Fishery Report No. 107

2012

GENETAG:

Genetic Mark-Recapture for Real-Time Harvest Rate Monitoring:

Pilot Studies in Northern Australia Spanish Mackerel Fisheries

R C Buckworth, J R Ovenden, D Broderick,
G M Macbeth, G R McPherson and M J Phelan

FRDC Project No. 2002/011

Northern Territory Government

Australian Government
Fisheries Research and Development Corporation

Queensland Government

Fishery Report No. 107

GENETAG: Genetic Mark-Recapture for Real-Time Harvest Rate Monitoring: Pilot Studies in Northern Australia Spanish Mackerel Fisheries

R. C. Buckworth, J. R. Ovenden, D. Broderick, G. M. Macbeth, G. R. McPherson and M. J. Phelan

Australian Government

Fisheries Research and Development Corporation

FRDC Project No. 2002/011

2012

Fishery Report No. 107

GENETAG: Genetic Mark-Recapture for Real-Time Harvest Rate Monitoring: Pilot Studies in Northern Australia Spanish Mackerel Fisheries

R. C. Buckworth[1, 4], J. R. Ovenden[2], D. Broderick[2], G. M. Macbeth[2], G. R. McPherson[3] and M. J. Phelan[1]

1. Northern Territory Fisheries, Department of Resources, GPO Box 3000, Darwin NT 0801, Australia
2. Molecular Fisheries Laboratory, Queensland Department of Employment, Economic Development and Innovation
3. Northern Fisheries Centre, Queensland Department of Employment, Economic Development and Innovation
4. Current address: CSIRO, Marine and Atmospheric Research, GPO Box 2583, Brisbane Qld, 4001, Australia

FRDC Project No. 2002/011

Disclaimer

The authors do not warrant that the information in this document is free from errors or omissions. The authors do not accept any form of liability, be it contractual, tortious, or otherwise, for the contents of this document or for any consequences arising from its use or any reliance placed upon it. The information, opinions and advice contained in this document may not relate, or be relevant, to a reader's particular circumstances. Opinions expressed by the authors are the individual opinions expressed by those persons and are not necessarily those of the publisher, research provider or the Fisheries Research and Development Corporation.
The Fisheries Research and Development Corporation plans, invests in and manages fisheries research and development throughout Australia. It is a statutory authority within the portfolio of the federal Minister for Agriculture, Fisheries and Forestry, jointly funded by the Australian Government and the fishing industry.

While all care has been taken to ensure that information contained in this Fishery Report is true and correct at the time of publication, changes in circumstances after the time of publication may impact on the accuracy of its information.
The Northern Territory of Australia gives no warranty or assurance, and makes no representation as to the accuracy of any information or advice contained in this Fishery Report, or that it is suitable for your intended use.
You should not rely upon information in this publication for the purpose of making any serious business or investment decisions without obtaining independent and/or professional advice in relation to your particular situation.
The Northern Territory of Australia disclaims any liability or responsibility or duty of care towards any person for loss or damage caused by any use of or reliance on the information contained in this publication.

April 2012

Bibliography: preferred citation

Buckworth, R. C., Ovenden, J. R., Broderick, D., Macbeth, G. M., McPherson, G. R. and Phelan, M. J. (2012). GENETAG: Genetic mark-recapture for real-time harvest rate monitoring: Pilot studies in northern Australia Spanish Mackerel fisheries. Northern Territory Government, Australia. Fishery Report No. 107.

ISBN: 978-0-7245-4750-0

Corresponding author:

Dr Rik C Buckworth, CSIRO Marine and Atmospheric Research
GPO Box 2583, Brisbane Qld 4001 Australia
Phone: +61 7 38335902
Email: rik.buckworth@csiro.au

Table of Contents

NON TECHNICAL SUMMARY **12**

ACKNOWLEDGEMENTS **14**

BACKGROUND **15**

OVERVIEW 15
HARVEST RATE MANAGEMENT AND MONITORING 16
USE OF MICROSATELLITE DNA AS A 'GENE TAG' FOR HARVEST RATE ESTIMATION 16
AUSTRALIAN SPANISH MACKEREL FISHERIES AND ATTRIBUTES FOR GENETIC TAGGING 17

NEED **18**

OBJECTIVES **18**

METHODS **19**

METHODS OVERVIEW 19
LIAISON AND EXTENSION TO SUPPORT TAGGING PROGRAMS 22
COMBINATION MARK-RECAPTURE METHODS 23
ACOUSTIC TRACKING OF CONVENTIONALLY-TAGGED SPANISH MACKEREL 25
Acoustic environment *25*
10 kHz acoustic tags *25*
Propagation of acoustic tag signals *25*
Detection of acoustic tags *27*
GENETIC TAGGING METHODOLOGY: TISSUE SAMPLING 28
Sampling tissue for genotyping *28*
Genetag sample collection 28
Landed fish 30
DEVELOPMENT OF GENETIC LOCI FOR GENOTYPING 30
Building the genetic library for genotyping: theory *30*
Construction of a microsatellite library for Spanish Mackerels *31*
Genotyping with microsatellite loci 32
GENOTYPING PANELS 32
Development overview *32*
Panel design strategy *33*
M13 fluorescent labelling system 34
Panel composition 35
Evaluation of successive panel genotyping 35
IDENTIFYING GENETIC RECAPTURES: GENOTYPE MATCHES 36
Process overview *36*
DNA extraction 38
DNA extraction from hook-tips 38
Early problems with copper contamination 38
Routine DNA extraction from hook-tips and lancets 38
DNA extraction from tissue samples from the landed catch 39
DNA amplification 39
Genotyping of genetag and fishery samples *40*
Raw data handling 42
Genotype checking 42
Identification and removal of cryptic repeats 42
Identification and removal of non-Spanish Mackerel genotypes 42
Identification and correction of genotyping errors 44
Using a custom access database 44
Using SHAZA 44
Matching genotypes (i.e. identifying recaptures) and dealing with shadows to determine recaptures 45
Concept of shadows 45
Shadows – Sample size and siblings 46
Data Interrogation 47
Recapture rate 48
ESTIMATING ACTIVE FEEDING AGGREGATION SIZE AND FINE-SCALE HARVEST RATE FROM SHORT-TERM RECAPTURES 49
Molecular genetic analysis *49*
Determination of genotyping error rate *49*
Creating composite lure and fin genotypes prior to harvest rate analysis *49*
Estimation of putative recaptures between fins and lures *49*

Feeding and harvest model *49*

RESULTS/DISCUSSION **51**

CONVENTIONAL TAGGING 51
Releases of combination-tagged fish *51*
Conventional tag recaptures *52*
Combination tagging and recapture: Discussion *54*
ACOUSTIC TRACKING OF CONVENTIONALLY-TAGGED SPANISH MACKERELS 58
Estimated acoustic detection ranges based on likely propagation rates *58*
Acoustic tag releases *59*
Discussion: The potential for acoustic tracking of Spanish Mackerels *61*
Post-tagging survival *64*
Acoustic tag enhancements *65*
Tracking enhancements *65*
GENOTYPING 66
Microsatellite library *66*
Maximising information from partial genotypes 69
The effect of copper from hook bodies on DNA extractions 69
In-house or out-sourced genotyping? 71
Genotypes 71
Matching genotypes using a likelihood-based approach: SHAZA *75*
Determination of genotyping error rate 75
Empirical evaluation of genotype pairs as putative recaptures 75
Estimation of number of recaptures 76
SIZE OF ACTIVE FEEDING AGGREGATIONS AND FINE-SCALE HARVEST RATE 76
Estimation of genotype error rate from lure samples *76*
Estimation of genotype error rate from fin samples *77*
Removing lure and fin matches prior to lure by fin match analysis *77*
Estimation of short-term harvest rates from fishing trips (multiple day fishing data) *78*
Estimation of harvest rates from fishing days (single day fishing data) *79*
DISCUSSION: IDENTIFICATION OF RECAPTURES AND THE ESTIMATION OF ACTIVELY-FEEDING AGGREGATION SIZE AND HARVEST RATE 80
Genotyping, recaptures and the feasibility of the Genetag approach *80*
GENERAL DISCUSSION 83

BENEFITS **85**

FURTHER DEVELOPMENT **86**

EXPERIMENTAL DESIGN, MONITORING SYSTEM AND MANAGEMENT CONTROL DEVELOPMENT AND EVALUATION 86
TECHNICAL APPLICATIONS AND DEVELOPMENT 86
Tissue sampling *86*
Information capture, logistics and coordination *87*
SHAZA 88
Recreational tagging and acoustics *88*
Integrated studies *89*
A GENETAG APPROACH FOR MONITORING NORTHERN AUSTRALIA'S SPANISH MACKERELS 91
Costing *92*
FURTHER APPLICATIONS: WHICH SPECIES ARE MOST SUITABLE FOR GENETAG? 93
FISHERIES IN WHICH GENETAGGING COULD BE USEFULLY APPLIED 94
Examples *94*
Orange Roughy 94
Black Jewfish and other croakers 94
Commercial shark fishery of northern Australia 95
Gulf of Carpentaria populations of Barramundi 95
Queensland's east coast and Torres Strait Spanish Mackerel fishery 95
Red and Gold-band Snapper in the Timor/Arafura Sea 96
Invertebrate species 96

PLANNED OUTCOMES **97**

CONCLUSION **97**

REFERENCES **100**

APPENDIX 1: INTELLECTUAL PROPERTY **106**

SOME PROJECT-RELATED PUBLICATIONS AND PRESENTATIONS 106

APPENDIX 2: PROJECT STAFF 109

NORTHERN TERRITORY 109
QUEENSLAND 109

APPENDIX 3: DNA EXTRACTION 110

SALTING OUT TO EXTRACT DNA FROM FINS 110
Materials 110
Reagents 110
Equipment 110
Procedure 110
Critical steps 110
Troubleshooting 110
Notes 110
Acknowledgment 111
Reference 111
CHELEX-BASE SPANISH MACKEREL FIN/OPERCULUM DNA EXTRACTION – LAB PROTOCOL 111

APPENDIX 4: ESTIMATING WILD FEEDING SCHOOL SIZE (*W*) FROM FIN SAMPLES AND GENETAGS 112

APPENDIX 5: STRENGTHS, WEAKNESS, OPPORTUNITIES AND THREATS ANALYSIS (SWOT) OF GENETAG 114

STRENGTHS 114
WEAKNESSES 115
OPPORTUNITIES 116
THREATS 116

List of Tables

Table 1. Locus composition and panel fluorescent colour (M13 label) for the four panels used to genotype Spanish Mackerel samples 35
Table 2. PCR cycling conditions for the amplification of panels 1 – 4 - denaturation, annealing and extension stages were repeated 37 times 40
Table 3. PCR composition per well for panel one loci 41
Table 4. PCR composition per well for panel two loci 41
Table 5. PCR composition per well for panel three loci 41
Table 6. PCR composition per well for panel four loci 42
Table 7. Genotype error rate from fin samples 45
Table 8. Monthly releases of combination tagged Spanish Mackerels in the Genetag project, 2004-06 51
Table 9. Recaptures of conventionally-tagged Spanish Mackerels by year of release and year of recapture 53
Table 10. Mean and standard deviations of times at liberty for recaptured Spanish Mackerels by year at release and year recaptured 55
Table 11. Results of assessments of seven releases of acoustically-tagged Spanish Mackerels 60
Table 12. Amplification success of 34 primer pairs across closely related or co-distributed species of fish .. 67
Table 13. Summary data for microsatellites tested in Spanish Mackerels 68
Table 14. The numbers of full seven loci genotypes and partial genotypes across fins, lures and lancets ... 69
Table 15. Success rate of DNA extraction from Genetag hook-tips containing varying amounts of visible tissue midway through the project (June 2004) 70
Table 16. Copper concentration (ug/L) using graphite furnace flame atomic absorption spectroscopy of Spanish Mackerel DNA extracted from gene-tag hooks that was a good (good quality) or poor (poor quality) template for PCR of mtDNA control region 5 70
Table 17. Allele frequencies for 6071 seven-locus Spanish Mackerel genotypes from NT waters 72
Table 18. Summary statistics across loci for 6071 Spanish Mackerel seven-locus genotypes from NT waters representing hook-tips, lancets and fins 73

Table 19. Number of hook-tips (T) /lancets (H) /fins (F) that produced multi-locus genotypes compared to the number of those samples from which DNA was extracted 74
Table 20. By-lure aggregation of genotyping success where 0 genotyped is maximum success 75
Table 21. Recapture estimates ($\hat{r}$) in 14 of 90 sampling events for narrow-barred Spanish Mackerel genetic mark-recapture data 77
Table 22. Number of fish harvested, lures successfully deployed and fins sampled during fishing trips lasting more than one day - recaptures between lure and fin samples with standard error of genotype assignment 79
Table 23. Estimates of the number of Genetagged fish remaining at large, feeding aggregation size and harvest percentage from data pooled over a fishing trip lasting more than one day 80
Table 24. Number of fish harvested, lures deployed and fins sampled on the same day (or adjacent days) of fishing trips recaptures between lure and fin samples with standard error of genotype assignment 81
Table 25. Estimates of the number of Genetagged fish remaining at large, feeding aggregation and harvest percentage from data pooled over single day fishing trips 82
Table 26. An example of annual costing for northern Australia's Spanish Mackerel project based on deployment of 500 Genetags and screening of 7000 landed fish 93

List of Figures

Figure 1. Conceptual overview of the mark-recapture process: (a) Conventional and (b) Genetic 21
Figure 2. Lancets used to collect tissue simultaneously with the placement of conventional tags: (a) Cut and ground from stainless steel wire, 5 cm long (b) Laser-cut from sheet stainless steel, 4 cm long 24
Figure 3. Snapping shrimp showing large appendage for generating short bursts of broadband sound to stun and dazzle with light generated with the acoustic pulse 28
Figure 4. A Genetag hook, in double hook configuration, mounted on a Smith's jig 29
Figure 5. Nucleotide sequence of oligonucleotides used to extend the primer sequences to increase allele lengths 33
Figure 6. Diagrammatic explanation of the primer extension and fluorescent M13 labelling system used in this project 34
Figure 7. Hook-tip Sc272 showing a large amount of tissue with green colour that could have been a result of copper contamination 38
Figure 8. Hook-tips showing small (left) and large (right) amounts of retained tissue 39
Figure 9. Q-Q plot of observed and expected ln(genotype probability) frequencies 44
Figure 10. A histogram of pairwise allelic matches among 250 Spanish Mackerels that were genotyped at seven loci (2 x 7 alleles) 46
Figure 11. The relationship between the expected number of shadows and sample size for different PIDs . 47
Figure 12. Feeding and harvest model with relationship between total feeding (*W*), number harvested (*H*), harvested fish with fins genotyped (*F*) and the number of lures genotyped (*L*) 50
Figure 13. Location of releases of combination tagged Spanish Mackerels during 2003-06 52
Figure 14. Distribution of the numbers of narrow-barred Spanish Mackerels tagged on successful tagging days 52
Figure 15. Approximate vectors of movement of combination-tagged Spanish Mackerels 53
Figure 16. Calculated straight-line distances moved for recaptured Spanish Mackerels 54
Figure 17. Frequency distribution of time at liberty for recaptured Spanish Mackerels 56
Figure 18. A comparison of distance moved with days at liberty for recaptured Spanish Mackerels 56
Figure 19. Estimated acoustic propagation of 10 kHz tone bursts signals from an acoustic tag given most likely propagation models 58

Figure 20. Acoustic Tag 2 immediately after release at first detection at approximately 16 m away from a hydrophone at 5 m 61
Figure 21. Acoustic Tag 4 at 34 seconds after release 62
Figure 22. Estimated range to the acoustic tag signal shown in Figure 20. 62
Figure 23. Sequence of shark depredation on a Spanish Mackerel (upper), with fish remains in a fish tagging cradle (lower) 64
Figure 24. Data flow in the Genetag project with electronic data recording implemented 90

2002/011	**GENETAG: Genetic mark-recapture for real-time harvest rate monitoring - Pilot studies in northern Australia Spanish Mackerel fisheries**

PRINCIPAL INVESTIGATOR: Dr Rik C Buckworth
ADDRESS: Department of Resources
GPO Box 3000
Darwin NT 0801
Telephone: +61 8 89992144 Fax: +61 8 8992002

OBJECTIVES:

1. Confirm the technical basis of *in situ* genetic tagging for large pelagic fishes.
2. Provide initial estimates of harvest rates in the Darwin area Spanish Mackerel fisheries, to develop protocols and scenarios for the monitoring of harvest rate in Australia's Spanish Mackerel fisheries using genetic and conventional tagging.
3. Compare genetic and conventional tag mortality and retention for *Scomberomorus commerson.*
4. Provide information on movement rates of *S. commerson* in northern Australia.
5. Develop a general methodology for the use of genetic mark-recapture as the basis for fishery harvest rate monitoring.

Additional objectives approved as a variation to the project were:

6. Undertake genetic analysis of material collected during 2002, 2003 and 2004 sampling years in the Genetag (FRDC 2002/011) project.
7. Undertake Genetag sampling during 2005-06.
8. Undertake genetic analysis of material sampled during 2005-06.
9. Undertake pilot sonic tracking for conventionally-tagged *S. commerson* for the estimation of initial mortality rates.
10. Communicate progress, concepts and results at the 2006 FRDC Australian Society for Fish Biology workshop on "The Cutting Edge in Fisheries Science and Management".

NON TECHNICAL SUMMARY

OUTCOMES ACHIEVED

Genetic mark-recapture has been developed in this project, as a novel methodology which, with further development, will support monitoring the status of a variety of fisheries, particularly those for narrow-barred Spanish Mackerel (*S. commerson*) and similar large pelagic fish.

The project provided a set of protocols for the application of genetic mark-recapture, including remote tissue collection, protocols and software for the design of panels for genotype elaboration as well as simulation of genetic mark-recapture experiments (LOCUSEATER and SHADOWBOXER). A major advance was the development and provision of protocols and corresponding software (SHAZA), for the comparison of genotypes, including partial genotypes, and quantification of recaptures and errors in mark-recapture studies. The protocols and software have application in wider contexts, including forensic analysis.

Effective protocols were also developed for combined genetic/conventional tagging, indicating the strong value of recreational fishery participation in experimentation and monitoring. In a preliminary assessment of the survival of conventionally-tagged fish, we demonstrated simple acoustic tracking protocols that should have wider application for species such as Spanish Mackerel.

The project provided the basis for development and management of large scale genetic mark-recapture studies, concurrent conventional and combined genetic/conventional mark-recapture studies and the ability to evaluate experimental or monitoring mark-recapture programs. The project has demonstrated the potential for the genetic mark-recapture for fished species that are difficult or expensive to tag in more conventional manners. Careful experimental design and planning will be needed to maximise the information yield given the logistic and analytical requirements of a genetic tagging project. Nevertheless, with further development of the Genetag approach, where it can be routinely applied to monitor mortality and harvest rates in fisheries for species such as Spanish mackerel, the primary outcome will be to reduce uncertainty in assessment and management of those fisheries, thereby improving management performance in Australian and other fisheries.

We developed a new monitoring method (“Genetag”), for application to a wide variety of fisheries. Although we report the development and evaluation of protocols specifically for Spanish Mackerel (*S. commerson*) fisheries of northern Australia, the protocols have much wider potential utility.

Gene-tagging provides the basis for mark-recapture estimation of two important fisheries assessment statistics: the fishery harvest or exploitation rate and catchability, over time. Fish are ‘marked’ via remote, *in situ* tissue sampling and subsequent genotyped. Remote tissue samples are collected using ‘Genetag’ hooks. With *in situ* sampling, fish do not need to be captured and landed for tagging as they do in conventional tagging, thus avoiding some of its serious limitations, such as mortality and behavioural changes associated with the tagging and release process. Furthermore, tags are not lost. Under-reporting, which otherwise may lead to a dangerous optimistic bias in estimated fishery exploitation rate, is avoided, as a known proportion of the commercial catch is screened for the Gene-tagged fish. In many contexts, genetic mark-recapture will actually be a more cost-effective approach to estimating these important parameters of fishery performance than conventional tagging.

Adult Spanish Mackerels in northern Australia are thought to form highly localised sub-populations that have low levels of mixing. Consequently, obtaining information on harvest rates for stock assessment is difficult using traditional monitoring methods, such as abundance surveys and age structure analyses. In addition, these large pelagic predators are highly unsuitable for conventional tagging as they may suffer high mortality rates being line-caught and brought aboard vessels. Genetic tagging overcomes these problems and the

value of individual Spanish Mackerels is sufficiently high to justify the associated costs of this individual-based approach.

Pilot projects provided a hook that retained a small piece of tissue when it was attacked by a Spanish Mackerel, and a set of candidate genetic loci (markers for DNA fingerprinting) were identified. A protocol and software (LOCUSEATER/ SHADOWBOXER) were designed to choose amongst the candidate markers to create optimum genotyping panels. A set of 7 polymorphic loci was chosen.

Most genetic tagging was during commercial fishing operations, mostly in the western Top End of the Northern Territory (NT), based in Darwin. Of 1372 Genetag lures deployed during 2002-06, useable genotypes (>3 loci, suitable for analysis) came from 491. These were “Gene-tagged” animals. To estimate the proportion of these Genetagged fish recaptured, we collected fins from nearby fishing operations. More than 9000 fin samples produced 7528 useable genotypes.

We also conducted a separate program of combined tagging, in which fish were simultaneously genetically and conventionally tagged, with recreational fishery volunteers. Combined tagging potentially provided for the measurement of the shedding rate of conventional tags. Conventional tagging used protocols maximising the survival of tagged fish (fish were brought rapidly to the boat and tagged in the water). We achieved 842 releases. Tissue was sampled simultaneously with tagging, using lancets designed within the project producing up to 95% useable genotypes (2005). Additionally, 16 angler-caught fish were genetically but not conventionally tagged. The highly successful conventional tagging component of the project provided a gross return rate of 3.6% and allowed engagement with the recreational sector. The mean time at liberty was 320 days (342.2 days S.D.). Recaptures were up to four years after release. With low mean distances moved by these fish (< 30 km), and little sector overlap in fishing effort, there was a low probability of recapture of the conventionally–tagged fish in the commercial Spanish Mackerel Fishery: only one conventional recapture was reported and there were no genetic recaptures between the sectors. Given effort distributions and apparent movements, this is likely due to spatial separation of the different stocks targeted. The results also provided information on the relative harvest by the Offshore Net and Line Fishery.

Pilot acoustic tracking examined survival of conventionally-tagged fish, demonstrating the utility of the acoustic methods and, with the in-water pole tagging methods of the project, good initial survival. Tagged fish apparently moved rapidly (< 8.5 mins) away from the tagging boat, to the edge of audibility (> 500m).

The project genotyped and compared more than 11 000 tissue samples. Quality control was paramount. The variable quality and large number of samples led to many challenges in genotyping and comparison of samples. The SHAZA (shadow zone analysis) protocol entailed the development of new theory and procedures and was implemented as a new publicly-available software package. The protocol increased the proportion of useful genotypes from 72.5% to 94%, enabling a 3.2 fold increase in pairwise comparisons. This conceptual and procedural breakthrough allowed complex comparisons of genotypes and the estimation of recapture and error rates. There were no long-term recaptures detected. However, 21 short term recaptures (within two days) were identified. Feeding aggregations encountered during tagging operations were estimated to comprise 64 (48-137) and 1382 (713-78418) fish. The percentage of active feeders harvested on single fishing days ranged from 6% to 90% (average of 41%).

The focus of this project was the development of the suite of resources required for the technical feasibility of remote Gene-tagging, for use as a tool for generating data to estimate harvest rates of such species as Spanish Mackerel. We have generated a protocol that can provide such information. However, further careful planning and development will be required to implement the approach as a routine monitoring tool.

KEYWORDS: Spanish Mackerel, *Scomberomorus commerson*, genetic tagging, mark-recapture, Genetag, acoustic tracking, movement rates, movement distances, microsatellites, genotype matching, genotyping error rates, SHAZA, harvest rate estimation.

ACKNOWLEDGEMENTS

We are grateful for the considerable financial and in-kind support provided by the NT and Queensland Governments, including a grant from the NT Innovation and Research Fund, as well substantial in-kind support from the Spanish Mackerel Licensee Committee of the NT Seafood Council and the Amateur Fishermen's Association of the NT (AFANT). CSIRO Marine and Atmospheric Research supported the completion of reporting by Rik Buckworth.

We thank staff from NT Fisheries of the Department of Resources (DoR), especially Charles Bryce, Adrian Donati, Paul de Lestang, Chris Errity, Julie Martin, Rachael Hunter, and Kylie Higgins, who undertook much of the field work, administration and liaison associated with the project, as well as Stephanie Boubaris, Roland Griffin, David McKey, Quentin Allsop, Danny Argent, Ian Dunn, Trixie Trojan, Paul Davies, Bruce Young-Smith, Richard Sellers, Nick Rayns, Bill Flaherty and Heather Brayford, who all contributed to, and were hugely supportive of, this project. HockSeng Lee plotted the conventional tag releases and provided distance calculations.

Thor Saunders and Andria Handley, at NT Fisheries, and Matt Barwick (Recfishing Research) were especially helpful in revision and proofing of the Final Report.

From the Queensland Government, Simon Hoyle, David and Samantha Peel, George Leigh, Rick Officer and Mike Potter all deserve particular thanks for their inputs and encouragement.

Steve Newman, of Fisheries WA, participated in the project as a "sounding board", provided review and his input was especially appreciated in the formulation of the project.

Further conceptual guidance and encouragement was provided by Professors Carl Walters and Steve Martell (University of British Columbia), Sean Cox (Simon Fraser University) and Norm Hall (Murdoch University).

The contributions to this project from the fishing industry were substantial. From the NT Spanish Mackerel Fishery, we especially thank Norm Hedditch and Milton Miller. Peter Manning, Iain Smith and Katherine Sarneckis of the NT Seafood Council, and other licensees and fishers were also supportive.

In the recreational fishing community, John Harrison, Peter and Julie Fisher and Wayne Baldwin championed this project, as did many other members of our recreational 'tag team'. We also received excellent support from Chris Makepeace and the AFANT executive, while Denis Lillee and Andrew Ettinghausen added encouragement and excitement.

The generosity of sponsorship for the recreational taggers was remarkable. This came from *Happy Mick's* (Michael and Charlie Chambers), *Aussie Outback Supplies* (Darren Spencer), *Reidy's Lures, Halco Lures, Classic Bluewater Lures, In & Outboard Marine, Crocodylus Park, CMAX Theatre* (Palmerston), the *Lewis Group* and others. These were all very much appreciated. Dennis Lillee generously provided a signed caricature of himself and Christopher May donated a logo for the project.

Ian Dunn of NTTAGS manufactured the Genetag hooks. Jim Ottley and Charles Bryce devised lure alarms.

The kind support of the Fisheries Research and Development Corporation (FRDC) staff, in particular Carolyn Stewardson and Crispian Ashby, is also greatly appreciated.

Contributors to cover art

Sam M. Buckworth created the front cover design. Christopher May provided the project logo (back cover)

Colourings of Spanish Mackerels were provided by:

Camille Bradshaw, Ella and Tim Bryden-Brown, Manisha and Soni Buick, Jasper and Paige Cusie, Ben and Oliver De Lestang, Elarose Donnely, Sam and Kym Errity, Isabella Handley, Lesley and Tamicah Jerry, Louis Kenyon, Evie and Ivy Kernke, Erica and Mihaelia Kurnoth, Jemilla Sasha and Lilly Halliday, Olivia and Emma Harm, Emma and Laura Macbeth, Sabyne McCallum, Lachlan and Lillie Phelan, Ruby Taylor; and,

The "Holy Trinity Early Learning Centre" Curtin, Canberra ACT, guided by teachers Iva Plessnitzer and Rachael Hansen:

Aiden, Amelia, Angela, Anita, Casey, Cecilia, Charles, Chihiro, Chloe, Claire, Daniel, Ella, Emily, Georgia H., Georgia O., Henry, Hugh, Ioanna, Isabella, Joshua, Katherine, Leo, Liam, Molly, Noah A., Noah P., Olivia, Paddy, Patrick, Ross, Sofia, Sophie, Tayah, Timothy, Toby, Willow, and Zari.

BACKGROUND

OVERVIEW

In this project, we combined several novel approaches to develop a new monitoring method based on genetic mark-recapture, called “Genetag”, which has potential application in a wide variety of fisheries. We developed protocols specifically for Australian Spanish Mackerel fisheries because they appeared particularly suited to the pilot work and have a strong need for the development of an appropriate monitoring method for stock assessments. The approach was directed at the simple mark-recapture estimation of fishery harvest rates (U) and tracking of catchability (q). Instead of conventional tagging, we used remote (i.e. *in situ*) tissue sampling for the identification of individual fish, using “DNA fingerprints” (microsatellite DNA genotypes), as the basis of mark-recapture monitoring. Tissue samples and biopsies were collected using special hooks or lancets – i.e. fish were not usually captured and landed for tagging in the conventional manner. This approach avoids the serious problems of mortality and behavioural changes associated with the conventional capture, tagging and release process, as well as tag loss. Under-reporting, which leads to a dangerous optimistic bias in estimated U, is also avoided, as a known proportion of the commercial catch is screened. We included conventional tagging methods both as a control methodology and for the development of conventional tagging as a source of additional information. The genetic tagging approach would be useful in any fishery where more usual monitoring methods, such as survey, age structure analyses, and conventional tagging are problematic, especially where the value of individual fish is sufficiently high relative to population size to justify a significant cost.

The status of Spanish Mackerel fisheries in Australia is largely uncertain because there are no monitoring measures that can adequately provide estimates of relative biomass or harvest rates, at least within the technical and economic capacity of most fisheries and fishery management agencies. The objective of this project was to develop a genetic mark-recapture approach for mackerel as an approach that might be applied to other fisheries. This work is highly suitable for Spanish Mackerels as they are biologically and operationally well- known and have other attributes (such as presumed small stock size and fairly restricted movements), which would maximise feasibility.

HARVEST RATE MANAGEMENT AND MONITORING

The fundamental role of fishery management is to control the impact of fishing so that targeted populations remain sustainable. One simple measure of this impact is the harvest rate U, which we define as the proportion of the population vulnerable to fishing that is caught annually, so that the annual Harvest Rate = Catch/ Fishable Biomass. Target harvest rates may be chosen to provide a sustainable maximum catch, determined from equilibrium calculations or other rules (Walters and Parma 1996; Patterson 1992). Management thus needs to monitor U. The quality of monitoring - the accuracy and precision with which U is known - has a strong impact on the performance of the fishery. If U is poorly known, precautionary management may ensure sustainability but will also reduce economic or recreational benefit. At the same time, unaccounted change in monitoring measures may of course render apparently conservative fishing levels detrimental. Impacts of fishing are assessed by monitoring catch rates, conducting surveys to track changes in abundance or track the effects of fishing through changes in age and size structures, all of which provide indirect measures of harvest rates. Uncertainty in the monitoring of U is a strong contributor to the risks of either under- or over-fishing. In contrast, if harvest rates are known in real time, managers can react quickly.

Harvest rates can be estimated directly from mark-recaptures studies (Ludwig et al. 1993). In the simplest scenarios, the proportion of tags returned from annual releases is a direct measure of the harvest rate. Recent work has shown that monitoring U with such mark-recapture methodologies can be quite effective, even with relatively small annual releases of tags (Martell and Walters 2002; Buckworth et al. 2002; Buckworth 2004a).

The virtue of this approach is that unbiased estimates of U from small annual tagging experiments can be employed in a monitoring program. Repeated annually, information from the tagging program provides an annual initial estimate of U, as well as catchability, which is used to update a U estimate based on the full time series. However, these simulations have considered idealised tagging, not limited by the difficult problems of tag loss and lack of information about (unknown) post-release mortality rates and under-reporting of recaptures (Buckworth 2004a). These can be a source of large error. The high cost of catching undamaged fish for marking and release must also be considered. Although they provide definitive growth and movement information (e.g. McPherson 1992; Govender 1993), conventional tagging methods have not been particularly successful in monitoring *S. commerson*. Even in the highly competitive east coast sports fishery, the Sunfish program (an angler-based mark-recapture program in Queensland) has achieved a maximum return rate of only about 5% for Spanish Mackerels (Bill Sawynock, pers. comm.).

To overcome the issues of conventional tagging Spanish Mackerels, genetic tagging, whereby an *in situ* biopsy harvests a small amount of tissue without actually catching the fish – so that a fish may be identified - can be used in its place. A pilot project (Buckworth et al. 2002; Buckworth 2004a) demonstrated the feasibility of this remote collection of tissue from Spanish Mackerels.

USE OF MICROSATELLITE DNA AS A 'GENE TAG' FOR HARVEST RATE ESTIMATION

Genetic methods have had extensive application in fisheries for the description of spatial stock structures, with allozyme and mitochondrial DNA techniques being applied to establish the degree of relatedness between spatially and temporally separate components of fish stocks. However, the identification of individuals by analysis of their DNA has its most familiar application in forensic science – popularly known as "DNA fingerprinting". Because they may be highly variable within a population, DNA microsatellites (msDNA) potentially identify individuals with relatively few loci (i.e. requiring the examination of relatively little DNA). Because it also requires a small amount of tissue, the technology has been used in terrestrial biology for estimating population abundance with non-intrusive sampling methods that are preferred for rare or cryptic species, such as bears (Paetkau and Strobeck 1994), wolves (Davies et al. 1999) or wombats (Taylor et al.

1994). DNA from tissue sampled with biopsy darts has been widely used for stock structure studies and population estimation in marine mammals (e.g. Barrett-Lennard et al. 1996). A naturally occurring genetic variation was used as a marker for red drum stocking in Texas (Ward et al. 1995), and the genetic identity of the parental stock is similarly used to identify prawns stocked as part of an enhancement experiment in Exmouth Gulf (Loneragan et al. 2002). However, to the authors' knowledge, there has been no previous effort to employ genetic-based mark-recapture estimation of harvest rates in fisheries.

Pilot studies show that msDNA sequences can be readily established for Spanish Mackerels (Buckworth et al. 2002); initial experiments also showed that genetically 'tagging' fish by obtaining an *in situ* biopsy is also feasible (Buckworth 2004a). The declining costs of the genetic technology are now such that this method is also economically feasible.

AUSTRALIAN SPANISH MACKEREL FISHERIES AND ATTRIBUTES FOR GENETIC TAGGING

Spanish Mackerels are the subject of important commercial and recreational fisheries in Australia (at the inception of this project in 2002, commercial catches of Spanish Mackerels were around 1500 tonnes, valued at around $9 million, with recreational catches of a similar magnitude, and growing). The biology of the species in Australia is relatively well known with key population processes of growth, reproduction and feeding described (see McPherson 1992), and management and monitoring programs in place in most fisheries (e.g. Buckworth and Clarke 2001; Welch et al. 2002).

A series of recent projects addressed key management and assessment problems for the species (e.g. Welch et al. 2002; Hoyle 2002; Mackie et al. 2003). The FRDC project 98/159 titled The Stock Structure of Northern and Western Australian Spanish Mackerel (Buckworth et al. 2007a), provided information to scale the management of the species. This has shown that, rather than being a highly migratory, well-mixed stock, Spanish Mackerels in northern Australia consist of a mosaic of adult assemblages, which have very little mixing - at least as adults - yet there is a small degree of genetic interchange. A meta-population may be susceptible to local depletion and serial overfishing, which remains undetectable in log book information except when examined at fine scales, or when depletion is strong.

We emphasise that the Genetag project was about developing a new methodology that might be applicable in many fisheries in Australia and globally. However, there are several attributes that made Spanish Mackerels a good candidate for pilot work on this methodology. Normally fished with trolled lures or baits in a highly targeted fishery, Spanish Mackerels lend themselves to remote tissue collection. A pilot project (with NT Fishing Industry Research and Development Trust Account funding) indicated the feasibility of remote tissue collection for the species. Previous work (Buckworth et al. 2007a) also indicated that the small spatial area occupied by Spanish Mackerel adult assemblages – which might be considered as sub-stocks - allowed for the 'tagging' component of the operation to be concentrated, allowing a large proportion of a small population to be tagged. Because adult fish do not move great distances, there was a good chance of recapture by fishers conducting operations in the original tagging area. Since Spanish Mackerels are large fish, the catch is composed of few animals (the annual NT catch of typically 300-400 tonnes is numerically small at only 30 000 to 60 000 fish). This meant that it was feasible to collect tissue and screen a substantial proportion of the catch from the Darwin area. These fish are taken by few operators, and the monitoring and research programs conducted in the fishery meant that there was a strong awareness and collaborative support for this research program.

Spanish Mackerels are a popular target of anglers, especially chartered game fishing, and of recreational tagging clubs. The Suntag club passed the 250 000 tagged fish mark in 2002, indicating that recreational taggers had the potential to contribute substantially to monitoring of fishery status. Recreational tagging was an important component of earlier FRDC-funded studies into the fisheries for the smaller *Scomberomorus*

species (Begg et al. 1998a, 1998b). We proposed in this project to collaborate with recreational taggers to provide methodological control and begin the incorporation of recreational tagging into monitoring programs. We envisaged a modified conventional tagging program in which survival of the fish would be emphasised. The absence of hard scales in the species makes the fish especially suitable for pole tagging. Australia's recreational taggers are a group whose efforts would be so much more useful if their work could more often produce valuable information for fishery management, such as mortality rate estimation. They are a useful resource and they could be made much more valuable.

Additional support for the project became necessary when technical issues with outsourced genotyping, as well as protocols for tissue storage and recruitment difficulties compromised the completion of the project. Additionally, acoustic tracking methodologies developed by FRDC Project 2003/016 titled Reduction of Interactions by Toothed Whales with Fishing Gear, created the opportunity to test the feasibility of simple sonic tracking approaches and to thereby estimate the initial mortalities imposed on Spanish Mackerels by conventional tagging.

NEED

Effective fishery management requires knowledge of the impact of fishing on stocks i.e. effective methods for monitoring harvest rates (U). However, there are no reliable indices of U or of abundance for many fished species. Most methods are flawed; some, such as catch per unit effort CPUE), might only indicate the impact of fishing when populations are depleted. A lack of effective monitoring means loss of catch, income and recreation, and jeopardizes sustainability. Although conventional tagging is an obvious method of measuring U, it is strongly limited by post-release mortality, tag shedding and non-reporting of tags. The attraction of genetic "tagging" – with individuals identified from tissue collected with special hooks, or perhaps with spears, by SCUBA divers, and subsequent screening of the commercial catch to establish a "recapture rate" – is that it largely overcomes the problems of conventional tags. Even a modest increase in monitoring information quality translates to real economic and sustainability benefits. Anglers provide useful information on movements and growth (e.g. through SUNTAG tagging). But the information they provide for monitoring U is limited by the general tagging problems. By coupling conventional with genetic tagging, this project provides a direction for improvement of recreational tagging, by calibrating and quantifying problems and developing them as a source of auxiliary information. This could substantially increase the value of recreational tagging. There is currently no effective monitoring method for Spanish Mackerels; they are not amenable to survey and because they shoal, CPUE is a poor abundance measure. They have poor post-tagging survival, with low return rates (<5% even in the highly-publicised Queensland Suntag program, Sawynock, pers. comm.). Lack of quality monitoring information is of concern for all Australian mackerel fisheries; in several of them there are real concerns about the status of the stocks. It was concluded in stock assessment workshops conducted in Darwin in August 2000, led by Professors Carl Walters and Norm Hall, that the primary research direction for mackerel and several other fisheries should be the development of new tagging approaches. The Genetag project met that recommendation and it may provide the basis for effective monitoring for a wide range of fisheries in which current status is otherwise very uncertain.

OBJECTIVES

1. Confirm the technical basis of *in situ* genetic tagging for large pelagic fishes.
2. Provide initial estimates of harvest rates in the Darwin area Spanish Mackerel fisheries so as to develop protocols and scenarios for monitoring harvest rates in Australia's Spanish Mackerel fisheries using genetic and conventional tagging.
3. Compare genetic and conventional tag mortality and retention of *S. commerson*.
4. Provide information on movement rates of *S. commerson* in northern Australia.

5. Develop a general methodology for the use of genetic mark-recapture as the basis for fishery harvest rate monitoring.
6. Undertake genetic analysis of material collected during 2002, 2003 and 2004 sampling years in Genetag (FRDC 2002/011) project.
7. Undertake Genetag sampling during 2005-6.
8. Conduct genetic analysis of material sampled during 2005-06.
9. Undertake pilot sonic tracking for conventionally-tagged *S. commerson* to estimating initial mortality rates.
10. Communicate progress, concepts and results at the 2006 FRDC Australian Society for Fish Biology workshop on "The Cutting Edge in Fisheries Science and Management".

METHODS

METHODS OVERVIEW

The objectives of this project were principally about developing and demonstrating the feasibility of genetic mark-recapture to provide information for estimation of fishery harvest rates.

Conventional tagging is a familiar concept: fish are labelled or marked in some way that is usually visually detectable. For example, the dart tags commonly used for tagging many fish species are simply labelled streamers or tubes of plastic that are anchored in the dorsal muscle of the fish. When the fish are later sought for recapture, they are detected because the tags are obviously visible. The tags are usually highly coloured for this purpose and individually numbered, so that information on movements and other attributes of individual or groups of fish is available. This process is summarised in Figure 1(a).

Genetic tagging is a more complex process, largely because the genetic identity of a fish is not visible, and therefore many fish need to be examined chemically to distinguish those that have been “tagged”, from those that were not tagged. This project required the achievement of certain elements, summarised in Figure 1(b). They included firstly, that fish could be tagged; we must reliably take tissue from Spanish Mackerels *in situ*, without landing the fish, and that this tissue sample could be used to generate a “DNA fingerprint”, a genotype of the tagged fish, thus identifying that fish. Conceptually, this is the same process as tagging a fish with a numbered plastic tag where a fish has an individual, recognisable identity.

Secondly, to detect genetically-tagged fish if they were subsequently caught required genotyping a significant proportion of the landed catch. Genotyping, or DNA fingerprinting, is the determination of the genes that a particular sample possesses at a particular set of genetic loci that is at particular positions on a DNA molecule. Given that a large number of samples would need to be processed, the development of cost-efficient, high-throughput genotyping protocols was an equally fundamental requirement of the project. The project plan also depended heavily on the cooperation of commercial and recreational fishers for tagging the fish, for collecting tissue from landed fish, and for the return of tags from conventional tag recaptures.

In situ tissue sampling was successfully developed in a preliminary study, with the development of special hooks that collected tissue without it being necessary to land the fish. In fact, it is not necessary even to bring the fish to the boat.

Protocols were needed, too, for collecting samples from the landed catch so that these could also be genotyped; recaptured fish would then be detected as matches between these two sets.

In order to undertake large-scale genotyping, the project successfully achieved two important steps. Firstly, the identification of sets of genetic loci that could provide a high degree of resolution between individuals was accomplished in another preliminary study. Secondly, a protocol was developed within this project to

choose from amongst the sets of loci, the optimum set: those that were both highly informative and most amenable to the laboratory processes involved in genotyping (see Hoyle et al. 2005).

We planned that many thousands of samples would be collected. Thus the theoretical and practical capacity to deal with the considerable data sets and huge numbers of comparisons between samples was necessary and was successfully developed. This framework would then provide the tag and recapture information typically used in mark-recapture studies to estimate fishery harvest rates.

There are important limitations to conventionally tagging Spanish Mackerels, as described in the Background Section of this report. In particular, the cost per fish of conventional tagging by commercial fishers or by a research team was considered prohibitive for the commercial sector of the fishery, where individual fish are worth $50 or more, where catches are numerically small and where there may be a high incidence of shark mortality in line-caught fish. Such constraints, however, are less applicable in a recreational fishery, particularly when there is a strong “catch and release” ethos.

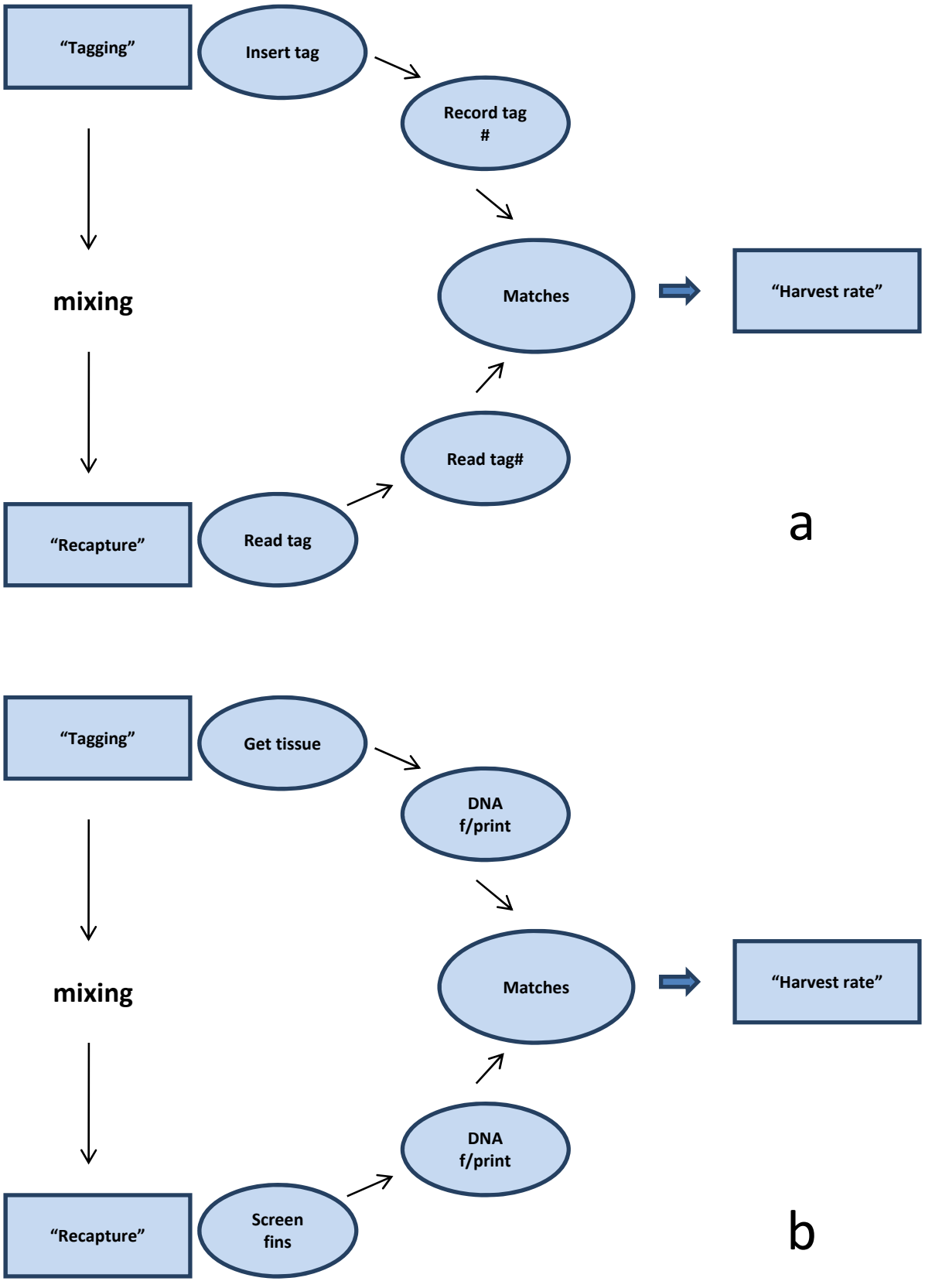

Figure 1. Conceptual overview of the mark-recapture process: (a) Conventional and (b) Genetic

We therefore considered that there was a valuable opportunity for the recreational sector to contribute extra information to the project, with voluntary conventional tagging. Conventionally-tagged fish could provide information on movements, especially between spatial areas fished by commercial and recreational sectors and, if also genetically-marked, could potentially provide data on loss rates of conventional tags (Buckworth 2004a). The technical feasibility of conventional tagging was demonstrated in previous work with the species (McPherson 1992). We also made initial observations on the impact of the initial capture and tagging operation on the behaviour and survival of the tagged fish, under our particular tag and release protocols. This became feasible given other FRDC research (Project 2003/016 *Reduction of interactions by toothed whales with fishing gear*; McPherson et al. 2006). We were able to make a preliminary estimate of the initial mortality imposed on Spanish Mackerels by conventional tagging, using sonic tracking. The estimate was primarily to consider the effect of predator-induced mortality immediately after release. Sharks were primarily the species of interest, although toothed whales have been implicated internationally in this type of fishery.

Given the untried nature of the Genetag approach, an important role of conventional tagging was as a control. Some unobserved failure in the genetic tagging method might be possible, and a lack of recaptures might be misinterpreted as simply being due to a very large population size. Recaptures generated with a conventional tagging component in the project would prevent this potential error.

LIAISON AND EXTENSION TO SUPPORT TAGGING PROGRAMS

Considerable community support was necessary to undertake both the genetic and conventional tagging programs. With potential benefits accruing to all sectors of the fishing community, and requiring the cooperation of quite disparate groups of people, the Genetag project emphasised communication.

The project was initiated with significant consultation with the commercial and recreational sectors. It was supported with continuous liaison about the project objectives, procedures and results. Opportunistically, this involved wharf-side liaison with individual fishers, communication through the NT Seafood Council and AFANT, and the fishing clubs. More formally, public workshops were held in March 2003 and June 2004 to provide information to all stakeholder groups (including the scientific community) and in May 2007, to provide feedback to the fishers.

A highlight in the development of the stakeholder relationships was the adoption of *Genetag* by Mr Norm Hedditch as his project for the *Advance in Leadership Development Program*. An NT Spanish Mackerel Fishery licence holder and, at that time, Chair of NT Spanish Mackerel Fishery Troll Fishermen's Association, Mr Hedditch's aim was to ensure the participation of the NT professional mackerel trollers in the FRDC project. Rik Buckworth acted as his project mentor.

Publications and other extensions of the project to the scientific community were produced steadily (e.g. chapters of the thesis by Buckworth (2004a), a poster at the 4th World Fisheries Congress (Buckworth 2004ab) and publication of the software for optimising the panels of loci for genetic screening (Hoyle et al. 2005; Macbeth et al. (2011); see Appendix 1). There has been substantial media coverage and general interest in the project and opportunities arose for broad media coverage on TV fishing shows and magazine news items (see below). Aspects of the project methodology were trialled in other fisheries, such as in a study of population sizes and movements of Pacific Rockfish (*Sebastes* spp.) relative to fishery closed areas in the vicinity of Vancouver (Hague 2006).

To ensure knowledge of the project and to maximise returns of recaptures, project staff invested considerable effort increasing the public profile of the Genetag project. This involved:

- Wharf-side liaison with individual fishers and communication through the NT Seafood Council.

- Extensive liaison with AFANT, fishing clubs and fishing tour operators, including the provision of several evening presentations at club meetings.
- The production of posters advertising the Genetag project. The posters were distributed across the Top End to tackle shops, bait and ice outlets, and at boat ramps.
- The designing and printing of fishing shirts for our volunteer taggers. The shirts displayed the project's name and logo as well as the sponsors' logos.
- The production of a series of articles for visual and audio media. The Genetag project was the focus of over 30 media articles during 2004 alone.
- The regular production of the 'Genetag Rag' during 2004-06, a newsletter providing a regular update on the progress of the project; it was widely distributed to interested persons and organisations.
- In 2004 and 2005 the Genetag project featured in articles in the *Northern Australian Fishing Annual.*
- In 2005 and 2007, the project was highlighted on Channel Nine's *Escape with ET*.
- Former Australian Test bowler Dennis Lillee participated in the 2005 NT Spanish Mackerel Tagging Tournament, donating a caricature of himself as a prize for the tagging team.
- The invention of the Genetag hook was featured on ABC TV's *New Inventors*, in July 2005, winning both the judges' and peoples' choice for the episode.
- The Genetag project was featured on ABC's *Stateline* and *Landline* radio programs in September 2005.

COMBINATION MARK-RECAPTURE METHODS

A tagging panel was instituted by canvassing skilled recreational fishers who were known to target Spanish Mackerels and to practise catch and release. These anglers were identified with the assistance of AFANT and fishing clubs, and included DoR Fisheries staff. The panel was limited to about 30 fishers to ensure quality control. However, panel members were often accompanied by other fishers who contributed under their supervision and new members were recruited while some members left over the course of the study; so there were many more than 30 taggers over the course of the study. The panel had a goal of tagging 200 Spanish Mackerels conventionally each season (around June-November) in 2004, 2005 and 2006. Tissue samples for later genotyping were usually collected simultaneously.

Upon recruitment, taggers were briefed to ensure the adoption of a standard procedure. Fishers were requested to capture mackerels using relatively heavy lines (10-12 kg) and place tags with a pole-mounted insertion needle in the dorsal muscle. Using this approach, it was not necessary to remove fish from the water. However, some anglers removed fish from the water for measurement in agreement with the rules of fishing competitions or clubs in which they were engaged. We found that when mackerels were floated upside down in the water, they became completely still (tonic immobility), enabling the ready removal of hooks. Also, to facilitate hook removal, taggers were encouraged to use barbless hooks or crush the barbs on the hooks on their lures. Not all did this. These protocols helped to minimise injury to fish and fishers.

Only fish in good condition (moving easily and without obvious bleeding or damage) were usually tagged. The status of fish at release was recorded. It was emphasised to the taggers that the survival of tagged fish was paramount. Tissue samples (usually small pieces of fin) for subsequent genotyping were usually retained from fish that were unsuitable for release.

The conventional tags were Hallprint, which are small plastic-tipped intramuscular dart tags with stainless steel wire core attachments (type PIMS-W). The tags were bright yellow and were consecutively numbered. They were printed with the word "REWARD" and a phone number to notify recaptures. When recaptures were notified, information was collected of the person, the data and the location of the recapture.

Tissue sampling for genotyping the fish was simultaneous with placement of the conventional tags. Small, barbed stainless steel needles or blades, hereafter termed "lancets", were also mounted into the end of the

tagging pole. A lancet pierced the skin and dorsal muscle and retained tissue (up to 2 g) when a conventional physical tag was placed on the fish. Immediately after tagging, the lancet and adhering tissue were placed in preservative. The preservative was, in most cases, 20% dimethyl sulphoxide in 5M sodium chloride solution (DMSO), or 80% ethanol in some cases, in a vial containing the corresponding tag number. The vial containing the lancet with the tissue sample was placed on ice and thereafter kept refrigerated until DNA extraction and genotyping occurred.

The lancets used during 2004 and 2005 were ground and shaped from lengths of stainless steel wire (Figure 2a). After tissue processing, some lancets were autoclaved and re-used. During 2006, a new lancet design (Figure 2b) was introduced. Laser-cut from stainless steel sheet in batches of several dozens, this design and manufacturing approach considerably reduced the amount of labour in the preparation of lancets.

As well as the tag number, taggers recorded the date, time, physical condition of the fish and line class used. Taggers were asked to provide location (as a descriptive site name and/or latitude and longitude). In practice, most supplied only site names. For mapping and calculation of distances moved, latitudes and longitudes were assigned to standard sites. Taggers were also asked to record the approximate fork length of the fish (using measurements marked off on the tagging pole or size stickers mounted on the gunwale of the boat so that information could be accumulated on any obvious trends in survival or movements of tagged fish relative to size.

Figure 2. Lancets used to collect tissue simultaneously with the placement of conventional tags: (a) Cut and ground from stainless steel wire, 5 cm long (b) Laser-cut from sheet stainless steel, 4 cm long

At initial annual briefings, taggers were issued with kits consisting of 10 tags, tagging poles, lancets, data sheets, storage vials and preservative. Each tag was tied together with a sample vial containing a label indelibly marked with the tag number, so that the conventional tag and any tissue sample could be matched. Additional kits were provided to taggers on request.

To focus the interest of the participating anglers, tagging tournaments were organised each year in June-July). Substantial sponsorship was generated to provide incentives for the participating anglers and considerable resources were martialled to publicise the tagging exercise and to ensure that the wider fishing community was informed to anticipate captures of tagged fish (see details in the Liaison and Extension section above).

Rewards were provided for return of tags when accompanied with information on the date and location of the recapture. Details related to the recapture, including the original date and place of release, were provided. AFANT voluntarily managed the collection of recaptures and their information during 2003-06.

An audit of issued tags was conducted annually at the end of the fishing season (from January to April, 2005-07). We attempted to account for all the tags issued, collect any unsent data and, where possible, collect any unused tags and equipment.

ACOUSTIC TRACKING OF CONVENTIONALLY-TAGGED SPANISH MACKEREL

Acoustic environment

This section refers to the tag developed as part of FRDC Project 2003/016. It considers the applicability of the tag output in terms of modelled tracking range using simple acoustic monitoring systems that could be made by local electrical technicians and could also utilise existing radio systems speakers currently on board recreational fishing vessels.

Field work was undertaken from 7 to 9 October 2008 in conditions varying between calm (wind speeds < 4 km/h) to rough (20 km/h gusting to 30 km/h), and seas varying up to 2 m. No fish could be tracked on 7-8 October due to a mix of rough conditions, damage to tracking gear or an inability to catch fish in a suitable condition for tagging. However, a conjunction of relatively calm weather and compliant fish allowed tracking work on 9 October.

Narrow-barred Spanish Mackerels were located in waters of 20 m average depth 30 km off the coastline of Fog Bay, NT. Fishing occurred in the vicinity of low areas of rock rubble 1-2 m above the substrate, although the bottom depth averaged 20 m. The substrate was thought to be of mud/sand composition.

10 kHz acoustic tags

The acoustic tags used for this project are described in detail in Chapter 16 of FRDC Project 2003/016. The tags are low cost (<$40 components) and are based on a transducer made available to FRDC 2003/016 and *OceanWatch* (Australia) by RAAF/RAN. The tag transducer was compared with a commercially available acoustic tag transducer by Kingsberry (2005). Using the same battery and electronic circuit at frequencies between 17 and 100 kHz the RAAF/RAN transducer outperformed the commercial tag transducer generating tag signals at up to the frequency commonly used in commercial acoustic tags, namely 70 kHz. At the lower frequencies of comparison the FRDC project transducer generated a tag signal 15 dB higher than the commercial, an attribute that would ensure four times the tracking range.

An early production model was made in early 2007 for FRDC Project 2003/016. The tag has a fundamental acoustic output at 10 kHz (two frequency versions are available at 0.5 kHz apart) and the Source Level seems to average approximately 140 dB re 1µPa at 1 m.

The tags feature a 10 kHz fundamental signal chosen to be detectable by an unaided ear provided a simple hydrophone system was available. The hydrophone system utilised here is also from the RAAF/RAN. The hydrophone utilised is no longer available in defence inventory although an alternative hydrophone system is now part available at low cost.

The intention of the project was to generate low-cost, non-commercial systems for recreational and research organisations in the NT so as to conduct their own research into fish survival after tag release.

Propagation of acoustic tag signals

Available acoustic propagation models for this area are limited. Schulkin (1968) observed thousands of measurements in shallow waters (effectively constrained sound ducts referred to in FRDC Project 2003/016 for oceanic surface situations) with various substrates ranging from sand to sound-absorbing mud, at frequencies up to 10 kHz. However, the measurements were taken over kilo-yard ranges (thousand yard

increments) and available advice from a FRDC Project 2003/016 co-author in the Defence Science and Technology Organisation was that locally determined acoustic estimates would be far better.

McPherson et al. (1999) determined acoustic propagation rates in shallow coastal waters of north Queensland, with comparable depths and substrates to that in Fog Bay, and used them to determine appropriate acoustic alarm (2.9 kHz fundamental frequency) and acoustic pinger (10 kHz fundamental frequency) deployment distances to provide whales, dugong and dolphins with variable (and often estimated) hearing capabilities. The most appropriate models for 300 millisecond pinger tone burst propagation, comparable in time and frequency to acoustic tag tone bursts, included the spherical spreading model (Urick 1983; McPherson et al. 2006, Chapter 11) where sound drops away as the surface of an expanding sphere irrespective of any water surface or substrate effects. This model was proposed for detection of acoustic tags in relatively deeper Spanish Mackerel fishing grounds in the Great Barrier Reef waters and is still seen as the most appropriate for that acoustic environment.

The other model utilised for alarm and pinger tone burst propagation was a transitional model where acoustic spreading changes from a greater transmission loss spherical rate (*i.e. 20 LOG [range]*) to a lower transmission loss cylindrical rate (*i.e. 10 LOG [range]*) with an increasing range and is well described by Urick (1983), Richardson et al. (1995) and outlined in Chapter 11 of McPherson et al. (2006). The distance at which the transition occurs is highly variable, usually depending on increments of water depth. The transition is rarely a 'knife-edge' transition and a smooth transitional propagation model between spherical and cylindrical spreading was originally suggested by Urick (1983). McPherson et al. (1999) developed a transitional model for the shallow northern Australian acoustic environment and modelled a transition range procedure generating transitional ranges up to 16 times the shallow water depths.

The transitional models of both Urick (1983) and McPherson et al. (1999) require input data to determine the transitional parameters for the specific acoustic environment under investigation. McPherson et al. (1999) tested 12 models against data determined for shallow tropical waters with mud substrates. The spherical and transitional models fitted the available data best. However the model with the next best residual sums of squares variability had an arbitrary parameter suggested by Urick (1983) where sound would spread spherically to a range at least equivalent to water depth (i.e. transmission loss of *20 LOG [range]*), and then spreading at a combination of spherical and cylindrical spreading (*i.e.* transmission loss of *15 LOG [range]*).

The 15 LOG (range) model could also be improved with prior assessment to determine a substrate signal absorbing co-efficient although it was useful as a stand-alone model. The 15 LOG (range) model diverged from observed data with increasing water depth >15 m and with mud substrate, clearly include factors present in the Fog Bay acoustic environment. It is therefore likely that the estimates for the 15 LOG (range) model for Fog Bay represent an over-estimate for sound propagation.

Cato (1978, 1997 and 1999) reviewed aspects of acoustic propagation in Australian waters. While advocating direct experimentation for specific acoustic monitoring sites Cato referenced the calculations of Urick (1983) and Etter (1996) for mud substrate with signals with low grazing angles (i.e. signals in a shallow duct 20 m deep over ranges to 500 m. Signal losses of 0.025dB/m at 10 kHz were advanced; therefore, 2.5 dB over 100 m and 12.5 dB over 500 m were significant losses in terms of an acoustic tag tracking range within the Fog Bay environment. As a co-author in McPherson, Cato and Gribble (1999) and McPherson et al. (2004), Dr Doug Cato provided valuable input to ambient noise assessment and sound propagation for these papers.

Acoustic propagation in shallow water is also influenced by the state of the sea surface and various wind/wave height parameters are usually factored in most models (reviewed in Chapter 11, McPherson et al. 2006). On the day of narrow- barred Spanish Mackerel acoustic tagging in Fog Bay, zero wind conditions were experienced with a near flat sea surface.

In smooth sea conditions where source and receiver are close to the surface, the surface reflection of the sound is likely to strongly interact with the direct sound radiation generating positive and negative interference effects. This effect is known as the Lloyd mirror effect; it is most common with narrowband tonal signals, such as toothed whale whistles and acoustic tag signals. Richardson et al. (1995) provide a means of estimating the likely range and magnitude of interference effects. Reflection off the sea surface results in acoustic signal loss to a maximum of 6 dB within a model determined range in addition to loss described by other appropriate models (Urick 1983; Chapter 11 FRDC Project 2003/016). The acoustic loss due to Lloyd mirror effects may be calculated using parameters such as the frequency of the signal, the depth of recording hydrophone (i.e. 5 m) and the depth of the transmitter. In the Fog Bay sampling, Spanish Mackerels were seen swimming in loose schools at an approximate depth of 5 m on the fishing grounds; therefore, the depth of transmitter was assumed to be 5 m.

Detection of acoustic tags

Initial detection of acoustic tags was by a rewired AN/SSQ57 omni-directional sonobuoy hydrophone with an enhanced frequency response up to 10 kHz over commercial hydrophones. The hydrophone signals associated with each acoustic tag release were split between a recording system (for post processing) and a monitoring system (suited to the requirements of the project). The signal was recorded by a SONY DAT TCD8 recorder and monitored through a small project-made amplifier system using headphones. The analogue DAT tapes were played to an ESI 48 kHz sound card and saved as digital WAV files with a degree of signal loss due to damage sustained by the recording system in an open boat travelling at high speed.

Initial detection was also available using a similar hydrophone with a small Marshall acoustic amplifier (approximately $80) with a headphone monitoring option. The Marshall amp and headphone is seen as the most cost effective method for monitoring acoustic tags with 10 kHz signals. Detection was also possible using the AUX input of many vessel radios. Detection of acoustic signals at higher frequencies would require more expensive and difficult to maintain systems in open recreational vessels.

Apart from the strength of the acoustic tag signals, the other major determinant for acoustic detection is the ambient noise level. In calm sea conditions, the major determinants of ambient noise are usually snapping shrimp and fish.

The loudest biological signal came from snapping shrimp (Figure 3). These would have been present in high concentrations around the rocky rubble that was the focus point for fishing activities.

Estimates for noise levels from snapping shrimp at the shallow Fog Bay fishing sites were particularly loud at around 90 dB at 4 kHz and around 85 dB at 10 kHz in 20 m of water with a hydrophone deployed 5 m below the surface. In deeper water, or with hydrophones located further away from the substrate, broadband estimates of noise levels as detected by ear could reduce to around 80 dB. Noise levels would be expected to vary on a diurnal basis with loudest signals occurring before dusk till about midnight.

Figure 3. Snapping shrimp showing large appendage for generating short bursts of broadband sound to stun and dazzle with light generated with the acoustic pulse

Snapping shrimp generate the bulk of biological noise in shallow tropical seas.

Ambient noise levels have been reviewed by Urick (1983) and Cato (1978; 1997; 1999). McPherson et al. (2004) considered ambient noise levels in northern waters of the Gulf of Carpentaria up to 20 m in depth. At broadband levels biological signals on mud-bottom fishing grounds of around 80 dB were considered. The 85 dB level estimated for the Fog Bay site was attributable to the closeness of the hydrophone to the substrate. This was primarily rock rubble, an optimal substrate for snapping shrimp.

The loudest non-biological signal in the 10 kHz range was from nearby outboard powered vessels also trolling for mackerels. These signals would dominate acoustic tag signals at vessel ranges less than 200 m from the recording hydrophone. Detection of larger vessels with acoustic signatures <<2 kHz could be detected at distances > 5000 m although they did not interfere with tag signals. The acoustic output of commercial fishing vessels with diesel engines, mother ships and dories would not interfere with acoustic tag signals.

GENETIC TAGGING METHODOLOGY: TISSUE SAMPLING

Sampling tissue for genotyping

Genetag sample collection

Genetag hooks were mounted on simple plastic jigs, typical of lures used by commercial fishers in northern Australian Spanish Mackerel fisheries. The Genetag hooks comprise a hook formed from a shaft of pliable copper tube or wire, on which is mounted a hollow stainless steel tip containing small barbs. When struck by a fish, the tip of the hook scrapes the skin of the fish and tissue is retained in the barbed tip. The weight of the fish on the line straightens the pliable hook, thus releasing the fish *in situ* with minimal injury. The straightened hook indicates that the hook has been struck. A double hook configuration was usually employed, as earlier trials indicated that this was an effective design (Buckworth 2004a). In this fishery, commercial fishers mostly use baited hooks (usually baited with garfish), switching to lures when strike rates are high. However, we used lures to avoid contamination of genetic samples with bait tissue, and for logistic ease.

Kits consisting of Genetag lures, plastic bags and labels were distributed to NT commercial Spanish Mackerel trollers throughout the project. Around 2000 lures were distributed to commercial fishers in these kits during 2003-06. The planned experimental approach was that each boat would Genetag one fish per day

of fishing. The lure was to be frozen in a plastic bag as soon as possible after it was struck by a fish, with a label indicating the date and position at which the strike was made. Rewards and other encouragement for struck lures included 'Scratchies' (instant lottery tickets), hats and shirts.

This operational plan had several advantageous attributes, including:

1. Genetic tagging would be distributed spatially and temporally in proportion to commercial fishing effort, so reducing potential for spatial or temporal biases in the estimation of overall harvest rates.

2. With lures of a type typically used in the fishery and deployed with the gear used in normal practice, contemporaneously with normal fishing, the gear and fishing behavioural selectivity characteristics of the fishery and the experiments would be matched.

3. It provided efficient use of project resources and a workable model for continuing monitoring post-project.

Figure 4. A Genetag hook, in double hook configuration, mounted on a Smith's jig

Although a number of Genetag lures were deployed by fishers in this way, it became clear early in the project that we would not be able to maintain a sufficient tagging effort. Most Genetagging was consequently undertaken by DoR Fisheries personnel during monitoring trips. This was usually achieved by 'chartering' lines during commercial fishing operations. For example, two of a vessel's six or eight lines would be dedicated to Genetagging and fishermen were compensated for the loss of the fish. This approach agreed with the second attribute above and additionally, could provide information on the species, sex and size composition of the fish caught simultaneously, improved quality control of samples and data, and could accountably provide reasonable compensation for lost catch.

Frozen lures were collected when vessels returned to Darwin, where they were maintained in a frozen state until the hook tips were removed and placed in a preservative. Typically, when a fish struck a Genetag lure, one or both of the hook shafts would be straightened, and rendered inoperative. We observed that after a strike, if one of the hook shafts was straightened, the lure would not usually "swim" properly. Nevertheless, if just one hook was straightened, a second fish could attack the lure and leave a sample in the second hook. Thus, in order to establish whether more than one fish had attacked a lure, and thus potentially leaving multiple tissue samples, each hook tip was stored in a separate numbered vial. Additionally, both hook tips

were retained for DNA extraction and genetic analysis, even when just one of the tips appeared to have been struck.

Hook tips from Genetag lures struck when deployed by DoR Fisheries staff were placed in a preservative immediately after they were struck.

Many struck lures collected during 2002 and 2003 were removed from frozen storage, inspected for the presence of tissue in the hook tips and then stored in 20% dimethyl sulphoxide in 5M sodium chloride solution (DMSO). They were then maintained at room temperature. This procedure was discontinued when it was discovered that contamination with copper from the hooks interfered with the genotyping (likely inhibition of the PCR) when the hook tips containing the samples were stored in highly reactive DMSO (see further detail below). Thereafter, after removal from the freezer, hook-tips were placed in vials of 80% ethanol and were then kept refrigerated (c. 4 °C) until DNA extraction (see below). The practice of inspecting hook tips was also discontinued at the same time. This was to ensure the quality of retained tissue; it was also realised that sufficient tissue to provide DNA was often not evident in a visual inspection of a hook tip.

Tissue samples were taken from Spanish Mackerels in waters adjacent to the NT from 2003 to 2006. After DNA extraction and autoclaving, a small number of lancets were re-used.

Landed fish

Commercial fishers were asked to collect fin samples; the procedure being to remove and retain the paired ventral fins during fish processing (in the NT fishery, Spanish Mackerels are usually prepared as trunks or fillets on board the vessel). The ventral fins were chosen because they are relatively small, moderately identifiable as having come from Spanish Mackerels and it is fairly easy to remove and store both fins, providing a discrete piece of tissue. A pair was a unique piece of anatomy so that fishers would be unable to provide replicates. Fins were accumulated in a plastic bag labelled with date and position of fishing, all of which were maintained on ice until the end of a fishing session (such as morning, day), after which they were frozen. Fishers were asked to keep as many fins as possible. In those instances when DoR Fisheries biologists were aboard, other tissue was often retained instead of fins. For example, when heads from landed fish were retained for later otolith extraction, pieces of operculum or muscle provided the tissue for genetic samples. Fishers received a small reward for each fin retained. In an effort to limit cross-contamination, DoR Fisheries staff rinsed knives and cutters in ethanol and wiped them dry on paper towel between samples.

DEVELOPMENT OF GENETIC LOCI FOR GENOTYPING

Building the genetic library for genotyping: theory

Genotyping is the determination of the genes that a particular sample possesses at a set of genetic loci. For diploid species (like Spanish Mackerel and the majority of fish species) there are two gene copies at each locus. The gene copies are referred to as alleles. Numbers are used to describe alleles; for example at locus one the alleles could be 100/102. In this case the sample is heterozygous (two different alleles). A homozygous sample would, for example, be 100/100. Among samples in a population, loci may have numerous alleles (e.g. locus one could have alleles 100, 102, 104, 106 and 108) which are termed “polymorphic alleles”.

The alleles are referred to as numbers because the numerical value refers to the size of the allele in base pairs[1]. So, allele 100 is 100 base pairs in length. Alleles at a locus differ in size by an amount that is

1 Base pair is shorthand for nucleotide pair. Nucleotides are the building blocks of the linear DNA molecule. The length of pieces of DNA is measured in numbers of nucleotide, or base, pairs.

characteristic of the locus. For the loci in this study, the alleles differ by two base pairs (so called dinucleotide locus). The loci that we have used here, and which would be generally used in Genetagging, are called microsatellite loci.

Loci have different sets of alleles that can vary in size and number between loci. For example, locus one has alleles 100, 102, 104, 106 and 108. Locus two has alleles 205 and 207 only. Generally, allele length ranges from 100 to 450 base pairs, and allele number varies between two alleles and maybe up to 30 alleles.

The genotype of a sample (a fish) is indicated by the alleles that are present at a set of loci. For example, at locus one and locus two, the genotype of animal one could be "100/102 205/205". This animal is heterozygous for locus one (100/102) and homozygous for locus two (205/205). An animal's genotype is fixed over its lifetime. The set of loci at which samples are genotyped are project-specific and are determined by a team of geneticists against a set of criteria determined by the project. In this project, the genotyping set consists of seven loci.

To a large extent, loci are specific to a species. For example, Spanish Mackerel loci will not be useful for any other species. There are some exceptions; loci from closely-related species can be useful in the target species (cross-amplification). For example, some of the loci used in this study on Spanish Mackerel came from another mackerel species, the north American King Mackerel (*Scomberomorus cavalla*).

Loci normally need to be developed in the laboratory for each new species. The development of new loci is called "making a microsatellite library". This process takes a researcher between three and 12 months to complete. The completed library can consist of between five to 50 loci and the genotypes of 20 to 30 animals at those loci. The animals are normally selected from several locations in the distribution of the species to fully describe genetic variation of the species. It is possible to out-source this set, but considerable pre-existing knowledge is needed to successfully set this up.

Most importantly, the library contains the information needed to work with the loci in the laboratory on the target species. This includes the PCR [2] conditions needed to amplify alleles from that locus, including the locus-specific sequence of primer pairs. It also contains information about the 'reliability' of the locus; in other words, what proportion of time the PCR for that locus fails and the ease at which alleles at that locus can be determined (scored) by the researcher. Reliability is very important in projects where thousands of samples are to be genotyped as it significantly reduces the amount of raw data checking. Reliability is a more important feature than the number or size of alleles at that locus.

Construction of a microsatellite library for Spanish Mackerels

Microsatellite loci for *S. commerson* were prepared using the method of Fischer and Bachman (1998). RNA-free high molecular weight DNA was extracted from 15 mg of liver tissue and purified using a DNeasy tissue kit (QIAGEN). Adapter primers were ligated to RSA1 digested DNA fragments and then hybridised in two pools containing 10 μM of each biotin-labelled oligo [Pool 1: $(TAGA)_{10}$, $(AAG)_{10}$ and $(TC)_{15}$, Pool 2: $(ATG)_{10}$ and $(AC)_{15}$]. The hybrid complexes were bound to streptavidin-coated magnetic beads (Dynabeads®; Dynal GmbH, Hamburg, Germany) and washed twice at 25 ^{o}C in 2x SSC, 0.1% SDS for 5 minutes, twice at 25 ^{o}C in 2x SSC for 5 minutes and twice in 1x SSC for 2 and 5 minutes at 58 ^{o}C for pool 1 and 70 ^{o}C for pool 2 on a slowly-rotating wheel. The enriched DNA was eluted into MQ water and PCR-amplified prior to cloning into plasmid vectors (pGEM®-T easy vector; PROMEGA). Colonies were picked and sequenced bi-directionally using ABI bigdye. From 140 sequences, 40 clones (GENBANK accession numbers AY700808-849) had adequate flanking sequence and microsatellites to design primers using OLIGO primer analysis software v 6.3 (Molecular Biology Insights Inc., Cascade, CO., USA).

[2] PCR is an abbreviation of the polymerase chain reaction.

PCRs typically comprised 5-10 ng of genomic DNA, 0.5 μM of each primer, 200 μM of each dNTP, 0.05 units of Taq DNA polymerase (QIAGEN) and 1.5 mg of $MgCl_2$ supplied with the 10x PCR buffer in 10 μl reaction volume. Amplification cycles (35) were performed in a 9700 Perkin Elmer thermocycler over a range of annealing temperatures (55 to 60 ^{o}C) and magnesium concentrations (1-4mM) to assess whether loci could be reliably amplified and the extent of non-specific banding. Amplicons were separated on a 1.5% high resolution agarose gel and visualised using ethidium bromide staining. We also assessed the utility of King Mackerel (*Scomberomorus cavalla*; Broughton et al. 2002; Gold et al. 2002), Coral Trout (*Plectropomus laevis*; Van Herwerden et al. 2000a) and Red-throated Emperor (*Lethrinus miniatus*; Van Herwerden et al., 2000b) microsatellite primers in *S. commerson*.

Genotyping with microsatellite loci

Amplicons were resolved using capillary electrophoresis on an ABI 3130xl. Genemapper software was used to bin alleles. Microsatellite genetic diversity was characterised by the number of alleles, expected (H_E) and unbiased (UH_E) heterozygosity, observed heterozygosity (H_O) and fixation index using GenAlex 6.1 (Peakall and Smouse 2006).

Cryptic duplicate hook-tip, lancet and fin genotypes were identified using the matching routine of SHAZA (see below and Macbeth et al. 2011). Genotypes were removed such that each fish was represented only once.

Genepop v4.0 was used to check for deviation of genotype proportions from the Hardy Weinberg equilibrium and to check for genotypic linkage disequilibrium.

Samples that had a higher probability of not being taken from Spanish Mackerels were identified and removed from the data-set on the basis of the probability of their genotype, where genotype probability (GP) is the product of the population- wide allele frequencies for those alleles present in that genotype. Samples with *ln*(GP) values outside three or four standard deviations were assumed not to be Spanish Mackerels. This was confirmed by simulating a random mating population of *N* genotypes based on the allele frequencies in the data-set representing 8050 individuals. A small proportion (0.15%) of the simulated genotypes had *ln*(GP) values outside three or four standard deviations, which justified the removal of samples with *ln*(GP) greater than three standard deviations. The procedure was repeated twice to identify and remove additional outliers because the removal of genotypes at each round changed the mean and thus the standard deviation of GP. This method is conservative in that it is likely to remove all non-Spanish Mackerel samples but may inadvertently remove some Spanish Mackerels.

GENOTYPING PANELS

Development overview

A genotyping panel is a subset of loci being used for genotyping. Loci are grouped into panels for practical reasons; they allow genotyping to occur in a resource- effective way. There may be two to several panels of loci in a routine genotyping project.

Loci are placed in a panel according to their range of allele sizes across a population, so that the allele sizes do not overlap. For example, panel one may contain three loci. The first locus has alleles that range from 100 to 150 base pairs, the second locus may have alleles that range from 200 to 270 base pairs and the last locus may have a size range of 310 to 320 base pairs. Loci are arranged so that there is at least a 50 base pair gap between allele ranges. The Genetag project has produced software *Locuseater* (Hoyle et al. 2005) that assists the researcher in combining loci into panels for efficient screening.

Loci are arranged with non-overlapping allele sizes so that the allele lengths in base pairs can be determined simultaneously. Length determination using capillary electrophoresis is described in the next Section. Simultaneous length determination is called “lane-multiplexing”.

Loci from a given panel can also be amplified simultaneously in the same amplification reaction. This is called “PCR-multiplexing”. The reaction would contain locus-specific ingredients (e.g. primer pairs) as well as generic PCR requirements (e.g. dinucleotide tri-phosphates, magnesium chloride, thermo-stable DNA polymerase etc.). Suitable PCR reaction conditions would have been previously determined. PCR-multiplexing significantly reduces the resources needed to genotype samples, but it is time-consuming to determine the appropriate laboratory protocols and occasionally it is not possible to combine the amplification of a set of loci. If this occurs, loci can be amplified in single reactions and reactions can be physically combined after the PCR step is completed to achieve lane-multiplexing.

Panel design strategy

It was a major challenge to place the selected Spanish Mackerel loci into panels. Loci within panels have to have non-overlapping allele sizes and amplify reliably in the same PCR multiplex.

This project developed a unique system for allowing loci with overlapping allele sizes to be combined into a single panel. For a particular locus, allele lengths were altered by 10 – 100 base pairs by redesigning primers for that locus. But changes to the allele size range are limited by characteristics of that locus (e.g. flanking sequence length and base composition for primer design). This information is often not available if the loci are from other investigators; so redesigned primers for loci not developed in-house are often not possible.

Allele sizes were altered by extending the 5’ end of each primer with a random oligonucleotide tail (Figure 5 and), thus changing the resulting allele size. Thirty oligonucleotides were designed with random sequence. They ranged in length from 10 - 60 bp at 10 bp intervals. The software *Oligos* (Kalendar 2002) was used to check that they were free of complementary sequences that would promote primer dimer formation.

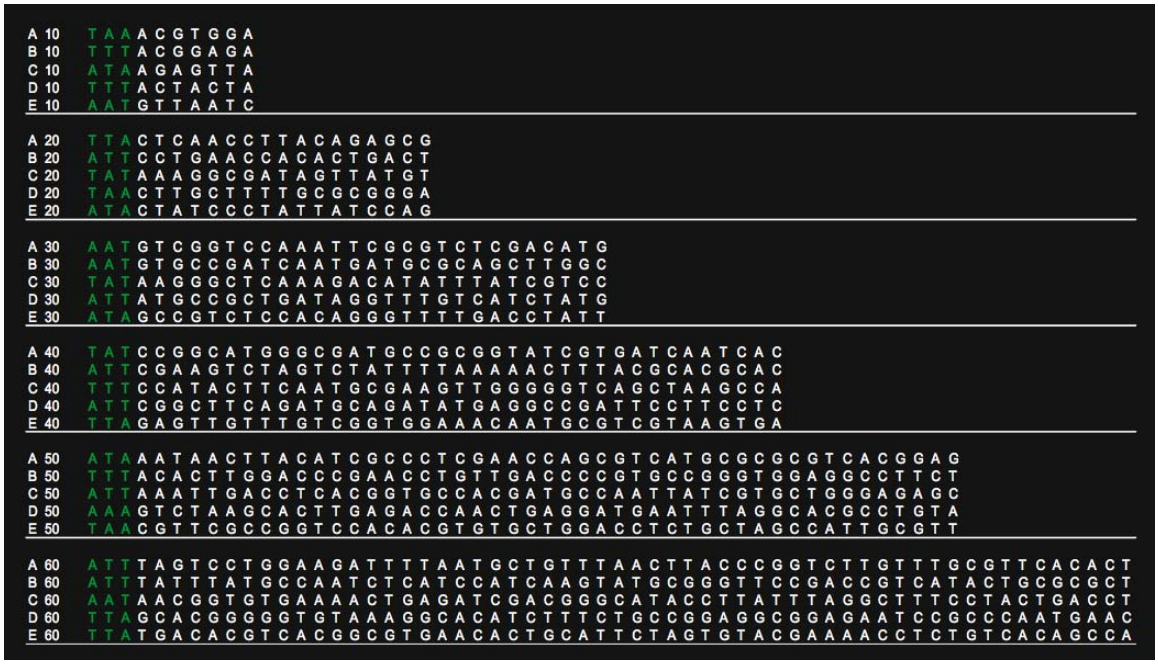

Figure 5. Nucleotide sequence of oligonucleotides used to extend the primer sequences to increase allele lengths

Primers were lengthened by the addition of these 5' random tails and then all pairwise primers combinations were again checked for primer dimer formation in *Oligos* (Kalendar 2002). This strategy increased allele sizes up to 120 bp (both forward and reverse primer extension) and its judicious use allowed the degree of overlap in allele sizes among loci to be controlled. Alleles can only be lengthened (i.e. not shortened) using this process.

M13 fluorescent labelling system

In this project we used an M13 fluorescent labelling system, which increased flexibility in panel design and reduced costs. This is different from the normal approach where amplified alleles are tagged with a fluorescent colour (FAM, VIC, NED, PET or HEX) that allows them to be detected using laser technology following capillary electrophoresis. The forward primer is normally labelled with this fluorescent tag, which ensures that the amplified product is fluorescently labelled. However, labelling primers in this way is expensive and the label cannot be changed at will.

In the M13 fluorescent labelling system, forward primers are modified with an addition of M13 complimentary sequence to their 5' ends. The fluorescent tag is placed on an oligonucleotide with the M13 sequence and PCR reactions are performed with the modified forward, reverse and fluorescent-labelled M13 primers (Figure 5). The annealing efficiency of the fluorescent-labelled M13 primer is such that it can be diluted ten-fold compared with the forward and reverse primers without a loss in signal intensity. This strategy is flexible as the user can change the dye colours of amplified loci by using FAM, VIC, NED, PET or HEX labelled M13 oligonucleotides.

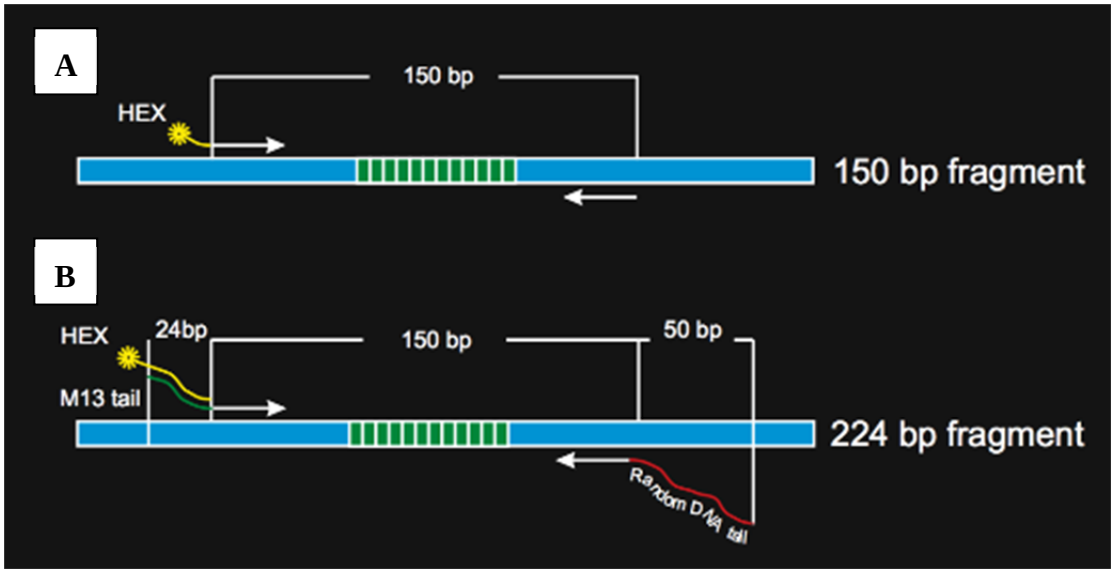

Figure 6. Diagrammatic explanation of the primer extension and fluorescent M13 labelling system used in this project

Forward and reverse primers are represented by inward facing arrows. Before primer extension and M13 labelling, the locus has an allele of 150 base pairs (A). When an M13 – Hex labelled tail is added to the forward primer and a random DNA tail is added to the reverse primer the allele size has increased to 224 base pairs (B).

Locuseater and *Shadowboxer* (Hoyle et al. 2005) use both Probability of Identity (PID) scores and loci size ranges to compare the discriminating power of different panels of loci. These packages are also designed to determine the precision of harvest rate estimates in mark recapture studies using genetic tags. Highly

diagnostic panels were then tested to see if all loci could be reliably amplified and scored in a multiplex environment.

Panel composition

To effectively combine loci into panels, the allele size range was determined on a sample of 100 fish from the north Australian population of Spanish Mackerels. A 50 base pair gap was allowed to separate the largest allele of one locus from the smallest allele of the next locus.

Four panels (seven loci total) were used in this project. Annealing temperature (56 °C) and number of cycles (37) were constant across panels. Panels were labelled with FAM, VIC, NED or PET fluorescent dyes (Table **1**). Alleles for these loci were amplified for a sample and the products combined for length resolution in a single “lane” (lane-multiplexing).

Table 1. Locus composition and panel fluorescent colour (M13 label) for the four panels used to genotype Spanish Mackerel samples

Panel	Locus	M13 label
1	SCA30, SM3	FAM
2	SCA47, SCA49.A50	VIC
3	SCA8, 90RTE.A20	NED
4	SM37	PET

The seven loci selected had numerous alleles and high heterozygosity. Their combined PID was 9×10^{-11}, considered at the time to provide sufficient power to individually identify fish. We underestimated the effect of sample size (actually number of pair-wise comparisons between samples) on PID. By the end of the study, the actual PID (i.e. the probability of observing a chance match between *any* samples in the dataset) was significantly closer to 1.0 than we anticipated. But shadows (false-positive matches of genotypes) did not occur among matches where seven-locus genotypes were compared. Shadows did occur, however, if genotypes were matched that contained information from less than seven loci. About 28% of genotypes produced in this project were genotyped at four, five or six loci only. These types of genotypes were called ‘partial genotypes’. The implementation of project-specific software SHAZA (Shadow Zone Analysis) corrected for the presence of false-positives in this type of data.

Genotyping cost was a significant factor in designing the genotyping system. Costs increase with each locus added; seven loci was considered an adequate set on the basis of their low combined PID and the genotyping strategy that we planned to implement. We planned to successively genotype groups of samples with one panel after another, discarding samples that were unlikely to be recaptures at each round. We predicted that this would reduce the total amount of genotyping and hence control the overall cost. We also predicted that this approach would control the number of false-positives generated with seven locus genotypes. As discussed below, this approach was not feasible.

Evaluation of successive panel genotyping

This project tested a genotyping strategy, successive panel genotyping, based on the successive use of panels as a way to minimise the number of loci genotyped per sample and total number of samples genotyped. This strategy was applied to harvested samples, which were screened for the presence of recaptures. It was not suitable for genotyping tissue from the Genetag hooks.

In the first round, samples from the harvested catch (fin tissue) were amplified and length of alleles determined from the first panel of loci. These three-locus fin genotypes from panel one were compared to the

panel one genotypes of Genetag hook samples. Fin samples that did not match were excluded as possible recaptures. In the second round, fin samples that matched in the first round were genotyped with the second panel of loci. The matching process was repeated and fins that could not be recaptures were excluded. This process was repeated until the remaining fins had been genotyped with all panels and a match between them and one or more of the lure genotypes was confirmed.

We discontinued this strategy for a number of reasons. Firstly, there was a significant resource cost in identifying and carrying forward ("cherry-picking") fin samples from one round of genotyping to the next. The DNA from fin samples was stored in plastic plates in sets of 96 (a standard genotyping laboratory procedure) and there were hundreds of such plates in this project. To recover specific DNA samples from the plates was time-consuming and the chance of human error was high. Secondly, the strategy was discontinued due to the 'shadow' problem. As the number of fin samples increased, the proportion of genotypes matching also increased to a rate where only a small number of samples were excluded in the panel-one round. This problem was made worse because fin genotypes were being matched against each other, as we needed to identify and exclude fins that had been inadvertently sampled from the same individual fish. Lastly, the successive panel strategy was more time-consuming than predicted because of the raw data processing and genotype matching that needed to occur after each round.

The approach that was used in the remainder of the project entailed simultaneous amplification and allele resolution across all panels.

IDENTIFYING GENETIC RECAPTURES: GENOTYPE MATCHES

Process overview

The aim of genotyping is to find matches between fish 'tagged' with the Genetag hook and fish that are subsequently harvested: recaptures. As the genetic identity of each fish is unique, a match between the genotypes of two samples indicates that they are from the same fish. If one sample is from a Genetag hook and the other from a harvested fish, then we assume that the same fish was 'tagged' with the hook and subsequently recaptured. The process involves genotyping each hook and each fin sample and making pairwise comparisons between the genotypes to search for matches.

This project has made significant advances in the process of searching for genotype matches. It has made major theoretical and practical advances in defining and solving challenges that are common to large-scale mark-recapture studies using genotyping. These developments are also applicable beyond the use of Genetag in fisheries management, in the realms of wildlife and human population genetics and forensics. A provisional patent was lodged to extend the use of this project technology into other fields.

The two major challenges identified and overcome in this project are the presence of 'false-positives' among recaptures and secondly, the incorporation of partial genotypes in the search for recaptures. There are some circumstances in which two samples have matching genotypes, but where the samples have not come from the same fish. This kind of match is a false positive and the genotypes are referred to as shadows. It occurs when the multi-locus PID of the genotyping panel used is compromised by large numbers of pairwise comparisons of genotypes. PID refers to the probability that two samples drawn at random from the population will have identical genotypes. As more comparisons are made (10^3 to 10^6 in a large-scale genotype mark-recapture study), some pairs of samples will have identical genotypes by chance. It is critically important to distinguish these false-positives from genuine matches. This project has developed a method to do this based on partitioning genotype probabilities (GP) and has provided software to implement the method called "SHAZA". More detail is given below.

In this study, a seven-locus genotype was a 'full' genotype, whereas a 'partial' genotype consists of data from less than seven loci. Partial genotypes arise when loci fail to amplify or cannot be resolved with capillary electrophoresis.

Partial genotypes are common in most genotyping studies, dealt with in a variety of ways: the laboratory analysis can be repeated; that sample can be dropped from the study; or, that locus can be dropped from the panel. In this project, none of these strategies were feasible. It was too time-consuming to go back and repeat failed loci from selected samples. If we omitted all samples with partial genotypes from the study, then the chances of observing recaptures in the remaining samples would be unacceptably low. We could not omit loci from the genotyping panel as it was fixed for the entire study. Using partial genotypes to search for recaptures was challenging as there are a considerable number of combinations in which partial genotypes can be compared with each other from a seven-locus panel, assuming the minimum number of loci is four. For some comparisons among four loci genotypes, they may only have one locus in common. Matches can be found within, but not between, these classes using conventional matching processes. The SHAZA software developed by this project uses an enabling process to accumulate within class matches to find between class matches. More details are given below.

When performing genotyping in the laboratory, it is essential to resolve the size of PCR amplicons [3] to a high level of precision and accuracy. Accuracy is provided by the use of DNA fragments of known length (size standards) that are mixed with each sample during capillary electrophoresis. Precision must be within one base pair of the true length and the capillary electrophoresis process provides this capacity. At some stage, the raw data of PCR amplicon sizes must be converted into genotypes. This is normally done by 'binning', where raw amplicons are grouped into allele bins based on within and between group variance of length in base pairs. Commercial software packages perform this with varying degrees of success and their performance is checked manually (i.e. graphically).

Before the binning process, all raw data from the capillary electrophoresis hardware is checked by eye for ambiguities. Genotyping can only be semi-automated because it requires a judgement to score consistently (i.e. recognising complicating factors/artefacts, such as interpretation of stutter, bogus amplification, and variation in shape of peaks between loci). Judgement is needed to 'clean-up' or verify automated scores. Judgement also has to be consistent across all samples (i.e. thousands) within a project. When genotyping protocols are well developed, the amount of judgement that has to be used declines and automated scores become more likely to be accurate. The data checking process also includes the identification and removal of genotypes from non-target species. In this project, tissue samples were taken using the Genetag lure from fish *in situ* and usually at depth and the species identification of fish could not be checked. This project has developed unique methods of identifying outlying genotypes (more information given below). This significantly reduced the work involved in identifying non Spanish Mackerel samples. In other projects, mitochondrial (mt) DNA sequence would usually be used to exclude non-target samples before genotyping.

Genotyping can be out-sourced to a commercial provider. Most commercial operations have the ability to handle large sample numbers and outsourcing avoids having internal genotyping facilities, which are expensive to purchase, maintain and operate. In-house genotyping requires considerable technical and genetic expertise. However, the control provided by in-house genotyping over each step in the process is invaluable.

[3] PCR amplicon is the DNA fragment, corresponding to an allele, that is amplified in a PCR.

DNA extraction

DNA extraction from hook-tips

Early problems with copper contamination

In mid-2004, it was noticed that, after storage in the DMSO/NaCl solution, tissue retained in some hook-tips developed a green tinge (Figure 7). Copper would be present in the DNA extraction because the entire hook-tip, snipped off the copper Genetag hook, was placed in solution for DNA extraction.

The success rate of DNA recovery from hook-tips was experimentally tested by the usefulness of the extracted DNA to produce a PCR product (amplicon) when that DNA was used as a template in a PCR for the control region of Spanish Mackerel mtDNA. MtDNA, rather than micro-satellite DNA, was used in these tests as the suite of microsatellite loci for the project was still under development.

Success rates were compared between groups of hook-tips with varying amounts of visible tissue. Copper concentrations in Spanish Mackerel DNA extracted from hook-tips, were measured using graphite furnace flame atomic absorption spectroscopy[4].

Figure 7. Hook-tip Sc272 showing a large amount of tissue with green colour that could have been a result of copper contamination

The Genetag lure design was changed so that the lure tips were threaded onto the copper hook and could be separated from the copper. Hook-tips were subsequently stored in 70% ethanol, which is less reactive than the DMSO salt preservative solution, and stored at 4 °C.

Routine DNA extraction from hook-tips and lancets

The stainless steel hook or lancet tip was placed in a 1.5 mL eppendorf tube and digested with proteinase K from the Qiagen DN easy tissue kit ® extraction kit. The tip was removed after incubation at 50 °C prior to column purification of the DNA.

DNA extractions were made from tips regardless of the visible presence of tissue inside the tip (Figure 8). DNA was recovered from hook-tips where tissue could not be observed under the dissecting microscope.

Fin (~3-5 mm^2) and operculum (~3-5 mm^3) samples were defrosted and washed in Milli-Q water and air-dried. DNA was extracted using chelex (Walsh et al. 1991) or salting out (Sambrook et al. 1989) (Appendix 3).

[4] Copper concentrations courtesy of Professor Graham Pegg of Central Queensland University.

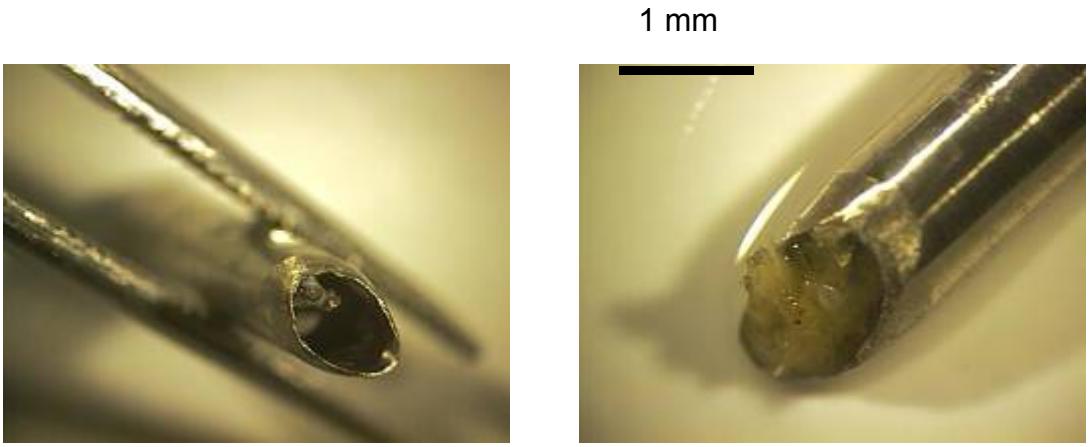

Figure 8. Hook-tips showing small (left) and large (right) amounts of retained tissue

The small amount of tissue is retained on the internal barb of the tip.

DNA extraction from tissue samples from the landed catch

Tissue samples taken from harvested Spanish Mackerels were ventral fins or operculum tissue. Ventral fins were chosen because they were easily distinguished as tissue from this species and could not be confused with other fins from the same fish. This was important as we were paying the fishermen per fin and we needed an easy way to ensure that we received only one fin per fish.

The fishermen found it easy to remove the ventral fins as a pair. However, the pair often fell apart and this caused downstream problems. The fins collected per day on a trip were placed in the same, labelled plastic bag. If the paired fin collapsed, then we had no alternative but to perform genotyping on both fins. This led to redundancy in the fin database, but as fins from the same fish had the same genotype, they could be identified using the software SHAZA and one of the two could be removed.

To minimise sample cross-contamination, fin or operculum tissue was washed in distilled water and dried on filter paper prior to DNA extraction.

Over the course of the project, two methods were used to extract DNA from fins and operculae. The two methods were (Walsh et al. 1991) or salting out (Sambrook et al. 1989) (see Appendix 3). Salting out was more resistant to the oil content of the tissue and was used for the majority of samples. The presence of oil reduced the performance of the DNA as template in subsequent amplifications. DNA extractions were performed in 96-well plates in a Corbett liquid handling robot. About 200 samples were processed per week (DNA extracted, genotyped and raw data checked).

DNA amplification

DNA amplification was performed in 96-well plates. Four wells per plate were reserved for controls, two of which were known DNA. These controls were used in all plates genotyped in this project. These DNA controls yielded the same genotype on each plate and were used to trouble-shoot the PCR for that plate. For example, if the PCR reaction for that plate failed due to lack of polymerase activity, then all genotypes would fail (i.e. from the control samples and for the remainder of the samples). Alternatively, if the DNA extraction for that plate failed, then genotypes for the control samples would be normal and the remainder of the samples would fail. Other uses of the control DNA samples included checking on the allele size determination of the capillary electrophoresis process; the alleles for the controls should be identical from plate to plate.

One control was an extraction control. An extraction control is prepared as a hook-tip or fin DNA extraction (i.e. contains all extraction reagents) but contains no tissue. The extraction control should not lead to a genotype that can be scored. If it does, then it shows contamination of fish tissue or PCR amplicons in the extraction reagents and DNA extractions prepared with those reagents should be discarded as potentially contaminated. The remaining control was a post-extraction control. It was prepared alongside DNA extractions beginning where the DNA is resuspended in milli-Q (ultra-pure) water. The control tested whether fish tissue contamination or PCR amplicons had contaminated the milli-Q water, PCR reagents or plastic ware. Genotypes from a plate with contaminated post-extraction (i.e. displayed a genotype when it should have been blank) were discarded.

One 96-well plate PCR was performed for each panel of loci. This means four plate-PCRs were needed to genotype 90 (i.e. 96 samples less four controls) Spanish Mackerel samples for the seven loci. The primer annealing temperature was 56 °C for 45 seconds (Table 2). Four 96-well PCR machines (two Perkin Elmer 9600 series and two Perkin Elmer 9700 series) were used. To minimise variation between machines, the same PCR machine was used for each panel. For example, the same PCR machine was used for all panel-one amplifications.

Table 2. PCR cycling conditions for the amplification of panels 1 – 4 - denaturation, annealing and extension stages were repeated 37 times

PCR stage	Temp. (°C)	Time (minutes, seconds)
Initial Denaturation	95	15, 0
Denature:	94	0, 30
Anneal:	56	0, 45
Extension	72	1, 30
Final extension	72	45, 00
Hold	20	∞

The PCR volume per well was six microlitres (Table 3). Qiagen master mix (containing Taq polymerase and magnesium chloride) and Qiagen Q-solution (i.e. to facilitate locus multi-plexing) was used. Mineral oil was used to control evaporation during cycling.

Genotyping of genetag and fishery samples

Samples were genotyped with loci SM3 and SM37 (Broderick et al. (in preparation)), SCA30, SCA47, SCA49, SCA8 (Broughton et al. 2002 and Gold et al. 2002) and 90RTE (Van Herwerden et al. 2000a). Reverse primers for loci SCA49 and 90RTE were lengthened by a random oligo tail of 50 (SCA49) or 20 (90RTE) bases on their 3' end to increase amplified allele length to facilitate locus pooling. Amplification conditions have been described (Broderick et al. (In preparation)).

Table 3. PCR composition per well for panel one loci

Reagent	Microlitres
Milli-Q H20:	0.85
Primer SCA30M13f (5 uM):	0.02
Primer SCA30r (10 uM):	0.10
Primer SM3M13f (5 uM):	0.03
Primer SM3r (10 uM):	0.15
M13 FAM (10 uM):	0.25
5xQ solution	0.60
2x QIAGEN Master mix 6 mM $MgCl_2$	3.00
Template (~10 ng):	1.00
Total:	6.00

Table 4. PCR composition per well for panel two loci

Reagent	Microlitres
Milli-Q H20:	0.87
Primer SCA47M13f (5 uM):	0.03
Primer SCA47r (10 uM):	0.16
Primer SCA49M13f (5 uM):	0.02
Primer SCA49.A50r (10 uM):	0.08
M13 VIC (10 uM):	0.24
5xQ solution	0.60
2x QIAGEN Master mix 6mM $MgCl_2$	3.00
Template (~10 ng):	1.00
Total:	6.00

Table 5. PCR composition per well for panel three loci

Reagent	Microlitres
Milli-Q H20:	0.78
Primer 90RTEM13f (5 uM):	0.016
Primer 90RTEr.a20 (10 uM):	0.08
Primer SCA8M13f (5 uM):	0.04
Primer SCA8r (10 uM):	0.20
M13 NED (10 uM):	0.28
5xQ solution	0.60
2x QIAGEN Master mix 6 mM $MgCl_2$	3.00
Template (~10 ng):	1.00
Total:	6.00

Table 6. PCR composition per well for panel four loci

Reagent	Microlitres
Milli-Q H20:	0.96
Primer SM37.56.u17.M13f (5 uM):	0.04
Primer SM37.302L20.r (10 uM):	0.20
M13 PET (10 uM):	0.20
5xQ solution	0.60
2x QIAGEN Master mix 6mM $MgCl_2$	3.00
Template (~10 ng):	1.00
Total:	6.00

Raw data handling

Genemapper ABI software, which integrated with the ABI genotyping platform, was used to score alleles, assign them to bin classes and store and export genotype information in a database environment.

It was important to perform re-binning on the entire data set for consistency. One reason for this is that the Genemapper software is inflexible to changing the binning as we progressed through the project. Software has a limitation to the number of samples that can be binned at once (certainly not all samples in this project). We developed custom Microsoft Excel applications to view the relationship between bin class and amplicon size. This allowed us to change the size classes of bins across all samples in the dataset, which is absolutely important for consistency. Binning issues are more prevalent in large datasets due to the statistical spread of raw allele sizes within bins. A bin is a way of reflecting the average size of alleles to reflect two, three or four base pair repeats allele size.

Genotype checking

Identification and removal of cryptic repeats

When a fish strikes at a lure, it can leave tissue behind in one or both hook-tips (double strike one fish). Although it is also possible that a different fish can strike each hook-tip independently (double strike two fish) we did not identify any instance in which a double strike was validated as coming from more than the one fish. All samples are genotyped blind and only when they match do we interrogate the database to determine if they are on the same lure or not. A previously reported high incidence of independent double strikes was due to a database error. If matching genotypes are from a single lure, then it is inferred that they represent a single fish and one genotype is removed from the database. In cases where genotypes have different loci missing, composite genotypes can be created.

Likewise, when fin genotypes match, it is likely that they represent two samples from the same fish. Often, the paired ventral fin sample separated and both fins are genotyped independently. This is confirmed when the fins came from the same boat on the same day. In such a case, one of the genotypes was removed from the database.

Identification and removal of non-Spanish Mackerel genotypes

The routine pre-genotyping assay of DNA extracted from lures and lancets alerted us to the possibility that two DNA samples may not be from Spanish Mackerels. One assay showed a fragment that was 100 base pairs (approx.) smaller than the expected Spanish Mackerel fragment. The other fragment was 20 base pairs (approx.) larger than the expected band. At the time, we expected that the samples may represent two non-target species, or possibly unusual Spanish Mackerel DNA that we have not seen before. Both fragments were sequenced. The sequence identification corresponded to records that were taken at the time of sampling. The first sample was a Barracuda (*Sphyraena barracuda* Walbaum, 1792) physically tagged on

1 April 2004 by Chris Errity. The second sample was Genetagged on 10 July 2004, on the Taroona II. The captain of the vessel reported that, at that time, about 10% of the catch was Giant Trevally (*Caranx ignobilis)*.

It is possible that contamination from non-Spanish Mackerel species could make rare genotypes appear more common with the effect of diminishing the power of detecting recaptures. Non-Spanish Mackerel species will have other alleles that will not amplify and will not contribute to allele frequencies. It was recognised that non-target species could add error to the analysis and the genotypes were examined in an attempt to exclude those genotypes from the analysis.

Non-target species are expected to have genotype probabilities (GP) that are much lower than expected from the normal sampling distribution of *ln*(GP). GP is the product of the allele frequencies (observed in the sampled population) for those alleles present in that genotype. Samples with *ln*(GP) values outside three or four standard deviations are likely to be non-target species. This was confirmed by simulating a random mating population of N genotypes based on the allele frequencies in the data-set representing 8050 individuals. It was observed that only 0.15% of the data had *ln*(GP) values outside three or four standard deviations. This gave us justification to remove samples with *ln*(GP) greater than three standard deviations. This procedure was repeated in a further effort to identify and remove additional outliers. The procedure requires at least two rounds because the removal of non-Spanish Mackerel genotypes at each round will change the mean GP and thus the standard deviation of each sample.

A quantile-quantile plot can provide a visual representation of departures from normality. Sorted normal deviates from observed *ln*(GP) data and expected values from normal deviates based on cumulative proportion are plotted as a quantile-quantile plots (Figure 9). This plot is from a selected example data set containing 1202 full genotyped samples. The observed samples had a larger deviate than expected at the lower *ln*(GP) range, which could be due to the presence of non-target species. A search for outliers from all combinations of loci (partial genotypes) was made with the largest outlier having a standard normal deviate of seven standard deviations.

Fins, hook-tips and lancets were analysed separately because of the prior expectation that the percentage of bycatch (non-target species) varies amongst the three groups. For example, fishermen were requested to send Spanish Mackerel fins only. For the hooks, both Spanish Mackerel and non-target species strike the hook but are not observed, hence species sorting needs to occur in the laboratory. For lancets, recreational fishermen were occasionally likely to tag pelagic fish other than Spanish Mackerels. Analysis showed that the fins contained 0.63% bycatch. The hook-tips contained 2.68% bycatch and the lancets contained 3.94% bycatch. The average incidence of non-Spanish Mackerels in this species catch in northern Australia was estimated to be around 3%, based on on-vessel observation of bycatch (Handley 2010). The genetic estimates are biased downwards because non-Spanish Mackerel bycatch can only be counted if the sample produces a genotype at four or more loci.

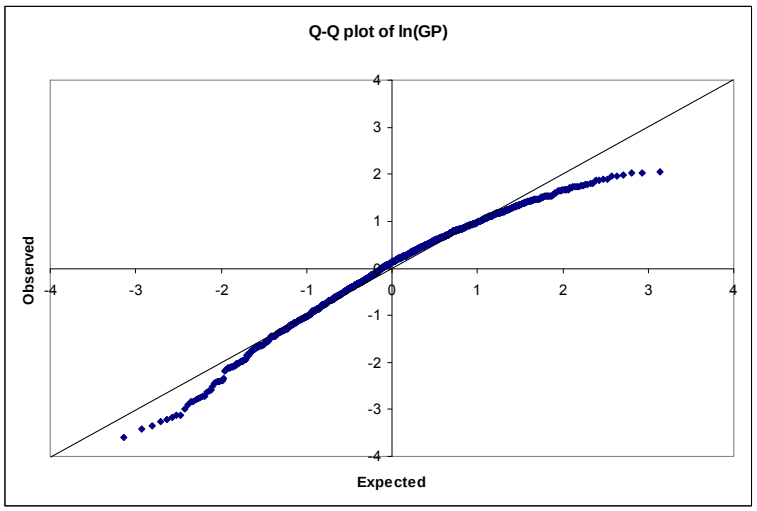

Figure 9. Q-Q plot of observed and expected ln(genotype probability) frequencies

Identification and correction of genotyping errors

Using a custom access database

The project produced an Access database for storing and analysis of data prior to exporting to SHAZA for recapture analysis. Established by Samantha Peel and built upon by Damien Broderick, the database checked incoming genotypes for consistency with existing data. This is particularly important when samples are repeated. If incoming data is not identical with existing data, the user needs to address this issue before proceeding. The database also attaches fisheries information to genotypes, which is used for estimation of harvest rate in the context of catch and effort for the relevant fishing event.

Using SHAZA

Genotype errors could occur in hooks by DNA contamination with other fish, or through any number of processes (e.g. labelling samples, DNA extraction and interpretation). When two or more samples from the same individual occur, given that genotyping and matching are “blind” processes, it is an opportunity to quantify potential genotype errors. The process of finding errors on one or more loci is described in high level detail in Macbeth et al. (2011). Briefly, samples can be compared in all combinations of loci including the exclusion of those alleles with errors. If there is sufficient power to identify two samples being from the same individual when the erroneous alleles are excluded from pairwise matches, then the error can be detected. The alleles with errors are unknown so it was necessary to search for the combinations that exclude the errors in this blindfold manner. It can be computationally demanding to test for all allele combinations but the search was minimised by searching for all allele combinations with samples that differed by up to three alleles. Recaptures that differed by genotype but which had sufficient power to be identified with SHAZA as a match, were investigated further to determine if they were real recaptures (two samples from the same individual caught at different times similarly for duplicate sampling of the same fin).

Table 7. Genotype error rate from fin samples

Common alleles	Number of samples	No. in match category	Match category: Alleles different	Match category: Possible dropout
8	3	3	0	0
10	4	4	0	0
12	8	6	0	0
		1	1	0
		1	1	1
14	40	34	0	0
		6	6	6

Total replicates	55
Total match	47
Allelic dropout rate per locus	1.9%
Total error rate per locus	2.2%

As an example, genotype errors in fin samples determined through SHAZA matches from unlabelled samples from the same batch are provided in Table 7. It is important to realise that there was sufficient power with 14 alleles to detect up to four allelic errors using SHAZA, but all six errors with fully genotyped samples were due to possible allelic dropout. The implication of this finding is that potential matches based on SHAZA loci combination analysis could also be excluded on a mismatch allele criterion.

Matching genotypes (i.e. identifying recaptures) and dealing with shadows to determine recaptures

Concept of shadows

There are both theoretical and empirical indicators to help us distinguish between recaptures and shadows (cases in which separate individuals have the same genotype). Pairwise comparisons from our seven loci (14 alleles) data-set can be used to generate a histogram of the number of alleles that match between any pair of samples (Figure 10). This histogram comprises two discrete distributions. The first distribution is of comparisons among individuals that share from 0/14 alleles (highly unrelated fish) through to 10/14 alleles (highly related fish). The second distribution is of fish that share 14/14 alleles and are identified as recaptures. Because the two distributions are non-overlapping we can be confident that 14/14 matches represent true recaptures. Contained within this distribution is information telling us that we need at least five loci (10 alleles) to distinguish shadows from recaptures. That is, because 10/14 matches do exist, so too could 10/10 matches. Thus we would expect a small proportion of our 10/10 matches to be shadows rather than recaptures.

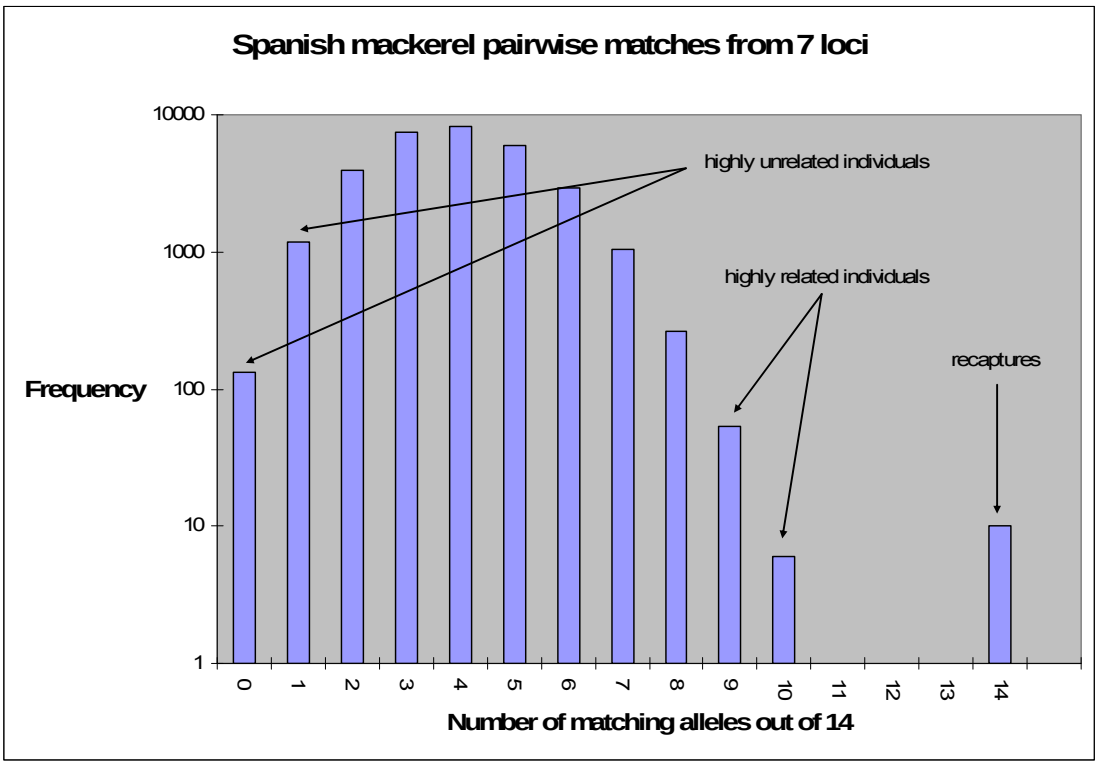

Figure 10. A histogram of pairwise allelic matches among 250 Spanish Mackerels that were genotyped at seven loci (2 x 7 alleles)

Shadows – Sample size and siblings

The frequency at which shadows occur is a function of the power of our microsatellite loci (commonly expressed as probability of identity - PID) and sample size *N* (Figure 11). PID is the probability that two samples match and the number of samples determines the number of pairwise comparisons that need to be made. The expected number of shadows in a dataset is described by the following relationship.

$$PID \times N(N-1)/2$$

Thus as sample size increases, so too does the number of shadows. Figure 11 indicates that we expect to see shadows when we genotype 4000 fish. Likewise, this figure indicates that at 4000 fish genotyped at five loci, we will expect to see 100 shadows. At first glance, these predictions are alarming, especially for a project that intends to genotype 10 000 fish; but because they occur at a predictable rate, we can correct for their occurrence. Fin-fin and other combinations from the data, non-feasible fin-lure or fin-lancet matches (i.e. where the match is for a fin caught before tissue from a lure was collected) all provide information for estimating and understanding PIDs.

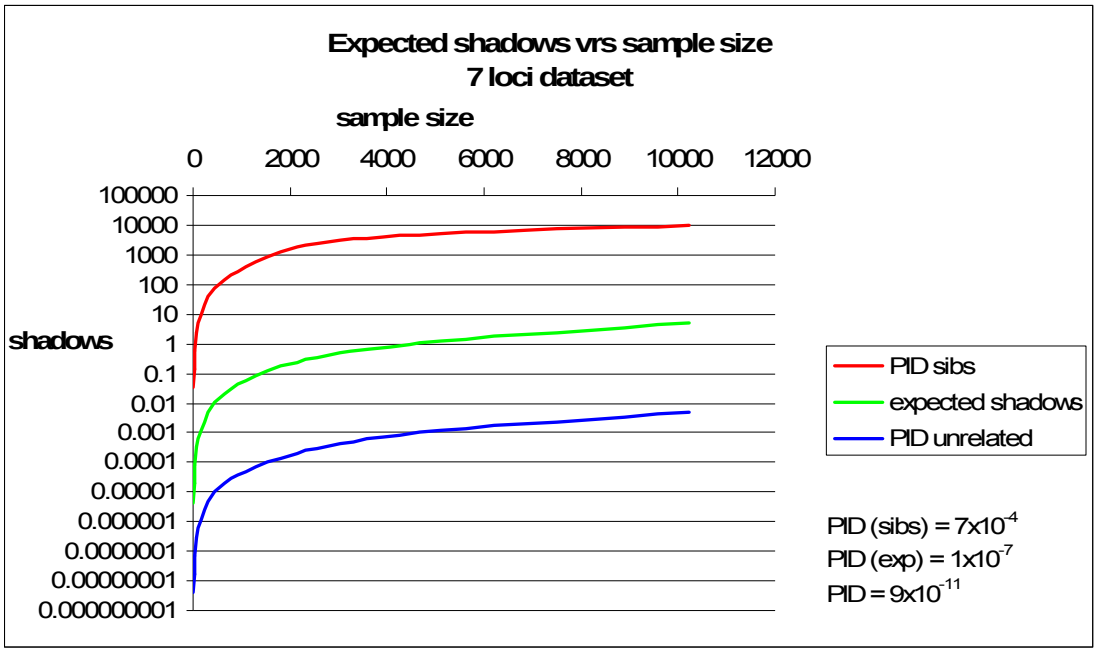

Figure 11. The relationship between the expected number of shadows and sample size for different PIDs

PIDs are calculated differently among unrelated (PID) and related (PID_{SIBS}) fish but are both derived from the allele frequencies of 1914 fish genotyped at seven loci. PID_{EXP} lies somewhere between PID and PID_{SIBS} because the real population comprises of both related and unrelated fish. The expected shadow curve (derived from PID_{EXP}) describes the number of shadows we are likely to encounter for a given sample size.

Data interrogation

Our strategy was initially to periodically interrogate the database and re-genotype pairs of samples matching at five loci or more to determine whether or not they represent true recaptures. We found, however, that this ad hoc approach challenged the limits of the database implementation and became increasingly inefficient as the number of samples grew and the numbers of comparisons and potential shadows both grew exponentially.

We developed mathematical solutions to determine the probability of recaptures in the presence of shadows, so that the data collected can be interrogated to its fullest potential. The program 'SHAZA' (SHAdow Zone Analysis) was specifically developed to conduct genotype match analysis using the methods described in Macbeth et al. (2011). It conducts the comparisons and evaluates the likelihood of matches between genotypes. Details of the process by which SHAZA can estimate recaptures, as well as nomenclature, are provided in Macbeth et al. (2011).

Apart from being an analytical tool, SHAZA was also developed to be a versatile simulation package. In the context of the Genetag project, we address the limits of our methods in data with low statistical power. SHAZA is, however, also a very powerful tool for finding matches in more powerful data with high error rates (Example 3, SHAZA user manual). The SHAZA user manual and software (including ANSI C source code) are available via the internet from the authors on: http://www.dpi.qld.gov.au/28_6899.htm

The current version of SHAZA determines the shadow-free zone using iterations of random data. The number of iterations is critical in maximising the information provided by the data and minimising potential shadows. Preliminary SHAZA simulations were used to assess the criteria for scanning potential data for further examination. Simulating 100 genotypes in each of two blocks using Genetag allele frequencies and

400 SHAZA iterations, the minimum genotype probabilities with a shadow were determined for each loci combination. This provided a false positive rate (shadows) of approximately one in 40 runs and as a result 400 iterations were considered reasonable for initial scanning of the data. Repeated SHAZA runs on the same data can reduce the rate of shadows, by excluding them as matches if they did not match in each additional run. As the number of iterations is computationally demanding the SHAZA analysis was repeated to reduce the rate of false positives in blocks of data where recaptures occurred. Potential shadows were also eliminated based on the knowledge that (i) fins between trips should not be a biological match and (ii) lures cannot match fins that were harvested earlier.

Recapture rate

After eliminating potential shadows in the analysis, additional calculations were performed to determine recapture rate. As mentioned above, known recaptures are determined from pairwise comparisons enabled within the shadow free zone. From the number of enabled comparisons determined over multiple allele combinations an effective sample size (*E*) is estimated using SHAZA. The estimate of *E* will be lower than the total number of genotyped samples (*N*) when samples within the shadow zone exist (i.e. pairwise comparisons will not be enabled if the genotypes of at least one sample fall outside the shadow free zone for all loci combinations). A probability of individual resample *PIR* is estimated from the number of recaptured animals identified (*R*) within the enabled pairwise comparisons. A corrected number of pairwise comparisons (*R**) is estimated when the sample size is *N* using the *PIR* estimated. The end result is that *R* observed recaptures have been positively identified with additional (*R*-R*) recaptures estimated to be outside the shadow – free zone.

The process of identifying a recapture in a conventional mark-recapture study involves two steps: firstly, finding a match between a marked and re-sampled animal; and secondly, confirming that the marked animal does not match any other re-sampled animal. This is the ‘yes/no’ approach. In this study, this approach can be adopted with seven-locus genotypes as there are no shadows in the dataset at that level. If we had readily found recaptures in this study using this approach, we would have restricted our data analysis to seven-locus genotypes and discarded genotypes consisting of less than seven genotypes. However, in this study recaptures were rare so we used partial data to search for recaptures.

The SHAZA approach allows us to work with partial data. It uses locus combinations to deal with the non-overlapping nature of partial genotypes and employs the shadow-free zone concept to identify recaptures in the data set. However, it cannot use the ‘yes/no’ approach to find recaptures. SHAZA can provide an answer to the ‘yes’ part of the question, but not to the ‘no’ part. This is a result of the inherent weakness of partial genotype data but also due to the search strategy used by SHAZA that is needed to implement the shadow-free zone methodology. The shadow-free zone approach is more powerful for smaller sample numbers, because shadows are rarer, so SHAZA uses a ‘block’ search strategy. Instead of searching among all pairwise combinations of the entire data set, it searches for recaptures within and between blocks of data, where a block is a subset of the data. As well as controlling sample size and hence the number of shadows, it makes sense to adopt a block approach. For example, block #1 could be all lure genotypes from location #1 and block #2 all fins from location #1; and, if so, the aim would be to find recaptures among fins. Thus, setting up blocks tests explicit hypotheses about the occurrence of recaptures among the dataset. A block strategy could also be used to search for recaptures among seven-locus genotypes, not because the rate of shadows needs to be controlled, but to make the search routine more efficient.

When SHAZA finds a recapture based on partial genotypes, the raw data for those genotypes can be re-examined. If re-examination increases the number of loci in the partial genotype and the match persists, then the certainty of recapture may increase to the extent of being able to apply the ‘yes/no’ approach. If not, that recapture cannot be confirmed. However, the unique attributes of the shadow-free zone approach mean that the proportion of recaptures in that block of data can be estimated. The proportion of recaptures is entirely consistent with using Genetag data to estimate harvest rates.

ESTIMATING ACTIVE FEEDING AGGREGATION SIZE AND FINE-SCALE HARVEST RATE FROM SHORT-TERM RECAPTURES

The partial charter approach for release of Genetagged fish, in which Genetag and normal commercial fishing operations were conducted jointly, provided an opportunity to examine the fine scale feeding dynamics of Spanish Mackerels.

Molecular genetic analysis

Allele frequencies were pooled from both lure and fin samples as they were sampled from the same population. Genetic analysis was performed using SHAZA version 1.00 (Macbeth et al., 2011). Genotype differences between putative matches were used to estimate the genotype error rate.

Determination of genotyping error rate

In this analysis, both lure and fin genotype error rates were determined. Replicate genotypes from a single strike of the same lure with tissue samples lodged in the tips of both of the two hooks were used to estimate the error rate from the lure tissue samples. Duplicate fin samples were sampled from fin pairs split in two within the same sample bag which also enabled the error rates per locus to be determined. Genotypes of fin pairs matching between sample bags are known false positives or "shadows" (Mills et al. 2000).

A prior estimate of the error rate of 1% ('-e 0.01' option in SHAZA), using a maximum number of shadows equal to 1.0 ('-z 7' option in SHAZA) was used to investigate error rates in putative matches in fin and lure samples, analysed separately. The error rate was adjusted according to the error rate estimated within the set of putative matches found. The likelihood ratio (*LR*) of a match (Macbeth et al. 2011, their equation 3) was modified to account for the different error rate per locus within lure (ε) and fin (ε') samples as:

Equation 1 $$LR_{lure\ to\ fin} = (1-\varepsilon)(1-\varepsilon')T(g_a \mid g_b)/P(g_a) + \varepsilon - \varepsilon\varepsilon' + \varepsilon'$$

Creating composite lure and fin genotypes prior to harvest rate analysis

Matches with the highest likelihoods were found within fin and within lure samples using SHAZA by setting shadows less than 1.0. If they involved partial genotypes, then these matches were joined to create one composite genotype prior to fin by lure recapture analysis. The composites were formed by adding alleles in paired samples that had loci missing between them. Where there was a different allele at any locus between matching pairs the electropherogram was re-examined to resolve the difference. In this process, heterozygote loci were assumed where there was a potential allelic dropout.

Estimation of putative recaptures between fins and lures

Using Equation 1 the total number of lures by fin matches was determined using SHAZA (Macbeth et al. 2011), which minimised the variance of estimated putative recaptures by accounting for phantoms and shadows. The estimated recaptures were simulated one hundred times using SHAZA with option '-b 2'. This bootstraps missing loci combinations in the real data according to those distributed in the different sample groups of fins and lures. This simulation generated a standard error of recapture estimates which reflects the precision of detecting matches from the statistical power available from the genotype data.

Feeding and harvest model

The model in the form of a subset diagram is shown in Figure 12. The number of fish harvested (*H*), the number of harvested fish with fins genotyped (*F*) and the number of lures genotyped (*L*) are determined directly from sample collection numbers. The total number of recaptures *Y* and *Z* were determined using

program SHAZA (Macbeth et al. 2011). This program estimates the corrected number of genotype matches by accounting for genotype error rates and missing loci in genotype matching with resulting estimates therefore not necessarily equal to whole numbers.

The number of wild fish feeding prior to harvest (*W*) is of most interest. Assuming lures are deployed prior to fins being sampled, then *W* could be estimated using: *W*=*L*.*F*/*Y*. This equation is equivalent to mark-recapture using the Petersen Method (Seber 1982) where the number marked is equivalent to *L*, the number captured is *F* and the number of animals with a mark that were captured is *Y*. The Petersen Method was modified to account for simultaneous lure deployment and harvesting. The time of individual harvested fish and lure deployments were not recorded. As Genetag lures were deployed at the same time as commercial lures used to capture fish, we assumed the proportion of lures deployed was approximately the same as the proportion of harvested fish at any given time within each harvesting session. Using this assumption, *W* was estimated using Equation 1 which uses information from both lure by fin recaptures as well as lure by lure recaptures. Solutions for *W* were determined by iteration using *T*=100 time periods. The derivation of Equation 1 is detailed in Appendix 4 with solutions for *H*/*W* representing the proportion of fish harvested on any given harvest period.

Equation 2

$$\frac{Y+Z}{F+L} \approx \frac{1}{T}\sum_{t=1}^{T}\left[\left(\frac{t.L}{T}-\sum_{t'=0}^{t-1}\left(\frac{H}{T}P_{t'}\right)\right)\Big/\left(W-\frac{tH}{T}\right)\right]$$

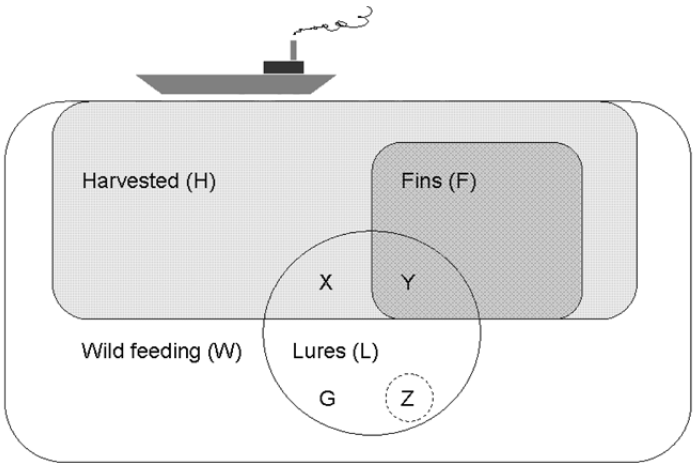

Figure 12. Feeding and harvest model with relationship between total feeding (*W*), number harvested (*H*), harvested fish with fins genotyped (*F*) and the number of lures genotyped (*L*)

Genetag fish are partitioned between those harvested without a fin sample (*X*), those lure by fin recaptures (*Y*) and those lures remaining at large (*G*) with (*Z*) equal to the number of lure by lure recaptures.

RESULTS/DISCUSSION

CONVENTIONAL TAGGING

Releases of combination-tagged fish

Between 2004 and 2006, 842 combination-tagged Spanish Mackerels were released (Table 8). Most fish were tagged and released without being removed from the water but a substantial number, 162 (19.2%), were boated first. Most releases were in the Darwin area (Figure 13), particularly in the area encompassing Fog Bay and the outer Darwin Harbour, where the tagging panel was most active. Tags were also opportunistically released in the vicinity of Melville and Bathurst Islands, Coburg Peninsula and Gove Peninsula.

During 2003 to 2006, a further 18 Spanish Mackerels were conventionally-tagged but released without taking tissue samples for genotyping; in 16 cases, tissue samples were collected for genotyping and the fish were released without applying a conventional tag. This occurred, for example, when the fish was struck a glancing blow with the tagging pole and was released before the conventional tag could be properly applied. At least two additional fish were tagged and released in the period but no release information was provided by the anglers involved (these fish were later detected as recaptures). Tissue samples from 38 unreleased fish were also retained for genotyping, a very small proportion (4.5%) of the total number tagged and released. These were captured during tagging sessions but were judged to be in too poor a condition to survive the tag and release process. Several of these were recorded as having been attacked by sharks during capture.

Most releases were in the June to August period in each year of the study (Table 8), reflecting the seasonality of the fishery for Spanish Mackerels. Although this period of the year, known as the Dry Season (austral winter), is when the species is most available to NT anglers, it is also a period during which the strong South-East Trade Winds can be very disruptive. This was manifest in the relatively low number of tags released and recaptured during 2006 (Table 8), when windy weather disrupted most planned fishing activities.

No records were collected of actual days on which anglers fished for Spanish Mackerels in order to tag them, nor the number of anglers involved, so that total tagger effort cannot be estimated. However, over the 2003-06 period, 118 anglers tagged fish in the project. There were 353 angler-days when fish were successfully tagged; if unsuccessful angler-days were included, the total effort estimated might be considerably larger. Most successful anglers tagged just one or two fish in a day. Few achieved more than three in a day (mean number tagged = 2.6, S.D. = 2.5) but the maximum achieved was 17 (Figure 14). Most participants tagged fewer than 10 fish in total. Some, however, put considerable personal effort into this project: four of the anglers tagged more than 50 fish, including one personal total of 129 fish over the three years.

Table 8. Monthly releases of combination tagged Spanish Mackerels in the Genetag project, 2004-06

	Month											
Year	2	3	4	5	6	7	8	9	10	11	12	**Annual total**
2004	3	1	1	6	21	183	39	7	35	19	3	318
2005	1	1	2	26	335	12	10	23	8	1		419
2006					5	51	24	24	1			105
Monthly total	4	2	3	32	361	246	73	54	44	20	3	842

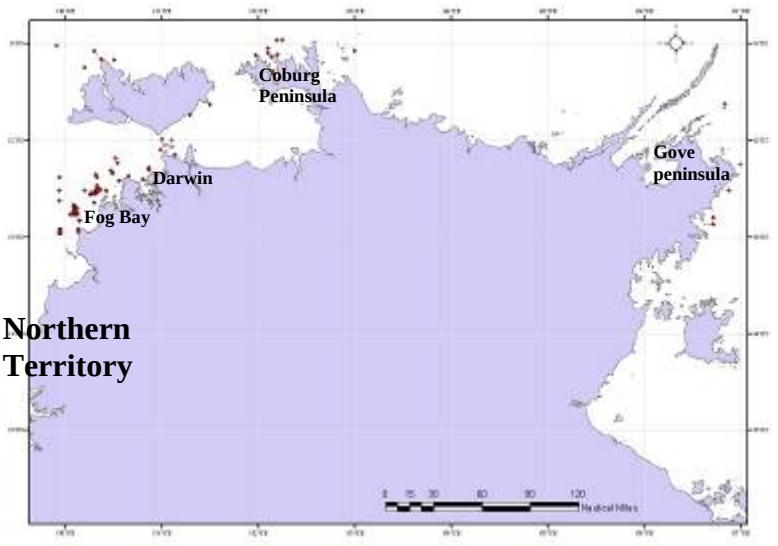

Figure 13. Location of releases of combination tagged Spanish Mackerels during 2003-06

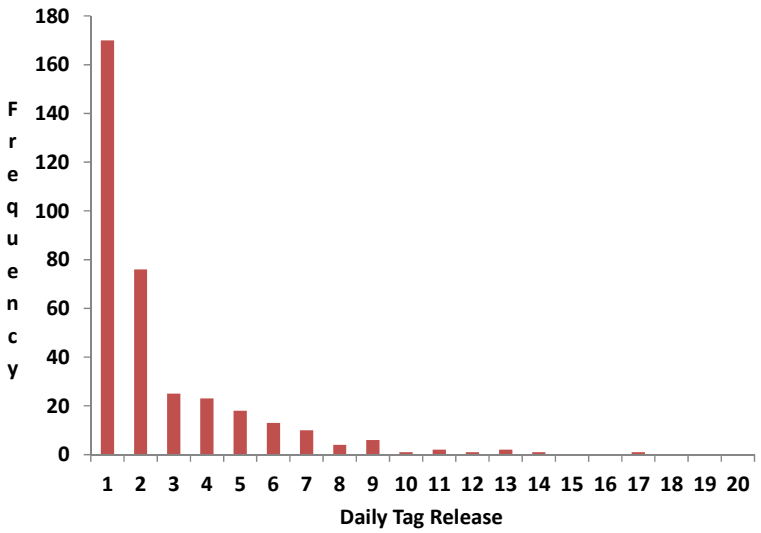

Figure 14. Distribution of the numbers of narrow-barred Spanish Mackerels tagged on successful tagging days

Conventional tag recaptures

By the end of 2010, there were 31 confirmed recaptures from the 860 Spanish Mackerels conventionally-tagged and released between 2003 and 2006 (Table 9), providing a gross return rate of 3.6%. Six of these fish were re-released immediately after recapture but none were recaptured a second time. None of the 18 fish that were only genetically marked were subsequently detected.

It is interesting to note that the first fish tagged in the study, on 4 December 2003 near Vernon Island (near Darwin), was recaptured one day later at precisely the same site. This was also the only recapture (3.4% of recaptures) that was recorded as having been boated before tagging and release; 28 had been pole-tagged in the water and there was no such release data recorded for two.

Table 9. Recaptures of conventionally-tagged Spanish Mackerels by year of release and year of recapture

	Year recaptured							Total recaptured
Year tagged	2003	2004	2005	2006	2007	2008	2010	
2003	1							1
2004		5	8	2	1			16
2005			8	2	1			11
2006							1	1
Unknown			1	1				2
Grand total	1	5	17	5	2	0	1	31

Reported recaptures of conventionally-tagged narrow-barred Spanish Mackerels were fairly evenly divided between the commercial and recreational sectors. Fourteen Spanish Mackerels were recaptured by drift net vessels in the Offshore Net and Line Fishery and just one was reported by a commercial mackerel troller. The balance of 16 reported recaptures came from recreational fishers or project researchers and other DoR Fisheries staff.

There is no information available on the distribution of recreational fishing effort over the period of this study. Effort information from the commercial sectors cannot be provided due to confidentiality requirements for commercial-in-confidence data in the NT *Fisheries Act*. However, it can be stated that the spatial and temporal distribution of recaptures by the sectors basically reflected the distribution of effort. There is significant effort by the Offshore Net and Line Fishery, but little effort by the Spanish Mackerel Fishery, in the area in which most of the tag releases and recaptures were located.

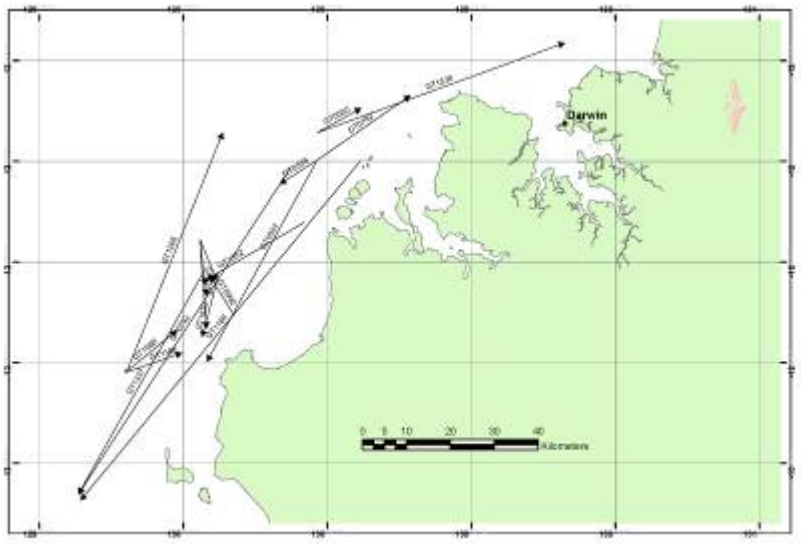

Figure 15. Approximate vectors of movement of combination-tagged Spanish Mackerels

All recaptures were reported in the area around Fog Bay and the vicinity of Darwin, with fish usually recaptured in moderate proximity to their point of release (Figure 15). Calculated straight-line distances ranged from zero to 100.2 km between release and recapture (Figure 16), and displacements were typically longshore (Figure 15). Although the mean distance was 28.0 km, the distribution of these recaptures was more of a lognormal (*ln*) form (Figure 16). The mean of *ln*(distance) was 2.32 (±0.45 S.E.).

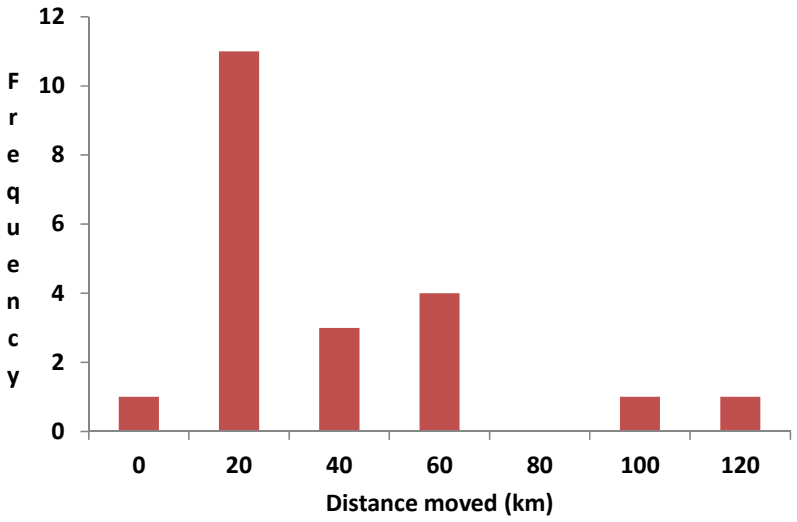

Figure 16. Calculated straight-line distances moved for recaptured Spanish Mackerels

As indicated in Table 9, a substantial proportion of recaptures from all tagging years occurred during fishing seasons in later years. The gross mean time at liberty for conventional tag recaptures was more than 320 days (S.D. 342.2 days; Table 10).

Recaptures of Spanish Mackerels that were released in 2004 actually increased from five to eight fish, between 2004 and 2005, and a few individuals were captured in 2006 and 2007. Mean time at liberty for the 2004 fish was close to one year, at 348.8 days (Table 10).

In 2005 to 2007, recaptures of 2005 releases were numerically the same as 2004 releases. The mean time at liberty for this group was 199.0 days (Table 10), while the single recapture from the 2006 releases occurred in 2010. The interval of 1510 days, more than four years, was the largest observed.

The time at liberty for most fish was measured as months or years, with just one fish recaptured at one day, one after each of 20, 33 and 57 days, and the remainder at 60 days or more. The mean time at liberty was substantial at 320 days (S.D. = 348.2). The distribution of time at liberty (Figure 17) is also described by a lognormal distribution, with the mean *ln*(time at liberty) = 4.96 (S.D. = 1.74).

The mean movement rate was 94.8 km per year (S.D. = 136.1). However, comparison of distance moved with the time at liberty (Figure 18) for the recaptures with sufficient information suggests that within-year (seasonal) movements tended to be greater than between-year movements.

Combination tagging and recapture: Discussion

The gross recapture rate of conventionally-tagged mackerels, 3.6% (5.0% for 2004 releases, higher when Darwin area releases alone are considered), is comparable with past mark–recapture studies of Spanish Mackerels (e.g. McPherson 1981, 1992) and given that time at liberty was usually large, proving good long term survival of the tagged fish and generating some significant long-term recaptures, it supports the technical feasibility of conventional tagging with the species.

Although there was little data to make strong conclusions, pole-tagging the fish without removing them from the water appears to have been a protocol that contributed to this success: although boated fish represented 19.2% of releases, they were represented by just one recapture (3.4% of recaptures). This result underlines the importance of handling protocols to the consequent survival of the fish and indicates that research in this

area could be an important contributor to better performance of mark-recapture programs. It is difficult to maintain rigorous protocols in voluntary programs, but it is an area where constant communication can be fruitful. The vigorous attack and fight attributes that make Spanish Mackerels an exciting game species also mean that they are likely to be in a state of exhaustion at the time of capture. Without a covering of large, hard scales, Spanish Mackerels are amenable to pole-tagging. Given their tonic immobility (i.e. they stop moving) when inverted, providing relative ease of hook removal and even measurement, removing the fish from the water for most mark-recapture purposes is probably not warranted.

Table 10. Mean and standard deviations of times at liberty for recaptured Spanish Mackerels by year at release and year recaptured

Time at liberty		Year recaptured						Grand total
Year tagged		**2003**	**2004**	**2005**	**2006**	**2007**	**2010**	
2003	Mean	1.0						1.0
	S.D.	0.0						0.0
2004	Mean		69.0	355.0	658.0	1079.0		348.8
	S.D.		47.7	92.8	125.0	0.0		277.0
2005	Mean			80.9	421.5	699.0		199.0
	S.D.			38.6	37.5	0.0		207.9
2006	Mean						1510.0	1510.0
	S.D.						0.0	0.0
All years	Mean	1.0	69.0	217.9	539.8	889.0	1510.0	320.0
All years	S.D.	0.0	47.7	154.4	150.0	190.0	0.0	342.2

A key element in the success of this part of the project was that it successfully harnessed the good will of the recreational and commercial fishing communities. More than 100 anglers, fishing over 350 successful angler-days (i.e. days on which fish were tagged and released) produced the modest number of releases (876) described in this project. This information emphasises the difficulty of mark-recapture work with a species such as Spanish Mackerel. Unsuccessful tagging days and unsuccessful taggers were not recorded; most tagging days involved few fish. The large number of days on which few fish were tagged, and the relatively few fish tagged by most anglers, indicates the difficulty of locating concentrations of mackerels that were behaviourally available to tagging. Clearly the input effort by the recreational community in the project was substantial. To duplicate such an effort by a research team alone, or on a commercial basis, would involve considerable cost. A nominal cost of say, $500 per successful angler tagging-day would generate a total cost of $175 000. Additionally, although the contribution of the volunteer taggers cost was not evaluated, it entailed a prodigious coordination and liaison effort by project staff. The close liaison maintained with the commercial sectors taking Spanish Mackerels was reflected in the fact that they were the source of around 50% of the recaptures.

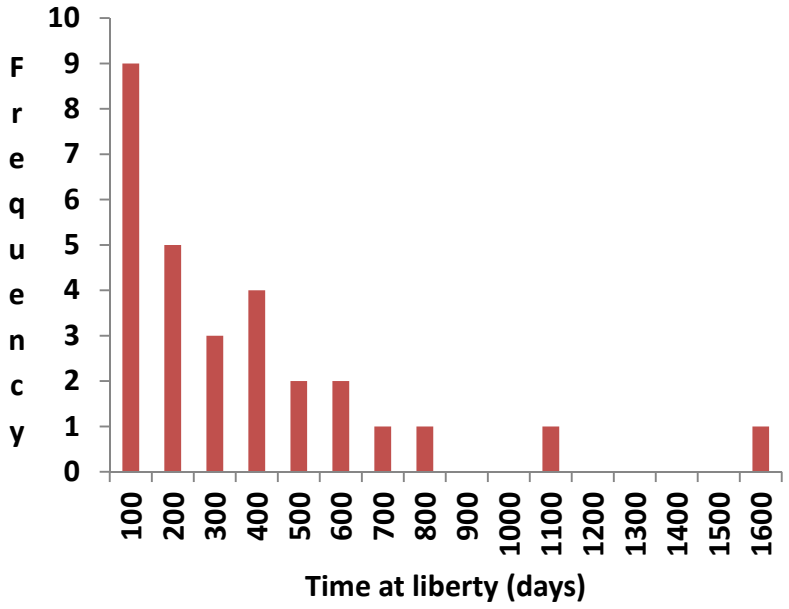

Figure 17. Frequency distribution of time at liberty for recaptured Spanish Mackerels

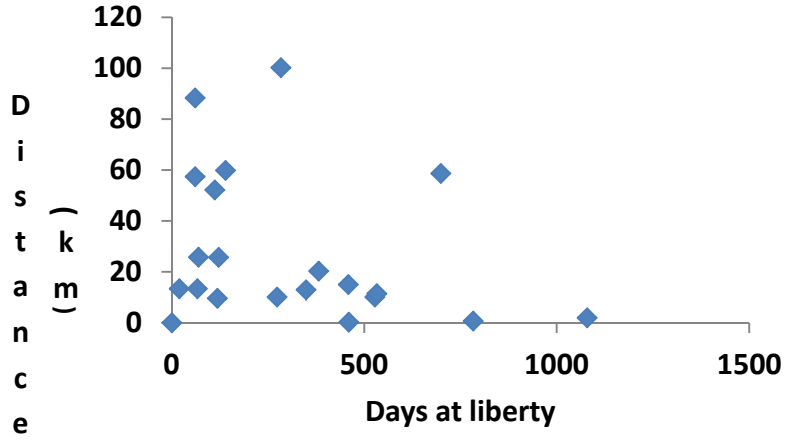

Figure 18. A comparison of distance moved with days at liberty for recaptured Spanish Mackerels

Although Spanish Mackerels are known in some populations to undertake large scale migrations, such as the east coast of Queensland (McPherson 1981) and the east coast of Africa (Govender 1993), the apparent distances moved by recaptured fish in this work, with a mean of about 30 km, were typically little greater than the error that might arise from assigning latitudes and longitudes to fishers' stated locations. It could be argued that the lack of recreational effort outside the range of the Darwin population centre is the main cause of these observations and more widespread effort would have generated more recaptures with greater distances moved by those fish. Nevertheless, a lack of recaptures in the commercial Spanish Mackerel and Offshore Net and Line fisheries, other than in the vicinity of the bulk of releases, adds some support to the conclusion that the movements of the fish were fairly restricted, as described by the pattern of recaptures.

Detailed studies across spatial and temporal scales with genetic, parasite and otolith isotopes techniques (Lester et al. 2001; Moore et al. 2003; Buckworth et al. 2007a; Ovenden and Street 2007; Newman et al.

2009) have indicated that adult Spanish Mackerels in northern Australia tend to form spatially-small functional groups, which tend not to mix at scales of as little as 100 km. The results of the current study have indicated that the general scale may even be smaller. There were indications in the data that movements may be seasonal and cyclical: long term recaptures (over years) tended to be at smaller distances compared with within-year recaptures. We emphasise, however, that apparent distances moved by recaptured fish represent interplay between the movements of fish, the spatial and temporal distribution of fishing effort and reporting (which may also have temporal trends). We have little information on the distributions of recreational fishing effort, or reporting rates.

A significant number of recaptures was achieved, so that the conventional tagging program also provided a control to the Genetag approach. This helped to ensure that a lack of genetic recaptures could not be misinterpreted as evidence of a very large, minimally-exploited fish population. A control would also be useful in any future genetic tagging projects.

This section of the Genetag project clearly demonstrated that a multi-sector, conventional mark-recapture program could effectively provide information on the recreational fishery for narrow-barred Spanish Mackerels in northern Australia. Information was provided particularly on movement and survival, which were project objectives.

At the same time, however, many of the constraints of a conventional tagging program were quite evident. For estimation of mortality and harvest rates, the impact of the capture/tagging operation on survival would need to be quantified. The use of a protocol of in-water tagging appears to have substantially reduced immediate post-release mortality, indicated by the relative return rates of the fish tagged in water versus those that were boated. Nevertheless, there was a lack of quantitative information on the effect on survival of the capture, tagging and release process on fish. This might be estimated with dedicated experiments and statistically using information from multiple larger releases.

We are also unable to provide any estimate of reporting rate. Although we emphasised communication, the surveys or experiments necessary to establish a reporting rate for recreational fishers were beyond the scope of the project. With most conventionally tagged fish also genetically tagged, one approach might be to screen landed fish in a creel survey approach. This would, however, be expensive.

Quite conceivably, the reporting rate in the recreational sector might be no better than 50%. No recaptures were reported by Fishing Tour Operators (FTOs) and given that the Darwin–Fog Bay area is the most heavily fished by this sector, it is improbable that they did not catch any tagged mackerels. Although there are very few operators in the commercial Spanish Mackerel and the Offshore Net and Line fisheries, we were able to maintain personal contact on a daily basis and we have confidence (not certainty) that the reporting rate for the operators in these sectors would be closer to 100%.

Without data on the distribution of fishing effort, quantification of survival and the estimation of fishery harvest rates are problematic. However, the approaches adopted in this project have been proven sound and could be the basis of a program to monitor recreational fishing. The results of the current study could be used with some knowledge of the distribution of recreational effort distribution for developing an appropriate experimental plan. The FTO sector, which has grown substantially in the last decade, could be a good industry partner. Our observation is that this sector prefers to release fish and tagging might provide a means of adding value to the experience for fishing tourists.

If the management need is for detecting long-term changes, such as increases in harvest rates, or changes in the spatial distribution of fishing and exploitation, then effort data might be teamed with a tagging program to provide effective long-term monitoring information. If the real intention is to establish the relative magnitude of change, then a relatively low-intensity program, in which effort data were gathered with, for example, small aerial surveys (describing the number and location of boats), automatic counts of boat ramp

use, or trailer counts, might be sufficient to team with conventional tagging to provide an effective tool to monitor the impact of recreational fishing.

ACOUSTIC TRACKING OF CONVENTIONALLY-TAGGED SPANISH MACKERELS

Estimated acoustic detection ranges based on likely propagation rates

The propagation of 10 kHz acoustic tags in narrow-barred Spanish Mackerels in Fog Bay in 20 m water in calm conditions is modelled in Figure 19. A spherical model, a transitional model and a Lloyd Mirror Effect enhancement of the transitional model are presented. To indicate likely detection ranges the ambient background noise estimated at the Fog Bay site (85 dB) and ambient noise levels assumed for a mud bottom site of comparable depth away from rocky habitat in Gulf of Carpentaria waters (80 dB) are presented.

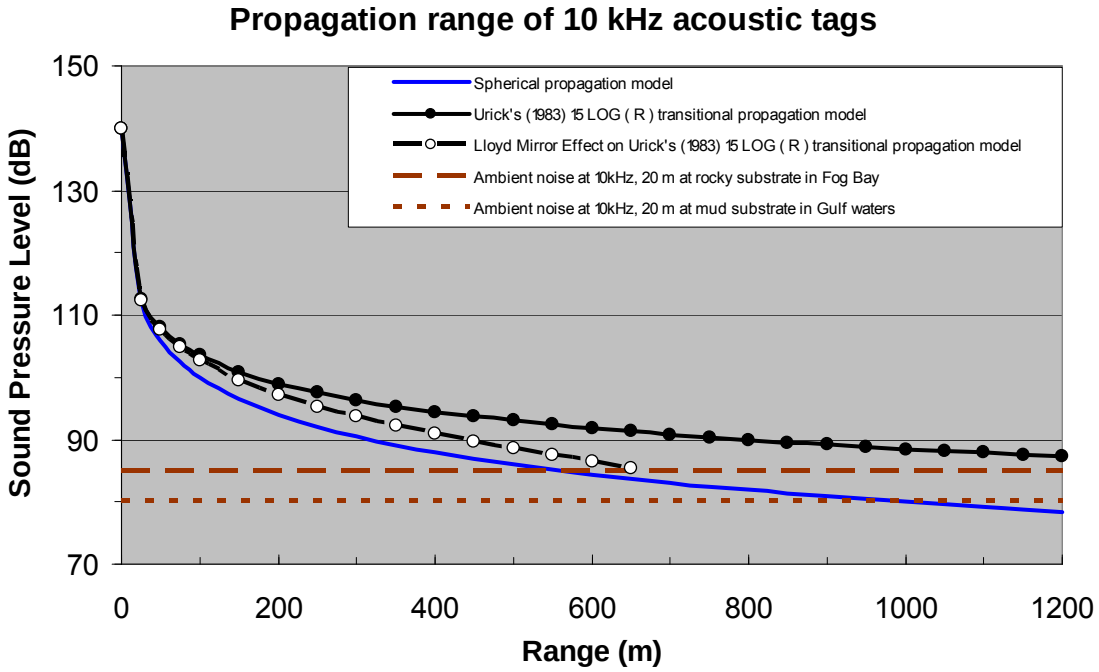

Figure 19. Estimated acoustic propagation of 10 kHz tone bursts signals from an acoustic tag given most likely propagation models

Detection is usually where the signal level of the acoustic source matched or exceeded the background noise level at the recording hydrophone although there are caveats to this:

- Acoustic detection using earphones may be enhanced by a feature of mammal hearing systems where signals below the noise floor may be isolated from the louder noise levels (i.e. the cocktail party effect) but may not show as signals in digital post-processing of signals.
- Acoustic detection using signals converted to digital sound files and processed with acoustic software offers multiple opportunities to detect a signal and particularly overlapping signals from other acoustic tags.

Given the high levels of snapping shrimp noise at Fog Bay, the likely detection range of an acoustic tag with a source level of 140 dB at 1µPa at 1 m for a 300 msec tone burst at 10 kHz could be expected to be:

- At a minimum of 500 m for the spherical propagation model.
- To approximately 600 m for an assumed 15 LOG (range) transitional model influenced by Lloyd Mirror Effects.
- To >1000 m for an assumed 15 LOG (range) transitional model where Sea State was >0.

The 15 LOG (range) model, whether incorporating Lloyd Mirror Effect or not, probably represented over-estimates for propagation range based on likely bottom absorption of signals grazing off the bottom. The estimates may reflect propagation for hard, acoustically-reflective substrates; the likely mud substrate in the Fog Bay area would contribute to reduced propagation.

The effect of mud substrate on propagation of 10 kHz tone bursts has already been discussed (Cato 1978, 1997 and 1999; Urick 1983; Etter 1996). The potential transmission loss up 10-12.5 dB reduces the propagation from the 15 LOG (range) transitional model of Urick (1983) shown in Figure 19 to the simpler spherical spreading model.

Off the rubble reef area if water depth increased, or if snapping shrimp densities decreased and noise levels reduced, the detection range could be expected to increase. The detection range would be greater in more open waters (such as Queensland's east coast Spanish Mackerel grounds, or offshore NT waters around Bathurst or the Wessel Islands) where water depth usually exceeds 30 m and propagation could be expected to be more of a spherical nature and snapping shrimp noise levels lower. Detection ranges of 1000 m could readily be achieved.

Detection at higher tag frequencies could be achieved, which is the case for commercial acoustic tags at around 60-80 kHz although that was not the objective of this project. The objective was for research and recreational acoustic taggers to monitor the immediate survival fortunes of tag releases using a minimal amount of specialist equipment.

Acoustic tag releases

Seven acoustic tags were released at the Fog Bay fishing site. Releases were distributed along an area of low rock rubble in 20 m of water over an approximate 4 km range. Summary results of detection times are provided in Table 11.

The audio signal of Tag #1 was lost within 12 seconds. There was a possibility that Tag #1 was attacked by a shark and the signal terminated although that scenario is still speculative as no large sharks were seen on the fishing grounds. Interference from the outboard motor to where the fish moved beyond ready detection range could not be discounted. Given this was the first tag finally assembled at sea (in a small dinghy with associated movement and driven salt water spray), an error associated with the activation of the signal through physical attachment and waterproofing epoxy application is thought to be the most likely explanation for tag signal failure.

Assessments by the audio monitoring method on the fishing grounds and by post-processing after the recorded signals were digitised to WAV files are presented.

Table 11. Results of assessments of seven releases of acoustically-tagged Spanish Mackerels

Tag number	Time monitored		Assessment
	Audio	**Post processing**	
1	12 seconds	Nil	Lost signal due to either shark attack or outboard interference.
2	6 minutes	6 minutes	Initial steady movement to limit of range then drifting in an out of detection.
3	2 minutes 30 seconds	1 minute 30 seconds	
4	2 minutes 20 seconds	1 minute	Rapid decline in signal strength.
5	1 minute 5 seconds	1 minute 5 seconds	Interference from outboard
6	8 minutes 30 seconds	7 minutes 30 seconds	Signal at maximum detection range then interference from outboard.
7	1 minute 18 seconds	1 minute 18 seconds	Interference from damaged audio recorder.

Tag #2 was tracked for approximately 6 minutes. The signal strength declined steadily for the first minute, declining rapidly after that. The signal was detected erratically as the fish seemed to vary its range from the monitoring hydrophone, remaining on the edge of acoustic detection. Tag #6 was tracked for a comparable period and the signal was lost in interference from an outboard motor.

Tags #3, #4, #5 and #7 demonstrated short tracks in the order of 1 minute irrespective of the method suggesting the fish rapidly moved away from detection at the tagging site.

At no stage were signals detected from more than one acoustic tag. Tagging operations were conducted over a 4-hour period.

The nature of the acoustic signal immediately after tagging is given in Figure 20. The time series domain view (upper panel) indicated the level of interference primarily from snapping shrimp. The impulsive nature of the shrimp signals is minimised in the frequency analysis domain view (middle panel) where the signal is analysed using 2048 FFT lines. The spectrum level of the signal (lower column) reflects the approximate Received Level of the 140 dB Source Level tag recorded by a hydrophone at 5 m depth at the other side of the vessel where tagging occurred. When the signal is first detected, the animal may well have moved off at a rate above human walking pace generating a tone burst peak of 107 dB at an approximate 16 m. Acoustic signals reduced by 6 dB for every doubling of distance from Source Level at 1 m, namely 6, 12, 18 and 24 dB at 2, 4, 8 and 16 m, respectively. The approximate 85 dB background noise level is highlighted.

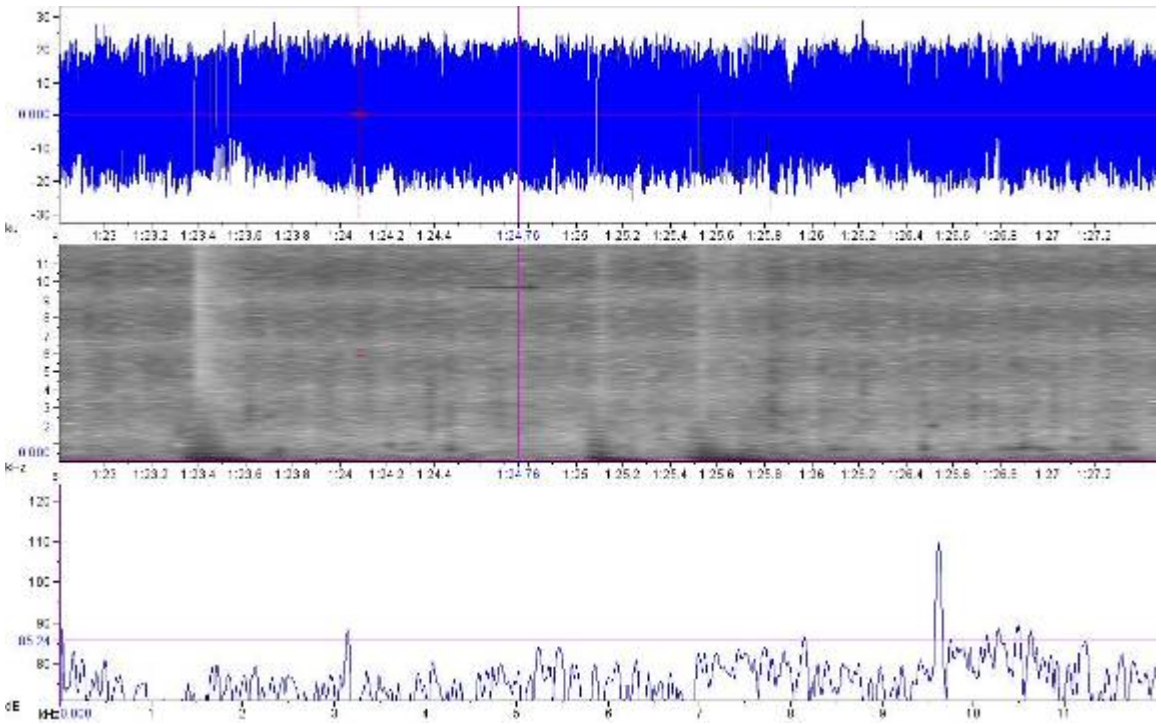

Figure 20. Acoustic Tag 2 immediately after release at first detection at approximately 16 m away from a hydrophone at 5 m

The SL of the tag was approximately 105 dB and the ambient noise level at 10 kHz 85 dB.

With increasing range to the acoustic tags, the signal strength declines. A recording of Tag #4 34 seconds after release is given in Figure 21.

The acoustic tag tone burst shown in the spectrum view of Figure 21 with a signal-to–noise ratio (i.e. tag signal over background noise level ratio) of 12.5 dB could be put into perspective of range to target for each of the selected acoustic propagation models. The spherical propagation model indicates the tagged fish was approximately 125 m from the hydrophone, the Urick (1983) 15 LOG (range) model approximately 200 m and the Lloyd Mirror Effect 15 LOG (range) model approximately 300 m (Figure 22).

Discussion: The potential for acoustic tracking of Spanish Mackerels

A problem with interpretation of recapture information from conventionally-tagged fish is that the impact of the initial capture and tagging operation on the behaviour and survival of the tagged fish is difficult to quantify. The intention of the work reported here was first to establish whether the approaches and technology adapted from Project 2003/016 could be adapted to application in the pole-tagging context used for conventional tagging of Spanish Mackerels in the Genetag project and to provide an initial estimate of the survival of tagged fish.

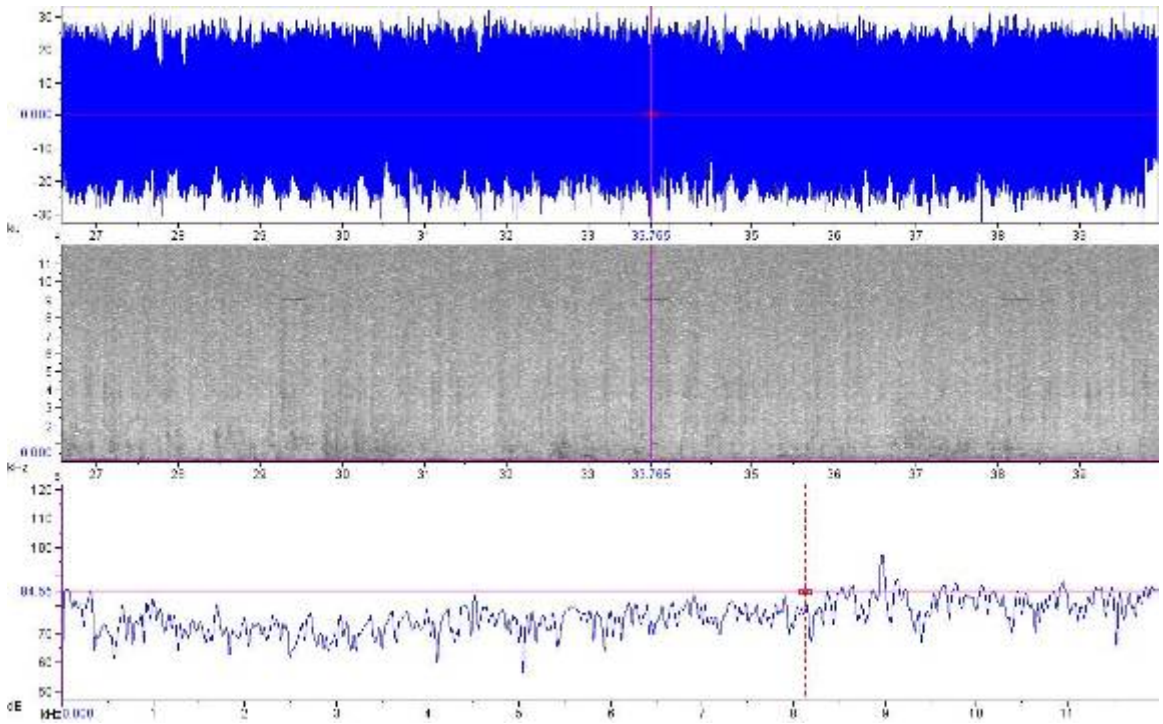

Figure 21. Acoustic Tag 4 at 34 seconds after release

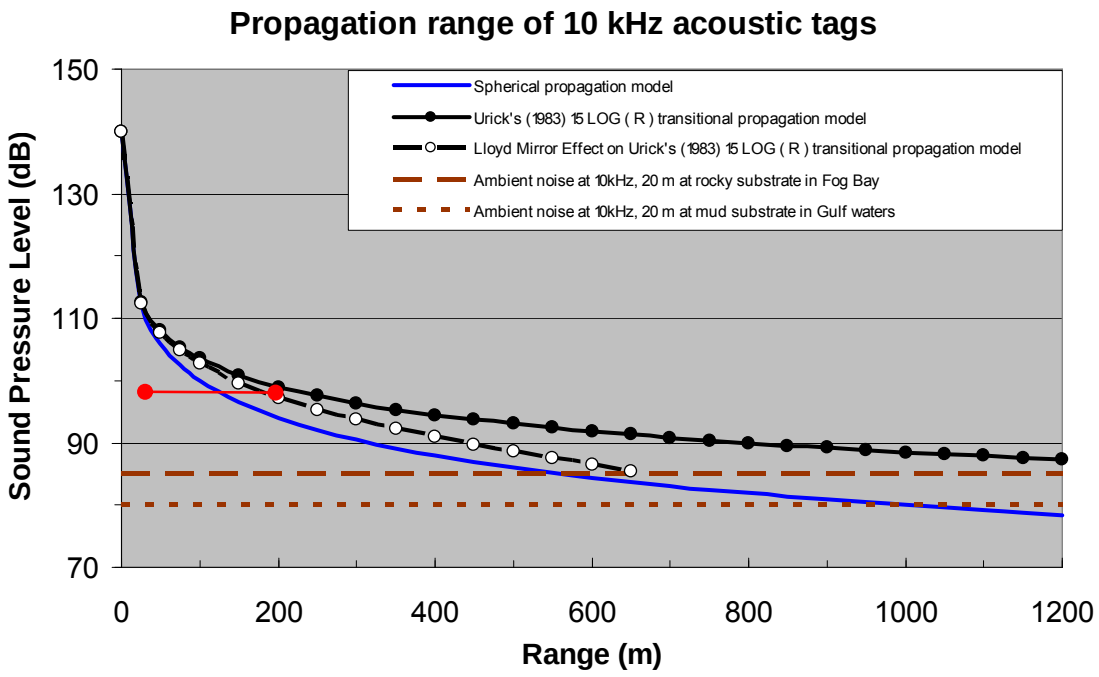

Figure 22. Estimated range to the acoustic tag signal shown in Figure 20.

Post-tagging mortality influencing tag recapture rates has been reviewed extensively. Essentially, the two main sources of mortality are:

- Tag loss. Tag attachments could fail as was demonstrated by McPherson (1981) due to tag depredation by other Spanish Mackerels and individuals rubbing tags against floating substrates/objects (that removed labelling) were component factors.
- Physiological mortality. Capture myopathy or physiological damage to fish that eventually led to fish death and tag loss.

One tag mortality factor not well-documented involves the immediate loss of a tag due to predation by other animals within a short time of fish release. Tag losses from surface-tagged deep-water fish have been investigated with variations of lowered cage release techniques. Such techniques are not readily suited for release of large scombrid fishes, such as Spanish Mackerel species.

FRDC 2003/016 demonstrated that shark depredation occurred during normal commercial and research tagging on fish present at spawning reefs of Spanish Mackerels and shark predation on fish soon after release was observed in the conventional tagging component of this project. The depredation was a real factor in commercial landings (Figure 23; McPherson et al. 2008). The depredation, if observed, was factored into effective tag release rates. If not observed, the depredation reduced the effective number of released fish and reduced the potential recapture rate. Shark depredation also occurred in non-spawning reefs. When tagging occurred where sharks were present and capable of post-tagging release attacks on disoriented or slightly injured fish returned to the water, there would be an immediate potential for post-tagging mortality.

Immediate shark predation on released Spanish Mackerels was observed during FIRTA/FRDC Spanish Mackerel tagging projects in Queensland waters (McPherson 1981). Mitigation methods involved:

- Throwing of remains of previously depredated fish to a different side of the vessel to distract the sharks to permit a safer release. This technique was only used in open-water situations when the presence of sharks only became known during the fish retrieval process.
- Release of fish in extremely shallow waters along a reef edge when the immediate presence of sharks could not be detected. This re-positioning of the fishing vessel would take time.
- Not to conduct fishing operations when sharks were known to be present on a ground. Fish had to be captured before they could be tagged and if fish concentrations corresponded with apparent shark abundance, risks had to be taken. The FRDC tagging dory (McPherson 1981) remained in company with mother vessels and if the mother vessel remained on the grounds where sharks were present, fishing operations were conducted off to the edge of commercial fishing operations where shark abundance could be expected to be lower.

Zollett and Read (2006) noted that depredation on King Mackerels (*S. cavalla*) occurred at a rate of 20% in the commercial troll fishery off eastern Florida. The top predators responsible for the depredation were Bottlenose Dolphins. Comparable depredation occurs to recreational fisheries for other *Scomberomorus* species in the Gulf of Mexico waters. Such depredation has rarely been reported in Australian waters but it could increase as it has in most other locations.

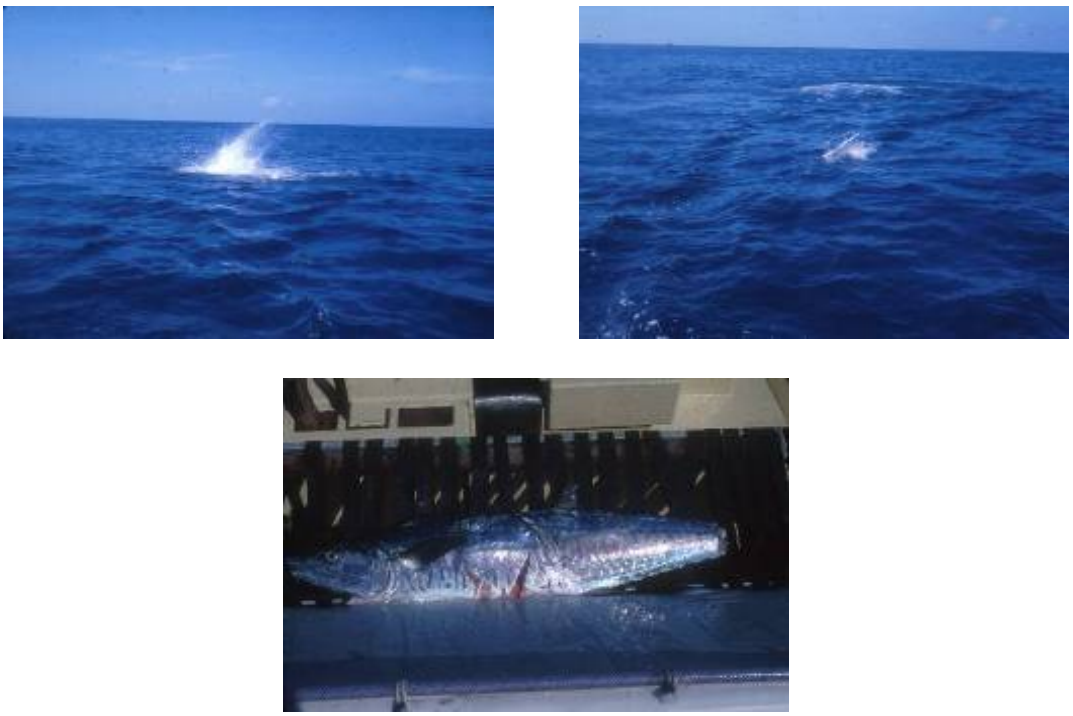

Figure 23. Sequence of shark depredation on a Spanish Mackerel (upper), with fish remains in a fish tagging cradle (lower)

Taken from McPherson et al. (2008) (Photos/fishing by G. R McPherson, 1979).

Jolley and Irby (1979) examined the effect on tag releases of immediate post-capture mortality. They followed the behaviour of an acoustically-tagged sailfish in Florida waters for some days, although it was not for some time that they realised their tagging project for a sailfish had morphed into a tagging project for a shark. The sailfish had perished during the post-tagging attack although the tag had continued to function in the stomach of the shark. Jolley and Irby (1979) were the first to monitor an acoustic tag externally-attached to a *S. cavalla* although no details are available.

Post-tagging survival

The tracking duration of all seven releases was relatively short. Initially, there was a suggestion that the first tag release may have been taken by a shark although mechanical construction issues associated with the first tag activation may well have been responsible.

All other tracks varied in duration up to six minutes after release. All fish appeared to have moved rapidly away from the site of tagging within the first minute of tagging at a most parsimonious 200-300 m radius. Two tag signals drifted in and out of reception suggesting a holding pattern at the limit of detection. Once a tag was eventually lost from reception, it did not return suggesting general continual movement from the area.

The tags were shown to operate for a period of 50 hours underwater in the lab but no tags were detected after four hours or less if the monitoring interval was less. Initial short term survival at close range to the tagging vessel was at least suggested, which was the project objective. The tagging method provided tentative support for the validity of recreational release using project or recreationally constructed equipment (meaning no reliance on non-commercial specialised equipment) of acoustic tags on grounds where sharks

were commonly reported and with a limited range of approximately 200 m within which most immediate mortality would be expected to occur.

Acoustic tag enhancements

This stage of the project effectively demonstrated that any tagging vessel could monitor tag releases. An obvious enhancement would be to manufacture higher frequency tags offering increased detection ranges. Higher frequency tags would also reduce interference from outboard motors and snapping shrimp and could increase tracking range. These could be accomplished using shareware software, older (i.e. inexpensive and expendable) laptops or moisture-protected laptops.

Full commercial acoustic tagging systems could be introduced at a later date when depredation by sharks, a source of data loss and substantial potential for wasting research funds, was eliminated.

The tag shape is substantially influenced by the flattened disc shape of the piezo bender that offers a cheap acoustic transducer, and the mini 12V battery. Minor tag size reductions could be achieved by:

- Using a double-sided circuit board with tag aerial connection incorporated into the tag body around the battery pack instead of the distal end of the tag that increased tag length.
- Placing the 12V battery in the middle of the circuit board would improve streamlining capability of the tag. James Cook University Electrical Engineering are continually improving acoustic tag designs.
- Making a latex mould for alarm resin pouring.
- Using a lower Shore A hardness two pack resin to enhance signal Source Level.

A further modification would be to produce a tag that would signal mortality of the tagged fish through predation or injury. McPherson et al. (2008) described a tag that would be readily damaged upon an attack, immediately stopping transmission. The immediate cessation of acoustic output could be interpreted as an attack provided the reliability of the tag was determined.

Tracking enhancements

We chose our Fog Bay study area as it is not a shark depredation fishery area. However, the methods demonstrated here offer the potential to demonstrate and estimate the tag mortality due to shark attack after release in areas where shark depredation is a problem.

Acoustic tracking would be more effective in deeper water areas to reduce the effect of background noise from snapping shrimp or outboard motors from recreational vessels. Improvements could include:

- monitoring restricted to non-fishing vessels only, or
- monitoring from anchored sonobuoys with acoustic data recorders.

General considerations for the assessment of acoustic tag tracking

- Current acoustic tag manufacturers provide Source Levels for their products. Detection ranges are usually provided for the performance levels of the tag. Few manufacturers, if any, provide acoustic propagation capability for their products as is the practice of manufacturers of many other acoustic products, such as transmission/detection hydrophones.
- Bycatch mitigation pingers generate sounds to warn marine mammals of the presence of the alarm/pinger and the net to which they are attached/associated. Few bycatch mitigation acoustic pinger manufacturers provide an indication of the variation of their product with the exception of STM products (Italy). Major discrepancies in output performance have been documented from a range of

acoustic pingers by McPherson et al. (2004), Kastelein et al. (2007) and Shapiro et al. (2009). The discrepancies usually relate back to the shape/type of the transducer type.

- A pinger style used in the Queensland Shark Control program to mitigate marine mammal bycatch was quoted as "world's best" with an assumption by some that the pinger would be effective. Independent acoustic assessment (Kastelein et al. 2007; McPherson pers. obs.) had demonstrated directional output variations from a piezo disc transducer between 10-25 dB that would render them totally ineffective at some approach directions.
- The generic style of transducer used in more effective pingers is referred to as a piezoceramic cylinder. The cylinder has a dominant Source Level output perpendicular to the cylinder attaining a minimum at each end. Shapiro et al. (2009) identified a 6 dB variation between the optimal perpendicular axis and the end axis. A decline in 6 dB equates to a halving of detection range of any acoustic source.
- Kingsberry (2005); McPherson pers.obs.) identified end axis deficiencies for thin walled piezoceramic cylinders used in commercial acoustic tags approaching 18 dB. That signal drop predicts three halvings of a signal strength equivalent to $1/8^{th}$ of the original signal. A signal variation of this magnitude would cause major deficiencies in acoustic tag detection. Acoustic tag manufacturers do not currently provide directionality performance curves for their products.
- A further cautionary note for analysis of acoustic tag data was highlighted by Cotton (2010). For a commercial tag Cotton (2010) determined that signal variation for the same tag varied between 133 and 2131 m depending in the same acoustic environment depending on bottom slopes. Many more parameters exist to modify acoustic detection range. Cotton (2010) highlighted the need for researchers to field-test equipment before conducting telemetry projects.
- The present FRDC pilot study determined that the soundscape in Fog Bay, NT did not present any impediments to signal propagation and the signal propagation models used would reasonably predict any variations in the acoustic soundscape. The acoustic transducer in the FRDC acoustic tag would have far better signal omni-directionality than might be expected for some commercial acoustic tags, given their acoustic characteristics and the fact that manufacturers do not publish their tags' omni-directional performance.

GENOTYPING

Microsatellite library

Thirty four loci amplified under multiple combinations of annealing temperatures and magnesium concentrations in Spanish Mackerels and were considered robust enough to test on an assemblage of closely related or co-distributed species. Under uniform cycling conditions (35 cycles, T_A = 58 ^{o}C, $MgCl_2$ = 1.5 mM), these 34 loci amplified in 62% of other mackerel, 51% of tuna and 36% of snapper species tested (Table 12). Our findings indicate that these loci and potentially others developed for Scombridae provide useful starting points for fisheries genetics applications in other species of tuna and mackerel.

Thirteen of the 34 loci that amplified the best and gave few non-specific products using the uniform cycling conditions above were chosen to assess levels of polymorphism in Spanish Mackerels. Genotypes at these loci were determined at the Australian Genome Research Facility (Melbourne) for 27 fish from representative populations throughout northern and eastern Australian waters using fluorescent labelled dNTPs and gel separated on an ABI 377 (Table 13).

Table 12. Amplification success of 34 primer pairs across closely related or co-distributed species of fish

Successful amplifications (+) are those with visible amplicons on a 1.5% high resolution agarose gel. Blanks indicate non-amplification. Loci with SM prefixes are from this study, SCA8 through to SCA47 are from Broughton et al. 2002, SCA23 through to SCA65 are from Gold et al. (2004), 90RTE is from van Herwerden et al. (2000a) and BST6.39TG is from van Herwerden et al. 2000b).

Scientific name	Common name	SM1	SM3	SM4	SM5	SM6	SM7	SM8	SM9	SM12	SM13	SM20	SM23	SM24	SM25	SM31	SM33	SM35	SM36	SM37a	SM37b	SM37c	SM38	SM40	SCA8	SCA30	SCA37	SCA44	SCA47	SCA23	SCA49	SCA61
Mackerel																																
Scomberomorus commerson	Narrow barred Spanish mackerel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Scomberomorus semifasciatus	Broad barred Spanish mackerel (grey mackerel)		+	+				+	+	+	+				+	+	+	+	+	+	+				+	+		+		+		+
Scomberomorus queenslandicus	School mackerel	+	+	+		+	+	+	+	+	+		+	+	+	+		+	+	+					+	+	+	+	+	+	+	+
Scomber australasicus	Slmey mackerel		+		+		+		+	+			+	+	+	+	+	+	+				+		+	+			+	+	
Rastrelger kanagurta	Indian mackerel		+		+					+						+		+	+						+	+					+	+
Scomberomorus guttatus	Indo-Pacfic king mackerel				+					+						+		+	+	+	+				+			+	+	+	+	
Tuna																																
Auxis thazard	Frigate tuna									+						+			+		+				+	+		+			+	
Euthynnus affnis	Mackerel tuna			+	+			+						+	+	+							+		+	+	+		+	+	+	+
Cybosarda elegans	Leaping Bonto	+	+	+	+		+	+		+			+	+	+	+	+	+	+	+	+		+		+	+		+	+	+	+	
Snapper																																
Pristipomoides multidens	Gold band snapper	+	+		+		+	+								+		+			+				+			+			+	
Lutjanus malabaricus	Red snapper		+													+	+	+			+				+			+			+	
Lutjanus argentimaculus	Mangrove jack	+	+						+	+				+	+	+	+	+			+					+	+				+	
	number of species amplfied	5	9	5	7	2	5	6	5	9	3	1	4	6	7	12	6	10	8	5	8	1	4	1	11	9	4	8	6	7	10	5
	proportion of species amplfied	0.42	0.75	0.42	0.58	0.17	0.42	0.50	0.42	0.75	0.25	0.08	0.33	0.50	0.58	1.00	0.50	0.83	0.67	0.42	0.67	0.08	0.33	0.08	0.92	0.75	0.33	0.67	0.50	0.58	0.83	0.4

Table 13. Summary data for microsatellites tested in Spanish Mackerels

Locus, repeat motif, source (a = this study, b = Broughton et al. 2002, c = Gold et al. 2002, d = van Herwerden et al. 2000a and e = van Herwerden et al. 2000b), primer name, primer sequence, number of individuals genotyped (n), observed number of alleles (Na), amplicon size range (size), observed (Ho) and expected (He) heterozygosity and uncorrected p-values for HWE (* = significant after correction for multiple tests).

Locus	Repeat Motif	Source	Primer name	Primer sequence (5'-3')	n	Na	Size	Ho
SM3	$(CA)_{24}$	*a*	SM3.AGRFf	GAAGGAGGAGGAGGAGCTGT	27	11	171-195	0.81
			SM3.AGRFr	GTTTCTTGGTCAGTCTGCCGG				
SM8	$(CA)_{9}$	*a*	SM8.73U24	GCTCTCTTCCCAGTATTTATCACT	27	8	166-212	0.78
			SM8.215L24	ATATCTTGCTCTATTTCTCATCCC				
SM9	$(CA)_{15}$	*a*	SM9.376U20	GTTTTTCTCCAGTCACACGG	21	2	346-350	0.29
			SM9.556L20	ACCCTGAGAACCAGACTGAG				
SM36	$(CT)_{5}$ TACT $(CA)_{25}$	*a*	SM36.46U22	AGTCAAGCTGTCACTGCACTCG	5	7	174-194	1.00
			SM36.202L22	GGTTTTCAATCATTTGGCTCCT				
SM37a	$(CA)_{22}$	*a*	SM37.93U17	AGCGGTTCTCCACCTCA	27	16	246-282	0.89
			SM37.339L19	CACCACAGCCCCTCTACAG				
SCA30	$(CA/GA)_{n\ compound}$	*b*	SCA30f	TGGCTGTCGGTCACTCTGCCTC	25	18	109-149	0.92
			SCA30r	ACACACACGGGTACACACAGGG				
SCA37	$(CA)_{n\ compound}$	*b*	SCA37f	GCGCCGTGACTTTTTATTGCTC	27	12	171-197	0.59
			SCA37r	CAACAATTAGTCGCAGCCCTAG				
SCA47	$(CA)_{n}$	*b*	SCA47f	CAAAGAGTGAAGCAGGTATTC	27	9	138-168	0.78
			SCA47r	GGGATCATGCAGCAAGGTAACA				
SCA49	(CA)17	*c*	SCA49f	AGATGTGACAACAGTGGG	27	11	147-177	0.78
			SCA49r	ATGGCAGCAGTAATAAAG				
SCA61	$(CA)_{6}$ TGTA $(CA)_{8}$	*c*	SCA61f	GGTACTGTCGGGAGAATGAGAT	27	2	211-213	0.44
			SCA61r	TGAATTTTATATGGAGGGTCTG				
SCA8	$(CA)_{n}$	*b*	SCA8f	CAGCTGTTCATTCCCATAGCCCA	25	13	133-175	0.96
			SCA8r	ATGAAGGAACAATGAGCCTCCAGC				
90RTE	$(CA)_{4}$ CTCATA $(CA)_{17}$	*d*	90RTEF	ATGCTGTCCACTTCCTCCAGC	27	7	175-190	0.63
			90RTER	TTTCTCAAACTCCTGCCCTTCC				
BST6.39TG	$(CA)_{17}$	*e*	BST6.39TGF	GCAGCATTAAGTGAGAGAGGC	27	4	132-141	0.22
			BST6.39TGR	GGATAATGTAGGGCCAGAGCG				

Observed and expected heterozygosities and tests for Hardy Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were assessed using GENEPOP (Raymond and Rousett, 1995; http://wbiomed.curtin.edu.au/genepop/). Overall heterozygosity was 0.70 and there were no loci with significant levels of LD after correction for multiple tests (Rice 1989). SCA37 was the only locus out of HWE and is reported as a compound CA repeat in *S. cavalla* (Broughton et al. 2002) whose allele sizes are 30-40bp larger in Spanish Mackerel. This suggests that departures from HWE may be due to null alleles. A robust panel of seven multiplexed loci (SCA30, SM3, SM37, SCA47, SCA49, SCA49, 90RTE and SCA8) was developed using ShadowBoxer and LocusEater software (Hoyle et al. 2005). Theoretically, this set provides sufficiently low probabilities of identity (PID = 8.76×10^{-11} assessed from 3000 fish) needed for genetic mark-recapture to estimate harvest rates in a Spanish Mackerel fishery (Buckworth 2004a).

Maximising information from partial genotypes

Full genotypes were more common from fin and lancet samples than from Genetag samples from hook-tips as the amount of tissue in the sample was much greater. Full genotypes are routinely recovered from nearly 90% of lancets (Table 14). However, particularly with fins, there was a high failure rate associated with certain fishing trips of industry-supplied fins. This is almost certainly due to DNA degradation from poor handling of fins between the time of capture and delivery at DoR Fisheries. When we removed this batch effect, >95% of fins were routinely genotyped at the full seven loci.

Table 14. The numbers of full seven loci genotypes and partial genotypes across fins, lures and lancets

All duplicates and failed genotypes (<4 loci amplified) were removed from the data set.

	Full genotypes	Partial genotypes	Total	Full %
Lures	291	182	473	61.5
Fins	5296	2053	7349	72.1
Lancets	501	70	571	87.7
Total	6088	2305	8393	72.5

With 8393 genotypes comprising of 6088 full genotypes and 2305 partial genotypes, SHAZA gives us an effective sample population size of 7892.59. Overall, 72.5% of samples were fully genotyped but, with the extra information provided by utilizing SHAZA methodologies, it was equivalent to 7892.59/8393 = 94%. Implementation of SHAZA limited information loss from partial genotypes to only 6% compared with 26.5% had the traditional approach of excluding partial genotypes from the dataset been implemented. The use of SHAZA therefore made a marked contribution to the power of detecting recaptures and providing harvest rate estimates.

The effect of copper from hook bodies on DNA extractions

At June 2004, we noted that the success rate of DNA recovery from hook-tips was 39% overall (Table 15). The success rate was tested by the usefulness of the extracted DNA to produce a PCR product (amplicon) when that DNA was used as a template in a PCR for the control region of Spanish Mackerel mtDNA.

We proposed that the presence of copper on the hook-tips prevented PCR from the extracted DNA. The body of the Genetag hooks is made of copper. Although the stainless-steel hook-tip was snipped off the copper hook after use, when placing it in preservative, small amounts of copper would be present in the DNA extraction because the entire hook-tip is placed in solution for DNA extraction. Copper is a known inhibitor of the DNA polymerase enzyme that is a key component of the PCR. Some hook-tips contained green coloured tissue (Figure 7) from copper oxidation. Copper contamination may have been exacerbated by storage of hook-tips in 20% DMSO solution in 5M sodium chloride.

Table 15. Success rate of DNA extraction from Genetag hook-tips containing varying amounts of visible tissue midway through the project (June 2004)

Success was determined by the presence of an amplicon following PCR of the control region of mtDNA.

Tissue	Number extracted	Number successful	% Successful
Trace	44	13	30
Small	60	21	35
Medium	2	1	50
Large	45	27	60
All	158	62	39

Table 16. Copper concentration (ug/L) using graphite furnace flame atomic absorption spectroscopy of Spanish Mackerel DNA extracted from gene-tag hooks that was a good (good quality) or poor (poor quality) template for PCR of mtDNA control region 5

Solution	Copper concentration parts per billion (=ug/L)
Control	
Double-distilled water #1	0
Double-distilled water #2	0
TE buffer lab reagent	4
10% chelex solution lab reagent	0
Good quality DNA	
Genetag lure Sc271-3	1436
Genetag lure Sc278-3	509
Poor quality DNA	
Genetag lure Sc883	1773
Genetag lure Sc906	1386
Genetag lure Sc712	3962
Genetag lure Sc566	1990

There was no consistent relationship between the presence of copper in a DNA extraction and its performance as a PCR template. DNA that was a good template had copper present, as did extracted DNA (without copper) that was a poor template. However, the range of copper concentrations in high performing DNA (509 – 1436 parts/billion) was lower than in poorly performing DNA (1386 – 3962 parts/billion Table 16), suggesting that there was an impact of copper.

Given that the copper contamination and reaction with the DMSO preservative was likely to be reducing the success rate of DNA extraction, we immediately changed the method of attachment of hook-tips to the hooks and lure bodies, and handling and preservation procedures. The improved genotyping success for hooks from 2004 onwards indicates that this was an appropriate decision. The excellent success rate for the lancets indicates the value of DMSO in NaCl as a preservative, at least in the absence of copper. Moreover, it is stable and, unlike ethanol, is not classed as a dangerous chemical. This makes for easy storage, transport and distribution and is an important consideration when dealing with volunteers and when operating in alcohol-restricted areas (this is especially true in the NT).

In-house or out-sourced genotyping?

This project trialled outsourcing the genotyping process, where DNA was extracted and amplified in-house, but commercial providers determined amplicon sizes. Outsourcing is optimised for sample analysis where variation between samples is minimal, and where genotyping protocols are well established (e.g. medical testing). In our case, we found that quality of the tissue sample (and thus the quality of the extracted DNA) varied greatly. Flow of results back from the outsourcing facility was too slow for dealing adaptively with quality issues (i.e. adaptive protocol management). The nature of outsourcing operations required them to use fixed protocols, which did not assist with our quality control problems and limited our capacity to get the best out of our samples.

Further reliability issues arose with the third-party genotyping, creating project delays and imposing substantial additional staffing, operational and quality control costs. An alternative commercial laboratory was employed, but the bulk of the genotyping was undertaken in the genotyping facility at the Molecular Fisheries Laboratory after the acquisition of an ABI3130xl sequencing machine in mid-2005.

Genotypes

As expected, the seven loci used for genetic mark-recapture were highly polymorphic. The numbers of observed alleles ranged from 19 (locus 90RTE) to 37 (SM37) (Table 18). Observed heterozygosity ranged from 0.431 (locus 90RTE) to 0.913 (SM37) (Table 18). Some loci had alleles that deviated from the di-nucleotide repeat motif (one-base-pair allele, Table 18). Loci SCA30, SCA49 and 90RTE included one allele each that fell into this category. Locus SCA47 had three one-base-pair alleles and locus SM3 had seven.

Table 17. Allele frequencies for 6071 seven-locus Spanish Mackerel genotypes from NT waters

Genotypes from hook-tips, lancets and fins are combined. Allele names are in italics and alleles that deviate from the di-nucleotide repeat motif are highlighted in bold.

Locus SCA30		Locus SM3		Locus SM37		Locus SCA47		Locus SCA49		Locus 90RTE		Locus SCA8	
119	0.001	*170*	0.001	*247*	0.001	*154*	0.001	*217*	0.001	*184*	0.001	*143*	0.001
133	0.051	*176*	0.001	*259*	0.002	*156*	0.001	*219*	0.001	*186*	0.001	*149*	0.002
135	0.031	*180*	0.001	*263*	0.002	*158*	0.010	*221*	0.132	*190*	0.004	*155*	0.001
137	0.009	*182*	0.002	*265*	0.001	*160*	0.002	*223*	0.029	*192*	0.740	*157*	0.014
139	0.030	***185***	0.001	*267*	0.001	*162*	0.050	***224***	0.004	*194*	0.014	*159*	0.147
141	0.179	*186*	0.001	*269*	0.131	*164*	0.020	*225*	0.010	***195***	0.001	*161*	0.004
143	0.022	*188*	0.001	*271*	0.038	***165***	0.001	*227*	0.130	*196*	0.095	*163*	0.218
145	0.051	***189***	0.001	*273*	0.042	*166*	0.491	*229*	0.252	*198*	0.038	*165*	0.125
147	0.045	*190*	0.009	*275*	0.005	*167*	0.001	*231*	0.167	*200*	0.066	*167*	0.129
149	0.107	*192*	0.143	*277*	0.097	***168***	0.191	*233*	0.164	*202*	0.013	*169*	0.011
151	0.096	*194*	0.005	*279*	0.049	*170*	0.139	*235*	0.046	*204*	0.001	*171*	0.053
153	0.018	***195***	0.053	*281*	0.086	*172*	0.074	*237*	0.014	*206*	0.019	*173*	0.034
155	0.048	*196*	0.140	*283*	0.065	*174*	0.005	*239*	0.004	*208*	0.001	*175*	0.061
157	0.039	***197***	0.001	*285*	0.117	*176*	0.009	*241*	0.001	*210*	0.001	*177*	0.015
159	0.048	*198*	0.181	*287*	0.037	*178*	0.001	*243*	0.015	*212*	0.001	*179*	0.026
161	0.052	*200*	0.007	*289*	0.045	*180*	0.003	*245*	0.006	*216*	0.006	*181*	0.017
163	0.042	***201***	0.001	*291*	0.039	*182*	0.002	*247*	0.016	*218*	0.001	*183*	0.037
165	0.038	*202*	0.037	*293*	0.024	*184*	0.001	*249*	0.005	*220*	0.001	*185*	0.020
167	0.019	*204*	0.026	*295*	0.078	***185***	0.001	*251*	0.004	*222*	0.001	*187*	0.005
169	0.022	*206*	0.050	*297*	0.038	*188*	0.001	*253*	0.001			*189*	0.007
171	0.012	***207***	0.002	*299*	0.044	*190*	0.001	*255*	0.001			*191*	0.004
173	0.009	*208*	0.097	*301*	0.017	*194*	0.001					*193*	0.007
175	0.008	***209***	0.001	*303*	0.012	*196*	0.001					*195*	0.003
177	0.003	*210*	0.076	*305*	0.007							*197*	0.014
178	0.001	*212*	0.110	*307*	0.002							*199*	0.006
179	0.007	*214*	0.049	*309*	0.001							*201*	0.019
181	0.003	*216*	0.008	*311*	0.003							*203*	0.010
183	0.001	*218*	0.003	*313*	0.001							*205*	0.003
185	0.002	*220*	0.001	*315*	0.001							*207*	0.001
187	0.001	*224*	0.001	*317*	0.001							*209*	0.001
189	0.001			*319*	0.002							*211*	0.006
191	0.001			*321*	0.011							*213*	0.001
193	0.003			*323*	0.002							*215*	0.001
195	0.001			*325*	0.001								
				327	0.001								
				329	0.001								
				331	0.001								

Fixation indices (1 - (Ho /He) were close to zero and positive suggesting slight deficiency of heterozygotes. This was confirmed by Genepop, which showed that loci SCA30, SAC47, SCA49 and SCA8 were likely to have heterozygote deficiency. Genotype proportions were not in Hardy-Weinberg equilibrium at loci SCA47 and SCA49. Genotypic linkage disequilibrium was apparent between locus SCA30 and SCA47, SCA49 and SCA8 and SM3 and SCA49 and SCA8 and well as SM37 and SCA8.

Note the poor performance of samples collected in 2002 and 2003. This was a storage system failure, as noted above, due to copper contamination and reaction within the salt preservative (i.e. DMSO/NaCl). From early 2004 onwards, the hook-tips were stored in 80% ethanol.

Table 18. Summary statistics across loci for 6071 Spanish Mackerel seven-locus genotypes from NT waters representing hook-tips, lancets and fins

Number of alleles (Na), observed heterozygosity (Ho), expected (He) and unbiased expected heterozygosity (UHe) and fixation index (F).

	SCA30	SM3	SM37	SCA47	SCA49	90RTE	SCA8
Na	34	30	37	23	21	19	33
Ho	0.901	0.886	0.913	0.665	0.769	0.431	0.872
He	0.924	0.890	0.927	0.695	0.844	0.436	0.887
UHe	0.924	0.890	0.927	0.695	0.844	0.436	0.887
F	0.026	0.004	0.015	0.042	0.089	0.013	0.017

We note that for any lure, both hook tips were individually subjected to DNA extraction whether or not both hooks had apparently been struck: this was accommodate the possibility that more than one fish might strike a lure, and the observation (Table 15) that even when tissue was not always easily visible in hook tips, good genotypes might be produced. Thus, a large proportion of tips were from the unstruck hooks and probably did not contain tissue. This contributed to the relatively low genotyping success rate in Table 19.

When data was aggregated by lure, (Table 20) the success rate, measured as the proportion of lures producing genotypes with four or more loci, was more than 50% during 2004-06, with 2004 especially successful (62%). These per-lure success rates are substantially greater than the performance target of 40% and represent a roughly 50% increase in performance measured per hook-tip.

Lancets were stored in DMSO/NaCl at 4 °C. Fins were frozen on the fishing vessel and were shipped and stored frozen. We noted considerable variability between samples taken at different times from different vessels. Without further information, we can only surmise that this variability arose from treatment on board the vessel. For example, variations in the period between the landing of the fish and storage of the fin sample or the quality of freezer storage on-vessel could be responsible for substantial differences in quality. The tissue from lancets was better in genotype performance than either fins or hook-tips. We believe this is mostly due to the quality control inherent in the collection process. We suspect that the most important part of the process is the transfer of freshly collected tissue into preservative as soon as possible (i.e. within 5 minutes). In 2005, we changed the design of the lancets, which may have been responsible for the increase in genotyping success from 78% in 2004 to 95% in 2005.

Table 19. Number of hook-tips (T) /lancets (H) /fins (F) that produced multi-locus genotypes compared to the number of those samples from which DNA was extracted

		No of loci										
YEAR	**METHOD**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Extracted**	**>3 loci**	**% >3 loci**
2000	H							1		1	1	100.00%
2001	T		1		1					2	0	0.00%
2002	T	36	4						1	41	1	2.44%
2003	F	84	46	29	19	29	40	54	97	398	220	55.28%
2003	T	191	73	47	30	7	7	6	38	399	58	14.54%
2004	F	180	125	149	131	166	238	310	1610	2909	2324	79.89%
2004	T	122	103	106	96	43	67	42	187	766	339	44.26%
2004	H	28	9	15	10	10	22	15	186	295	233	78.98%
2005	F	349	139	192	186	242	385	575	3361	5429	4563	84.05%
2005	T	237	110	85	26	19	44	43	110	674	216	32.05%
2005	H	9	5	3	1	5	24	13	335	395	377	95.44%
2006	F	9		3	3	17	30	33	341	436	421	96.56%
2006	T	9	3				2	1	5	20	8	40.00%
		1254	618	629	503	538	859	1093	6271	11765	8761	74.47%

Table 20. By-lure aggregation of genotyping success where 0 genotyped is maximum success

	Year					
Loci	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**
0		22	78	30	62	4
1		4	48	23	44	2
2			40	49	51	
3	1		26	52	16	
4			5	27	10	
5			6	45	35	1
6			5	28	36	
7		1	36	154	96	6
Total	**1**	**27**	**244**	**408**	**350**	**13**
Summaries						
lures > 3 loci	0%	4%	21%	62%	51%	54%
lures 7 loci %	0%	4%	15%	38%	27%	46%
tips > 3 loci	0%	2%	15%	44%	32%	40%

Matching genotypes using a likelihood-based approach: SHAZA

Determination of genotyping error rate

In this study, the genotyping error rate was determined from tissue samples that were inadvertently genotyped more than once due to the anonymous separation of a pair of fins into two parts. The separation occurred within a sampling bag each holding samples from different commercial fishing trips. Preliminary analyses to search for recaptures across the entire fin sample data-set (8392 genotypes) (using ε=0.02 and α=0.5) found 116 matches all of which occurred within the same sampling bags. One hundred of the matches represented identical genotypes, but 15 of the 116 matches differed by one allele, and one match differed by two alleles. Thus, there were 17 allelic errors across a total of 1490 alleles in the 116 matches. This equated to a genotype error rate of 100 x 2 alleles/locus x 17 errors /1490 alleles = 2%. In all subsequent analyses of narrow-barred Spanish Mackerel data, ε was set at 2%.

Empirical evaluation of genotype pairs as putative recaptures

Preliminary simulations with the Spanish Mackerel genotypes indicated that estimates of $\hat{R}$ with the smallest standard error occurred with $\tilde{\alpha}$=1.0, and there were 21 pairs of genotype matches detected within this shadow threshold. This means that the expectation of the number of recaptures was 20 (i.e. 21, less one shadow).

Two additional quality control measures were implemented. Firstly, genotype pairs with their *LLR* close to, but below, the $LLR_{\tilde{\alpha}}$ threshold were examined as potential true recaptures. For example, the 22nd ranked match, with α =1.8 shadows, matched at four loci with no allele errors. The remaining three loci had failed initial quality control, but on re-examination of the electropherogram, the pair matched at all seven loci. Thus another true recapture was identified, bringing the total to 21. Secondly, pairs of genotypes that had their *LLR* above the $LLR_{\tilde{\alpha}}$ threshold but which had one or more alleles that did not match were re-examined. For example, the 18th ranked match, with α equal to 0.05 shadows, had two alleles that were different at a particular locus. Similarly, the 21st ranked match, with α equal to 0.19 shadows, had one allele that was different. Examination of the electropherograms suggested that the alleles were different. There are two explanations for this (i) the non-matching alleles were generated by amplification (e.g. Taq error, allelic

dropout) or systematic (e.g. scoring errors, inadvertent switching of genomic DNA) errors or (ii) they are shadows as a consequence of using a particular *LLR* threshold to define genotype matches.

Estimation of number of recaptures

The number and pattern of recaptures was determined within the total data set and between 90 groups (i.e. sampling events). The groups had mean, minimum and maximum sample sizes of 93, 4 and 1045, respectively. Pairwise comparisons between the 90 groups formed 90 diagonal and (90(90-1)/2) = 4005 lower diagonal blocks and recaptures ($\hat{R}$ =21) were identified in 14 of these (Table 21). Recapture numbers (m) presented here have been revised upwards ($\hat{r}$) to account for the presence of phantoms as the effective sample size was less than the actual sample size. The recapture estimates within each block ($\hat{r}$) were used as input for 100 simulation runs producing a simulation mean ($\bar{r}$) and standard error of recapture estimates (**Table 21**). The average simulated number of recaptures ($\bar{r}$) was not significantly different from their input value ($\hat{r}$) used in the 100 simulation runs (P > 0.05) providing evidence that the theory was sufficiently robust to provide unbiased recapture estimates from the narrow-barred Spanish Mackerel data.

Shadows (not listed in Table 21) occurred in the remaining 4095 – 14 = 4081 blocks from the 100 simulation runs. Over these runs and within these blocks, there were by chance 62 blocks with one shadow, 18 blocks with two shadows and one block with 3 shadows giving an average of 0.83 shadows. As expected, this was similar to the threshold number of shadows ($\tilde{\alpha}$ =1.0) used in the analysis.

SIZE OF ACTIVE FEEDING AGGREGATIONS AND FINE-SCALE HARVEST RATE

Estimation of genotype error rate from lure samples

Of the 664 lure samples, 58% were fully genotyped. There were a total of 79 putative matches detected by SHAZA when setting a total false positive rate of less than 1.0 and using a prior error rate per locus of 1.0%. Of the 79 matches detected, 41, 16, 13, 8 and 1 had 0, 2, 4, 6 and 10 missing alleles, respectively and came from 27 different day trips. The number of errors (allele differences between two samples) counted within these respective groups were 14, 6, 7, 2 and 2 of which 18 were potential allelic dropouts as they shared three alleles between the same two loci. All the detected match pairs were recorded on the same day, except one which was recaptured on the next day. The percentage error rate was 3.2%. The analyses were re-run with a 3.2% error rate ('-e 0.032' option in SHAZA) with two additional putative matches found giving a new error rate of 3.7% per locus. An additional SHAZA run with '-e 0.037' revealed no difference in matches.

Table 21. Recapture estimates ($\hat{r}$) in 14 of 90 sampling events for narrow-barred Spanish Mackerel genetic mark-recapture data

The values of $\hat{r}$ within each of the i^{th} and j^{th} group combination were used as a prior estimate for determining means ($\bar{r}$) from 1000 simulation runs. The standard errors (s.e.) are those estimated for $\hat{r}$ using equation 8. Effective block size (*b*) and effective pairwise sample size (*e*) were determined using the equations of Macbeth et al. (2011).

Sampling event		Group size		Effective block size	Effective sample size	Recaptures			
						Found	Data	Simulation	
i	j	n_i	n_j	*b*	*e*	m	¡ö	$\bar{r}$	s.e.
8	8	130	130	130.0	127.7	1	1.04	1.03	0.25
16	16	292	292	292.0	290.4	1	1.01	0.99	0.17
38	37	11	73	28.3	27.6	1	1.06	1.04	0.34
47	46	5	100	22.4	21.9	1	1.04	1.02	0.20
53	52	74	210	124.7	115.8	3	3.47	3.52	0.75
53	53	74	74	74.0	70.7	1	1.10	1.10	0.35
55	54	26	102	51.5	47.4	1	1.18	1.19	0.42
60	59	27	336	95.3	90.5	4	4.43	4.45	0.73
79	78	62	323	141.5	133.9	4	4.46	4.48	0.83
79	79	62	62	62.0	58.8	1	1.11	1.12	0.30
84	83	11	434	69.1	59.6	1	1.34	1.30	0.52
86	85	35	213	86.3	81.0	1	1.14	1.17	0.39
14	13	576	12	83.1	78.7	1	1.12	1.11	0.30
24	23	327	4	36.2	35.1	1	1.06	1.07	0.31

Estimation of genotype error rate from fin samples

Of the 5827 fin genotypes, 72% were fully genotyped with seven loci (14 alleles). This resulted in (5827 x 5826/2) = 16 974 051 pairwise comparisons in this dataset. Of these pairwise matches, a total of 113 putative matches were detected by SHAZA having a total false positive rate of less than 1.0 pairwise match amongst them when a prior error rate per locus of 1.0% was defined in SHAZA. Of the 113 putative matches, 70, 17, 18 and 8 had 0, 2, 4 and 6 missing alleles, respectively. The number of errors within these respective groups were 12, 3, 0 and 4 of which all but one were potential allelic dropouts as they shared three alleles between the same two loci. The percentage error rate was calculated 100 x (12 + 3 + 0 + 4)/ (70 x 14 + 17 x 12 + 18 x 10 + 8 x 8) = 1.3%. The analyses were re-run with a 1.3% error rate ('-e 0.013' option in SHAZA) with no difference in putative matches detected.

True false-negatives amongst the fin by fin matches could be detected if they were from different sample bags. No false-negatives were among the 113 putative matches detected by SHAZA, with the first false-negative occurring at the 128th highest likelihood ranked match. The cumulative number of real false-negatives was closely related to the predicted cumulative number of false-negatives from the highest likelihood ranked matches in SHAZA. When there were 10 and 20 real false-negatives, SHAZA estimated 10.3 and 21.7 putative false-negatives, respectively.

Removing lure and fin matches prior to lure by fin match analysis

Only single fish appeared to leave tissue samples each time when a lure was struck, so that tissue samples from each of the separate lure tips produced the same genotypes (subject to error). With composite

genotypes created using the information from both tips, the final count was 563 lure genotypes. Composite fin genotypes were created from the 113 matches to give a final count of 5714 fin genotypes.

Estimation of short-term harvest rates from fishing trips (multiple day fishing data)

The number of fish harvested, the number Genetagged and the number of fins sampled for each of the trips where recaptures were recorded, are shown in Table 22. The number of fish successfully Genetagged in the trips listed and the number of lures from which tissue was successfully genotyped, varied between 11 and 86. The number of lures that were struck depended upon a wide range of factors, including the success of the skipper at locating actively-feeding fish, the duration of the trip (those listed here varied between five to 14 days duration), the number and position of lines available to be used and the degree of activity on the vessel. We also noted that the baited hooks usually used by the fishers were struck at a higher but variable rate to the Genetag lures. The number of fins that were sampled also depended upon the activity of the vessel (at times our activities were constrained by their commercial needs), and was especially difficult when catch rates were high.

In this analysis, there were seven possible allelic dropout errors with one additional error that did not share loci in common between the pairwise match. Estimates of recapture numbers were determined using SHAZA (Table 22). The recapture estimates are a fraction higher than their observed count due to a correction being made by SHAZA to account for the number of Type II errors (Macbeth et al., 2011). The genotype matches were not determined with 100% precision due to the missing loci, the fractions of recaptures indicated in Table 22 reflecting the probabilistic basis of the matching method. The standard error of these estimates reflects the precision with which they could be estimated. Thus there appear to have been four lure-lure matches observed, (i.e. fish struck lures twice on four occasions), but estimation via SHAZA predicts that there may have been more such matches (Type II errors) which were undetected due to missing information in the genotypes. Similarly, while there were 16 observed lure-fin matches, in which Genetagged fish were subsequently caught and detected in the landed catch, calculation in SHAZA accounts for a small additional number of false negatives (Type II errors) and removes a small number of false positives (Type I errors).

Using H, L, F, Y and Z parameters tabulated in Table 22, the feeding and harvest model was applied (Appendix 3) to estimate the size of the pooled feeding aggregations (*W*) encountered over a fishing trip (Table 23). The number of actively feeding narrow-barred Spanish Mackerels encountered over the duration of a fishing trip could be several thousand fish, with the proportion harvested estimated to range between 7 % and 45%. An estimated 225 of the 260 Genetagged fish remained at large (87%) but this varied between trip with between 64% and 97% of the fish being uncaught.

Table 22. Number of fish harvested, lures successfully deployed and fins sampled during fishing trips lasting more than one day - recaptures between lure and fin samples with standard error of genotype assignment

Sample numbers				Recaptures	
Fishing trip code	**Harvest (H)**	**Lures (L)**	**Fins (F)**	**Lure x Fin (Y)**	**Lure x Lure (Z)**
29200404	297	86*	210	3.33±0.80	
		88			2.2±0.47
29200406	136	30	102	1.12±0.42	
29200407	452	29	281	5.39±0.80	
29200502	684	67*	244	4.28±0.79	
		68			1.08±0.39
29200505	248	37*	213	1.08±0.26	
		38			1.05±0.18
29200506	566	11	318	2.25±0.72	
Total	2383	260*	1368		

* Duplicate (lure x lure) recapture(s) removed prior to estimating (lure x fin) recaptures

Estimation of harvest rates from fishing days (single day fishing data)

To contain biases in this analysis, we used the eight fishing days on which at least five fish were Genetagged and a recapture identified. We additionally included four other occasions on which there were recaptures (all less than three days from Genetagging). The number of fish harvested, the number Genetagged and the number of fins sampled for each of these trips is shown in Table 24. As before, the recapture estimates are reported a fraction higher than their observed count due to a correction of Type I and Type II errors. Thus there were an estimated 4.4 lure-lure recaptures and approximately 14 lure-fin recaptures on the same day. Again, the standard error of these estimates indicates the precision with which genotype matches were estimated.

The feeding and harvest model was applied (Appendix 4) to estimate the size of the pooled feeding aggregations (*W*) encountered during the single days of fishing, using the information tabulated in Table 24. The number of fish encountered in these aggregations was estimated on most of these days to be in the hundreds, and ranged between 64 (with low and high estimates of 48 and 137, respectively) and 1382 (713 to 78418). The large high estimate in the latter was because the estimated number of recaptures less twice the standard error of the estimate was only 0.02 recaptures. Another day trip with more sensible confidence limits of recapture numbers was 800 (1312 to 4022). The percentage of active feeders harvested on single days ranged from 6% to 90%, with an average of 41%.

Table 23. Estimates of the number of Genetagged fish remaining at large, feeding aggregation size and harvest percentage from data pooled over a fishing trip lasting more than one day

Low and high estimates were derived from +twice the standard error of the mid estimate of (Y) and (Z) values.

Fishing trip code	Genetags at large (*G*)	Feeding aggregate(s) (*W*)	Harvest (%) $(100H/W)$
	Mid	Low Mid High	Low Mid High
29200404	80	1692 2421 4385	7 12 18
29200406	29	1066 1831 7189	2 7 12
29200407	21	818 1006 1357	33 45 55
29200502	55	1592 2203 3862	18 31 43
29200505	33	1635 2276 3936	6 11 15
29200506	7	725 1024 2452	23 55 78
Total	225		

DISCUSSION: IDENTIFICATION OF RECAPTURES AND THE ESTIMATION OF ACTIVELY-FEEDING AGGREGATION SIZE AND HARVEST RATE

Genotyping, recaptures and the feasibility of the Genetag approach

The Genetag project included the collection, processing, genotyping and comparison of more than 11 000 samples. Field sampling was frequently difficult and at times arduous; sample quality could be variable. The organisational challenge of managing the many thousands of samples and subsamples generated through field to laboratory to analysis, posed data management and interpretation difficulties. These challenges were all met; in doing so, the Genetag approach was clearly demonstrated to be feasible.

In contrast to this project, a typical (terrestrial) genetic tagging study has small sample sizes and the shadow effect is likely to be minimal, at least among individuals with complete genotypes. A geneticist working in such a project may have the opportunity to re-genotype sample material, to ensure maximum quality of each and every genotype in the data set. He or she would carefully, individually compare genotypes in the matching process. In small samples, decisions about whether or not genotypes might represent a true match are not obscured by the shadow effect. With increased sampling effort, however, the shadow effect will inevitably present itself and be amplified. The problem will also be manifest where there is a need for the inclusion of partial genotypes or through augmentation with other studies.

Table 24. Number of fish harvested, lures deployed and fins sampled on the same day (or adjacent days) of fishing trips recaptures between lure and fin samples with standard error of genotype assignment

Data was pooled when recaptures were on consecutive days.

	Sample numbers				
Day code (mark/capture)	**Harvested (*H*)**	**Lures (*L*)**	**Fins (*F*)**	**Lure x fin (*Y*)**	**Lure x lure (*Z*)**
3009 / 3009	46	22	45	1.04±0.28	
3010 / 3012	18+22+53	16+8+7	4+5+50		1.11±0.45
3012 / 3012	53	7*	50	1.12±0.46	
		8			1.09±0.32
3013 / 3013	95	33	61	1.16±0.57	
3069 / 3070	45+26	13+1	11+14	1.08±0.36	
3151 / 3151	140	11	100	4.30±0.85	
3156 / 3156	42	3	30	1.05±0.32	
3838 / 3838	143	34*	128	1.08±0.40	
		35			1.12±0.35
3841 / 3841	71	15	54	3.21±0.68	
4016 / 4017	112+26	3+0	76+23	1.06±0.38	
4021 / 4021	93	3	38	1.12±0.46	
4168 / 4168	42	11	0		1.03±0.16
4170 / 4171	64+26	10+0	53+26	1.03±0.36	

* Duplicate (lure x lure) recapture removed from count prior to estimating (lure x fin) recaptures

It was necessary in this study to develop the theory and practical application for undertaking the comparisons between a huge number of genotypes and establishing the likelihood that any two genotypes, many of which being partial genotypes, might match.

The genetic analysis component of the Genetag project firstly developed an approach for choice of an efficient genotyping panel (Hoyle et al. 2005). The development of SHAZA (Macbeth et al. 2011) not only facilitated the huge number of comparisons necessary to identify recaptures, but also enabled the capture of the information contained in partial genotypes, massively increasing the feasible number of comparisons between genotypes. The inclusion of partial data has been previously problematic and might typically be excluded. However, inclusion of partial data, especially when recapture rates are low, is highly desirable because of the exponential increase in the chance of observing recaptures with increased sample size.

Overall, 57% of samples were fully genotyped. The other 43% that were not fully genotyped could represent a large loss of information, but the application of SHAZA increased the effective number of informative samples to 85%. Although this difference was only (85% - 57%) = 28%, the total number of pairwise comparisons was increased by 3.7 times using SHAZA. The use of SHAZA therefore made a strong contribution to the power of detecting recaptures.

Table 25. Estimates of the number of Genetagged fish remaining at large, feeding aggregation and harvest percentage from data pooled over single day fishing trips

Low and high estimates were derived from +twice the standard error of the mid estimate (Y) with Z values remaining constant.

Day code (mark/capture)	Genetagged fish at large (G)	Feeding aggregate (W)	Harvest per cent (100 H / W)
	Mid	Low Mid High	Low Mid High
3009 / 3009	21	481 731 1566	3 6 10
3010 / 3012*	28	296 456 1119	8 20 31
3012 / 3012	5	74 110 328	16 48 71
3013 / 3013	32	713 1382 78418	0.1 7 13
3069 / 3070*	12	179 279 790	9 25 40
3151 / 3151	6	162 198 288	49 71 86
3156 / 3156	2	48 64 137	31 66 88
3838 / 3838	32	800 1312 4022	4 10 18
3841 / 3841	12	141 188 307	23 38 50
4016 / 4017*	2	147 200 563	25 69 94
4021 / 4021	1	93 97 342	27 95 100
4168 / 4168	7	62 75 101	42 56 68
4170 / 4171*	9	288 467 1480	6 19 31

* Using pooled information from more than one day

Unfortunately, there were no long-term genetic recaptures detected; as such, the short-term harvest rates presented are the estimates of the proportion of actively feeding fish, within the localised area “swept by the fishing gear”, those that could potentially be caught by the fishing operation. Estimation of fishing mortality, harvest rates and catchabilities over the larger time and spatial scales that would be useful to fisheries management was not feasible. Such calculations require that the marked fish have sufficient time to mix with the fished population so that, over the long term, marked fish have probabilities of recapture equal to that of unmarked fish. The scale at which this mixing occurs is of considerable importance in subsequent analysis.

Although some Spanish Mackerels in some populations are known to undertake extensive movements (McPherson 1981; Govender 1993), studies of parasites (Lester et al. 2001; Moore et al. 2003), otolith isotope ratios (Newman et al. 2009) and genetics (Ovenden and Street 2007) in northern Australian Spanish Mackerels indicated that the fish form functional adult groups that largely do not mix at distances of a few hundred km or less: the spatial scale at which those studies were conducted. However, with a finer spatial resolution, the Genetag project’s conventional tagging component demonstrated that the movements of Spanish Mackerels may be even more restricted, with a mean distance between capture and recapture of less than 30 km. Longer term recaptures would require sufficient genetic tagging, fishing and catch sampling focussed at the same fine scales. The commercial Spanish Mackerel fishery exerts just a few hundred days of fishing effort each year (Handley 2010) which dispersed across the NT coastal waters is quite diffuse. It appears that more effort, and screening samples taken from catches during that fishing, would have been needed at fine spatial scales, close to release positions, to ensure long-term recaptures. A recent assessment (Grubert et al. 2012) of the NT fishery for Spanish Mackerel sets the annual harvest rate at around 5%, and given a screening rate of 10%, there would be a high probability of zero recaptures given the extensive experimental plan applied in this project. Clearly, for fisheries such as the NT Spanish Mackerel fishery, it would be appropriate to devise an experimental plan that maximises the probability of recaptures, by focussing on spatial and temporal peaks in catches and effort.

The Spanish Mackerel genetic mark-recapture pilot data here was ideal for testing the validity of the statistical methods underlying SHAZA developed by Macbeth et al. (2011) in a real world application. This study represents a special case of the application of genetic mark-recapture, as the majority of samples taken could not be recaptured because they were taken from harvested fish. Thus, the non-occurrence of pairwise genotype matches between harvested fish was a control for the SHAZA analysis using partially genotyped data (Macbeth et al. 2011). Despite the large number of pairwise comparisons among harvested fish (26 992 878), no recaptures were identified between them, providing a high level of confidence in the practical and theoretical aspects of the work.

GENERAL DISCUSSION

Determination of the impact of fishing is a central yet pervasive fisheries science challenge. Mark-recapture approaches are among the most informative tools available to address this challenge but have been unusable in many fisheries, simply because it has been technically or economically problematic to tag the species involved. The Genetag approach was developed to alleviate this problem: we developed genetic mark-recapture as a tool for harvest rate estimation.

The technical basis of *in situ* genetic tagging for large pelagic fishes was established. The Genetag project:

- Demonstrated the feasibility of *in situ* tissue collection applied at a fishery scale. The significant number of lure-lure recaptures indicated that fish were essentially unaffected in the short-term by *in situ* tissue sampling.
- Established screening panels of high resolution polymorphic loci, and a protocol and software for optimising multiplexing for maximum efficiency and economy. This enabled high-throughput genotyping of more than 11000 samples
- Developed a theory, protocols and software for the highly efficient and accurate comparison of genotypes for the identification of matches. The approach enables the efficient use of partial genotype information as well as quantification of errors in large genetic data sets.
- Detected recaptures amongst tissue collected from the landed catch, as well as fish that were Genetagged twice.
- Established a recreational-fishery-based, combined conventional and genetic tagging approach, demonstrating the capacity of the recreational fishing community to provide monitoring information and information on movements.
- Demonstrated the initial survival of the conventionally-tagged Spanish Mackerels using inexpensive acoustic tracking. The tracking provided supporting evidence for the effectiveness of the project approach of pole-tagging fish rather than removing them from the water. Further support was provided by the relative recapture rates of pole-tagged fish compared with those that were boated before tagging and release.

There were no long-term genetic recaptures and hence no capacity within the project to estimate fishery-scale harvest rates or catchabilities. This appears to be due to a combination of insufficient tags and catch sampling, and the dispersed fishing effort of the fishery. Genetic mark-recapture analysis of small populations of rockfish (*Sebastes* spp.), using a modified version of the hooks developed for this project, was successfully applied in British Columbia (Hague 2006). The extensive approach adopted here, while relatively inexpensive, meant that the probabilities of recapture and detection were just too low for the approach to be successful. Thus, if the underlying recapture rate of genetically tagged fish were 3.6% (i.e. the same as the conventionally-tagged fish), the expected number of detections of the 563 Genetagged fish at a screening rate of 10% would be just two fish. Nevertheless, the project has provided the basis on which much more effective experimental designs, spatially and temporally more intensive, can be developed and implemented.

The project made considerable achievements in the face of many challenges. The failure of outsourced genotyping confused protocol development and effectively delayed the availability of genotyping results by at least two years and necessitated a project variation. In terms of project performance, its effect was immense: it created a substantial time lag between sampling and genotyping that prevented feedback on the success or otherwise of sampling during the project. Adaptive changes to the experimental plan were not implemented, as it was not apparent that the extensive tagging approach was not producing sufficient recaptures. Nor was it possible to adopt obviously desirable protocols (such as improving on-board tissue storage protocols, jointly analysing both tips from a lure, or using baited hooks), or to take steps to enhance preservation, simply because information was not available to support decisions to make the changes. In a project management sense, it also meant that issues such as staff turnover had a bigger impact than would be apparent in a shorter project.

While inappropriate preservation protocols were fortunately detected and rectified, they also caused a loss of samples in the important first sampling year. These problems were nevertheless part of the experience of developing a new sampling technique. As pilot work, the principal challenge of the Genetag project has been to move from concept to application, to confirm feasibility and to uncover various issues and problems for which there is little or no previous experience. We emphasise that the various barriers encountered in this project were overcome and the principal challenge met. The project developed tools for future experimental or monitoring programs based on genetic tagging. In the Section ‘Further Development’ (see below), we provide recommendations on how they can be implemented.

The development of SHAZA overcame theoretical and practical problems that simply had not been clearly anticipated. Project planning was built on the expectation that nearly all samples would provide full genotypes. This reflected a lack of prior experience anywhere with such a project, in which a very large number of samples of varying quantity and quality were analysed; the “standard approach”, with discarding of samples with incomplete genotypes would represent the loss of unique information. To our knowledge, there were no previous genetic mark-recapture projects of the sampling scale attempted in the Genetag project and the resolution of the problems that are engendered by addressing the huge number of comparisons required in this project is really an impressive achievement. These developments are also applicable beyond the use of Genetag in fisheries management in the realms of wildlife and human population genetics and forensics. Comparable problems have become apparent in forensic science: increasing data sets of poorly-informative genotypes and similar difficulties in identifying genotype matches are generating serious analytic and legal issues in forensic science.

Communication within the project, within the scientific community and with stakeholders was very effective throughout the project. The recreational fishing community in particular, took the project to heart and some continued recreational tagging, which has been supported since by the recreational community and DoR Fisheries.

Conventional tagging by recreational fishers was included in the project not only as a methodological control but as an additional source of information on fish movements and survival.

Movements were small relative to those observed in some fisheries for the species, but were consistent with results of other FRDC-funded research in northern Australia (Buckworth et al. 2007a). Just one of the combination tagged fish from the recreational fishery was detected in the commercial NT Spanish Mackerel Fishery and no Genetagged fish were detected in the recreational tagging samples. This effectively demonstrated a high degree of spatial separation between the fish targeted by the commercial Spanish Mackerel fishery and by the recreational fishery, which is useful information for management. Several conventional tags were, however, detected in the Offshore Net and Line Fishery, which takes Spanish Mackerels as a byproduct and which fishes in the same inshore areas targeted by anglers. The recapture of

these fish indicates that the Offshore Net and Line Fishery and the recreational community have a similar impact on Spanish Mackerels in the Darwin area, where these sectors overlap spatially.

BENEFITS

Primary beneficiaries of this work will be all sectors of the fishing industry and conservation bodies associated with the Spanish Mackerel fisheries. The general public will benefit from the economic benefit of improved fishery performance and sustainability and increased security of food supply.

Generally, it will benefit the wider fishing industry and the community at large. The Genetag project has provided an integrated technical and analytical framework that effectively provides for planning and implementation of a genetic mark-recapture program. The novel approach adopted has thereby provided new operational tools for use in the monitoring and assessment of fishing and may thereby support more effective fishery management. These new tools can support additional effective harvest rate and abundance monitoring. Thus management strategies entailing the development of new key fishery reference points and indicators, and controls will be available for many fisheries, where current monitoring and management tools are inadequate. In turn, this can support increased sustainability and enhanced economic performance of those fisheries, as even modest improvements in monitoring of fisheries will provide improved catches and enhanced sustainability.

The technical basis of the project is immediately applicable to a range of species and fisheries: those for the Spanish Mackerel species and other large pelagics, such as sharks, tunas or billfish. With a little technical development for sampling of tissue, and experimental design to capture the characteristics of individual fisheries, the approach can be applied more widely. It might be applied, for example, to other species for which application of mark-recapture methods are limited by barotrauma, including benthic or mid-water species, such as snappers or Orange Roughy, or where tag loss is a problem (e.g. rock lobsters), or where capture of species of conservation concern might be problematic.

The study has added to understanding of the spatial dynamics of Spanish Mackerel stocks in northern Australia and in other areas of the distribution of this species. In particular, the small scale of movements shown by conventionally- tagged fish added support to the hypothesis of a meta-population structure for Spanish Mackerels. This adds to the probability that similar spatial dynamic structures may exist for other *Scomberomorus* species and scombrid fishes in general. This knowledge provides the basis on which fishery managers might allow for the scales of spatial dynamics in fishery management.

Potentially, the largest benefits of the Genetag project are in the stimulus it has provided to explore new genetic mark-recapture and related approaches, and the development of new theory and tools. The ShadowBoxer/Locus-Eater and SHAZA packages developed in the project have provided potent new analytic and planning tools to the benefit not only of the fishing industry and management communities, but to broader scientific and wildlife management, and potentially forensic stakeholders.

The methods and results from the conventional tagging parts of this project also have direct application in abroad range of future studies. The protocols developed for in-water application of intra-muscular tags and tissue sampling, have value for scientific studies of this species and other pelagics and for the management information this will generate. The input of recreational fishers in the conventional tagging component has shown not only the potential of the sector to contribute to similar studies but also that participation in experimental and monitoring programs can add to the recreational fishing experience.

Although simple pilot work was undertaken in this project, the demonstration of the ready application of acoustic methods in this work should encourage the use of acoustic approaches to address evaluation of

post-tagging behaviour and survival. The approaches shown here could be readily expanded for more detailed tracking to describe post-tagging behaviours.

FURTHER DEVELOPMENT

In this Section we explore a suite of directions in which development of genetic tagging should occur. We deal firstly with general issues of experimental design and development of appropriate monitoring and management control systems, and various suggested technical applications and development. We then address specific applications of the Genetag approach, offering a general design for monitoring northern Australia's Spanish Mackerels, including a costing, briefly suggesting several fisheries in which a Genetag approach might be applied.

EXPERIMENTAL DESIGN, MONITORING SYSTEM AND MANAGEMENT CONTROL DEVELOPMENT AND EVALUATION

The prime motivation for this study was the difficulty and cost of effectively monitoring Spanish Mackerels using approaches such as conventional tagging or otolith-based age structure assessment. Given the feasibility of genetic tagging, adaptive experimental designs can be developed to maximally exploit the attributes of the approach. There would be considerable merit in simulation of experimental approaches, development of monitoring systems and management controls, in a full management strategy evaluation (MSE) approach. The potential power of a monitoring system based upon mark-recapture estimates of mortality rates and catchabilities was demonstrated in MSE by Buckworth (2004a).

The power of long-term genetic tagging studies, in which tagging is continued over several years and recaptures of fish may similarly be after several years, has yet to be explored but is potentially rich. As a logical extension of conventional mark-recapture theory, the approach has the potential to provide very valuable information on natural mortally rates, including their variation with size, age and sex as well as locality, and under changing environmental conditions and variation in catchability, also in relation to factors varying in time and space.

Approaches must nevertheless be adaptive: in this project we encountered many unanticipated issues. A major challenge with long-term and large genetic tagging work will be the development of protocols to cope with shadows, the number of which grows exponentially with sample size (see elaborations of the problem in the Methods and Results/ Discussion Sections).

TECHNICAL APPLICATIONS AND DEVELOPMENT

Tissue sampling

There is considerable room for elaboration and testing of *in situ* techniques for tissue collection for genotyping. Specifically for Spanish Mackerels, the Genetag hooks in this project were satisfactory. In ad hoc trial with sharks, Genetag hooks baited with squid have been very successful (based on visual inspection for tissue retention).

Once preservation issues had been addressed, the Genetag hooks deployed for Spanish Mackerels produced successful genotypes for more than 50% of the struck lures analysed. Given our prior ignorance of whether more than one fish would strike a lure and so be genetically sampled, the two hook-tips from any lure were separately analysed. Genotyping performance (the number of successful genotypes from the samples) should be improved simply by jointly analysing the two tips, as there would, in some instances, be an increased amount of tissue available to the PCR process. The work and cost in genotyping would

correspond to the number of lures, rather than hook-tips. In our study, that would have been a considerable saving in work.

The number of successful genotypes and the number of fish genetically marked would also be improved simply by generating an increased strike rate. We were constrained in this project by a lack of experience in genotyping large numbers of Spanish Mackerels. However, given the experience of this project, the genetics team is confident that the careful use of baited hooks could be accommodated. Preliminary testing with baited Genetag hooks has been successful. We believe deployment of baited hooks rather than lure-mounted hooks would, at most times, substantially improve strike rates for Spanish Mackerels and thus reduce the cost of genetic tagging per fish marked. It considerably widens the field of potential candidate species, given that some species are much more likely to attack baits rather than lures.

There is also an opportunity to improve the design of the hooks, using cheaper materials (e.g. appropriate plastics in lieu of copper components) which, without the potential for contamination by copper, would probably also provide opportunities to enhance the protocols for the preservation of tissue.

With the intention of improving hook designs, we attempted in this project to observe the strike behaviour of Spanish Mackerels, using relatively inexpensive towed video systems. We found, however, that the strikes by the mackerels were simply too fast for these inexpensive systems to capture elements of behaviour that might be exploited to improve hook design or deployment methods. There is potential to inform gear design by observing behaviour, but for Spanish Mackerels, high speed and high quality systems are required.

Conceivably, a suite of hook designs could be developed to address the suite of contexts and fish behaviours that are encountered among fisheries for different species. New designs of hooks would be appropriate for smaller or less aggressive fish. The current hook design depends upon the momentum of the fish to firstly scrape tissue, then to straighten the hook out to release the fish. Depending on the size and attack behaviour of a species, the sampling mechanism may require an alternative. There is thus ample opportunity for innovative design engineering and use of observed fish behaviour, to expand the range of potential species and fisheries that might use *in situ* tissue sampling and a genetic mark-recapture approach.

Information capture, logistics and coordination

In this project, records of lure numbers, hook numbers, vial numbers, line numbers, latitude, longitude, time and date of deployment, and time, date and position of strikes, vessel, etc. were made in the field by hand and later recorded in computer databases.

This was time-consuming and, in adverse conditions, it was quite difficult to ensure accuracy. It potentially introduced transcription-errors into the database. We strongly recommend the use of hand-held devices that automatically capture date, time and the GPS coordinates when lures are deployed or fish landed. Lures (or baited hooks) could contain an RFD that was recognised by the hand-held devices. The device could also include a bar-code reader to record vials used to hold hook-tips and fin tissue samples. The device would need to be robust for at-sea conditions, be easily and readily backed up in the field and be able to download to a computer. The device could transmit data back to base using the mobile internet from a lap-top computer or sat-phone using electronic logbook technology (an unsuccessful application was made to the NT Innovation and Research Fund to develop such a protocol).

Considering the large number of samples and handling steps for each sample that might be entailed with future genetic tagging projects, it would be valuable to employ sample tracking and inventory methods to create a sample audit trail as detailed in Figure 24. We recommend the development of a barcode tracking system. In a Genetag project, a sample is represented by a genotype in the final data set, a DNA extraction from a particular fish and a fin sampled from a vessel. One reason that sample-tracking is important is for verification. For example, a genotype might be an outlier suggesting that the fish is not a Spanish Mackerel.

To corroborate this, we may need to know if the sample was taken from a fisheries catch where other species were being landed. Another example of the need for verification is where genotyping has been repeated on the same tissue sample and the second genotype is different to the first and we want to know why this is so. Sample audit methods can assist with project management. For example, how many fins from 2004 were received and where are they and how many have been analysed? Another example is being able to link poorly-resolved genotypes with particular years, vessels or shipments.

Methods that are being used by diagnostic or forensic laboratories would be appropriate for sample tracking provided they connected between field and laboratory components of the work. We experienced a great deal of frustration and wasted valuable time locating particular batches of samples for processing or trouble-shooting and cross-referencing samples with databases. The use of barcode labelled sample collection tubes and barcode readers would be a very valuable component of sample handling.

Robotic liquid handling methods in the laboratory would facilitate high sample throughput and many include bar-code readers. Sample management needs to interface with databases that contain all information needed to make harvest rate estimates.

In future Genetagging projects, given the multiplicity of samples and the complexity of the information chain, a project officer could be employed to coordinate all aspects of the project, including data management and data entry. If the project officer did not have these skills, then a dedicated data-basing officer would need to be employed consistently across the entire project.

It is essential that Genetag workers have access to a relational database to store genotypes and other results. We used Microsoft Access in this project. However, it is not possible to use Access to search for recaptures because of the exponential increase in the number of pairwise comparisons as sample numbers increase. For example, if a database contained 10 000 genotypes, then the number of pairwise comparisons to be made is [n (n-1)]/2 or approximately 50 million Access should also be used as a data entry tool using its automatic error checking facility.

Archiving Genetag samples and data is an important issue. Genotypes should be archived with the appropriate metadata in permanent electronic storage. Tissue samples can be stored for long periods of time in low temperature freezers. The cost of collecting the tissue samples outweighs the on-going cost of low-temperature storage. Extracted DNA can be stored as ethanol-precipitates in sealed 96 well plates also at low temperatures.

SHAZA

There is as yet little experience in genetic mark-recapture with large databases. There is substantial opportunity for the development of both theory and practical application of the SHAZA approach. While the software has been developed for fisheries Genetagging, it has features that would make it suitable for grooming and analysing large genotype data sets beyond Genetagging (e.g. close-kin analysis) and fisheries (e.g. wildlife studies on whales, dugongs, pinnipeds and terrestrial carnivores).

Recreational tagging and acoustics

Especially in a culture where catch and release is practised, there is an excellent opportunity to engage the recreational fishing and FTO (fishing charter) sectors in coordinated monitoring programs that involve conventional and genetic tagging.

This project demonstrated both the utility and potential shortcomings of using conventional tagging approaches. Limitations here were a lack of information on the magnitude and distribution of recreational fishing effort and reporting rates, which constitute vital information if recreational mark-recapture were to be used in the estimation of harvest rates. Protocols were difficult to ensure, with information losses from

tagged fish for which no data was provided and probably more cryptic losses simply due to handling practices. We were also unable to determine the tag loss rate – the losses due to tags falling out of fish and fishing mortality or behavioural change after capture and tagging. While recapture rates and acoustic tagging results gave some reassurance that these problems were minimised, they could not be quantified.

There is considerable potential, therefore, to improve the utility of recreational tagging in such projects as the Genetag project, or as independent projects. These range through improved protocols for capture and tagging (e.g. all tagging done in the water), improved information control (requiring better liaison but also tighter information management) to exploration of better reward and incentive structures, to maximise returns. The management requirements of the conventional tagging program were largely related to good coordination and there is thus scope to make use of such projects more broadly.

The limited acoustic tracking in this project was inexpensive. It served as pilot work demonstrating the potential of this approach for building information on the post-release survival of tagged fish and would be valuable as part of studies into improved techniques for conventional tagging, as well as providing information on movements and survival in its own right.

Integrated studies

In situ collection of tissue samples provides genetic information that can be used in studies other than mark-recapture. For example, tissue from the current project could potentially be used for studies of spatial population genetic structure and connectivity, for estimation of genetic effective population size and for aging studies using telomeres, and for applications examining the relatedness between population components. Close-kin approaches for estimation of spawning stock abundance (Bravington and Grewe 2007) could be a potent combination with full genetic mark-recapture. Tissue could also be used for toxicological studies such as heavy metal accumulation and so on.

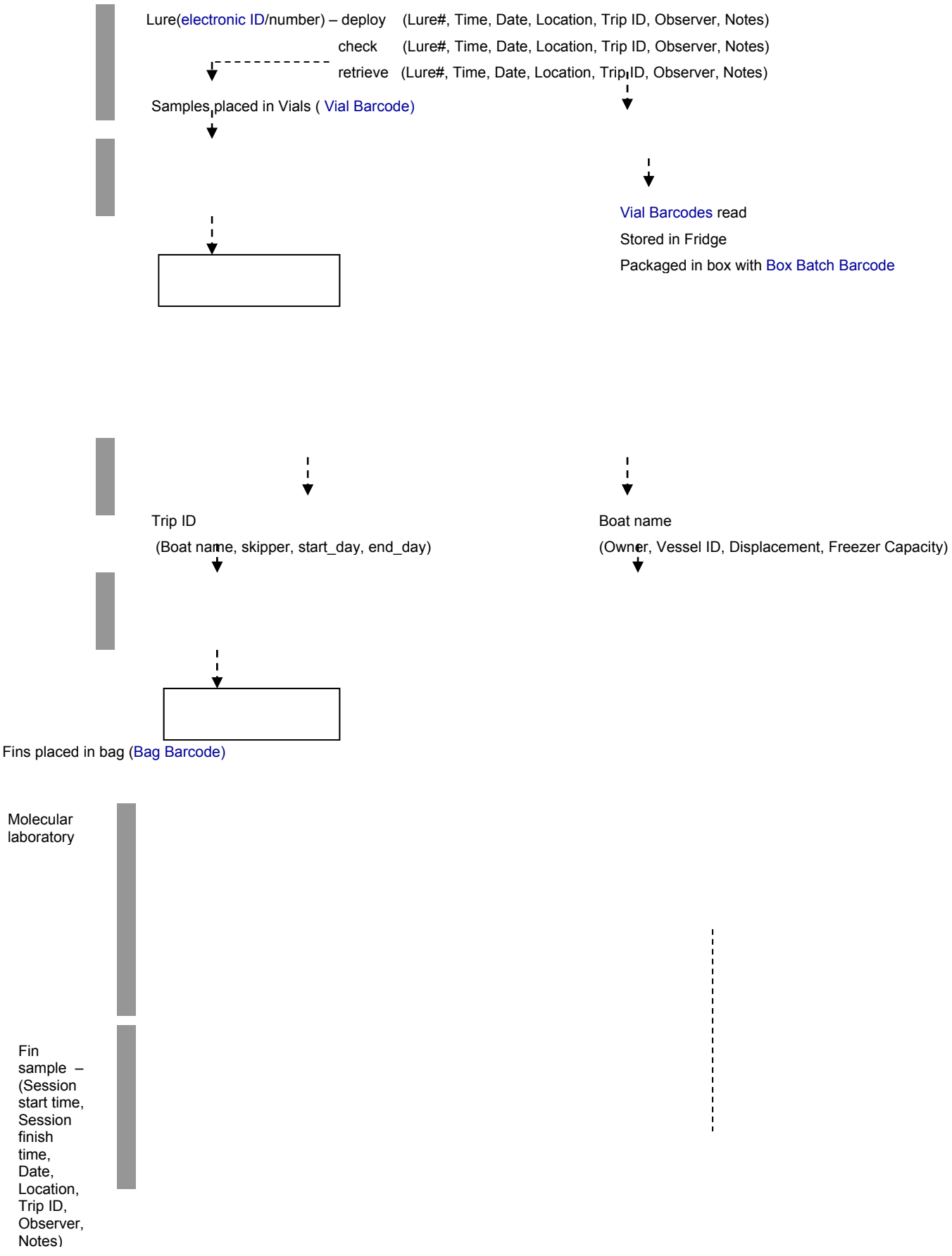

Figure 24. Data flow in the Genetag project with electronic data recording implemented

A GENETAG APPROACH FOR MONITORING NORTHERN AUSTRALIA'S SPANISH MACKERELS

Although this project has comprehensively provided the technical basis of genetic tagging for monitoring Spanish Mackerel fisheries, whether or not genetic tagging is adopted as a monitoring method for any fishery will depend upon decisions that weigh the perceived benefits of using the methodology against its costs. Our purpose in this Section is to provide the cost basis for such a decision for northern Australia's Spanish Mackerel fisheries and similar fisheries.

In this project, the experimental approach was based on the plan that each boat would Genetag one fish per day of fishing. However, we found this plan unworkable as there was no real incentive for crew members to undertake the work with the care necessary to ensure sufficient samples of good quality. Thus most Genetagging was undertaken by DoR Fisheries personnel during monitoring trips. The frequency and collection of fins by vessels' crew was highly variable.

To overcome the problems encountered in this project, we suggest an intensive monitoring approach. Generally, this would have the following elements:

1. Areas (preferably two or more) in which annual programs would be undertaken. These would be among the more heavily fished areas of the fishery and spatially distinct.
2. Genetic tagging operations would be undertaken by scientific staff on chartered commercial vessels, using otherwise standard fishing practices and gear.
3. The tagging operations would employ informative mark-recapture designs and so would be timed to precede a period of regular heavy fishing effort at a location that is regularly fished over the season. We strongly recommend inclusion of multiple release periods as required by informative statistical designs.
4. For Spanish Mackerel fisheries, collection of tissue samples from the landed catch (fins) would be concentrated in the monitoring areas in the months following the tagging operation. This would require an intensive liaison program and good incentives for the crew to ensure tissue and data quality. For other species and fisheries, there will be a need to account for anticipated movements of the fish.
5. Given logbook programs in which catches and fishing effort are spatially referenced, harvest rate (U) and catchability (q) for the monitoring areas would be estimated from recaptures identified in screening and effort information from logbooks and vessel monitoring systems.
6. An estimate of harvest rates and catchability derived from the monitored areas could be applied to catch and effort information from logbooks to provide estimates of harvest rates for the other areas of the fishery.
7. Information from successive years could be accumulated and applied, for example, using the catchability tracking approach described by Buckworth (2004a).

This approach still requires strong cooperation from the industry in providing fins from landed fish; but restricting the project spatially and temporarily would allow the liaison effort to be correspondingly focussed and provide an increased proportion of landings sampled for smaller spatial and temporal strata.

This approach retains many of the advantages of the original approach adopted for the Genetag project. Although the direct proportion between fishing effort and the distribution of tags would be lost, the heavily fished areas chosen for the intense monitoring are likely to be the most important for management. Relatively small increases in numbers of fish Genetagged, would particularly improve precision of estimates for the monitored areas. Monitoring of those areas would provide data-based rather than model-based performance measures (calculated harvest rates). Estimates of catchability, or ratios of catchabilities, may be applicable over much wider areas since the troll fisheries for Spanish Mackerels are conducted with

similar gear and in similar conditions. Given the effort information in compatible units, the information on catchability could then be applied to other fisheries. The merits of this approach are also an area for investigation and should be addressed by a combination of review and testing via MSE.

Without reliance on long-time series, harvest rate and catchability measures would reflect any changes induced by fishing or environmental trends (such as climate change).

Costing

An initial cost benefit analysis based on Genetagging by commercial fishers was provided by Buckworth (2004a, Chapter 6). He concluded that costs of monitoring the NT Spanish Mackerel fishery using genetic tagging on the planned basis would be around $63 000 per year compared to $42 000 for age structure analysis, based on 600 genetic tags, 10% of the catch screened (6429 fish), plus 200 conventional tags, versus 2000 otoliths sampled, sectioned and interpreted. Thus the cost for genetic tagging would be significantly more than gathering of age structure information, but comparable nevertheless. This would especially be so in cases where large number of otoliths would be necessary to accommodate for spatial heterogeneity in age structure. Based on the experience of this project, we offer an updated costing (Table 26).

A recreational combined tagging component with 400 tags released annually could be a valuable addition and could be achieved at relatively little marginal cost. Such a component, based on voluntary tagging and using sponsored prizes, could be included for around $10 000.

Table 26. An example of annual costing for northern Australia's Spanish Mackerel project based on deployment of 500 Genetags and screening of 7000 landed fish

Component	Unit cost ($)	Number	Total cost ($)
Genetag lures/ hooks (double hook)	10	500	5000
Charter vessel (per trip) and field costs for deployment of lures by research team	10 000	2	20 000
Payment to commercial fishers for fins (per fin)	0.25	7000	1750
Sample collection, labelling, packing (including cost of consumables, per sample)	0.5	8000	4000
Freight costs to Brisbane (per shipment)	500	3	1500
DNA extraction costs, including labour and consumables (per sample)	0.05	8000	400
Amplification using PCR, including labour and consumables (per sample)	0.10	8000	800
Allele length determination using capillary electrophoresis, including labour and consumables (per sample)	1.50	8000	12 000
Raw data processing and data basing (labour only, per 96 well plate)	100	80	8000
Recapture analysis using software SHAZA (per year)	2000	1	2000
Analysis and Reporting	10 000	1	10 000
Total per year			**$65 450**

FURTHER APPLICATIONS: WHICH SPECIES ARE MOST SUITABLE FOR GENETAG?

The Genetag approach can be used for any species that can be tagged with physical tags, as well as a number of others where physical tagging is challenging (e.g. Bêche-de-mer). For those species that can be conventionally tagged, genetic tagging may be preferred if there is a cost benefit: in conventional tagging, there is not only the cost of tagging itself, but also of accurately quantifying tag losses and non-reporting, to be considered.

The important driver for the implementation of the Genetag method is the lack of alternative fisheries assessment methods that can be used to provide data for management. This need is heightened by the commercial value of the species.

Species that have larger individual sizes are well suited to the Genetag approach. These species are also likely to be more commercially valuable, have smaller census population sizes than species with smaller individual sizes and tend to be longer-lived. Smaller population size is an advantage as fewer individuals will have to be tagged, and hence genotyped, and fewer individuals will have to be genotyped to search for recaptures. Minimising genotyping means minimising cost. Genotyping fewer samples to search for recaptures is also an advantage because it reduces the shadow problem (described above). The Genetagging approach on long-lived species (i.e. relatively low natural mortality) maximises the information return for a unit of tagging effort as genetic tags last for the life-time of an individual (i.e. are not shed).

Existing microsatellite information for a particular species would be a useful starting point, although not essential (see Section below). If a particular species would benefit from other information that could be extracted from genotyping (e.g. effective population size) or DNA analyses (telomeric DNA estimation of age), then investment returns in Genetagging would be maximised.

Additional investment would be avoided if the species could be biopsied using the existing Genetag lure. However, the Genetag lure could be re-designed if necessary.

If the biology of the proposed target species was well known, that would assist with the experimental design of the Genetagging program and interpretation of results, but it is not essential (see Section on fisheries below).

FISHERIES IN WHICH GENETAGGING COULD BE USEFULLY APPLIED

The driver for the application of the method to various fisheries could be the lack of alternative methods for gathering useful data for fisheries management (e.g. Orange Roughy), the difficulty in using conventional tagging (e.g. tropical sharks, Bêche-de-mer) and where reporting of conventional tags may be perceived as a problem (e.g. Southern Bluefin Tuna, other international fisheries and northern Australia Barramundi fisheries). The method will work best if these fisheries also have relatively small population sizes, high rates of harvest and consist of a single species, although Genetagging is relatively robust to deviations from these conditions (for example, *post-hoc* DNA sequencing can sort tissue samples into species).

Implicit in the design of a Genetagging program is that the component of the population that is tagged (i.e. sampled with the Genetag lure or by some other means) is the same component of the population that subsequently is harvested by fishers.

Examples

Orange Roughy

The main driver for implementing Genetagging on this species is its high commercial value and the difficulty of acquiring status information for its management. Several areas of the fishery for the species in southern Australia have been over-fished but are not currently subject to overfishing; its status is poorly defined; however, as there are many potential biases associated with the acoustic techniques of determining abundance and stock spatial relationships are poorly known (Wilson et al. 2010). Monitoring of fishing mortality rates using a genetic tagging approach might be a feasible alternative to current methods. With low natural and low current fishing mortality rates, Genetagged fish would accumulate in the population if a regular monitoring approach were adopted, which is an advantage. A low fishing mortality rate, however, has the consequence that tagging work would need to be highly focussed to ensure a reasonable probability of recapture. The species is not amenable to conventional tagging. It occupies depths from 800 to 1500 m and individual fish are dead by the time they are brought to the surface.

However, there are significant challenges. The existing Genetag hook and how it is deployed would have to be adapted for this species. For example, baited Genetag hooks could be deployed on long lines or drop lines to obtain remote tissue samples from fish at depth. Population sizes per seamount are large (i.e. ~ 0.5 x 10^6), so large numbers of fish would need to be Genetagged in order to achieve recaptures with an acceptable genetic screening rate of the landed fish. The cost of implementing Genetagging would need to be balanced against the value of the information obtained in terms of supporting the economic value of the fishery.

Black Jewfish and other croakers

The Black Jewfish (*Protonibea diacanthus*) is a prime candidate for Genetag approaches. Phelan (2008) reported that 46% and 100% of Black Jewfish landed from 10-15 m and 15-20 m, respectively had injuries that rendered them unlikely to survive if released. Conventional tagging should thus be limited to depths of <10 m to promote survival. This precludes many of the areas where the species is heavily fished. The species, like other croakers, tends to aggregate and tends not to move between aggregations (Phelan 2008)

so that catch rates are poorly related to abundance but as aggregations are spatially and temporally predictable, it is possible to efficiently target catches in very large numbers. Due to vigorous attacks by fish on baits and because they can be specifically targeted, the existing Genetag hooks and methodology would be suitable.

Commercial shark fishery of northern Australia

Genetagging overcomes the difficulty of handling landed sharks for conventional tagging studies as tissue samples for genotyping are taken remotely. Genetic analyses also circumvent the difficulty of species identification using morphology. DNA is a powerful tool for species identification. Tropical sharks have relatively restricted movement (Stevens et al. 2000), so the spatial scale of a Genetagging project is finite. Additionally, genetic information on tagged individuals can be used to follow products into and through the market. This could be important for shark products, such as fins, which may be recovered on vessels fishing illegally within Australia's economic fishing zone or from market surveys (e.g. Hong Kong).

The challenge of using a Genetagging approach in shark fisheries is primarily the species diversity. Single species cannot be targeted with the Genetag biopsy lure, but individual biopsies can be sorted into species using DNA sequencing. However, to apply Genetagging to a single species, a large number of biopsied samples would need to be screened to ensure an adequate number of genetically-tagged individuals. Alternatively, individuals could be landed and be genetically (i.e. tissue sampled) and conventionally tagged. Simultaneous tagging allows the estimation of the rate at which conventional tags are lost. Sharks are robust to handling and post-tagging mortality is thought to be low.

Gulf of Carpentaria populations of Barramundi

Genetagging may be useful to the management of Barramundi populations. This species occurs in freshwater habitats and moves to estuarine waters to breed. As such, their census population size is relatively small, which is an advantage as recaptures are more likely to be found with modest tagging and screening effort. A Genetag approach overcomes issues with reporting rates.

One option would be to use the cooperative and numerous Barramundi recreational fishers to catch and release fish, perhaps as part of an organised competition. Fish could be "combination" tagged - a tissue sample for genotyping could be taken from each fish, and each fish tagged conventionally. Barramundi do not experience barotrauma (they are caught within a few metres of the water surface) and have low catch and release mortality, which means that it may not be necessary to use the Genetag lure to obtain a remote tissue biopsy. However, capture is nevertheless stressful for the species (de Lestang et al. 2004)

The biggest challenge to obtaining harvest rate estimates using tagging (conventional or otherwise) is the general reluctance of the commercial fishers to allow biologists access to their catch. A proportion of the landed Barramundi need to be sampled to screen for recaptures, and estimates of effort need to be made for the downstream interpretation of the number of recaptures.

Queensland's east coast and Torres Strait Spanish Mackerel fishery

The northern Australian Spanish Mackerel Genetagging experimental design (see above) could be applied to the Queensland fishery. The distinction between the fisheries is the proportion taken by recreational fishers (high in Queensland) and the observed pattern of southwards migration and return. McPherson et al. (1981) have shown that some fish undertake long-distance movements in the summer, possibly to increase their feeding range when warm-water currents extend to southern New South Wales in summer. There is some evidence that travelling fish return to the tropics in winter, but it is unknown what proportion of the population has this movement pattern. Long-shore movements are thought not to occur in northern Australian populations. The Genetagging methodology should be applicable to the Queensland Spanish Mackerel population despite the presence of a travelling component.

In the Queensland population, Genetag lures could be placed intensively in selected regions along the coastline. Fisheries biologists should deploy the lures. As for the northern Australian fisheries, selected regions should coincide with maximum fishing effort and encompass what is likely to be the home range of the local Spanish Mackerel population. The advantage of this design is that the incidence of between region recaptures provides examples of individual movements on fine and broader scales depending on the regions chosen.

Random samples of landed commercial and recreational catch could be genotyped to search for recaptures. This would allow estimates to be made of the harvest rate of each sector. Once again, accurate catch data from each sector would be needed for the downstream interpretation of this information, such as estimates of biomass.

Red and Gold-band Snapper in the Timor/Arafura Sea

The Red Snappers (*Lutjanus malabaricus* and *L. erythropterus*) and Gold-band Snapper (*Pristipomoides multidens*) resource in the Timor/Arafura Sea straddles the international boundary between Australian and Indonesian territorial waters. In Australian waters, exploitation is regulated and believed to be light relative to sustainable limits. On the Indonesian side, exploitation is heavy and largely unregulated (Blaber et al. 2005). Australia populations might be seen as the source (or spawning) population upon which both fisheries depend.

Genetagging would be an excellent method to assess the degree of fine and broader-scale movement of individuals within and between fishing zones, as well as estimating local harvest rates. This information could be used to predict the sustainability of stocks under the existing management arrangements. Remote tissue samples from all species could be taken using a modification of the Genetag hooks (baited hooks and design modification to accommodate differences in snapper and Spanish Mackerel behaviour). *Post-hoc* DNA sequence analysis would be needed to assign samples to species. Genetic analyses of population structure suggests that Gold-band Snapper is more sedentary than red snapper (Ovenden et al. 2002b; Salini et al. 2006), so experimental design would need to accommodate the differing degree of movement and mixing between the species. An intense, spatially-focussed approach should be taken to the placement of genetically tagged fish and sampling of catches for recaptures.

Invertebrate species

Genetagging methodology can be applied to invertebrate fisheries species. Genetic mark recapture has been applied to Bêche-de-mer (Uthicke and Benzie 2002) to label individual animals for estimation of growth parameters in naturally occurring populations. The driver for this work was the extreme difficulty of conventionally tagging individuals of this species. Our Genetagging protocol may provide greater certainty of individual identification for a minimally-invasive tissue sample, although this remains to be evaluated.

The main challenge for the application of Genetagging to invertebrate species is the method used to collect non-lethal tissue samples for genetic tagging. Unlike fish, they probably cannot be remotely biopsied using the Genetag hook or similar. One alternative is to catch, sample tissue and then release. For example, abalone could be brought to the surface and a small piece of tissue sampled non-lethally from their mantle. The animals could be released and preferentially returned to their original microhabitat. Alternatively, it could also be feasible to biopsy them *in situ*. Similarly, divers on SCUBA could locate and biopsy trapped lobsters without imposing dangers of barotrauma and potential mortality from surface release.

PLANNED OUTCOMES

The project has demonstrated the potential for the genetic mark-recapture for fished species that are difficult or expensive to tag in more conventional manners. With appropriate experimental design, fishing mortality and harvest rates and catchabilities could be estimated. This provides the basis for development and application of monitoring systems and control rules that relate fishing effort or catch to an optimum fishing mortality rate through estimated fishing mortality rate, catchability, or abundance. By potentially improving information on fishery status, that status could be evaluated against management reference points. Commercial fisheries for Spanish Mackerels and many other species across northern Australia are currently managed with a mix of input and output controls that are based essentially on expert judgement and monitoring of performance indicators, such as catch rates and size composition that, in many cases, are in reality very poorly informative.

In the case of the recreational and FTO (charter) sectors, better knowledge of the impact of fishing would allow for the more careful and informed management of these fisheries. We have demonstrated an appropriate protocol for combined conventional and genetic tagging for use by these groups. The actual act of tagging in a catch and release culture may add value to the fishing experience. In the case of the recreational sector, combination of a similar protocol with measures to estimate the magnitude and distribution of fishing effort would be a very effective basis for estimating the impact of the sector. Logbook systems in the various management agencies already provide such information for the FTO and charter sectors. Plans that provide for mark-recapture based monitoring systems for these fisheries are described above.

The potential for genetic mark-recapture is not restricted to Spanish Mackerels. Quite clearly, cost-effective experimental designs might be developed for species subject to barotrauma (such as deep water snappers), tag loss (e.g. rock lobsters), or tag non-reporting (tunas), and improve the knowledge-base on which management of these species is based.

CONCLUSION

The over-arching objective of the project, which was to develop and demonstrate the feasibility of genetic mark-recapture as a monitoring tool has been achieved. The project provided a rich set of experiences and outputs on which further development of the approach can be based. The main conclusions are summarised against the specific project objectives.

1. **Confirm the technical basis of *in situ* genetic tagging for large pelagic fishes**

The objective was achieved. The *in situ* genetic tagging of Spanish Mackerels was demonstrated. More than 1200 lures mounted with Genetag hooks were used in the NT Spanish Mackerel Fishery between 2003 and 2006. These yielded nearly 500 remotely-collected, successfully-genotyped tissue samples. After problems with sample storage were overcome, struck lures produced genotyped samples more than 50% of the time. Recommendations have been made that should further improve this success rate. Genotyping of the large number of tissues required development within the project of a high throughput genetic analysis system. Purpose-developed software (SHAZA) identified 22 recaptures of these from the genetic screening of landed fish. This method, facilitated by software developed within the project, provides for maximal use of the genetic information within samples, including partial genotypes.

2. **Provide initial estimates of harvest rates in the Darwin area of Spanish Mackerel fisheries to develop protocols and scenarios for monitoring harvest rates in Australia's Spanish Mackerel fisheries using genetic and conventional tagging**

Harvest rates could not be estimated for the Spanish Mackerel fisheries in the Darwin area. Although a significant number of recaptures were identified in screened catches, all were from fish which had been tagged and then landed within short time periods – between 15 minutes and two days. This result principally reflects the field sampling protocols and the dispersed effort of the NT Spanish Mackerel fishery. It was found early in the project that the most efficient way to Genetag a significant number of fish was to undertake our operations during monitoring of normal commercial fishing. It appears that a significant proportion of the fish Genetagged in these operations were caught by the synchronous fishing operations. With few vessels working in the NT Spanish Mackerel fleet, and restricted movements of the fish, the extensive approach adopted for this project meant that additional recaptures from a tagging operation of this kind were unlikely. The rapid capture of a significant number of Genetagged fish is a unique result, indicating that commercial fishing may at times take a large proportion of the behaviourally-available fish within a location over small temporal and spatial scales. However, the results were not able to support an estimation of harvest rates imposed by the NT commercial troll fishery for Spanish Mackerels; some further development of the approach and implementation will be necessary to achieve this objective. The recreational tagging program was not designed to produce harvest rate estimates as there were no programs to quantify recreational fishing effort or tag reporting rates.

3. **Compare genetic and conventional tag mortality and retention for *Scomberomorus***

No fish Genetagged in the commercial fishery were detected among the genetic samples from conventionally-tagged fish in the recreational fishery. This indicates minimal movement between the spatial areas typically fished by the two sectors. Minimal movement of Spanish Mackerels was indicated by conventional tag recaptures and the recapture of just one of those fish in the commercial troll fishery for Spanish Mackerels. Spatial separation of the two fishery sectors, combined with minimal movements of fish, had the consequence that no information was generated on the relative survival of genetically versus conventionally-tagged fish.

4. **Provide information on movement of *S. commerson* in northern Australia**

This objective was achieved. The conventional tagging component indicated that tagged Spanish Mackerels moved small distances (on average less than 30 km), most being recaptured at much the same localities as where they were tagged. As noted above, there was minimal apparent spatial overlap in effort exerted by the commercial troll fishery and the recreational fishery. Spatial overlap between the recreational fishery and the Offshore Net and Line Fishery (ONLF), which takes Spanish Mackerels as a byproduct, was reflected in the recapture of 15 conventionally tagged fish in ONLF gillnets in the areas most fished by recreational taggers. The lack of recaptures of conventionally-tagged fish except within proximity to release sites (most recaptured fish had moved less than 30 km) also indicated minimal movements of tagged Spanish Mackerels.

5. **Develop a general methodology for the use of genetic mark-recapture as the basis of fishery harvest rate monitoring**

This objective was achieved. A general methodology for applying the Genetag approach for harvest rate monitoring is detailed in this report. The experience of this project indicates that a protocol for successful Genetagging to supply harvest rate and catchability information in the NT fishery for Spanish Mackerels and similar fisheries would include intensive genetic tagging experiments in areas where subsequent heavy fishing effort is predicted. This method would maximise both the number of fish Genetagged and the probability of their recapture in following months, allowing for subsequent, focussed collection of tissue samples from landed fish and maximum recaptures. Results from this work could then provide estimates of harvest rates and catchability with maximal precision for heavily-fished areas, where collection of this information is most pertinent. Careful experimental design and planning will be needed to maximise the information yield given the logistic and analytical requirements of a genetic tagging project. Generally,

experimental Genetag-like approaches will need further, customised development, and to be based upon good statistical mark-recapture design while accommodating the trade-offs between the various costs of genetic tagging, sampling the landed catch and genetics processing.

6. **Undertake genetic analysis of material collected during 2002, 2003 and 2004 sampling years in Genetag (FRDC 2002/011) project**

7. **Undertake Genetag sampling during 2005/6**

8. **Undertake genetic analysis of material sampled during 2005/6**

All of objectives 6-8 were achieved. More than 11 000 tissue samples were processed, yielding 8393 useable genotypes.

9. **Undertake pilot acoustic tracking for conventionally-tagged *Scomberomorus commerson*, for the estimation of initial mortality rates**

This objective was achieved. During the project, seven Spanish Mackerels were acoustically tagged and tracked. The results from this pilot work indicated firstly that acoustic tracking is feasible for narrow-barred Spanish Mackerels and that fish initially survive the conventional tagging process, at least in areas where sharks are not active. The method showed significant promise and opportunity for further application, and could be used to further quantify post-tagging and release survival rates.

10. **Communicate progress, concepts and results at the 2006 FRDC Australian Society for Fish Biology workshop on "The Cutting Edge in Fisheries Science and Management"**

This objective was achieved. Project results were communicated in two papers at the conference: Broderick et al. (2006) and Buckworth et al. (2007b). Papers were also presented at the *Advances in Fish Tagging and Marking Technology International Symposium*, in Auckland NZ in 2008 (Buckworth et al. 2008) and the *Australian Society for Fish Biology Conference/ Indo-Pacific Fish Congress*, in Fremantle, 2009 (Broderick et al. 2009; Buckworth et al. 2009).

REFERENCES

Barrett-Lennard, L. G., Smith, T. G. and Ellis, G. M. (1996). A cetacean biopsy system using lightweight pneumatic darts and its effect on the behavior of killer whales. *Marine Mammal Sci*ence **12**: 14-27.

Begg, G. A., Keenan, C. P. and Sellin, M. J. (1998a). Genetic variation and stock structure of school mackerel and spotted mackerel in northern Australian waters. *Journal of Fish Biology* **53:** 543-559.

Begg, G. A., Cappo, M., Cameron, D. S., Boyle, S. and Sellin, M. J. (1998b). Stock discrimination of school mackerel, *Scomberomorus queenslandicus* and spotted mackerel, *Scomberomorus munroi*, in coastal waters of eastern Australia by analysis of minor and trace elements in whole otoliths. *NOAA Fishery Bulletin* **96**: 653-666.

Blaber, S. J., Dichmont, C., Buckworth, R. C., Badrudin, Bambang, S, Fegan, B., Ramm, D. C. and Salini, J. (2005). Shared stocks of snappers (Lutjanidae) in Australia and Indonesia: Integrating biology, population dynamics and socio-economics to examine management scenarios. *Reviews in Fish Biology and Fisheries* **15**: 111-127.

Bravington, M. and Grewe, P. (2007). A method for estimating the absolute spawning stock size of SBT, using close- kin genetics, CCSBT-SC/0709/18. Working Paper to the Scientific Committee of the Commission for the Conservation of Southern Blue fin Tuna, September 2007, Hobart, Australia.

Broderick D., Ovenden, J. R. and Street, R. Isolation and characterisation of microsatellite markers in narrow-barred Spanish mackerel (*Scomberomorus commerson*) and their use in other mackerel and tuna. (In preparation for submission to *Molecular Ecology Notes*).

Broderick, D., Ovenden, J. Buckworth, R., Lanyon, J. and McHale, M. (2006). Application of genetic mark-recapture in large and small populations: Case studies from Spanish mackerel and dugong. Presentation, Australian Society for Fish Biology Annual Conference, Hobart. Tasmania, August 2006.

Broderick, D., Macbeth, M., Ovenden, J. R. and Buckworth, R. C. (2009). The Feasibility of 'Genetag': Genetic Tagging for Monitoring Mortality Rates and Catchability. Presentation. 8th Indo-Pacific Fish Conference and Australian Society for Fish Biology Workshop. Fremantle, Western Australia, 31 May- 5 June, 2009.

Broughton, R. E., Stewart, L. B. and Gold, J. R. (2002) Microsatellite variation suggests substantial gene flow between King Mackerel (*Scomberomorus cavalla*) in the western Atlantic Ocean and Gulf of Mexico. *Fisheries Research* **54:** 305-316.

Buckworth, R. C. (2004a). "Effects of Spatial Stock Structure and Effort Dynamics on the Performance of Alternative Assessment Procedures for the Fisheries of Northern Australia." PhD Thesis, University of British Columbia, 226 pp.

Buckworth, R. C. (2004b). Genetic tagging to monitor catchability and fishing rates. Poster Presentation, 4th World Fisheries Congress, Vancouver, Canada, 2-6 May 2004.

Buckworth, R. C., Ovenden, J., Hoyle, S. and McPherson, G. (2002). The technical feasibility of genetic tagging for monitoring mortality and catchability in northern Australian fisheries: a new technique with potential broad application. Marine Research in Northern Australia. Australian Marine Sciences Association Symposium, Museum and Art Gallery of the Northern Territory , Darwin, Australia 28-29 November, 2002.

Buckworth, R. C., Newman, S. J., Ovenden, J. R., Lester, R. J. G. and McPherson, G. R. (2007a). The Stock Structure of Northern and Western Australian Spanish Mackerel. Final Report, Fisheries Research & Development Corporation Project 1998/159. Fisheries, Department of Primary Industry, Fisheries and Mines, Northern Territory Government, Australia. *Fishery Report* **88**, 225 pp.

Buckworth, R. C., Ovenden, J. R., Broderick, D., McPherson, G. R., Street, R. and McHale, M. (2007b).Genetag: Monitoring fishing mortality rates and catchability using remote biopsy and genetic mark-recapture. *In*: Lyle, J. M., Furlani, D. M., and Buxton, C. D. (Eds.) Cutting-edge technologies in fish and fisheries science. *Australian Society for Fish Biology* Workshop Proceedings, Hobart pp. 44-49. Tasmania, August 2006, Australian Society for Fish Biology.

Buckworth, R. C., Ovenden, J. R., Broderick, D. and Macbeth, M. (2008). Proof of Concept for 'Genetag': Genetic tagging for monitoring mortality rates and catchability. Presentation. International Symposium on Advances in Fish Tagging and Marking Technology. *American Fisheries Society, Australian Society for Fish Biology* and *New Zealand Marine Sciences Society*. Auckland, New Zealand, 24-28 February, 2008.

Buckworth, R. C, Ovenden, J. R., Broderick, D. and Macbeth, M. (2009). The Feasibility of 'Genetag': Genetic Tagging for Monitoring Mortality Rates and Catchability. 8th Indo-Pacific Fish Conference and *Australian Society for Fish Biology* Workshop. Fremantle, Western Australia, 31 May- 5 June, 2009.

Buckworth, R. C. and Clarke, R. (2001). Fishery Assessment Report for the Northern Territory Spanish Mackerel Fishery – 1999: Summary of Assessment Information. Department of Primary Industry and Fisheries, Darwin, Australia. *Fishery Report* **52**, 28 pp.

Buckworth, R. C. and Martell, S. J. D. (2003). Adding value to recreational tagging: combined genetic and conventional tagging to estimate fishing mortality rates. *In* Regional Experiences for Global Solutions. *In*: The Proceedings of the Third World Recreational Fisheries Conference. Edited by A. P. M Coleman. Department of Business, Industry and Resource Development, Northern Territory Government, Darwin, Australia. pp. 43-47.

Cato, D. H. (1978). Review of ambient noise in the ocean: non biological sources. *Bulletin of the Australian Acoustical Society* **6:** 31-36.

Cato, D. H. (1997). Ambient sea noise in Australian waters. *In:* Proceedings of the Fifth International Congress on Sound and Vibration, Adelaide, Casual Productions, Adelaide **5**: 2813.

Cato, D. H. (1999). The ambient noise in the ocean. *In:* 'Basics of Underwater Acoustics' 21pp. Acoustics and Vibration Unit, Australian Defence Force Academy, 23-24 June, Canberra.

Cotton, C. F. (2010). Factors Affecting Reception Range of Ultrasonic Tags in a Georgia Estuary. *Marine Technology Society Journal* **44:** (5), 17-24.

Davies, N., Villablanca, F. X. and Roderick, G. K. (1999). Determining the source of individuals: multilocus genotyping in non-equilibrium population genetics. *Trends in Ecology and Evolution* **14**: 17-21.

de Lestang, P., Allsop, Q. A. and Griffin, R. K. (2004). Assessment of the post-release survival of Barramundi (*Lates calcarifer*). *Fishery Report* **72**. Northern Territory Department of Business, Industry and Resource Development Darwin, Australia.

Etter, P. C. (1996). Underwater Acoustic Modeling: Principles, Techniques and Applications, 2nd Edition, E&FN Spon.

Fischer, D., and Bachman, K. (1998). Microsatellite enrichment in organisms with large genomes (*Allium cepa* L.). *BioTechniques* **24**: 796-800.

Gold, J. R., Pak, E. and DeVries, D. A. (2002). Population structure of King Mackerel (*Scomberomorus cavalla*) around peninsular Florida, as revealed by microsatellite DNA. *Fisheries Bulletin* **100**: 491-509.

Govender, A. (1993). Growth of King Mackerel (*Scomberomorus commerson*) off the coast of Natal, South Africa - from length and age data. *Fisheries Research* **20:** 2063-79.

Grubert, M.A., Martin, J.M., Saunders, T.M. (2012). A summary of Northern Territory fisheries stock assessments undertaken by Professor Carl Walters in June/July 2011. Northern Territory Department of Resources, Fishery Report (In press).

Hague, M. J. (2006). The use of genetic tagging to assess inshore rockfish populations within a marine conservation area in the Strait of Georgia. MSc. Thesis. Simon Fraser University, 129 pp.

Handley, A. J., (Editor) (2010). Fishery Status Reports 2009. Northern Territory Government Department of Resources. *Fishery Report* **104** (Spanish Mackerel, pp. 91-203).

Hilborn, R., Walters, C. J. and Jester, D. B., Jr. (1990). Value of fish marking in fisheries management. *American Fisheries Society Sym*posium **7** pp. 5-7.

Hoyle, S. D. (2002). Management Strategy Evaluation for Spanish Mackerel. Fishery Assessment Report. Agency for Food and Fibre Sciences, 60 pp.

Hoyle, S. D., Peel, D., Ovenden, J. R., Broderick, D. and Buckworth, R. C. (2005). LocusEater and ShadowBoxer: programs to optimise experimental design and multiplexing strategies for mark-recapture experiments using genetic tagging. *Molecular Ecology Notes* **5**: 974-976.

Jolley, J. W. and Irby, E. W. Jr. (1979). Survival of tagged and released Atlantic sailfish (*Istiophorus platypterus*: Istiophoridae) determined with acoustic telemetry. *Bulletin of Marine Science* **29:** (2) 155-169.

Kalendar, R. (2001). A computer program “Oligos” for PCR primers design 2001. The third major international bioinformatics meeting in Scandinavia, "Bioinformatics 2001", March 29 - April 1, Skövde, Sweden.

Kastelein, R., van der Huel, S., van der Veen, J., Verboom, W. C., Jennings, N., der Han, D. and Reijnders, P. J. H. (2007). Effects of acoustic alarms designed to reduce small cetacean bycatch in gillnet fisheries on the behaviour of North Sea fish species in a large tank. *Marine Environmental Research* **64:** 160-180.

Kingsberry, M. (2005). Design and construction of an ultrasonic transmitter for marine tracking applications. Bachelor of Engineering with Honours thesis (Electrical and Electronic). James Cook University, Townsville, Australia, 118 pp.

Lester, R. J. G., Thompson, C., Moss, H. and Barker, S. C. (2001). Movement and stock structure of narrow-barred Spanish Mackerel as indicated by parasites. *Journal of Fish Biology* **59:** 833–842.

Loneragan, N., Die, D. J., Kailis, G. M. Watson, R. and Preston, N. (2002). Developing and assessing techniques for enhancing tropical Australian prawn fisheries and the feasibility of enhancing the brown tiger prawn (*Penaeus esculentus*) fishery in Exmouth Gulf. Final Report, FRDC 1998/222. CSIRO Marine Research, Brisbane, Australia, 53 pp.

Ludwig, D., Hilborn, R. and Walters, C. J. (1993). Uncertainty, resource exploitation and conservation: lessons from history. *Science* (Washington, D.C.). **260**: 17-36.

Macbeth, G. M., Broderick, D., Ovenden, J. R. and Buckworth, R. C. (2011). Likelihood-based genetic mark-recapture estimates studies when genotype samples are incomplete and contain typing errors. *Theoretical Population Biology* (in press). http://dx.doi.org/10.1016/j.tpb.2011.06.006.

Mackie, M., Lewis, P. D., Gaughan, D. J. and Buckworth, R. (2003). Stock assessment of Spanish Mackerel (*Scomberomorus commerson*) in Western Australia. Final Report, Fisheries Research and Development Corporation Project 1999/151. Fisheries Department of Western Australia 240 pp. .

Martell, S. J. D. and Walters, C. J. (2002). Implementing harvest rate objectives by directly monitoring exploitation rates and estimating changes in catchability. *Bulletin of Marine Science* **70**: 695-713.

McPherson, G. R. (1981). Identification of stocks and migration routes of Spanish Mackerel. FIRTA (Fishing Industry Research Trust Account) Report 1981, 44 pp.

McPherson, G. R. (1992). Age and growth of the narrow-barred Spanish Mackerel, *Scomberomorus commerson* (Lacepède, 1800) in north-eastern Queensland waters. *Australian Journal of Marine Freshwater Research* **43**: 1269-1282.

McPherson, G. R., Cato, D. H. and Gribble, N. A. (1999). Acoustic properties of low cost alarms developed to reduce marine mammal bycatch in shallow coastal waters of Queensland., Australia. Paper SC/51/SM36 presented to the IWC Scientific Committee, May 1999, 16 pp.

McPherson, G. R., Ballam, D., Stapley, J., Peverell, S., Cato, D. H., Gribble, N., Clague, C. and Lien, J. (2004). Acoustic alarms to reduce marine mammal bycatch from gillnets in Queensland waters: optimising the alarm type and spacing. *Proceedings of ACOUSTICS 2004*. Gold Coast, Australia, 3-5 November 2004, pp. 363-368.

McPherson, G. R., Clague, C., McPherson, C., Turner, P., Madry, A., Bedwell, I., Cato, D. H. and Kreutz, D. (2008). Reduction of interactions by toothed whales with fishing gear. Phase 1. Development and assessment of depredation mitigation devices around long lines. Final Report, FRDC 2003/016. Department of Primary Industry and Fisheries Information Series QI 06079.

Mills, L., Citta J., Lair, K., Schwartz, M. and Tallmon, D. (2000) Estimating animal abundance using non-invasive DNA sampling: promise and pitfalls. *Ecological Applications* **10:** 283–294.

Moore, B. R., Buckworth, R. C., Moss, H. and Lester, R. J. G. (2003). Stock discrimination and movements of narrow-barred Spanish Mackerel across northern Australia as indicated by parasites. *Journal of Fish Biology* **63:** 765-779.

Newman S. J., Buckworth R. C., Mackie M.**,** Lewis, P., Trevor, I., Bastow, P. and Ovenden, J. R. (2009)**.** Spatial subdivision among assemblages of Spanish Mackerel, *Scomberomorus commerson* (Pisces: Scombridae) across northern Australia: implications for fisheries management. *Global Ecology and Biogeography* **18:** 711-723.

Ovenden, J., Hoyle, S., Peel, D. and Broderick, D. (2002a). Genetagging for fisheries sustainability. *Today's Life Science* **14:** Sept/Oct 2002, pp. 50-52.

Ovenden, J. R., Lloyd, J., Newman, S. J., Keenan, C. P. and Slater, L. S. (2002b). Spatial genetic subdivision between northern Australian and South-east Asian populations of *Pristipomoides multidens*: a tropical marine reef fish species. *Fisheries Research* **59:** 57–69.

Ovenden, J. R. and Street, R. (2007). Genetic population structure of Spanish Mackerel. *In:* The Stock Structure of Northern and Western Australian Spanish Mackerel. Final Report for FRDC 98/159 (Buckworth, R. C., Newman, S. J., Ovenden, J. R., Lester, R. J. G. and McPherson, G. R., eds.), pp. 93–140. Darwin: Fisheries Group, Department of Business, Industry and Resource Development, Northern Territory Government, Australia. .

Paetkau, D. and Strobeck, C. (1994). Microsatellite analysis of genetic variation in black bear populations. *Molecular Ecology* **3:** 489–495. .

Patterson, K. (1992). Fisheries for small pelagic species: an empirical approach to management targets. *Reviews in Fish Biology and Fisheries* **2:** 321–338.

Peakall, R. and Smouse, P. E. (2006). GenAlEx 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6:** 288-295.

Phelan, M. J. (2008). Assessment of the Implications of Target Fishing on Black Jewfish (*Protonibea diacanthus*) Aggregations in the Northern Territory. FRDC Project 2004/004. Department of Primary Industry, Fisheries and Mines *Fishery Report* **91**.

Raymond, M. and Rousset, F. (1995). GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86:** 248-249.

Rice, W. R. (1989). Analysing tables of statistical tests. *Evolution* **43:** 223-225.

Richardson, W. J., Greene, C. R., Malme, C. I. and Thomson, D. H. (1995). Marine Mammals and Noise. Academic Press, San Diego, CA 576 pp.

Salini, J. P., Ovenden, J. R., Street, R., Pendrey, R., Haryanti and Ngurah (2006). Genetic population structure of red snappers (*Lutjanus malabaricus* Bloch & Schneider, 1801 and *Lutjanus erythropterus* Bloch, 1790) in central and eastern Indonesia and northern Australia. *Journal of Fish Biology* **68:** (Supplement B) 217–234.

Sambrook J, Fritsch E. F. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, 2nd edn. Cold Spring Harbour Laboratory Press, Cold Spring, New York.

Schulkin, M. (1968). Surface coupled losses in surface sound channels. *Journal of the Acoustical Society of America* **44 :**(4) 1152-1154.

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

Shapiro, A. D., Tougaard, J., Jorgensen, P. B. Kyhn, L. A., Balle, J. D., Bernardez, C., Fjalling, A., Karlsen, J. and Wahlberg, M. (2009). Transmission loss patterns from acoustic harassment and deterrent devices do not always follow geometrical spreading predictions. *Marine Mammal Science* **25:**(1): 53–67.

Stevens, J. D., West, G. J. and McLoughlin, K. J. (2000). Movements, recapture patterns, and factors affecting the return rate of carcharhinid and other sharks tagged off northern Australia. *Marine and Freshwater Research* **51:** (2) 127 – 141. http://dx.doi.org/10.1071/MF98158.

Taylor, A. C., Sherwin, W. B. and Wayne, R. K. (1994). Genetic variation of microsatellite loci in a bottlenecked species: the northern hairy-nosed wombat *Lasiorhinus krefftii*. *Molecular Ecology* **3**: 277-290.

Urick, R. J. (1983). Principles of Underwater Sound. (McGraw Hill, Baskerville, New York).

Uthicke, S. and Benzie, J. A. H. (2002). A genetic fingerprint recapture technique for measuring growth in "unmarkable" invertebrates: negative growth in commercially fished holothurians (*Holothuria nobilis*). *Marine Ecology Progress Series* **241:** 221-226.

Van Herwerden, L., Benzie, J., Peplow, L. and Davies, C. (2000a) Microsatellite markers for Coral Trout (*Plectropomus laevis*) and red throat emperor (*Lethrinus miniatus*) and their utility in other species of reef fish. *Molecular Ecology* **9:** 1919-1952.

Van Herwerden, L., Benzie, J., Peplow, L. and Davies, C. (2000b). Microsatellite markers for Coral Trout (*Plectropomus laevis*) and red throat emperor (*Lethrinus miniatus*). Townsville: Australian Institute of Marine Science Report 32.

Walsh, P., Metzger, D. and Higuchi, R. (1991). Chelex 100 as a medium for simple extraction of DNA for PCR-based typing forensic material. *BioTechniques* **10:** 506-513.

Walters, C. J. and Parma, A. M. (1996). Fixed exploitation rate strategies for coping with effects of climate change. *Reviews in Fish Biology* **6:** 125-137.

Ward, R., King, T. L., Blandon, I. R. and McEachron, L. W. (1995). Using gene marking to assess stocking success of red drum in Texas Bays. *American Fisheries Society Symposium* **15**: 551.

Welch, D. J., Hoyle, S. D. and McPherson, G. R. and Gribble. N. A. (2002). Preliminary assessment of the Queensland east coast Spanish Mackerel fishery. Southern Fisheries Centre, Deception Bay. Dept. of Primary Industry, Qld, Australia, Agency for Food and Fibre Sciences, Fisheries and Aquaculture.

Wilson, D. T., Curtotti, R. and Begg, G. A. (eds.) (2010). Fishery Status Reports 2009: status of fish stocks and fisheries managed by the Australian Government, Australian Bureau of Agricultural and Resource Economics – Bureau of Rural Sciences, Canberra.

Zollett, E. A. and Read, A. J. (2006). Depredation of catch by bottlenose dolphins (*Tursiops truncatus*) in the Florida King Mackerel (*Scomberomorus cavalla*) troll fishery. *Fishery Bulletin* **104:** 343-349.

APPENDIX 1: INTELLECTUAL PROPERTY

All intellectual property arising from this research project has been made publicly available. This includes publications and presentations (see below) and publicly available software. The latter include LOCUSEATER and SHADOWBOXER (see Hoyle et al. 2005), and SHAZA, (based on Macbeth et al. 2011). SHAZA was initially covered by a provisional Australian patent.

SOME PROJECT-RELATED PUBLICATIONS AND PRESENTATIONS

Anon. (2002). Gentle tag a world first? R & D News. **10** (4).6. October 2002. Fisheries Research & Development Corporation, Canberra, Australia

Anon. (2004). Fishing for a better solution. Catalyst Queensland Issue 9, March pages 4-5.

Broderick, D., Ovenden, J. R. and Street, R. Isolation and characterisation of microsatellite markers in narrow-barred Spanish mackerel (*Scomberomorus commerson*) and their use in other mackerel and tuna. (In preparation for submission to *Molecular Ecology Notes*).

Broderick, D., Ovenden, J. Buckworth, R., Lanyon, J. and McHale, M. (2006). Application of genetic mark-recapture in large and small populations: Case studies from Spanish mackerel and dugong. Presentation at the Australian Society for Fish Biology Annual Conference, Hobart, Tasmania, August 2006.

Broderick, D., Macbeth, M., and Ovenden, J. R. and Buckworth, R. C. (2009). The feasibility of 'Genetag': Genetic tagging for monitoring mortality rates and catchability. Presentation At the 8th Indo-Pacific Fish Conference and Australian Society for Fish Biology Workshop, Fremantle, Western Australia, 31 May - 5 June, 2009.

Buckworth, R. [C.] (2002). Genetic tagging Spanish mackerel – a new approach to monitoring fisheries...*In Depth*, 3rd & 4th Quarters 2002, p5. Amateur Fishermen's Association of NT. (Darwin, Australia).

Buckworth, R. C. (2004). "Effects of Spatial Stock Structure and Effort Dynamics on the Performance of Alternative Assessment Procedures for the Fisheries of Northern Australia." PhD Thesis, University of British Columbia, 226 pp.

Buckworth, R. C. (2004). Fish in Space: Effects of spatial stock structure and effort dynamics on the performance of alternative assessment procedures for the fisheries of northern Australia. Simon Fraser University Fisheries Research Group, Burnaby, British Columbia, Canada, 21 January 2004.

Buckworth, R. C. (2004). Genetic tagging to monitor harvest rates in a pelagic fishery. Fish 500 Seminar Series. University of British Columbia, Fisheries Centre. Vancouver, British Columbia, Canada, 30 January 2004.

Buckworth, R. C. (2004). Genetic tagging to monitor catchability and fishing rates. Poster Presentation, 4th World Fisheries Congress, Vancouver, Canada, 2-6 May 2004.

Buckworth, R. C. (2005). DNA, scientific method and other pleasures of the flesh. CSIRO Cutting Edge Lecture Series, Darwin, 9 November 2005.

Buckworth, R. C. (2005). The Australian fisheries for the Kingfish, *Scomberomorus commerson*: Status, Research and Management. Keynote address, the Regional Workshop on the Management of Kingfish Resources in the GCC and the Northwest Indian Ocean. 29-31 May 2005, May 2005. Ministry of Agriculture & Fisheries, and Sultan Qaboos University, Muscat, Sultanate of Oman.

Buckworth, R. C., (2005). Innovative Research in NT's Fisheries Group. Presentation to the Biotechnology Association of Japan. NT Museum and Art Gallery, Darwin, July 2005.

Buckworth, R. C. and Clarke, R. (2001). Fishery Assessment Report for the Northern Territory Spanish Mackerel Fishery – 1999: Summary of Assessment Information. Department of Primary Industry and Fisheries, Darwin, Australia. *Fishery Report* **52**, 28 pp.

Buckworth, R. C. and Martell, S. J. D. (2003). Adding value to recreational tagging: Combined genetic and conventional tagging to estimate fishing mortality rates. *In:* "Regional Experiences for Global Solutions – the Proceedings of the Third World Recreational Fisheries Conference, 21-25 May, Darwin, Australia (Edited by A. P. M Coleman), pp. 43-47 NT Department of Business, Industry and Resource Development. *Fishery Report* **67,** 269 pp.

Buckworth, R. C., Ovenden, J., Hoyle, S. and McPherson, G. (2002). The technical feasibility of genetic tagging for monitoring mortality and catchability in northern Australian fisheries: A new technique with potential broad application. Marine Research in Northern Australia. Australian Marine Sciences Association Symposium, Museum and Art Gallery of the NT, Darwin, 28-29 November, 2002.

Buckworth, R. C., Ovenden, J, Hoyle, S. and McPherson, G. (2002). GENEtag: Pilot studies using genetic tagging to monitor fishing mortality rates and catchability in northern Australian fisheries: a new technique with potential for broad application. Australian Society for Fish Biology Annual Conference, Cairns, Australia, August 2002. Poster presentation.

Buckworth, R. C., Ovenden, J. R., Hoyle, S., and McPherson, G. (2004). Genetic mark-recapture for monitoring mortality rates and catchability in pelagic fisheries. University of British Columbia, Ecology Group Seminar Series, Department of Zoology, Vancouver, British Columbia, Canada, 28 January 2004.

Buckworth, R. C., Ovenden, J. R., Broderick, D., McPherson, G. R., Street, R. and McHale, M. (2007b).Genetag: Monitoring fishing mortality rates and catchability using remote biopsy and genetic mark-recapture, pp. 44-49 *In:* Lyle, J. M., Furlani, D. M., and Buxton, C.D. (Eds.) Cutting-edge technologies in fish and fisheries science. Australian Society for Fish Biology Workshop Proceedings, Hobart, Tasmania, August 2006.

Buckworth, R. C., Ovenden, J. R., Broderick, D. and Macbeth, M. (2008). Proof of concept for 'Genetag': Genetic tagging for monitoring mortality rates and catchability. Presentation At the International Symposium on Advances in Fish Tagging and Marking Technology. American Fisheries Society, Australian Society for Fish Biology and New Zealand Marine Sciences Society, Auckland, New Zealand, 24-28 February, 2008.

Buckworth, R. C., Ovenden, J, Phelan, M., Broderick, D. and McPherson, G. (2005). Genetag: Monitoring fishing mortality rates and catchability using genetic mark-recapture. Fisheries Research and Development Corporation Workshop, " Monitoring". Australian Society for Fish Biology Annual Conference, Darwin July 2005.

Cox, S. P., and Buckworth, R. C. (2004). Genetic tagging for inshore rockfish assessment. Western Groundfish Conference, Nanaimo, British Columbia, January 2004.

Hedditch, N. (2003). Research with *Bite*: genetic tagging for the NT Spanish mackerel fishery. Project presentations, *Advance in: Leadership Development Program*, Canberra, 11 September 2003.

Higgins, K. L. (2004). Genetag: a Spanish Mackerel Tagging Project. Presentation Skills for Northern Territory Government, Darwin, 7 May 2004.

Higgins, K. L. (2004). The Genetag Spanish mackerel project, and the way we manage fisheries in the NT. The Department of Business, Industry and Resource Development Induction Course, Darwin, 27 May 2004.

Hoyle, S. D., Peel, D., Ovenden, J. R., Broderick, D. and Buckworth, R. C. (2005). LocusEater and ShadowBoxer: programs to optimise experimental design and multiplexing strategies for mark-recapture experiments using genetic tagging. *Molecular Ecology Notes* **5:** 974-976.

Macbeth, G. M., Broderick, D., Ovenden, J. R. and Buckworth, R. C. (2011). Likelihood-based genetic mark-recapture estimates studies when genotype samples are incomplete and contain typing errors. *Theoretical Population Biology* **80**(3): 185-196.

Macbeth, G. M., Broderick, D., Ovenden, J. R. and Buckworth, R. C. (2011). SHAZA User Manual and Software, Ver 2.0 July 2011: http://www.dpi.qld.gov.au/28_6899.htm

Ovenden, J. (2002). Where does Wanda wander? *Source* **1**, Department of Primary Industries, Queensland Government, Brisbane.

Ovenden, J., Hoyle, S., Peel, D. and Broderick, D. (2002a). Gene-tagging for fisheries sustainability. *Today's Life Science* **14:** Sept/Oct 2002, 50-52.

APPENDIX 2: PROJECT STAFF

NORTHERN TERRITORY

Rik Buckworth

Charles Bryce

Adrian Donati

Paul de Lestang

Rachael Hunter

Kylie Higgins

Michael Phelan

Julie Martin

Quentin Allsop

David McKey

Ray Clarke

Stephanie Boubaris

Chris Errity

Danny Argent

Ian Dunn

Paul Davies

Norm Hedditch (Taroona Pty Ltd)

QUEENSLAND

Jenny Ovenden

Raewyn Street

Damien Broderick

David Peel

Michael Macbeth

Samantha Peel

Geoff McPherson

George Leigh

Simon Hoyle

Marcus McHale

APPENDIX 3: DNA EXTRACTION

SALTING OUT TO EXTRACT DNA FROM FINS

Marcus McHale

From OpenWetWare http://openwetware.org/wiki/DNA_extraction_-_Salting_Out

* This is a simple procedure to purify DNA from diverse tissues.

Materials

1. Pipettes and tips
2. 1.5 mL microcentrifuge tubes

Reagents

1. Digestion buffer (pH 8.0): 10 mM NaCl, 10 mM TRIS (pH 8.0), 10 mM EDTA (pH 8.0), 0.5% SDS
2. Proteinase K 20 mg/mL
3. Sodium acetate 3M (pH 5.2)
4. Ethanol 70% and 98% (chill prior to use).

Equipment

1. Incubator/water bath: preferably shaking
2. Centrifuge: preferably refrigerated
3. Vortex.

Procedure

1. Add 5 µL proteinase K to each mL of digestion buffer
2. Homogenise (or simply place) tissue in solution
3. Incubate at 55 °C for 1 hour to overnight
4. Mix by vortexing then centrifuge at maximum speed in a benchtop centrifuge for 2 minutes
5. Transfer supernatant into a new tube
6. Add 1/10 volume of sodium acetate 3M (pH 5.2)
7. Invert to mix and incubate at -20 °C for ~30 minutes
8. Centrifuge (preferably at 4 °C) at maximum speed in a bench-top centrifuge for 20 minutes
9. Transfer supernatant to a new tube
10. Add >2 volumes of 98% ethanol
11. Invert to mix and incubate at -20 °C for 30 minutes
12. Centrifuge (preferably at 4 °C) at maximum speed in a benchtop centrifuge for 20 minutes
13. Wash pellet with 70% ethanol, dry and resuspend in water or TE What is TE?

Critical steps

* After adding sodium acetate and centrifuging be careful not to transfer any of the white solid (SDS define SDS) into the fresh tube
* Ensure to dry the pelleted DNA completely before attempting to resuspend

Troubleshooting

You may wish to kill the proteinase K (95 °C, 10 minutes)

Notes

* I routinely rely on the denaturation step of Hot-start Taq in my PCR to take care killing proteinase K (doesn't seem to affect TAq in this case).
* Successfully used on fish, mammal and insect tissue.
* Works well in 96 well plate format, to remove ethanol following precipitation/wash simply invert the plate over a sink then spin gently (~100 rcf) inverted over absorbent paper.

Acknowledgment

* Damien Broderick from Queensland Department of Primary Industries and Fisheries (Australia) supplied me with the foundation to this protocol.

Reference

* Miller, S. A., Dykes, D. D. and Polesky, H. F. (1988). A simple salting out procedure for extracting DNA from human nucleated cells*. Nucleic Acids Research* **16:** 1215–1215.

CHELEX-BASE SPANISH MACKEREL FIN/OPERCULUM DNA EXTRACTION – LAB PROTOCOL

Extraction buffer:
10 mM TRIS
1 m M EDTA
0.1 mg/mL ProK
10% Chelex

Prepare two stocks:
Chelex: 20% Chelex in 1 x TE (10 mM Tris-HCl, 1mM EDTA) and
ProK: 0.2 mg/mL Proteinase K in 1 x TE (make this fresh prior to use).

Procedure:

Digestion:

1. Aliquot 400 uL of chelex stock into each labelled (A1-H12) 1.5 mL tube, use a large weigh boat and magnetic stirrer to keep chelex suspended.
2. Aliquot 400 uL of ProK stock into each tube using a stepper pipette.
3. Rinse tissue under running distilled water and place on lint-free paper on an eski lid filled with ice and wrapped in Glad wrap.
4. Cut small portion (~3-5 mm^2 of fin and ~3-5 mm^3 of operculum) of tissue using forceps and scissors and put in tube. Rinse both utensils in two beakers of MQ-H_2O and then 100% ethanol followed by flaming to dry. *Leave tubes H11 and H12 empty for use as blanks and positive and negative controls in the PCR.*
5. Incubate at 55 ^oC shaking at 250 rpm for 3 hours. **WAIT**

Centrifugation:

6. Centrifuge at 4 °C, 18000 rpm for 15 minutes prior to transferring 200 uL of the aqueous phase to a 96 well plate.
7. Centrifuge again at 4 °C, 6200 rpm x 15 minutes prior to transferring 150 uL of the aqueous phase into a new 96 well plate using a multi-channel pipette.

Normalisation to 10 ng/uL:

8. Centrifuge again at 4 °C, 6200 rpm x 10 minutes and transfer 5 uL of each sample (including blanks) using a multi-channel pipette into a 96 well UV spectrophotometry plate containing 95 uL of MQ-H_2O.
9. Use Power-wave plate reader (Level 5) to measure absorption at 230 260 280 320 nm and save the output to an excel spread sheet.
10. Transfer this data to “Spec to normalisation template” and save page 3? as “Finbox** ROBOT INPUT.txt” (text, tab delimited).
11. Use Corbett Robot #1 in Ande’s Lab to perform dilutions to 10 ng/uL using this file.

APPENDIX 4: ESTIMATING WILD FEEDING SCHOOL SIZE (*W*) FROM FIN SAMPLES AND GENETAGS

Consider a small population of fish, actively feeding during a period *T*.

L of these fish are Genetagged i.e. their tissue is sampled and later genotyped, so that they are "marked" in the usual sense of a mark-recapture experiment.

H fish are subsequently harvested. Of these a randomly chosen subset *F* are sampled (fins retained) and subsequently genotyped, amongst which *Y* are detected as from the *L* Genetagged fish. These are recaptures. Amongst the harvested fish are a further *X* Genetagged fish which are not among those whose fins are sampled and genotyped. These undetected recaptures are analogous to unreported recaptures in a typical mark-recapture experiment.

The proportion of the *L* Genetagged fish that are harvested can be expressed in terms of total harvest size or as a subsample from fins that are sampled and genotyped:

Total recaptures/number in harvest = detected recaptures/fins genotyped

Appendix 4 Equation 1 $$(X+Y)/H = Y/F$$

Given the number of Genetagged fish below the boat is changing over time, the ratio in Appendix 4 Equation 1 can be expressed as:

Appendix 4 Equation 2 $$\frac{Y}{F} \approx \frac{1}{T}\sum_{t=1}^{T} P_t$$

where P_t is the proportion of fish with Genetag samples that are amongst the active feeding fish below the boat at time *t.* Assuming that the number of active feeding fish is unchanged except for harvest during the period *T*, then the number of active feeders at time *t*, $(W - H_t)$, is determined from the number of wild feeders before harvest, (*W*), less the number of harvested fish at time *t*, (H_t), giving:

Appendix 4 Equation 3

P_t = [(Lures deployed up to time *t*)-(Lures caught up to time *t*-1)]/(active feeders)

$$= \left(L_t - \sum_{t'=0}^{t-1}\left(\frac{H}{T}P_{t'}\right)\right) \Big/ (W - H_t) = \left(\frac{t.L_T}{T} - \sum_{t'=0}^{t-1}\left(\frac{H}{T}P_{t'}\right)\right) \Big/ (W - \frac{tH}{T})$$

where $H_t = t.H/T$ is the number of fish harvested up to time *t* and $L_t = t.L/T$ is the number of fish Genetagged up to time *t* where *L* is the total number of fish genetically marked during a fishing session *T.* By substituting Appendix 4 Equation 3 in equation Appendix 4 Equation 2 it follows that:

Appendix 4 Equation 4 $$\frac{Y}{F} \approx \frac{1}{T}\sum_{t=1}^{T}\left[\left(\frac{t.L}{T} - \sum_{t'=0}^{t-1}\left(\frac{H}{T}P_{t'}\right)\right) \Big/ (W - \frac{tH}{T}) \right]$$

Using *H*, *L* and the ratio *Y/F* the size of the feeding school (*W*) can then be determined from equation Appendix 4 Equation 4 which is solved by iteration using *T* increments in time.

Appendix 4 Equation 4 was developed further to include additional information from the number of lure by lure recaptures during a fishing session (*Z*). Assuming random sampling the expectation is that $Z/L \approx Y/F$ with a combined estimate of these ratios giving $(Y+Z)/(F+L)$ which from equation 4 leads to:

Appendix 4 Equation 5 $$\frac{Y+Z}{F+L} \approx \frac{1}{T}\sum_{t=1}^{T}\left[\left(\frac{t.L}{T} - \sum_{t'=0}^{t-1}\left(\frac{H}{T}P_{t'}\right)\right)\Big/\left(W - \frac{tH}{T}\right)\right]$$

The size of the feeding school (*W*) can then be determined as above by iteration using *T* increments in time.

Proof that $Z/L \approx Y/F$ is as follows: The expectation of *Z* is the product of lures sampled at time *t*, $(L_t - L_{t-1})$, by the proportion of Genetagged fish in the active feeders sampled at time *t*, (P_t), summed over time *T* giving:

$$Z = \sum_{t=1}^{T}(L_t - L_{t-1}).P_t = \sum_{t=1}^{T}\left(\frac{t.L}{T} - \frac{(t-1)L}{T}\right).P_t$$ which divided by *L* becomes

$$\frac{Z}{L} \approx \sum_{t=1}^{T}\left(\frac{t}{T} - \frac{(t-1)}{T}\right).P_t = \frac{1}{T}\sum_{t=1}^{T}P_t \approx \frac{Y}{F}$$ from Appendix 4 Equation 1.

APPENDIX 5: STRENGTHS, WEAKNESS, OPPORTUNITIES AND THREATS ANALYSIS (SWOT) OF GENETAG

STRENGTHS

Data-based rather than model-based measurement of the performance of the fishery

A mark-recapture-based monitoring system can provide a direct performance measure for the fishery. This means that deduction of the impact of fishing is data - rather than model - based, and therefore can be independent of past information and model assumptions (for example constant catchability over time) and structures (for example, assumptions about spatial dynamics that are reflected in model structure, such as a "unit stock" assumption). Unlike fisheries population modelling that is based on long time series of information, a Genetagging program is responsive to changes to the fisheries resource that occur over relatively short time scales, such as yearly or decadal perturbation driven by environmental change (for example, changes to recruitment as a response to global warming).

There is no tag loss

In a conventional tagging program, fish may shed tags over time and fish may either die or change their behaviour as a consequence of the capture and tagging process. These loss rates may be time-dependent (e.g. most shedding and mortality may occur within days of tagging), are difficult to observe and measure and, if uncorrected for, tend to produce optimistically-biased estimates of mortality rates. In a Genetag program with an appropriate in situ tissue sampling method, these problems are avoided.

Determination of reporting rates is replaced with a more tractable sampling problem

Unlike conventional tags, Genetags are invisible in the field. With conventional tags, the rate at which tags are reported is difficult to determine and at best can be addressed by extra experimentation. There may also be systematic errors where it is regarded by individuals or sectors that reporting of recaptures is a disadvantage, or where capture or processing reduces the observation of tags. In the case of a Genetag approach, this is translated to a sampling problem, where the rate of sampling of landed fish within a stratum determines the probability that the Genetagged fish are detected. A Genetag system also ensures freedom from researcher bias as Genetagging is essentially performed as a series of blind trials as field or laboratory workers have no knowledge that they are working with tagged samples.

Retrospective precision

Given that frozen fins or extracted DNA are inexpensive to store, increased precision of harvest rate and catchability estimates can be obtained by analysing past collections of samples. This may be important if there is a change in the need for management data; for example, this could occur if a resource becomes more valuable and catch rates increase. This could be a cost-saving approach in the design of projects for new species or areas, or could be a means by which costs could be minimised over a series of years of data. For example, while it might be desirable for a Genetag project to provide an annual measure of harvest rates, a project might entail three years of tagging and collection of tissue from landings, but genetic analysis might be concentrated in a single year to minimise staffing costs or take make best use of equipment availability.

WEAKNESSES

Mismatch between sample collection and genotyping

Not all samples produce a full genotype, so there is some wasted sampling effort. This could be addressed by improving sample collection protocols.

Slow processing speed

A Genetagging program is unlikely to be suitable for in-season harvest rate estimates. The complex nature of genotyping and matching is relatively slow.

Requires multidisciplinary partnerships

A Genetagging program requires a partnership between fisheries scientists (often with specialist information on one group of species and the operational and management idiosyncrasies of the fisheries in which they are caught) and population geneticists. Ideally, the geneticist will be familiar with the challenges of working on fisheries species. The partnership needs to be maintained with regular and detailed communication. This is particularly important if the team is spread across several locations. Reciprocal participation in field and laboratory work is a useful team building exercise.

Invisibility of tags and reliance on genetic technology

Unlike conventional tags, Genetags are invisible in the field. This slows down the flow of information about recapture rates to researchers and fishers.

This is because tags cannot be read and recaptures cannot be found without using genetic methods and the involvement of an experienced and qualified genetics team and the infrastructure that they require. Genetagging is exposed to genetics cost escalation or unavailability of essential components. Generally, genetics costs are decreasing with time. The method relies on expensive infrastructure (e.g. robotics). It could not easily be implemented in all nations, but samples are small and could be airfreighted around the world.

Relatedness between fish

The power of genotyping to identify individuals is reduced if the population contains individuals that share common past relatives. This may be the case for salmonid species in closed fisheries (e.g. lakes) or rock-fish (on deepwater reefs). This needs to be assessed as part of a pilot study. Spanish Mackerels from northern Australia show low relatedness. The Genetagging software (SHAZA) implements genotype matching with a given degree of population relatedness.

Relies on remotely sampled tissue

There are constraints in taking tissue samples from some species in a remote and non-lethal way. The design of the Genetag hooks is dependent upon the fish striking the lure with vigour, so that the momentum of the fish causes both the sampling (as the hook scrapes the fish's cheek) and the release of the fish (as the momentum of the fish straightens the hook). For smaller species or species which do not attack with the vigour of *S. commerson*, a different sampling apparatus would be needed

OPPORTUNITIES

Maximising information content from DNA

The DNA and the genotype data collected during a Genetagging project can be used to provide extra information about the fishery if required. For example, DNA from a sample can be used to track that sample through the processing, marketing and consumer stages. Spatial patterns in allele frequencies can be used to test for population genetic subdivision. Methods for the estimation of genetic effective population size (Ovenden et al. in preparation) could be applied to genotypes collected over successive years as it relies on the detection of change (via genetic drift). This would be a particularly valuable opportunity given the difficulty normally encountered in making these estimates in long-lived species. New methods that use DNA to estimate age (telomere analysis) could be feasibly applied to samples collected for Genetagging.

Encourages industry participation

Fish tagging programs have a high public profile. Commercial fishers can relate to the methodology and there are many ways in which the industry can provide operational support for a Genetagging program. Bringing industry into co-management has long-term benefits for all parties involved.

Opportunistic genetic information on by-catch species

The occurrence of non-target species in our Genetagging program was rare. They were recognised by the outlying nature of their genotypes and removed from the data analyses. But these outlying genotypes may represent cryptic, rare or undescribed species, which would have previously gone undetected. Identification of samples to species level could be a useful byproduct of Genetagging (e.g. identification of cryptic black-tip shark species; *Carcharhinus tilstoni* and *C. limbatus*).

THREATS

Inadequate desktop studies

A Genetagging program should be preceded by a desktop study that aims to assess feasibility, given feasible ranges across the census population size of the resource, a range of feasible harvest rates and the power of a set of genetic loci to identify individuals. A desktop study can fail if census population size and harvest rates are outside simulated ranges.

Change in fishing scale

Increasing fuel costs may focus fishing effort on near-shore resources, increasing the requirements for sound management plans. Conversely, reduced fuel costs might prompt rapid geographic expansion of the fishery. Changes in scale of spatial pattern may change if there is any other increase in profitability, due to market improvements or new products (such as a bycatch species that becomes marketable), or improved knowledge or technical efficiency. A Genetag program, like any other monitoring program, must adapt to these changes in scale.

Change in fishing techniques

A Genetagging program must also track changes to fishing methods over time. Genetagged fish must represent the same component of the population as fish being harvested. For example, if northern Australian Spanish Mackerel fishermen changed to net capture from lure capture, the Genetag researcher would need to determine that fish sampled with the Genetag lure were likely to be harvested with nets.