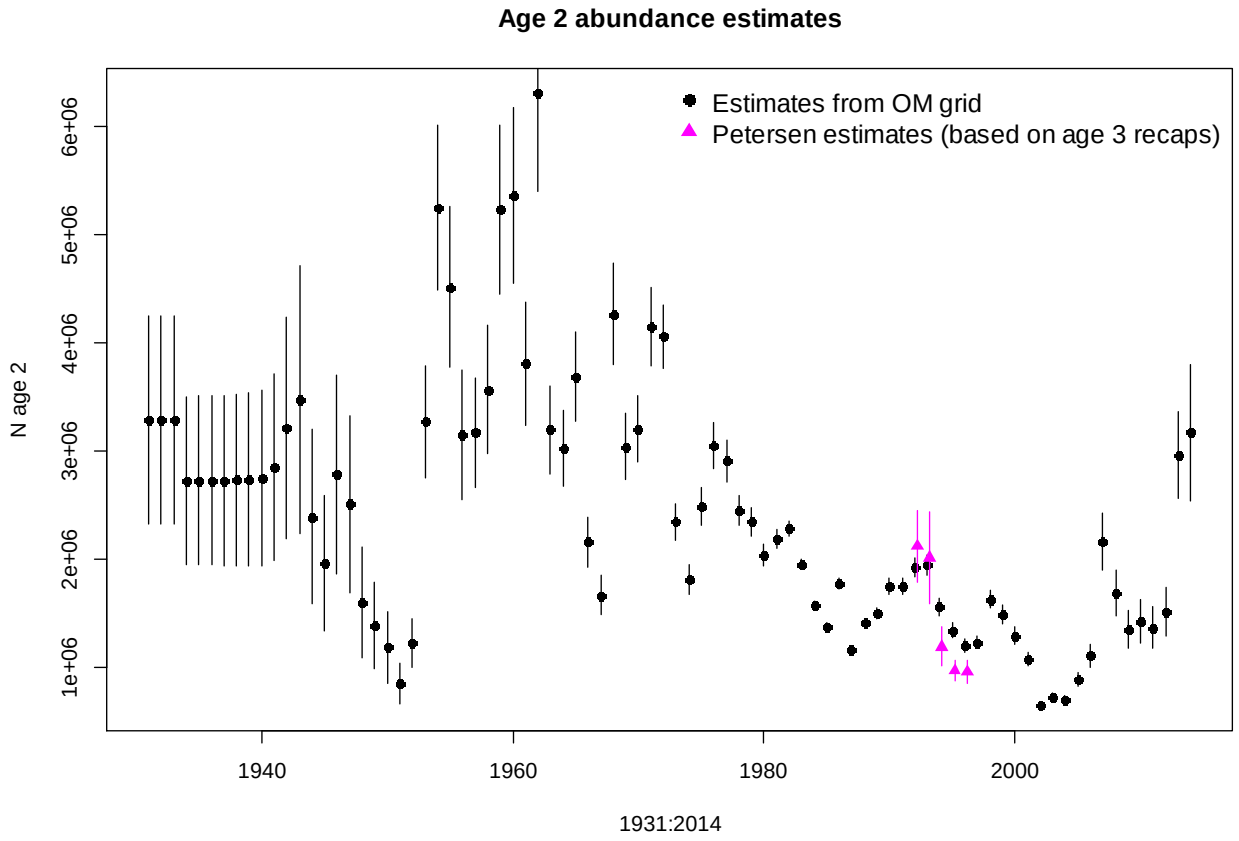

Figure 1. Comparison of Petersen age 2 abundance estimates (mean ± 2 SD) derived from the 1990s tag data using age 3 recaptures with the age 2 abundance estimates (mean ± 2 SD) from the 2013 OM grid runs. Note that the SDs are those estimated directly from the data and do not include any overdispersion.

Because the Petersen estimates are derived directly from the 1990s tagging data, it is not appropriate to include the 1990s tagging data through the Brownie likelihood as well. Thus, we ran the OM base grid with the Petersen estimates included but with the tag switch for the Brownie likelihood turned off. This allows us to evaluate how much effect using a Petersen design for the tagging study as opposed to a Brownie design can have on the results.

We specified a hypothetical overdispersion factor for the Petersen estimates to be 1.8. This factor is equal to the overdispersion value currently used in the OM for the 1990s tag data, but was chosen simply to illustrate the method for inclusion of gene-tagging data.

The q estimates over all grid runs ranged from 0.39 to 1.08, with a median of 0.82 (Figure 2). As noted above, this could be indicative of the proportion of the age 2 population that goes to the GAB, but could also indicate inconsistencies with other data sets (as we know that the 1990s

tagging data conflicts with other data sets, particularly with regard to natural mortality, when included in a Brownie framework; e.g. Eveson and Davies 2009).

The natural mortality vectors estimated from the new OM runs that include the Petersen estimates are quite different than those from the 2013 OM base runs (Figure 3). This is not surprising, but confirms our observation that the tag data do not contain the same information about natural mortality when included in a Petersen framework as a Brownie framework.

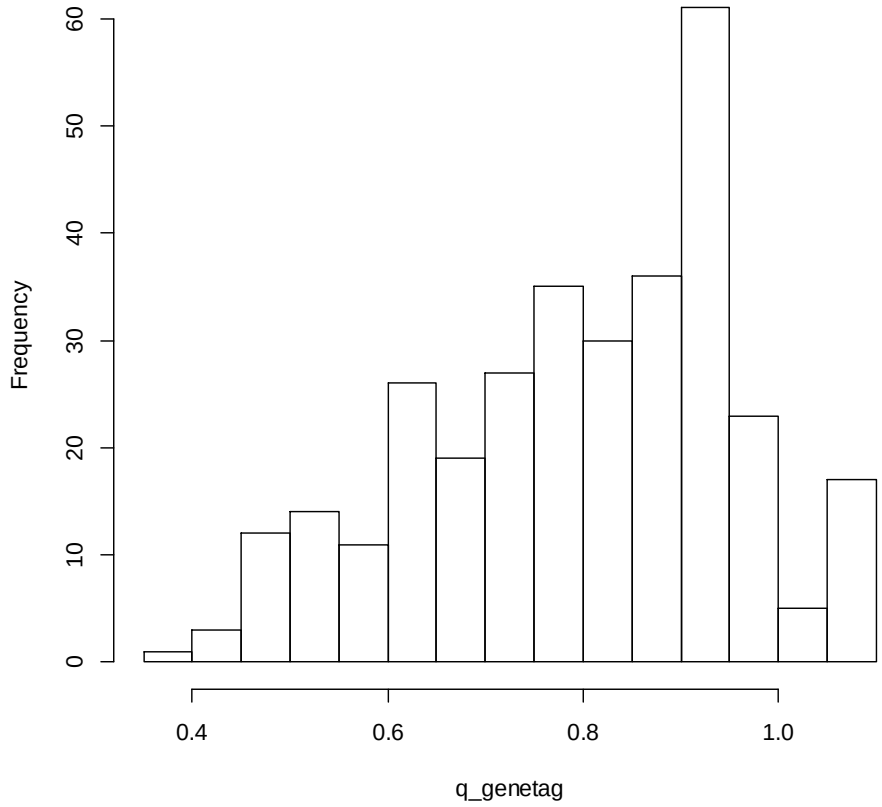

Figure 2. Estimates of the scaling ("q") parameter for the age 2 Petersen estimates across all OM grid run.

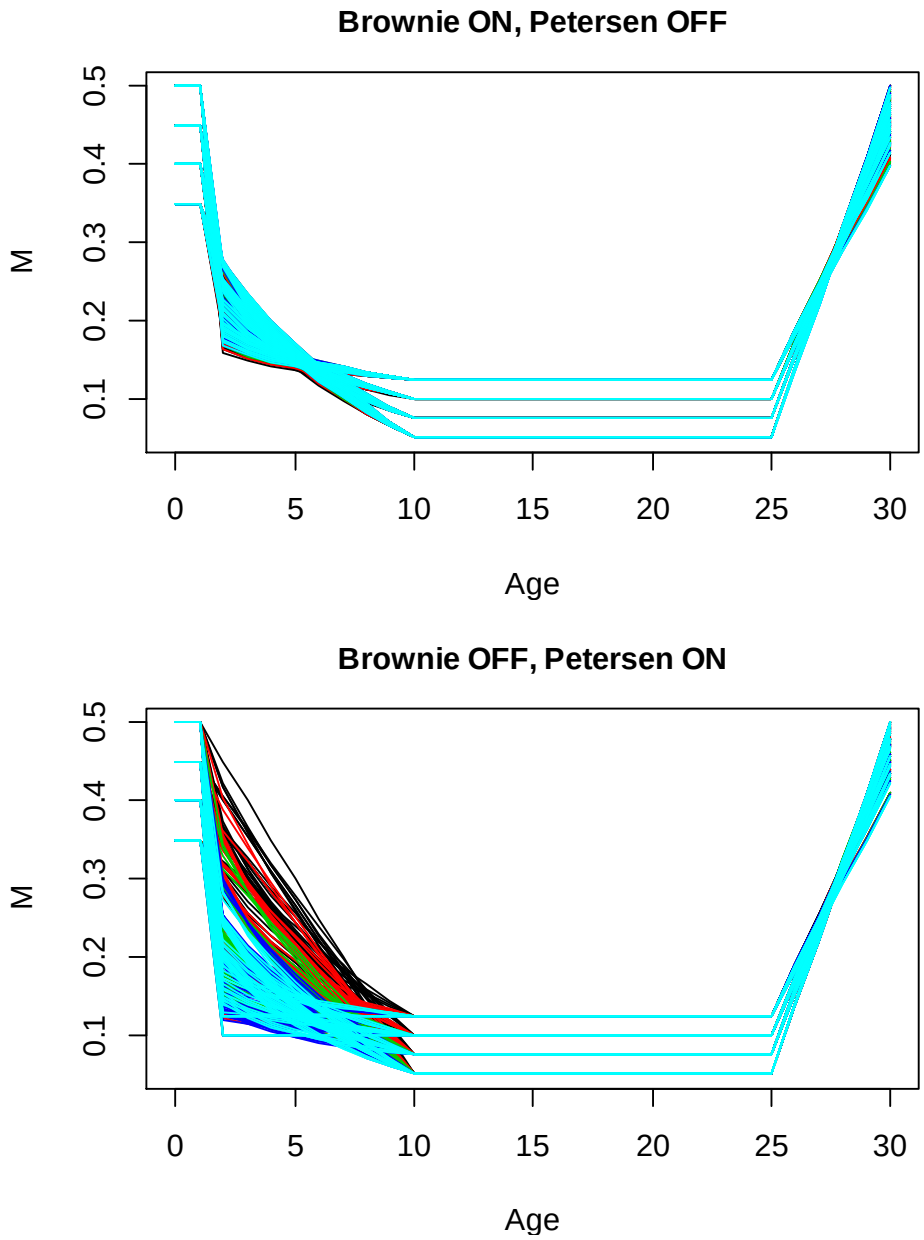

Figure 3. Comparison of estimated mortality rate vectors across all grid runs from: (a) the 2013 OM base runs (which include a Brownie likelihood for the 1990s tag data), (b) the new OM runs with the Petersen age 2 abundance estimates included (and the Brownie likelihood switched off). Note that the different colours correspond to different levels of steepness (black being lowest and light blue being highest).

9.2 Potential use in future MP

Any new MP developed to use the gene-tagging data will need to be fully MSE tested for performance, and tuned to the interim rebuilding target of the Commission before adoption. The data on absolute abundance will be different to the aerial survey based relative abundance index used in the Bali Procedure MP, but a similar type of biomass model of recruitment and growth could be considered as a candidate MP, amongst others. The gene-tagging abundance estimate could not just be appended to the aerial survey time series data in the MP, a new MP would need to be developed.

For a new MP a time series of abundance estimates would need to be acquired. The time series wouldn't necessarily need to be annual if adequate performance could be maintained with a less frequent index. However past recruitment monitoring and analysis has indicated an occasional strong cohort relative to previous and subsequent years, and the ESC may consider it essential to have an annual indicator of recruitment/cohort strength, especially during the rebuilding phase.

One advantage that the aerial survey has over gene-tagging for recruitment monitoring, and use of the data in models, is its immediacy. The aerial survey data can be collected and used in the same year. In comparison the gene-tagging estimates, which have other advantages, will take around 22 months from initial collection to obtaining the estimate. This time lag is made up of: 1) 12 months to allow for tag mixing between the summer in which the fish are tagged and the summer in which they are recaptured, 2) an additional 4-6 months in the farms before harvesting, and 3) a small amount of time for genetic processing, data quality control and analysis. The timing of harvesting in the South Australian farms (around July) would make it very difficult to obtain an abundance estimate that could be used in an OM or MP at the ESC in September of that year.

The timing issue and frequency of obtaining an estimate would be part of the development and testing of new MPs. The frequency issue for use of the data in the MP can't be resolved until a new MP is tested, relative to reference and robustness tests, to ensure that the performance would still be likely to meet the Commissions performance measures for stock rebuilding.

As noted previously, the gene-tagging abundance index will be more informative in terms of it being an absolute abundance estimate for a single age class compared with a relative abundance estimate over several age classes (from the aerial survey), and MPs that utilise this information most effectively should be developed. Gene-tagging data will also reduce the reliance on CPUE and catch composition data to estimate recruitment, which has large unresolved uncertainties in the underlying data. This was the aim of the 2000s SRP tagging program and it has been noted as an appropriate aim to continue to pursue (Itoh et al. 2007; Davies et al. 2007; Anon. 2007).

10 Conclusions

Gene-tagging can, like other tagging programs, provide highly informative data for use in stock assessment and management procedures on natural and fishing mortality, and absolute abundance of recruits. The key advantage is that gene-tagging resolves the reporting rates and tag loss problems that led to the cessation of the 2001-2006 CCSBT SRP conventional tagging project. The gene-tagging data are also fishery independent, and would help reduce reliance on CPUE, which can mask changes in abundance and are affected by historical over-catches.

The objectives of this gene-tagging design study were to refine the experimental design of a pilot program and, using simulated data, demonstrate methods for integration of the data into the SBT operating models.

The following work has been completed:

1. The design of a gene-tagging pilot study and extensions to this basic design have been evaluated.

2. Costs and precision estimates have been updated using the most recent estimates of the population size from the 2014 reference set of operating model results.
3. Updated genetics techniques and their costs, as evaluated in the close-kin design project, have been incorporated.
4. Potential sources of uncertainty and bias, and methods to address these, have been considered.
5. Methods for incorporating gene-tagging data in the SBT operating model have been demonstrated, and potential use in future SBT management procedures have been discussed.

The outcomes and recommendations from this design study are:

1. A pilot gene-tagging project, to test feasibility and logistics, should tag (take a small tissue sample and release) fish aged 2 years in the GAB and recapture (tissue samples taken at harvest) at age 3 after 12 months to allow for mixing in the full population.
2. Protocols developed as part of the 2000s SRP conventional tagging program should be followed to distribute tagging spatially and temporally across the population in the GAB to the extent possible. Genetic tissue sample quality control methods and tools will be used.
3. After the initial logistics have been demonstrated in the pilot program, extensions to the basic design, such as tagging and resampling fish from regions outside the GAB and tagging and resampling multiple age classes, should be considered, to provide more information on natural and fishing mortality rates of juveniles age classes.
4. The same genetic sequencing technologies evaluated as part of the future close-kin design project (Bravington et al. 2015) should be used. There is a small overlap in recapture samples which would reduce costs of the gene-tagging project slightly (the recapture sample size of age 3 fish required for gene-tagging is 5-10 times larger than the close kin sample of age 3 fish).
5. Otoliths should be collected from an appropriate sub-sample of fish in the recapture length class to allow for correction of length-based age estimates on the gene-tagging abundance estimate for the tagged cohort.
6. A method for integrating the gene-tagging abundance estimates in the SBT operating model has been developed and demonstrated. A time series of the data from gene-tagging can also be used in future management procedures, but a new MP will need to be developed and tested; these data cannot simply be added into the existing MP to replace the aerial survey recruitment index.
7. Tagging every year to develop an annual time series of age 2 abundance estimates should be considered for use in future management procedures, recruitment monitoring and stock assessment operating models. Bi-annual data can be integrated into the SBT OM, but the performance of a future MP using only bi-annual data will need to be evaluated and the MP tuned to meet the Commission objectives.

References

Anon. (2001a) Report of the CCSBT Tagging Program Workshop Canberra, Australia, 2 - 4 October 2001.

Anon. (2001b) Report of the Fifth Meeting of the Scientific Committee.19-24 March 2001,Tokyo, Japan.

Anon. (2007). Report of the Twelfth Meeting of the Scientific Committee. Commission for the Conservation of Southern Bluefin Tuna, Scientific Committee Meeting, 10 - 14 September 2007 Hobart, Australia.

Anon. (2009) Report of the Operating Model and Management Procedure Technical Meeting. 13 - 17 July 2009, Seattle, USA.

Anon. (2010) Report of the joint tuna RFMOs meeting of experts to share best practices on the provision of scientific advice. *Barcelona, Spain, May 31 to June 2, 2010.* KOBE2 – WS Report TRFMO2_W1-020/2010.

Anon. (2013) Report of the 18th Extended Scientific Committee meeting. 2-7 September 2013, Canberra, Australia.

Anon. (2014) Report of the 19th Extended Scientific Committee meeting. September 2014, Aukland, New Zealand.

Basson, M., Hobday, A.J., Eveson, J.P., Patterson, T.A. (2012) Spatial Interactions among Juvenile Southern Bluefin Tuna at the Global Scale: A Large Scale Archival Tag Experiment. FRDC Report 2003/002.

Bradford, R., Hill, P., Davies, C., Grewe, P. (In Press). A new tool in the toolbox for large-scale, high-throughput fisheries mark-recapture studies: Opening the door to Fisheries Genomics. (Submitted to Marine and Freshwater Research).

Bradford, R. W.; Hobday, A. J.; Evans, K.; Lansdell, M. (2009). CMAR code of practice for tagging marine animals. CSIRO Marine and Atmospheric Research Paper 028. CSIRO, Hobart

Bravington, M.V. and Davies C.R. (2013) Close-Kin; Where to now? CCSBT-ESC/1309/17.

Bravington, M.V., Grewe, P.G., Davies, C.R. (2014) Fishery-independent estimate of spawning biomass of Southern Bluefin Tuna through identification of close-kin using genetic markers. FRDC Report 2007/034. CSIRO, Australia.

Bravington, M.V., Grewe, P.G., Davies, C.R. (2015) Design of a Close-Kin Mark-Recapture program for monitoring SBT spawning stock. CCSBT-ESC/1509/19.

Chapman, D.H. (1951) Some properties of the hypergeometric distribution with applications to zoological censuses. University of California Publications in Statistics 1, 131-160.

Clear, N., Davies, C., Eveson, J.P. (2014) Identifying residence signals of SBT in the spawning ground and the Great Australian Bight: Initial insights from trace elements in otoliths. CCSBT-ESC/1409/22.

Davies, C., Clear, N., and Eveson, P. (2014). SRP Proposal: Identification of spatial distributions of fish by age from otolith microchemistry. CCSBT-ESC/1409/27.

Davies, C., Preece, A., and Basson, M. (2007). A review of the Southern Bluefin Tuna Commission's Scientific Research Program and considerations of current priorities and way forward. CCSBT-ESC/0709/16.

Davies C, Moore A, Grewe P, Bradford R and Basson M (2008). Report on the potential and feasibility of genetic tagging of SBT. CCSBT-ESC/0809/13

Eveson, J.P., and Davies, C.R. (2009) Re-conditioning of the CCSBT Operating Model: impacts of revised natural mortality schedule and interaction with steepness. CCSBT-ESC/0909/40.

Harley, S., Bradford, R., and Davies, C. 2008. Using passive integrated transponder (PIT) technology to improve performance of CCSBT's conventional tagging program. CCSBT-ESC/0809/14

Hillary, R.M., Preece, A., Davies, C.R., Bravington, M.V., Eveson, J.P., and Basson, M. (2012) Initial exploration of options for inclusion of the close-kin data into the SBT operating model. CCSBT-ESC/1208/21.

Itoh, T., Kurota, H., and Takahashi, N. (2007) Review of the CCSBT Scientific Research Program between 2001 and 2005. CCSBT-ESC/0709/41

Polacheck, T., Gunn, J., Stanley, C. (2001) Proposal for Conventional Tagging Program within the Scientific Research Program of the Commission for the Conservation of Southern Bluefin Tuna. CCSBT-SC/0108/15.

Polacheck, T., Eveson, J.P., Laslett, G.M. (2006) Estimation of mortality rates from tagging data for pelagic fisheries: analysis and experimental design. Final Report for FRDC Project No. 2002/015. Available at: http://frdc.com.au/research/Documents/Final_reports/2002-015-DLD.pdf

Polacheck, T., Eveson, J.P. (2007) Analyses of tag return data from the CCSBT SRP tagging program – 2007. CCSBT-ESC/0709/19.

Polacheck, T., Eveson, J.P., and Laslett, G.M. (2010) Classifying tagging experiments for commercial fisheries into three fundamental types based on design, data requirements and estimable population parameters. Fish and Fisheries 11: 133-148.

Preece A., Davies, C., Bravington, M., Hillary, R., Eveson, P., Grewe, P. (2013) Preliminary cost and precision estimates of sampling designs for gene-tagging for SBT. CCSBT-ESC/1309/18.

Sakai, O. and Itoh, T. 2014. Report of activities for conventional and archival tagging and recapture for southern bluefin tuna by Japan in 2013/2014. CCSBT-ESC/1409/30

Seber, G.A.F. (1982) The Estimation of Animal Abundance and Related Parameters, 2nd ed. Charles Griffin, London.

Appendices

A1. Protocols for tagging and recapturing

A code of practice and standard operating procedures for conventional and electronic tagging has been developed to guide tagging programs run in Australian waters (Bradford et al. 2009). These procedures ensure Australian animal ethics regulations are met, fish are handled carefully and tagging staff are safe.

Tagging protocols were developed during the experimental design stage for the CCSBT SRP conventional tagging program to ensure that tags are spread over as many schools and as great a time period as possible. It was recommended that the number of fish tagged within any school or area will be limited in order to ensure optimal spread of tags throughout the population; however, discretion will need to be exercised if fish availability is low in order to maximize the number of fish released within the available vessel charter time (Anon. 2001).

To tag fish in the right age class, a narrow range of lengths will be used to determine which fish to take a tissue sample from. The potential biases associated with using length to determine the age-class has been examined in the main document, and is no different to conventional tagging operations.

Finding sufficient numbers of fish for tagging can be difficult in some circumstances. The issues that can affect success include, experience of the fishing master and crew, weather, and number of days spent steaming to fishing grounds and between fishing areas. At-sea tagging and release of fish is expensive, so a minimal number of days of charter are included in the costings, but the number of days estimated to be required was based on the CCSBT SRP conventional tagging program in the GAB in the 2000s. The proposed 20 days sea time should allow some time for travel to fishing grounds, searching time and small amounts of bad weather, but capture and tagging 5000 fish cannot be guaranteed.

Field operations for “tagging” would involve capturing, measuring length (to determine if the fish is in the target age class), taking a tissue sample, and releasing thousands of fish. Sampling for recaptures is done by taking a tissue sample from a randomly selected sample of fish following capture by the fishery. Only fish that fall within the length range most apt to correspond to the age of the tagged cohort will be genotyped; however, it would be desirable to take tissue samples from a wider length range so that the more specific length range to be genotyped can be adjusted if necessary. As noted in the main document, it would be highly desirable to extract otoliths from fish within the target length range at the time of resampling so that they can be aged, and this information used, first, to refine the length range to be genotyped and, second, to estimate what proportion of the genotyped fish are likely to have been incorrectly aged based on their length.

A tissue sampling tool has been developed to assist in collection of clean, uncontaminated, tissue samples (Bradford et al. in press). The tissue sampling tool can be used in conjunction with an electronic fish measuring board that can automatically record length and weight, and a code that identifies the tissue sample vial. This approach also substantially reduces the labour costs associated with tissue handling for DNA extraction and geneotyping.

A2. Further information on the estimation of costs and sample sizes

Figure A2.1 and Table A2.1 provides information on the median abundance estimates of age 1 and 2 fish from the 2014 reference set of operating models. The most recent two points for age 2 are clearly above the average for much of the time series, and estimation of these cohorts is highly uncertain until additional information becomes available in future years as they age and are either measured through an abundance monitoring program or become fully recruited and fished in the longline fishery. The very high estimates here would have been influenced by the 2014 aerial survey data point. Hence the choice of 2 million fish as an approximate median estimate, and 3 million fish as a maximum estimate, of the current abundance of 2 year olds for the purpose of estimating the sample sizes required in the pilot gene-tagging program.

Figure A2.1. Median numbers of fish at age 1 and age 2 from the reference set of models from the 2014 stock assessment.

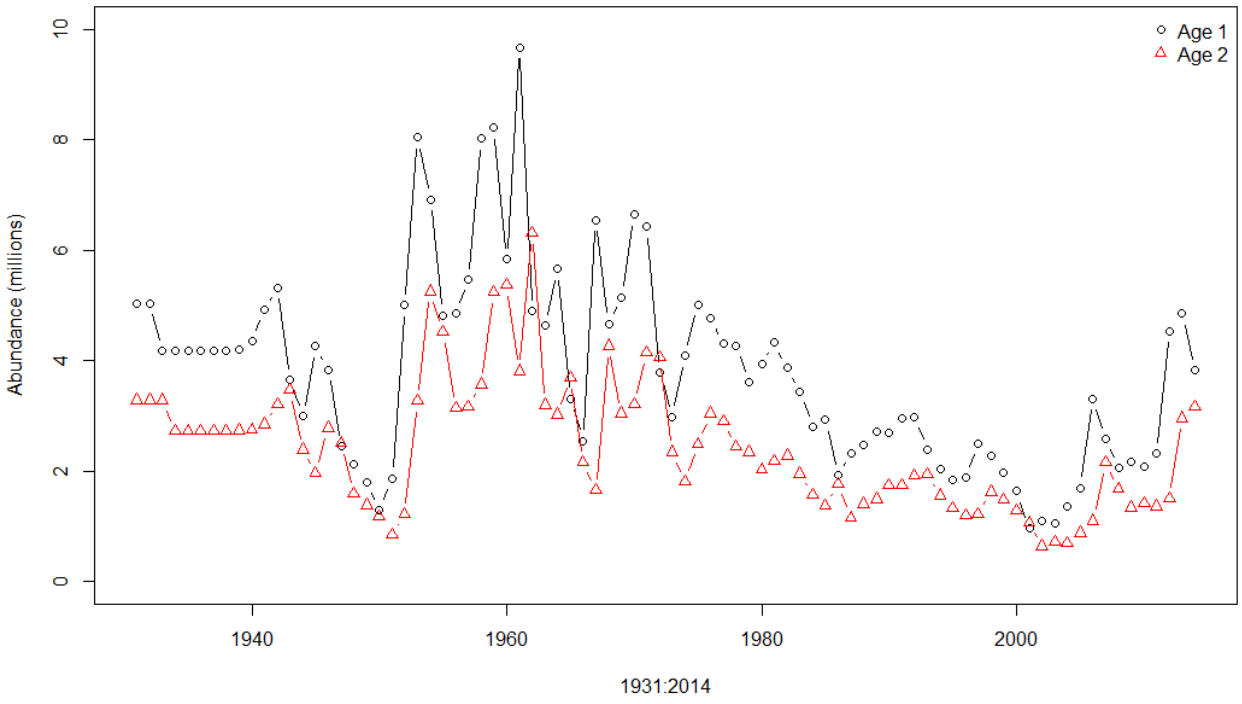

Table A2.1. Median numbers of fish at age 1 and age 2 from the reference set of models from the 2014 stock assessment from the last decade.

YEAR	AGE 1	AGE 2
2005	1685670	880582
2006	3303305	1099380
2007	2575850	2161305
2008	2053435	1678765
2009	2174390	1345510
2010	2085635	1422400
2011	2314660	1362610
2012	4518930	1513770
2013	4933620	2948070
2014	3891595	3228530

A3. Summer residency of juvenile SBT off South Africa (excerpt from Section 10.3.6 of Basson et al. 2015)

Summary:

Based on the analysis above we can provide the following answers to the questions originally posed.

Are there juveniles (age 2-4) that never visit the GAB in summer?

The answer to this question is still unknown and could still be "yes". One individual paid a very brief visit to the GAB in its first summer after tagging (age 2) and then migrated to the Indian Ocean and waters off South Africa where it remained for the subsequent two winters and summers.

Is there likely to be a large proportion of juvenile (age 2-4) SBT resident in waters off South Africa in summer?

We currently have no evidence to suggest that there is a large proportion of juvenile SBT resident in waters off South Africa in summer. Catches, and the proportions of age 2-4 SBT in those catches, have been very low in that area compared to catches in the GAB in the same months [*see Figure 10.19 below*]. Also, all but one of the tags in our dataset returned to the GAB in each subsequent summer during its deployment. Of course, lack of evidence does not constitute proof, so we cannot rule out the possibility.

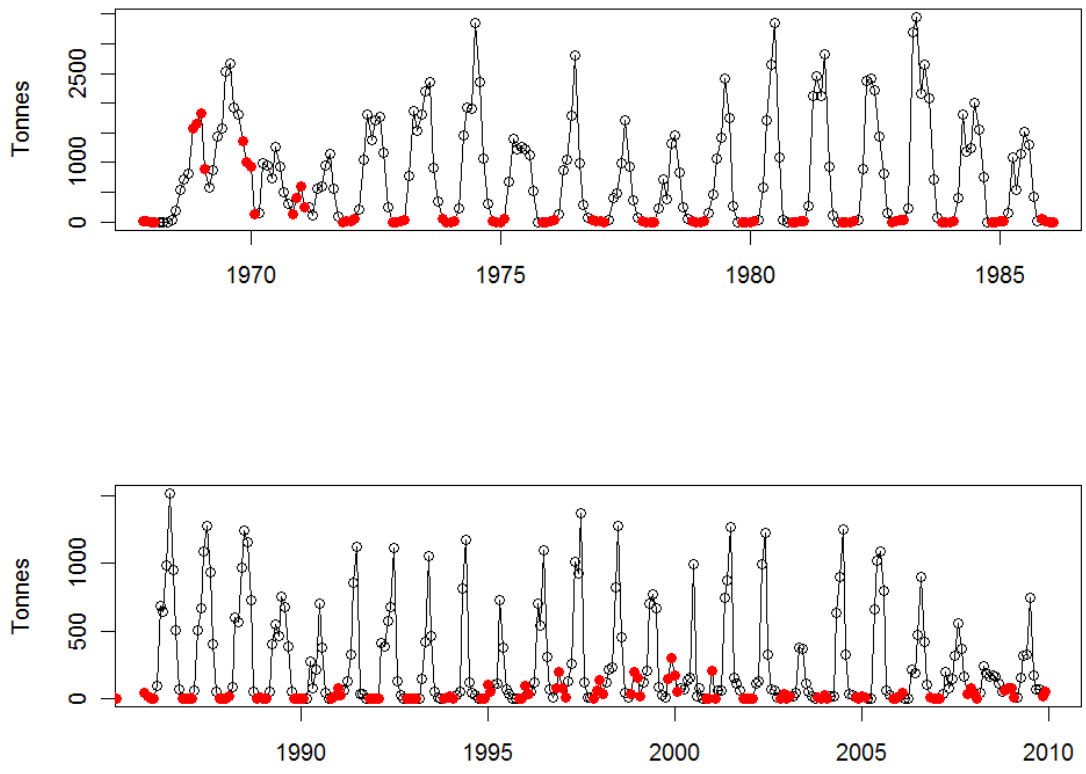

Figure 10.19. Monthly reported catches in tonnes in the area south and south-east of South Africa (20-55°E, 35-45°S) for 1968 to 1985 (top) and 1986 to 2009 (bottom). The summer months, November to February, are indicated by red solid dots. Note the difference in scales.

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