DETERMINATION OF MANAGEMENT UNITS FOR GREY MACKEREL FISHERIES IN NORTHERN AUSTRALIA

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Project No. 2005/010

Determination of management units for grey mackerel fisheries in northern Australia (FRDC 2005/010)

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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.

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Non Technical Summary

FRDC 2005-010	Determination of management units for grey mackerel fisheries in Northern Australia

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Objectives:

1. To determine the spatial and temporal stock structure of grey mackerel over its northern Australian range.

2. To use stock structure information in defining the geographic framework and appropriate management units required by Queensland and Northern Territory fisheries agencies for sustainable management planning of grey mackerel resources.

Outcomes achieved to date:

i) **The project has indicated that the appropriate spatial scale at which grey mackerel fisheries be managed is by state/territory, and by regions within these jurisdictions.** The project identified at least five separate stocks of grey mackerel throughout northern Australia for management purposes: a Western Australian stock, a north-western Northern Territory (Timor) stock, northern and southern Queensland east coast stocks, and a Gulf of Carpentaria stock. This information directly assists in compliance with the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* for net fisheries by the responsible agencies as it provides the basis for reliable and robust assessment of the status of grey mackerel stocks, identifies where grey mackerel stocks encompass shared jurisdictions, and helps deliver sustainable harvest and profitable utilisation of grey mackerel resources in northern Australian waters.

ii) **The project has provided the spatial and biological framework needed for more accurate stock assessment of grey mackerel fisheries.** Although not core to the project objectives, through co-contribution from QDPI&F and JCU, the project provides regional growth parameter estimates which will be critical input parameters for future grey mackerel stock assessments of the respective stocks identified during the study. Estimates of mortality, spawning seasonality, and maturity are also documented. The biological information we provide here makes greater use of the samples collected and

value-adds considerably to the project thereby further enhancing project outputs and management outcomes.

iii) This project addresses some of the major strategic research recommendations of the FRDC report of Ward and Rogers (2003). This review of northern mackerel research recommended stock structure determination and fisheries biology of grey mackerel as high priority research needs.

iv) The project results have influenced the development of monitoring strategies for grey mackerel fisheries on the Queensland east coast, and in the stock assessments for the Gulf of Carpentaria. The QDPI&F Long Term Monitoring Program has developed their monitoring program for grey mackerel based on the spatial dynamics identified during this project.

v) The project provided further evidence for the utility of holistic approaches in stock structure studies. Using the template provided for mackerel species by FRDC Project No. 1998/159, the use of multiple concurrent techniques has resulted in greater certainty and resolution in the identification of grey mackerel stocks. Further, to enhance interpretation in holistic stock structure studies this project has developed a simple tool for standardizing data integration, interpretation and presentation.

vi) The project helped develop relationships between community groups, research and management to address emerging fisheries issues. The project helped to inform emerging local community concerns of grey mackerel localised depletions in the Port Douglas region of the Queensland east coast through regular and direct communication of results to those communities, the inclusion of extra project sampling with continued industry participation, and analyses to better inform these concerns.

vii) The project further enhanced the link between research and management to maximize the uptake of research results by management. Due to the inter-jurisdictional nature of the project, and the possibility of the need for joint management between jurisdictions depending on results, fisheries managers from each jurisdiction were key partners throughout the project, including milestone reporting requirements (see Appendix 4). Managers were provided with regular progress reports throughout the project.

viii) The project provided significant human capital development opportunities. The project provided material for two BSc (Hons) projects (James Cook University, Nic Marton; University of Queensland, Robbie Charters). These student projects made significant contributions to the FRDC funded study and form the basis for Chapters 4 and 7 respectively.

Summary:
The requirement for Queensland, Northern Territory and Western Australian jurisdictions to ensure sustainable harvest of fish resources and their optimal use relies on robust information on the resource status. For grey mackerel (*Scomberomorus semifasciatus*) fisheries, each of these jurisdictions has their own management regime in their corresponding waters. The lack of information on stock structure of grey mackerel, however, means that the appropriate spatial scale of management is not known. As well, fishers require assurance of future sustainability to encourage investment and long-term involvement in a fishery that supplies lucrative overseas markets. These management and fisher-unfriendly circumstances must be viewed in the context of recent 3-fold increases in catches of grey mackerel along the Queensland east coast, combined with significant and increasing catches in other parts of the species' northern Australian range. Establishing the stock structure of grey mackerel would also immensely improve the relevance of resource assessments for fishery management of grey mackerel across northern Australia. This highlighted the urgent need for stock structure information for this species.

The impetus for this project came from the strategic recommendations of the FRDC review by Ward and Rogers (2003), "Northern mackerel (Scombridae: Scomberomorus): current and future research needs" (Project No. 2002/096), which promoted the urgency for information on the stock structure of grey mackerel. In following these recommendations this project adopted a multi-technique and phased sampling approach as carried out by Buckworth et al (2007), who examined the stock structure of Spanish mackerel, *Scomberomorus commerson*, across northern Australia. The project objectives were to determine the stock structure of grey mackerel across their northern Australian range, and use this information to define management units and their appropriate spatial scales.

We used multiple techniques concurrently to determine the stock structure of grey mackerel. These techniques were: genetic analyses (mitochondrial DNA and microsatellite DNA), otolith (ear bones) isotope ratios, parasite abundances, and growth parameters. The advantage of using this type of multi-technique approach was that each of the different methods is informative about the fish's life history at different spatial and temporal scales. Genetics can inform about the evolutionary patterns as well as rates of mixing of fish from adjacent areas, while parasites and otolith microchemistry are directly influenced by the environment and so will inform about the patterns of movement during the fishes lifetime. Growth patterns are influenced by both genetic and environmental factors. Due to these differences the use of these techniques concurrently increases the likelihood of detecting different stocks where they exist.

We adopted a phased sampling approach whereby sampling was carried out at broad spatial scales in the first year: east coast, eastern Gulf of Carpentaria (GoC), western GoC, and the NW Northern Territory (NW NT). By comparing the fish samples from each of these locations, and using each of the techniques, we tested the null hypothesis that grey mackerel were comprised of a single homogeneous population across northern Australia. Having rejected the null hypothesis we re-sampled the 1st year locations to test for temporal stability in stock structure, and to assess stock structure at finer spatial scales. This included increased spatial coverage on the east coast, the GoC, and WA.

From genetic approaches we determined that there at least four genetic stocks of grey mackerel across northern Australia: WA, NW NT (Timor/Arafura), the GoC and the east

coast. All markers revealed concordant patterns showing WA and NW NT to be clearly divergent stocks. The mtDNA D-loop fragment appeared to have more power to resolve stock boundaries because it was able to show that the GoC and east coast QLD stocks were genetically differentiated. Patterns of stock structure on a finer scale, or where stock boundaries are located, were less clear.

From otolith stable isotope analyses four major groups of *S. semifasciatus* were identified: WA, NT/GoC, northern east coast and central east coast. Differences in the isotopic composition of whole otoliths indicate that these groups must have spent their life history in different locations. The magnitude of the difference between the groups suggests a prolonged separation period at least equal to the fish's life span.

The parasite abundance analyses, although did not include samples from WA, suggest the existence of at least four stocks of grey mackerel in northern Australia: NW NT, the GoC, northern east coast and central east coast. Grey mackerel parasite fauna on the east coast suggests a separation somewhere between Townsville and Mackay. The NW NT region also appears to comprise a separate stock while within the GoC there exists a high degree of variability in parasite faunas among the regions sampled. This may be due to 1. natural variation within the GoC and there is one grey mackerel stock, or 2. the existence of multiple localised adult sub-stocks (metapopulations) within the GoC.

Growth parameter comparisons were only possible from four major locations and identified the NW NT, the GoC, and the east coast as having different population growth characteristics. Through the use of multiple techniques, and by integrating the results from each, we were able to determine that there exist at least five stocks of grey mackerel across northern Australia, with some likelihood of additional stock structuring within the GoC. The major management units determined from this study therefore were Western Australia, NW Northern Territory (Timor/Arafura), the Gulf of Carpentaria, northern east Queensland coast and central east Queensland coast.

The management implications of these results indicate the possible need for management of grey mackerel fisheries in Australia to be carried out on regional scales finer than are currently in place. In some regions the spatial scales of management might continue as is currently (e.g. WA), while in other regions, such as the GoC and the east coast, managers should at least monitor fisheries on a more local scale dictated by fishing effort and assess accordingly. Stock assessments should also consider the stock divisions identified, particularly on the east coast and for the GoC, and use life history parameters particular to each stock.

We also emphasise that where we have not identified different stocks does not preclude the possibility of the occurrence of further stock division. Further, this study did not, nor did it set out to, assess the status of each of the stocks identified. This we identify as a high priority action for research and development of grey mackerel fisheries, as well as a management strategy evaluation that incorporates the conclusions of this work. Until such time that these priorities are addressed, management of grey mackerel fisheries should be cognisant of these uncertainties, particularly for the GoC and the Queensland east coast.

Keywords: Grey mackerel, Scomberomorus semifasciatus, stock structure, spatial dynamics, otolith isotope ratios, population genetics, parasites, fisheries, management.

Acknowledgments

Photo credits: Front cover – commercial grey mackerel net fisher being assisted by F&F researcher Aaron Ballagh. Photo by D. Welch.
Underwater photo of a grey mackerel. Photo by D. Welch

1. INTRODUCTION

DJ Welch

1.1. Overview

The spatial dynamics of grey mackerel (*Scomberomorus semifasciatus*) populations within northern Australian and their relationships with one another were very poorly understood prior to this study being undertaken. Previous grey mackerel stock structure research across northern Australian focused primarily on the Queensland east coast (Cameron and Begg 2002). They used an integrated approach with genetic data to discriminate the east coast stock from fish found to the west in the Arafura Sea region of the Northern Territory, while otolith microchemistry data were inconclusive.

In this study we also used an integrated approach consisting of a complementary set of different techniques simultaneously applied to the same specimens sampled across the range of the species in Australia with the objective of discriminating stocks of grey mackerel over their entire Australian range. The techniques used were genetics (distribution of mitochondrial DNA and microsatellite DNA genotypes), otolith microchemistry (stable isotope ratios), and parasites (species and loadings), and comparisons were made between the same samples of fish collected from multiple locations determined by areas of highest commercial fishing effort. As a value-adding exercise to the project we also utilised the samples collected as an opportunity for a student project to generate estimates of growth for grey mackerel from each of the major regions as an additional technique for stock discrimination.

The project therefore set out to identify the appropriate spatial units of management for grey mackerel based on stock structure across northern Australia. Given that three separate management jurisdictions span the range of grey mackerel we also set out to identify where joint management arrangements between relevant agencies may be appropriate. In this report the results of each component of the study are laid out in individual chapters beginning with the current chapter which draws on the information used for the original proposal and presents the general project approach and

methodology. Chapter 2 presents biological information derived from the project samples to provide useful comparative information to that of Cameron and Begg (2002) and to maximise information content beyond the original project design. Chapter 3 presents the variation in growth rates between regions and discusses how these can be used to determine stock structure and forms the basis of a BSc (Honours project). Analyses of genetic information derived from mitochondrial DNA (mtDNA) and microsatellite DNA (msDNA) loci, provide complementary information on the current and past relationships between grey mackerel populations on an evolutionary time-scale and this is presented in Chapter 4. Otolith isotope ratio analyses (Chapter 5) and parasite analyses (Chapter 6) were used as different techniques that provided independent tests of finer scale stock structure and at temporal scales relevant to fisheries management. Chapter 7 provides a summary of the integration of all the different methods used to identify the appropriate management units for grey mackerel.

1.2. Background

Knowledge of the spatial dynamics of targeted marine fish species is essential for providing a framework for effective natural resource management. Of the northern Australian mackerel species, all which underpin important fisheries, this information is least understood for grey mackerel (*Scomberomorus semifasciatus*). Fundamental information on stock structure is therefore required so that the management interventions of grey mackerel fisheries in Queensland, the Northern Territory and Western Australia, are better informed and provide greater certainty in ensuring sustainable management. That is, where there exist discrete or semi-discrete units of grey mackerel, on which fishing effort is imposed, then ensuring the continued future harvest requires that management interventions reflect the level of harvest from that unit in the context of their biological attributes. It is this unit of individuals that we refer to as a stock. It is also important to define what a "stock" is, and although the literature abounds with such definitions (see Kutkuhn 1981; Waldman 2005), it is the questions being addressed that should dictate what that definition is (Buckworth et al. 2007). We set out here to define the management units for grey mackerel fisheries. This dictated that our definition of a stock, which is derived from the definition proposed by Hilborn and Walters (1992), was: a semi-discrete group of fish that are essentially self-reproducing with similar biological attributes. These groups of fish may or may not be genetically

distinguishable, but have other measurable differences that are essentially a gauge of their behavioural distinctiveness; they might be expected to respond differently to management. This project used a suite of complementary stock identification techniques designed to provide the necessary information on the spatial structure and effective management units of northern Australian grey mackerel populations that is required for management of this highly valued resource.

Grey mackerel are endemic to northern Australian waters and are an important targeted species taken across northern Australia predominantly by commercial offshore gill netters. They are also a highly prized light game fish in the rapidly-growing recreational and fishing tourism sectors, especially in the Gulf of Carpentaria. During the late 1990s, most of the Australian catch of grey mackerel was taken in the Gulf of Carpentaria by Queensland and Northern Territory commercial gillnet fishers. During this period the national commercial harvest of grey mackerel was approximately 800 tonnes and worth $6 million per year; thereby, adding significant value to the $12 million annual catch of northern sharks. In fact, during this period grey mackerel was the dominant single species in catches from the Northern Territory pelagic gillnet fishery and the Queensland Gulf N9 offshore set mesh net fishery. Coupled with these increased commercial catches was the establishment of valuable domestic and overseas markets for premium product. More recently annual commercial harvest of grey mackerel nationally have increased to approximately 1050 t (2006) largely due to increases in harvest levels on the Queensland east coast and in the Northern Territory.

Despite its importance to the commercial fishery, surprisingly little is known about the biology and stock structure of grey mackerel in northern Australian waters, where the understanding of this species is largely restricted to the FRDC-funded study of Cameron and Begg (2002). This study provided some information on grey mackerel, primarily in Queensland east coast waters. Further research was required to extend this knowledge westwards to provide information for profitable and sustainable management and to respond to the requirements of the Commonwealth *Environment Protection & Biodiversity Conservation* (EPBC) *Act 1999*. Information on grey mackerel is important to the EPBC Act export accreditation process for all the fisheries of which it is a component species. Studies into stock structure are an important step in the process for improving the basis for northern Australia fisheries management, and are a prerequisite for the

integrated multi-jurisdictional management arrangements being promoted by the Northern Australian Fisheries Management Forum (NAFMF).

The spatial extent of grey mackerel populations and the degree of interchange between them in Australian waters is unknown, although there is some evidence for large scale stock differences. Allozyme electrophoresis indicated that central Queensland east coast grey mackerel are genetically distinct from more westerly populations in the Arafura Sea and Gulf of Carpentaria (Cameron and Begg 2002). However, the relationships among grey mackerel populations in the Queensland Gulf of Carpentaria and Northern Territory waters are unknown. In both jurisdictions, grey mackerel fisheries are subject to management regimes designed to contain fishing effort. Nevertheless, commercial, and probably recreational, fishing effort has seen rapid growth in recent years.

The impetus for this project was provided by the FRDC-funded strategic review of northern Australian mackerel undertaken by Ward and Rogers (2003). In this review, it was recommended that research into the stock structure of grey mackerel across its Australian range be considered essential and of the highest priority for effective and cooperative fisheries management, especially where stocks may be shared between jurisdictions. It was also recommended that the techniques applied in FRDC Project 1998/159 for Spanish mackerel stock identification are adopted for research into the stock structure of grey mackerel; different from those used by Cameron and Begg (2002).

This project, therefore, firstly tested the hypothesis of broad scale spatial stock structure for grey mackerel in Queensland and Northern Territory waters. Secondly, the project tested finer scale spatial stock structure in areas of high fishing effort. As was recommended, we used the techniques applied in the FRDC Project 1998/159 on Spanish mackerel (genetic analyses, otolith microchemistry and parasite incidence) to provide a robust approach for investigating the northern Australia grey mackerel stock structure. This multi-technique approach follows the dictum of the FRDC-funded workshop in July 1997, "Taking Stock: Defining and Managing Shared Resources" (Hancock 1998), which concluded that an analysis of stock structure is most effective if several techniques are used because of the different population and temporal scales addressed by each. Genetic analyses typically identify stocks on large spatial and

temporal scales, where gene flow is minimal. In contrast, otolith microchemistry and parasite incidence reflect residence and movements of fish in different ways, and may be used to resolve a genetically homogeneous population into discrete units of adult fish that may be more appropriate for management (Buckworth 1998).

While responding to the strategic direction for priority mackerel research provided by the review of Ward and Rogers (2003), this project also addressed a long-standing and high priority requirement for resource status information driven by the Queensland Gulf of Carpentaria Fisheries Management Advisory Committee, the Queensland Fisheries Joint Authority and the Northern Territory Fisheries Joint Authority. During the development of the project strong support was received from the NAFMF (August 2003 and September 2004) with the recognition of the project's importance across northern Australian jurisdictions. The project was developed with the participation of fishery management authorities in Queensland (Department of Primary Industries and Fisheries), the Northern Territory (Department of Regional Development, Primary Industry, Fisheries and Resources) and Western Australia (Department of Fisheries), as well as industry groups in the Gulf of Carpentaria and along the Queensland east coast.

During the project implementation there were two developments that resulted in changes to the project's experimental design, particularly the sample collection and analysis schedules. The first change arose in 2006 when management of grey mackerel fisheries in WA introduced a grey mackerel Total Allowable Commercial Catch (TACC), effectively creating a new fishery. This greatly increased the potential for expansion of grey mackerel harvest in WA and made the inclusion of WA samples in the stock structure analyses a greater priority. The FRDC agreed and provided extra funding of $21,250 in late 2006 for the collection and inclusion of WA samples in the suite of analyses being carried out. The second development came about due to increasing local community concerns about the sustainability of grey mackerel on the Queensland east coast, primarily in the Port Douglas region. This necessitated several community and industry meetings that were attended by the PI and extra sample collections and analyses were carried out for the Port Douglas region (Snapper Island) across the respective analytical techniques. Funding for inclusion of these samples was provided by the QDPI&F in 2007.

1.3. Need

Queensland, the Northern Territory and Western Australia legislations require sustainable harvest of fish resources and their optimal use. Reliable and robust information on the status of fished resources are central to achieving these outcomes. Each jurisdiction has its own management regime for the mackerel fisheries in their corresponding waters. The lack of information on stock structure, however, means that the appropriate scale of management is not known. As well, fishers require assurance of future sustainability to encourage investment and long-term involvement in a fishery that supplies lucrative overseas markets. These management and fisher-unfriendly circumstances must be viewed in the context of recent 3-fold increases in catches of grey mackerel along the Queensland east coast, combined with significant and increasing catches in other parts of the species' northern Australian range. Such a scenario highlighted the urgent need for information on the stock structure of this species.

At its August 2003 meeting, the NAFMF signalled its intention to move from single jurisdiction-based fishery management towards a more integrated approach that reflected the management needs of species across their northern Australian range. In 2004, NAFMF progressed this undertaking for grey mackerel, with the development of an operational plan for sustainable harvest across northern Australia. In order to obtain the maximum benefit from this initiative, the underlying stock structure of grey mackerel had to be established. Furthermore, this project was consistent with the strategic directions of the Northern Territory Strategic Plan for Fisheries Research and Development, particularly those directives related to the sustainable harvesting of fish and other aquatic resources, and the optimum utilisation of fish and aquatic resources.

1.4. Objectives

1. To determine the spatial and temporal stock structure of grey mackerel over its northern Australian range.
2. To use stock structure information in defining the geographic framework and appropriate management units required by Queensland and Northern Territory fisheries agencies for sustainable management planning of grey mackerel resources.

1.5. Methods

This section provides an overview of the sampling approach and methods used during this project. Detailed methods for the respective analysis techniques are provided in the individual chapters of this report.

The approach taken was based on the management questions behind the project development, which largely dictated the sampling design (ie. when and where fish samples were taken for inclusion in the analysis regime). Since the vast majority of grey mackerel catches comes from the commercial sector, one of the main driving factors behind the development of this project was the management concerns of increased commercial targeting of grey mackerel (along with shark) in the Gulf of Carpentaria, and as such was deemed to be an area of focus for the identification of grey mackerel stocks. Also considered important was identification of whether the major commercial grey mackerel fishery areas across all state and commonwealth managed jurisdictions in northern Australia should be considered separate management units or, alternately, that these jurisdictions needed to adopt joint management in some areas. Sampling was therefore based on the major commercial fishery areas and as such utilised commercial fishery operations. As likely stock scenarios were also uncertain we also adopted a phased or exploratory approach to sampling (Abaunza 2008 – 104-113); an approach proven to be successful for *S. commerson*, a similar species to grey mackerel also with a tropical northern Australian range (Buckworth et al 2007). The major phases of the project, of which the first two were sampling phases, were:

PHASE 1 (Year 1): Broad spatial scale genetic and environmental influenced differences in grey mackerel populations were established over their northern Australian range using the Spanish mackerel stock identification methodology (Buckworth et al 2007) as recommended by Ward and Rogers (2003). Support for the notion of separate stocks would justify going to Phase 2, otherwise the project would cease after Phase 1.

PHASE 2 (Year 2-3): Finer spatial and short-term (inter-annual) temporal scale resolution of grey mackerel stocks were investigated at an increased number of locations.

PHASE 3 (Year 3): Project results were finalised across analysis methods and the management units for grey mackerel in northern Australia were defined in collaboration with the project team and fisheries managers from each of the major jurisdictions.

The project used three basic techniques to examine grey mackerel stock structure: 1) mitochondrial DNA and microsatellite genetic analyses; 2) whole otolith solution based microchemistry; and 3) parasite incidence. Growth parameters were also used as an additional method for determining stock structure. In the first year of the project (Phase 1), these techniques were applied to establish if broad spatial scale structural variation existed across the major fishing grounds, through the collection of samples from four primary locations in Queensland (East Coast and the Gulf of Carpentaria) and the Northern Territory (Gulf of Carpentaria and the North-West Coast). The east coast samples were initially required to provide an updated reference point for potential Gulf and Northern Territory stocks, and to compare results from the previous FRDC Project 1992/144 which was undertaken in the early 1990s (Cameron and Begg 2002). The samples were collected from commercial fishers and used to provide material for genetic, otolith and parasite analyses.

As the first year results supported the notion of separate stocks of grey mackerel, in the second year we undertook an extended sampling program to describe finer spatial scale population structure and temporal (inter-annual) variability in the short-term. This included sample collections from Western Australia as well as an additional sample collection from the Port Douglas region on the northeast Queensland coast. The Port Douglas region was explicitly added to the project experimental design due to emerging concerns from the local community that grey mackerel in the local area represented a separate stock from other parts of the Queensland east coast, and that the current harvest level of this stock was unsustainable. This resulted in four major regions for sample collection and included a total of 12 locations. The regions were: the Queensland east coast (4 locations overall), the Gulf of Carpentaria (6 locations), north western Northern Territory (1 location), and Western Australia (1 location) (Figure 1.1). Table 1.1 lists all of the locations sampled during the project and the acronyms used to describe the locations throughout this report.

Table 1.1. Summary of the locations sampled and their broad scale regions, the acronyms used to describe these sample locations, and the date/s they were sampled.

State	Region	Location	Location code	# fish sampled	Month/year sampled
WA	West Coast	Port Hedland	WA	40	08/2006
NT	North West Coast	NW coast	NW NT	50	05/2005
				50	08/2005
				26	10/2006
				81	04/2007
NT	Western Gulf of Carpentaria	Mid	WG mid	50	09/2005
				15	10/2006
NT	Western Gulf of Carpentaria	South west	WG SW	35	11/2006
QLD	Eastern Gulf of Carpentaria	South west	EG SW	53	03/2007
QLD	Eastern Gulf of Carpentaria	South east	EG SE	50	03/2007
QLD	Eastern Gulf of Carpentaria	Mid	EG mid	197	09/2005
				49	09/2006
				53	03/2007
QLD	Eastern Gulf of Carpentaria	North	EG N	146	05/2007
QLD	East Coast	Port Douglas	EC PD	58	08/2007
QLD	East Coast	North	EC N	17	11/2006
QLD	East Coast	Mid	EC mid	401	10/2005
				183	10/2006
QLD	East Coast	South	EC S	38	12/2005
				40	09/2006

At the completion of each Phase, the project results were assessed by the project team and progress reported to the FRDC, Northern Australian Fisheries Managers Forum (NAFMF), relevant Management Advisory Committees and other stakeholder groups. These progress reports included management responses to the information generated along the project timeline, directly linking research findings with management outcomes. Assessment of project progress was facilitated by annual team meetings held centrally in Darwin. At the final project workshop held in May 2008 the project team was able to integrate all components of the data analyses to identify grey mackerel management units. This final workshop was attended by fisheries managers from each jurisdiction, further facilitating direct transfer of research results to management outcomes.
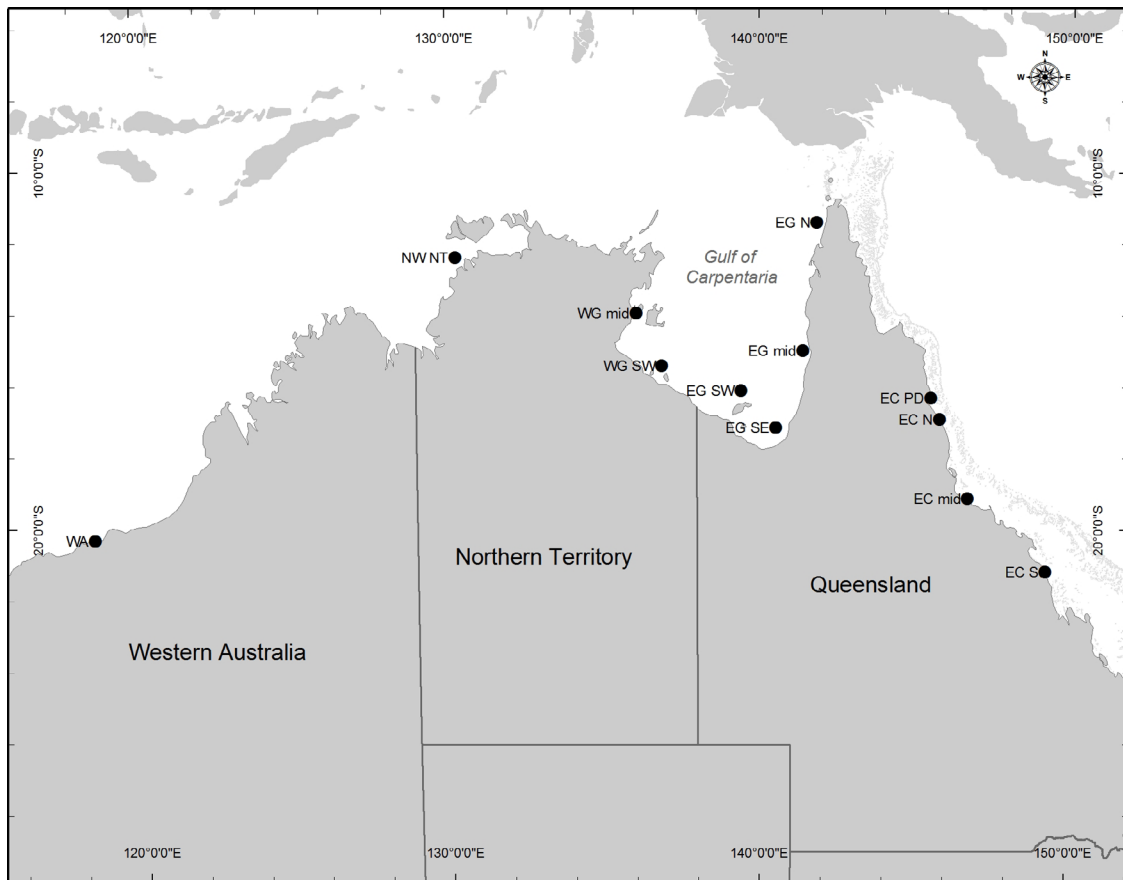
Figure 1.1. Map of northern Australia showing the locations sampled during the study.

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2. NORTHERN AUSTRALIA GREY MACKEREL FISHERIES

JM Stapley and DJ Welch

2.1. Fishery description

To describe the northern Australian grey mackerel fishery we present the catch characteristics for each northern Australian jurisdiction. For fine spatial scale interpretation of fishery data each jurisdiction is divided into fishery regions based on historic boundaries and physical coastal features such as peninsulas (Figure 2.1). For each of these regions we present annual catch data as well as seasonal catch data that may give understanding of the characteristics of the northern Australian grey mackerel fishery at regional scales. Further, catch histories examined at regional scales may be ascribed to particular stocks identified during the course of this project. Effort data is not presented due to difficulties in standardisation within and among jurisdictions.
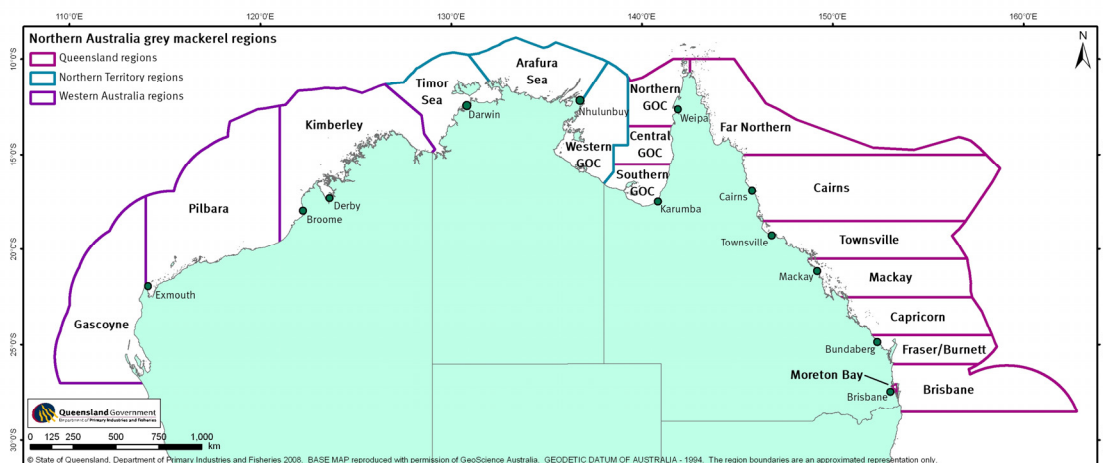
Figure 2.1. Regions used across Western Australia, the Northern Territory, the Queensland Gulf of Carpentaria and the Queensland east coast in presenting catch characteristics for northern Australian grey mackerel fisheries.

2.1.1 Queensland East Coast

Grey mackerel extends the length of the eastern coast of Queensland (Qld) and commercial mackerel landings are mainly from the East Coast Inshore Fin Fish Fishery (ECIFFF). The ECIFFF is a multi-species highly complex fishery, currently divided into six sub-fisheries. The small mackerel and shark sub-fisheries target grey mackerel intensively; in particular the offshore mesh net (>300t harvest in 2006 and 2007). The mesh net fisheries predominantly use 160-165mm mesh size for targeting grey mackerel. A small amount of grey mackerel is also landed from the pelagic troll line fisheries on the east coast of Qld, which contributed approximately 2 percent of the total east coast annual grey mackerel catch over the last seven years.

Management arrangements for the east coast inshore finfish fishery have undergone recent reviews, and changes will be implemented in early 2009. The results of the community consultation phase of the Regulatory Impact Statement (RIS), in particular, will determine specific management policy. Prior to this, no change to management had occurred to the grey mackerel take directly, except Great Barrier Reef Marine Park Authority (GBRMPA) closures (the Representative Area Program or RAP) that may have displaced some fishing effort. Various area and temporal closures also exist on the east coast, but none directly impact grey mackerel fisheries. Effort days where grey mackerel are targeted have quadrupled from 2000 to 2007 on the Queensland east coast.

The Queensland east coast was divided into 7 regions for fine scale investigation (Figure 2.1). Catch landings from the Commercial logbooks (CFISH) indicate that catches of grey mackerel have risen back to 1990 levels and are well in excess of 200t. The Townsville region has been the major contributor to grey mackerel landings along the entire east coast (Figure 2.2). However, no attempt was made to analyse the catch component of the logbook data reported as 'unspecified mackerel', and the potential grey mackerel component of this component is not included in Figure 2.2. Cameron and Begg (2002) estimated the grey mackerel component of the 1995 'unspecified mackerel' was 40%, which would contribute another 5t for that season. The logged 'unspecified mackerel' contribution was the largest between the early 90s to early 2000s, for both Line and Net sectors. Since 2004, the annual logged 'unspecified mackerel' component has significantly declined, especially in the Net fishery sector. A contributing factor to this would be the strong and stand-alone market established for grey mackerel since the late

1990s, which was mainly driven by the large catches in the Gulf of Carpentaria and specific marketing of this species by those commercial fishers. Also, the QDPI&F had floated the idea of further regulation in grey mackerel fishing, and operators were keen to establish a history of catch in the fishery.

Figure 2.2. Annual commercial net and line catches of grey mackerel from 1988 – 2007 for each region of the Queensland east coast.[1]

In the early years of logbook reporting a large proportion of the east coast catch was taken in the Fraser/Burnett region. Townsville however has been consistently the major fishery region for grey mackerel throughout the time series, although catch has increased in recent years for the Cairns, Mackay and Capricorn regions (Figure 2.2). For the east coast of Queensland, across the years 2005 and 2006, the average catch of grey mackerel per day was 767 kg for net and 183 kg for the line fisheries. Log book data indicate that in most regions grey mackerel were captured all year round; however there is very strong seasonality in the fishery with September and October the major months driven primarily by catches in the Townsville region (Figure 2.3). From regional monthly average catches for net and line (Figures 2.3 & 2.4 respectively) over the time series, peak catches in the Cairns region appear during the June-September period, whereas catches in the Fraser/Burnett region are relatively consistent throughout the

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[1] NB. 'Unspecified' in Figures refers to logbook grey mackerel catch records where no location data were provided.

year. Most other regions show peak catches in the months of September and October similar to Townsville.

Figure 2.3. Seasonality in commercial net catches of grey mackerel from each region of the Queensland east coast. The y-axis gives mean monthly catches in tonnes pooled across years.
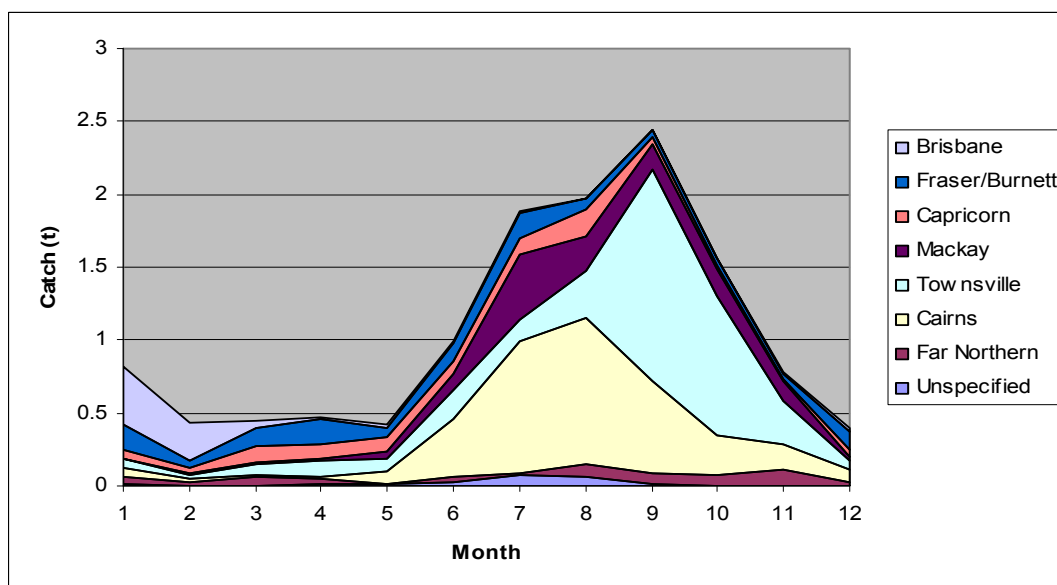
Figure 2.4. Seasonality in commercial line catches of grey mackerel from each region of the Queensland east coast. The y-axis gives mean monthly catches in tonnes pooled across years.

Catches and CPUE can be affected by fisher business decisions and accessibility to the resource, particularly due to weather constraints, and therefore may not represent the true dynamics of the grey mackerel population. CFISH data only indicate the potential of local and migratory and/or aggregation aspects for the grey mackerel population dynamics along the east coast of Queensland.

Based on logbook returns for the years 1995 – 2007 the charter or fish tour operator catch of grey mackerel was less than 0.01 t per year retained, although modelled estimates of grey mackerel charter catches within those recorded as 'unspecified mackerel' would bring this estimate to approximately 0.10 t per year (Begg et al. 2005). The recreational retained catch is greater than charter take, with estimates of 12 t for 1995 on the east coast (Cameron and Begg, 2002) and a Qld wide estimate of 19 t for 1999 (Williams 2002). Assuming similar catch characteristics and ratio of commercial to recreational catch has continued on the east coast, and applying the numbers provided in the McInnes (2008) report; grey mackerel retained could be roughly estimated at 26t for 1997, 14t for 1999, 4t for 2002 and 29t for 2005 by recreational fishers along the east coast.

2.1.2 Queensland and Northern Territory Joint management arrangements

For management purposes, grey mackerel in the Gulf of Carpentaria (GoC) are considered to be a shared resource with jurisdiction split between the Australian Commonwealth Government and two states; Northern Territory and Queensland. The mackerel stocks of the Gulf of Carpentaria have been fished commercially since the early 1960's. The Northern Territory (NT) identified and endorsed a separate "Shark/Mackerel" fishery in the early 1980's, but Queensland only officially initiated a limited entry offshore N9 "Shark" fishing endorsement in 1999. Both these net fisheries target grey mackerel intensively, using 160-165mm mesh size. The fishery(ies) interact between the two States, with some operators' licensed/endorsed in both jurisdictions, and with the fishing effort of the combined fishery being driven by the local market forces in either State. A small amount of grey mackerel is also landed from the line fisheries in the GoC.

Prior to the 1986 Offshore Constitutional Settlement (OCS) Agreement, which formed the Gulf Northern Territory Fisheries Joint Authority (NTFJA) and Queensland Fisheries

Joint Authority (QFJA), the shark and mackerel fisheries were controlled by the Commonwealth and reported to the Australian Fisheries Management Authority (AFMA). Due to an oversight at the time, grey mackerel was not listed as a state managed species in the OCS therefore management defaulted to the Commonwealth; that is, pre-OCS arrangements applied. Not only did the states not control the exploitation of the grey mackerel stock, they had no knowledge of, nor responsibility for establishing, the status of a fished stock that was caught across both State jurisdictions. In 2003 a resolution was made that grey mackerel would be jointly managed between the States and the Commonwealth, via the NTFJA and QFJA through permits to take the species in state waters.

2.1.3 Queensland Gulf of Carpentaria

For the purposes of this report the Qld GoC was divided into three regions for fine scale investigation; northern, central and southern regions (Figure 2.1). Catch landings from the Qld GoC commercial logbooks indicated a significant rise in grey mackerel landings from the mid 1990s onwards, with recent catches in excess of 600t (2007). Effort days towards grey mackerel from 2000 to 2007 have risen by approximately 30 percent, and a contributing factor to this increase was the diversification of inshore barramundi fishers utilising the offshore resources at limited times during the season. A four month netting closure, in line with barramundi spawning, exists in the Qld-managed GoC fisheries, usually from October to January, and extends out to 25 nautical miles from the shore.

Historically most of the grey mackerel catch from the Qld GoC has come from the central region however in the past two years catch has increased dramatically in the north with a concomitant decrease in catch from the central region (Figure 2.5). Historically the annual 'unspecified mackerel' logged take was minor in both line and net Qld GoC fisheries (<4t).
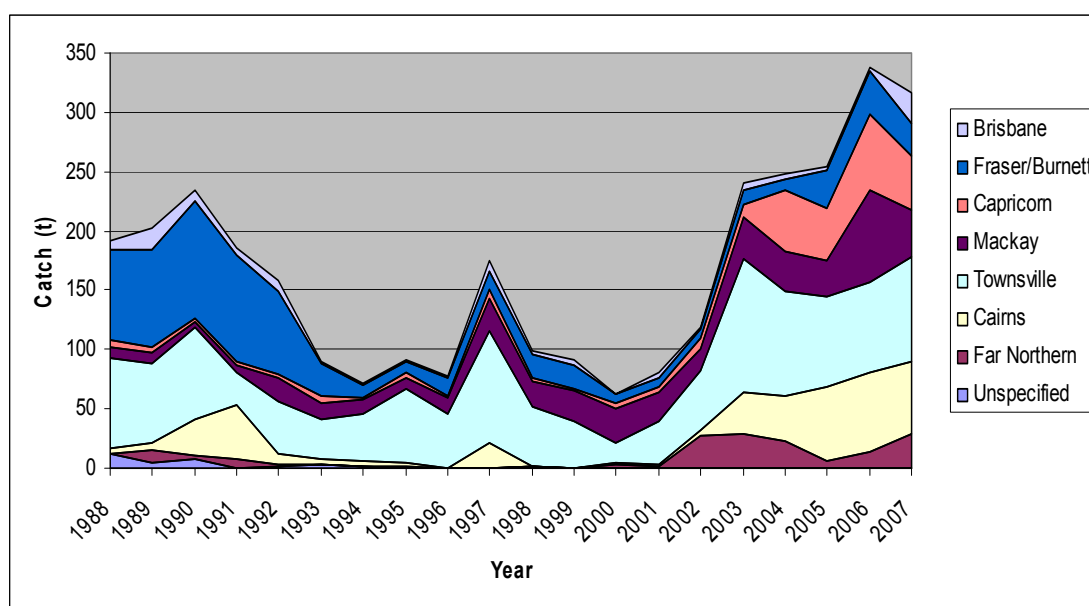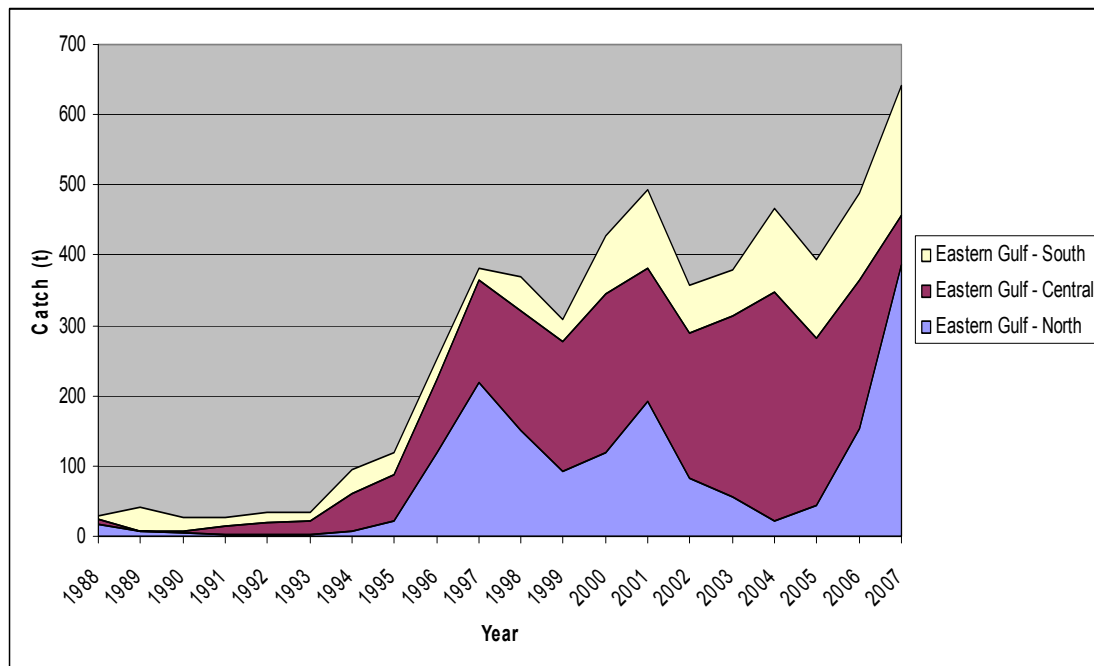
Figure 2.5. Annual commercial net and line catches of grey mackerel from 1988 – 2007 for each region of the Queensland Gulf of Carpentaria.

Across the years 2005 and 2006 the average catch of grey mackerel landed per day was 1.323t for net and 0.130t for the line fisheries. Average monthly catches for net and line (Figure 2.6 & 2.7 respectively) over the years 1988 - 2007 indicates that grey mackerel were captured all year round with a very strong overall seasonal peak evident for the months of August and September. This peak is driven primarily by catches in the northern region and to a lesser extent catches in the southern region. Most of the catch taken in the central region is during the May – August period. However, note that a seasonal closure exists for State net fisheries, whereas no such closures have been placed on State line and Joint Authority net fisheries that operate in Qld waters. The average catch per unit effort peaks were skewed by one month later for each region, compared to the catch landings. Thus data from CFISH indicates possible local migratory and/or aggregation aspects for the grey mackerel population dynamics along the eastern side of the GoC. Based on logbooks from 1995 – 2007 the charter or fish tour operator catch of grey mackerel was less than 0.15t per year retained. The recreational and indigenous take are unknown.
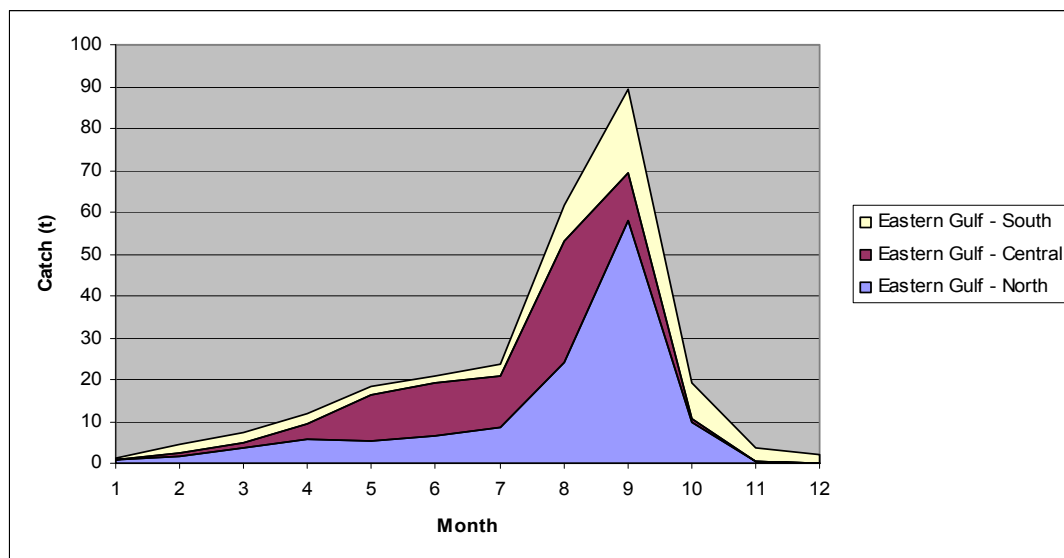
Figure 2.6. Seasonality in commercial net catches of grey mackerel from each region of the Queensland Gulf of Carpentaria. The y-axis gives mean monthly catches in tonnes pooled across years.
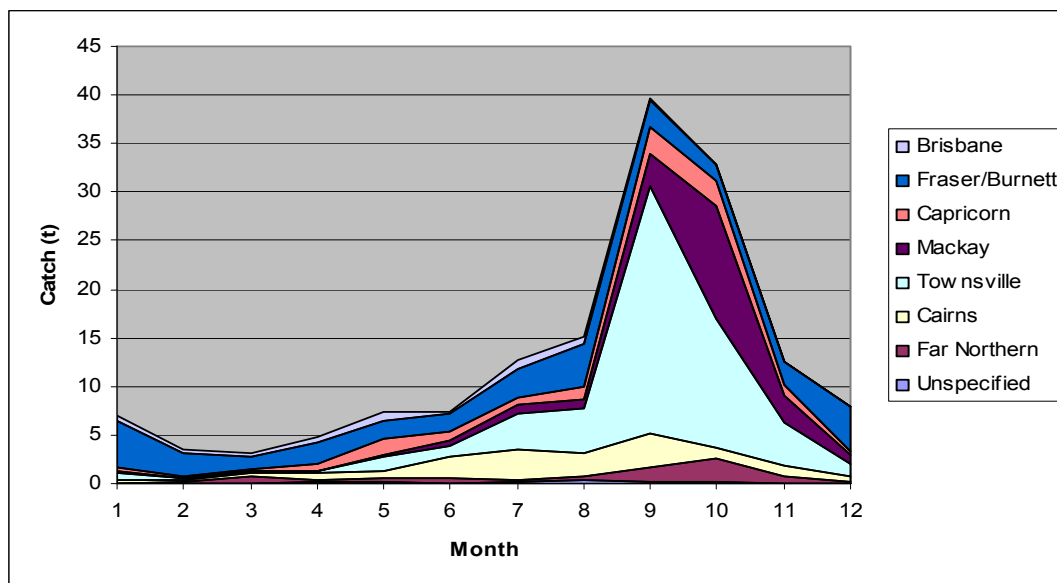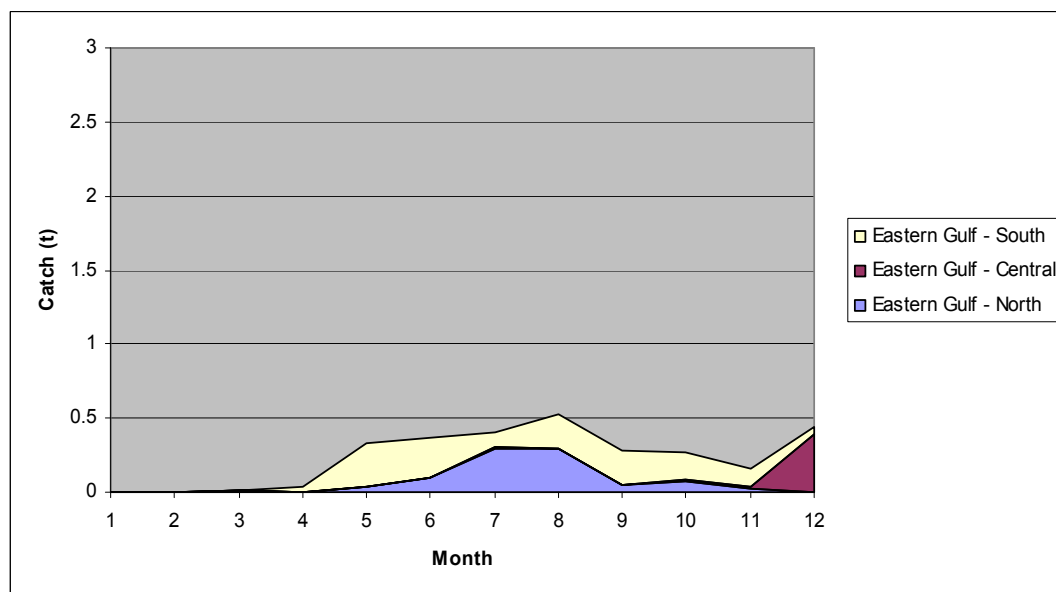

Figure 2.7. Seasonality in commercial line catches of grey mackerel from each region of the Queensland Gulf of Carpentaria. The y-axis gives mean monthly catches in tonnes pooled across years.

2.1.4 Northern Territory

The Northern Territory was divided up into three regions for similar fine scale

investigation: Timor, Arafura and western GoC (Figure 2.1). Catch landings from NT

commercial logbooks indicated a significant rise in grey mackerel landings from the late 1990s onwards, with recent catches fluctuating from a peak in 2003 of 760t and then progressively dropped to 240t for 2007 (Figure 2.8). This drop in catch is a reflection of management changes in NT towards reducing fishing effort in the offshore net and line fishery. The dataset incorporated the effects of an effort reduction program which included a three for one licence reduction scheme, setting of an annual cap in effort days, reducing the total net length and changes in mesh size. Historically the NT Timor and Arafura regions were the major catch regions. However these regions seem to be the most affected by the recent effort-reduction management changes.
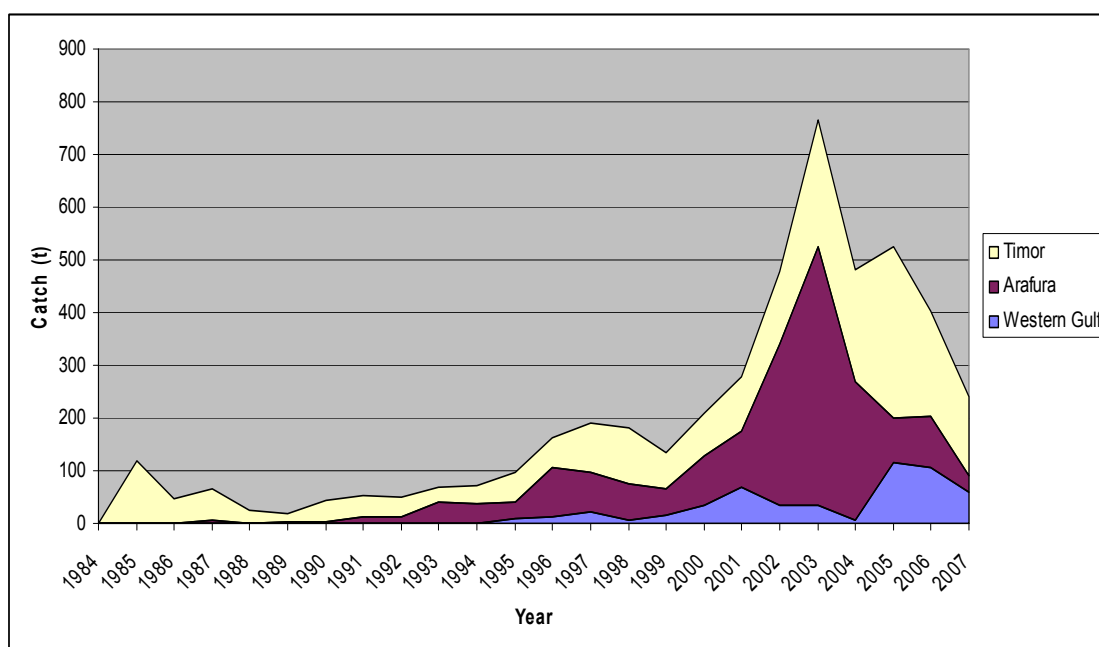
Figure 2.8. Annual commercial net and line catches of grey mackerel from 1984 – 2007 for each region of the Northern Territory.

From the 2005 and 2006 period the average catch of grey mackerel landed per day was 0.452t for the offshore net and line fishery predominantly from net fishing. Logbook monthly catches for the fishery averaged across the years 1984 – 2007 indicates that grey mackerel were captured all year round with varying seasonal peak catches within regions (Figure 2.9). Overall there is an extended grey mackerel season across the months of April – November. In the Timor region catches peak during August to November; April to August in the Arafura region; and October to November in the

western GoC region. However, the majority of the western GoC catches have been from recent years and the monthly catch dynamics are more than likely skewed from Qld fishers entering NT waters during Qld GoC fishing closures. As no temporal closures exist in NT, the fishery operates on capped effort and "fishing days" which can be utilised anytime during the season.

As stated previously, catches and CPUE, notwithstanding hyperstability, can be altered by fisher business decisions and accessibility to the resource. For the NT, the grey mackerel catch variations are driven by market and operational forces, rather than grey mackerel catchability (Fishery Status Report 2006).
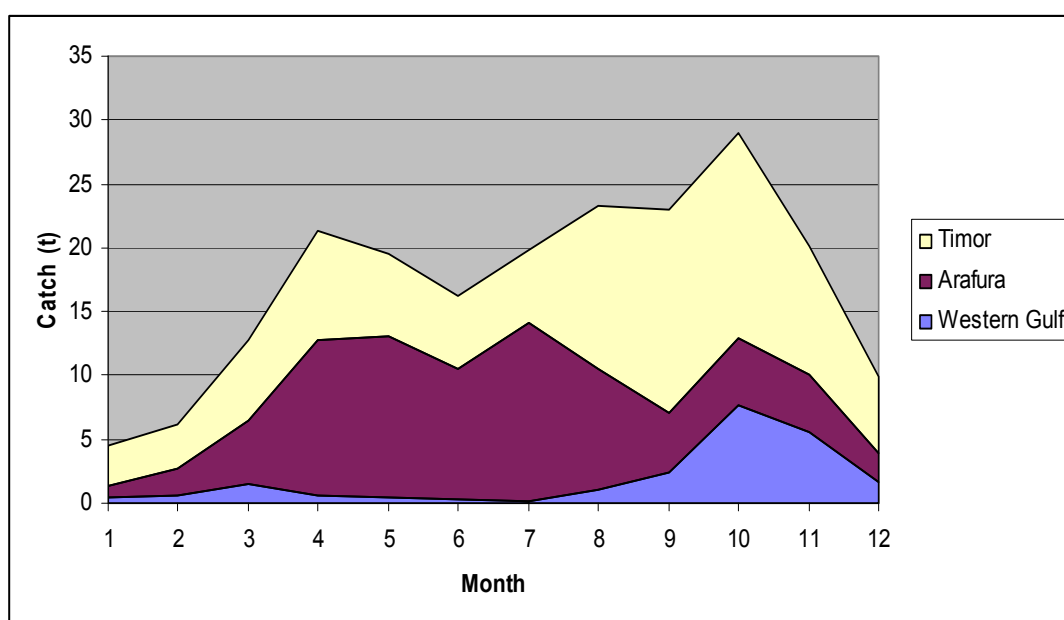
Figure 2.9. Seasonality in combined commercial net and line catches of grey mackerel from each region of the Northern Territory. The y-axis gives mean monthly catches in tonnes pooled across years.

The charter or fish tour operator catch of grey mackerel was not available but is assumed to be low. The estimated retained recreational catch of grey mackerel caught every year in NT has been estimated to be approximately 8,400 fish (Crofts and de Lestang, 2004; Coleman, 2004). With an assumed average grey mackerel recreational harvest weight of 3kg (usually 1-5kg) this puts annual recreational harvest of approximately 25t from NT waters.

2.1.5 Western Australia

Similar to the other states, Western Australia (WA) was divided up into three operational regions; Pilbara, Kimberley and Gascoyne (Figure 2.1). Catch landings from WA commercial line and net logbooks began recording grey mackerel in 2000, and are low (Figure 2.10) when compared with landings for the corresponding fisheries in Qld and NT waters. Grey Mackerel catches in WA have been declining from the 25t peak in the early 2000s due to new interim management arrangements implemented during 2004. This included the reduction in vessels numbers in each region with mackerel endorsements, the introduction of a 6 month closed season, a compulsory logbook program to record all commercial mackerel catches and a grey mackerel quota of 60t for each of the 3 regions.
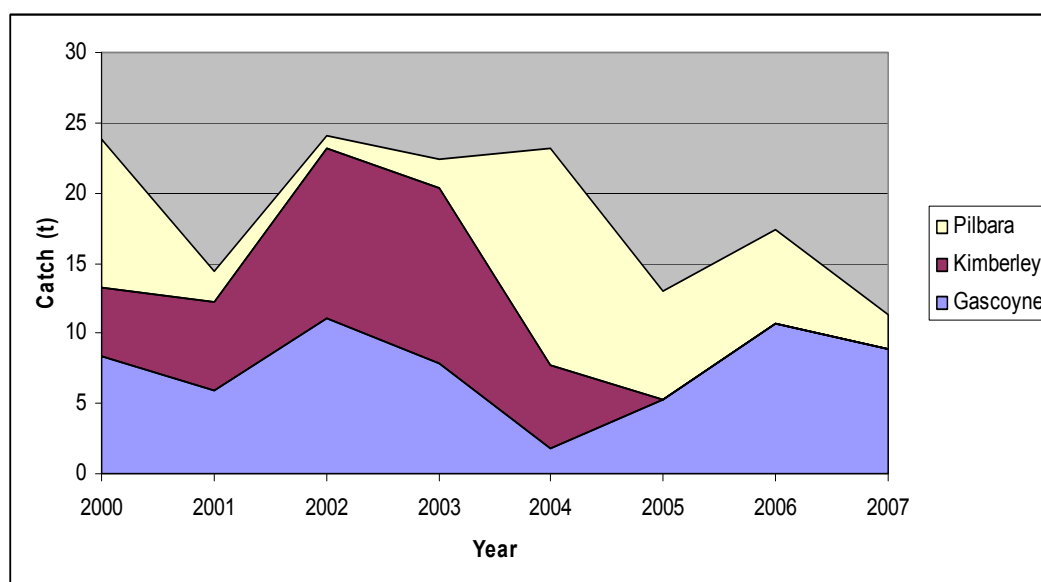
Figure 2.10. Annual commercial net and line catches of grey mackerel from 2000 – 2007 for each region of Western Australia.

Prior to the new management arrangements, the Kimberley region contributed the largest proportion of the grey mackerel catch (38%). The significant decrease in catch thereafter may be a result of the remaining boats targeting only Spanish mackerel during the reduced season to get their quota as grey mackerel attract a lower price. In the Pilbara a small number of commercial boats have continued to target grey mackerel, especially when they appear in large numbers at certain times of the year. For the Gascoyne, the collapse of the whole fish export market from Carnarvon in 2003 meant

that greys were no longer targeted and effort has been reduced; but catches have since returned to previous levels.

For the years 2005 and 2006 the average catch of grey mackerel per day was 0.112t combined for the offshore net and line fisheries; predominantly from line trolling. The overall seasonal catch data for grey mackerel (Figure 2.11) shows a distinct seasonal pattern for all regions but it must be noted that seasonal closures have been in place since 2004; from Oct-Feb (Gascoyne) and Oct-May (Pilbara), which may have had some influence on the pattern. Overall the peak season is from June - September, however the Kimberley region has a more protracted season from approximately May - November.
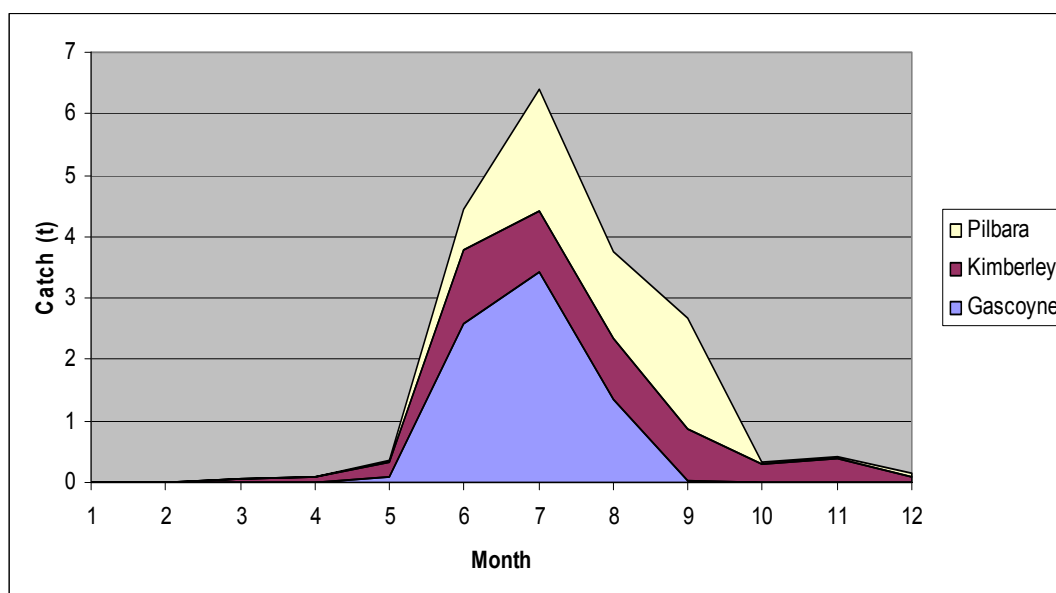
Figure 2.11. Seasonality in combined commercial net and line catches of grey mackerel from each region of Western Australia. The y-axis gives mean monthly catches in tonnes pooled across years.

The charter or fish tour operator catch of grey mackerel has been reported as negligible for all years and in WA it is reported separately from other mackerel species. The recreationally captured grey mackerel were not reported in the West Coast recreational survey from Augusta to Kalbarri in 2005-06 or in the Gascoyne 1998/99 survey. However, in the 1999-2000 Pilbara survey, the recreational catch included approximately 5 t of grey mackerel.

2.2 Fishery characteristics summary

The catch levels of grey mackerel fisheries differ among the different jurisdictions of northern Australia with the Northern Territory and the Gulf of Carpentaria supporting the largest catches where the predominant capture method is netting, although catches on the Qld east coast have increased in recent years. Catches in Western Australia in comparison were low where trolling using hook and line is the predominant method. Management changes during the respective catch time series appear to have influenced catches of grey mackerel. In Western Australia, since management changes were implemented in 2004, reported catches have declined despite not achieving the allowable quota. The increasing importance of grey mackerel products as markets have opened up is evidenced by dramatic increases in catches at various times since the mid 1990s in the Northern Territory, the Gulf of Carpentaria, and more recently on the Qld east coast. As discussed in Chapter 1, it is these increases that were a major impetus for this project. Seasonality also varies among the jurisdictions and among the regions within each jurisdiction. The Qld east coast, the Gulf of Carpentaria and Western Australia appear to have fairly distinct major seasons for catching grey mackerel across winter and spring; however the most northern regions in the Northern Territory do not. In many regions (for example much of the Queensland east coast) it is known that this is largely driven by seasonal availability of the fish due to movement and aggregating behaviour. This does not appear to be a factor in the Northern Territory. However as has been discussed earlier in this chapter, there are other contributing factors that influence seasonality among the different regions including market value, weather and access to the fishing grounds, and fishers shifting between different fisheries to target other species at various times. The vast majority of grey mackerel harvest in all jurisdictions of northern Australia is by the commercial sector.

2.3 References

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3. NORTHERN AUSTRALIA GREY MACKEREL BIOLOGY

DJ Welch and AC Ballagh

3.1 Introduction

Grey mackerel, *Scomberomorus semifasciatus* (Macleay, 1884), is one of several species of mackerel (Family Scombridae) that are popular with commercial, recreational and indigenous fishers throughout northern Australia. The species is endemic to the northern Australian region and ranges from Moreton Bay in south east Queensland, north along the Queensland coast to the southern parts of Papua New Guinea, and then west across the top of northern Australia to Shark Bay on the mid Western Australian coast line (Collette and Nauen 1983). Its known preferred habitat is inshore in the often turbid waters of tropical and sub-tropical areas where they feed on pelagic baitfish of sardines and herrings, and so become seasonally available to fishing operations. At certain times of the year they can also be found around rocky headlands and inshore reefs (D. Welch, pers. obs.). Larval and juvenile life history stages of grey mackerel are found inshore, often in estuarine environments, where they feed almost exclusively on other larvae with prey sometimes reaching up to 89% of the mackerel's own body length (Jenkins et al. 1984).

Current knowledge of the biology of grey mackerel is based primarily on a study in which samples were collected from areas across northern Australia including the NT and the Gulf of Carpentaria, but with a major emphasis on the Queensland east coast (Cameron and Begg 2002). This provided a limited spatial comparison of biological characteristics to primarily the east coast and Gulf of Carpentaria.

The productivity of fish populations and their likely responses to exploitation are determined by their life-history characteristics such as growth, reproduction and mortality. The determination of biological information for grey mackerel at different spatial scales is therefore essential for their sustainable exploitation, particularly where different stocks can be identified. Although not part of the objectives for the present study, during the course of sample collections we opportunistically collected further data

that provided useful biological information at different spatial scales. While these data proved to be sparse when examined among regions, they nevertheless provided an additional information source for assessing the population structure of grey mackerel, and at least preliminary biological information for grey mackerel management units identified during this study. This Chapter and Chapter 3 present these biological data. A description of the characteristics of the grey mackerel fisheries across northern Australia are also presented in the current chapter.

3.2 Biological data collection and analysis methods

Samples were collected as per the approach described in the overall methods presented in Chapter 1. Whole fish and fish frames (whole skeleton remaining after filleting) were retained by commercial gillnet fishers and stored frozen as soon as possible after capture. Fish samples were freighted to the respective agency's laboratory (WA Dept. of Fisheries, NT DRDPIFR, QDPI&F Northern Fisheries Centre, and the Fishing & Fisheries Research Centre at JCU). Samples were then thawed out and where possible, data recorded for individual fish. This data included a unique tag number, catch location and date, fork length (FL), total length (TL), head length, upper jaw length, sex, development stage of the gonad, and gonad weight (see Appendix 3). Head length and upper jaw length were measured to assess the reliability of these metrics as a proxy for fish length. Establishing a consistent relationship between these two measurements in the species would enable subsequent collections of grey mackerel samples for age and growth analyses to only require only collection of fish heads and viscera (for sex determination) rather than the whole body frame. Sex was macroscopically determined and the maturity stage was determined using a simplified macroscopic staging system developed for Spanish mackerel, *Scomberomorus commerson* (Mackie and Lewis 2001; see Appendix 3). Sagittal otoliths were also removed by a horizontal incision across the top of the head to expose the brain cavity, and otoliths were removed, washed in fresh water, dried and stored for ageing.

Age and growth analyses were carried out as part of a BSc (Hons) student project (N. Marton) and growth parameters were compared among regions as an additional technique for determining stock structure. The results of this work are presented separately in Chapter 3.

3.2.1. Data analysis

Morphometrics

Relationships for fork length–total length, fork length–weight and fork length–upper jaw length were estimated using regression analysis. Weight data were log transformed to standardise the residuals. Regressions fitted for females and males separately and pooled across regions were compared using Student's t-test (Zar, 1984). Regional comparisons were also performed where sufficient sample numbers (n ≥ 40) made it possible and separately by gender where appropriate using Analysis of Covariance (ANCOVA). This generally meant regional comparisons were only possible at broad spatial scales at best.

Size, Age and Mortality

The timing and validation of the assumption of annual formation of otolith opaque increments (annuli) was done by analysis of otolith margin categories and marginal increment measurements. The margin categories were determined while reading ages from otoliths based on the criteria presented in Table 3.1 (see Figure 3.1), and monthly margin categories were plotted. Mean monthly margin categories for the first four age classes were analysed using one-way ANOVA per age class to determine if there were any patterns in the formation of annuli. Tukey's Honestly Significant Difference (HSD) tests were used for post hoc pairwise comparisons (Zar 1984).

Age frequencies of the samples collected were plotted for each region and the instantaneous rate of total mortality (Z = fishing + natural mortality) was estimated for each region using age-based catch curves (Ricker 1975). These samples were pooled across gender for each region since sample numbers were not large enough for all regions, and instantaneous total mortality rate estimates for male and female grey mackerel have been shown to be similar (Cameron and Begg 2002). Catch curves were plotted as the $\log_e$ (frequency) for each age class for the descending right portion of the age frequency plots from the age of full recruitment to the sampling gear assuming constant recruitment and constant survival (Ricker 1975). The modal age class was assumed to represent the age at full recruitment. Catch curves were plotted to include all

age classes that were represented by at least one sample, and were compared between regions using ANCOVA.

Table 3.1. A description of the otolith margin categories used in assessing the timing of annulus formation (see Tobin and Mapleston 2004).

Margin category	Description
0	Complete and continuous opaque band formed around edge of otolith, with no translucent material beyond the last opaque band
I	Translucent band laid onto the outer edge comprising $^1/_4$ - $^1/_3$ the width of the previous translucent band
II	Translucent band laid onto outer edge comprising roughly ½ the width of the previous translucent band
III	Opaque band on edge, however is not continuous or complete
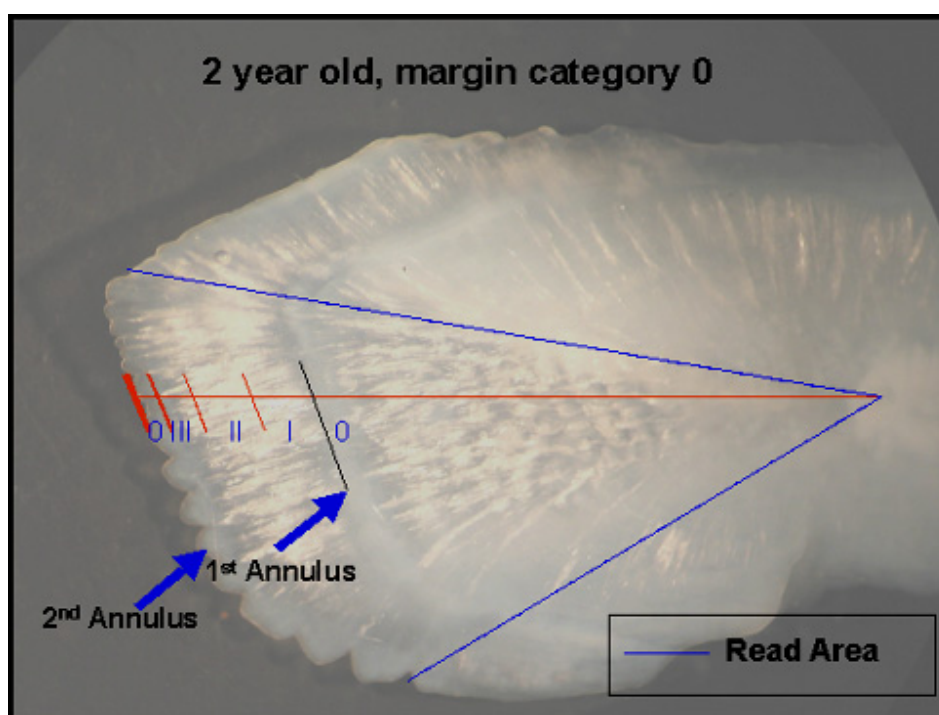
Figure 3.1. A mackerel otolith illustrating the margin categories used (see Table 3.1) in assessing the timing of otolith annulus formation. In this example the opaque band (annuli) is on the otolith margin (margin category = 0). Image by Amos Mapleston.

Reproduction

Seasonality in spawning was determined for each gender by plotting maturity stages by month, and by plotting mean monthly gonadosomatic indices (GSI) for females. GSI was calculated as:

$$GSI = (W / TW) \times 100$$

where W = total gonad weight (grams) and TW = total fish weight (grams). This was done by gender and by region where possible to assess the potential for different spawning times in northern Australia. As staging was done in the laboratory macroscopically and by different individuals, we also plotted the mean GSI for each stage to validate the macro-staging methods.

Length and age at which 50% and 95% of fish reach maturity (L_{50}, L_{95}, A_{50} and A_{95} respectively) were estimated for males and females separately. These estimates were determined using logistic regression analysis which plotted the relationship between fish length and age categories and the proportion of mature fish in each category (Maturity stage > 1).

Sex ratios were not analysed because sampling was opportunistic and usually by commercial fishers. This meant there was no way of knowing the representativeness of the catch from the samples collected; an issue applicable to all metrics.

3.3 Biological analyses results

3.3.1. Morphometrics

Fork Length-Total Length relationship

A significant linear relationship between fork length (FL) and total length (TL) was found for both male and female grey mackerel (Males: F = 8161, df = 1,305, $P < 0.001$; Females: F = 2952, df = 1,244, $P < 0.001$). Different regression slopes (t = 3.13, df = 549, P = 0.002) were estimated for each gender and the linear relationship for males and females were subsequently dealt with separately (Figure 3.2).
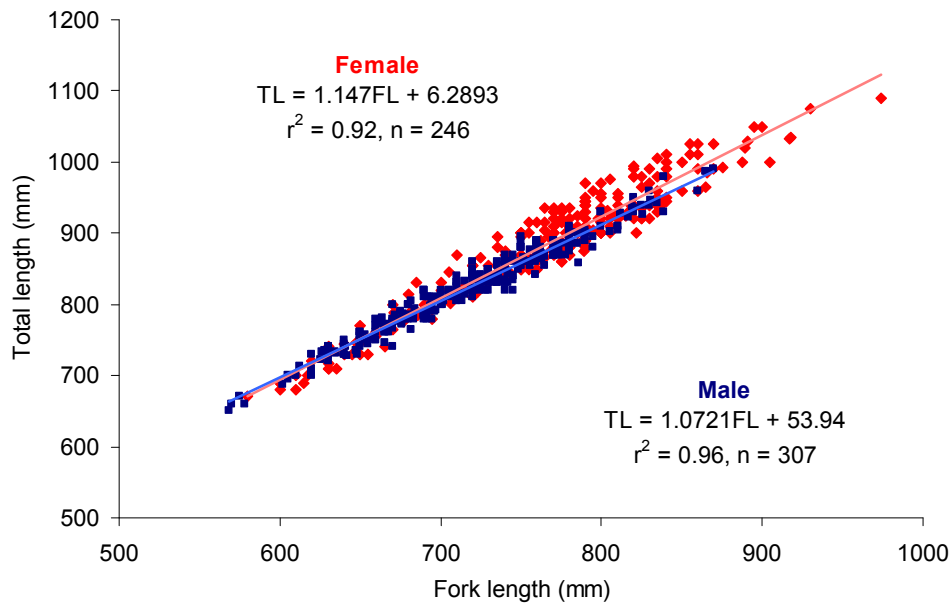
Figure 3.2. Total length (TL) – Fork length (FL) relationships for male (blue line and data points) and female (red line and data points) grey mackerel (regions combined).

Regional comparisons of the FL-TL regressions were only possible for 4 regions for females (EC, EG, WG, NW NT) and 3 regions for males (EC, EG, NW NT). For both sexes regression slopes were similar among regions (Males: F = 0.348, df = 2,300, P = 706; Females: F = 2.106, df = 3,239, P = 0.100) while regression intercepts were estimated to be different among regions (Males: F = 49.136, df = 2,301, P < 0.001; Females: F = 138.572, df= 3,242, P < 0.001). Multiple comparisons among elevations revealed that the EC and EG regions were similar for both males and females but were different for all other regional comparisons regardless of gender. Pooling across the EC and the EG still resulted in significantly different regressions among regions for females (Figure 3.3) while for males the pooled EC/EG regression was found to be similar to the NW NT. A common FL-TL regression relationship was therefore able to be used for male grey mackerel (Figure 3.2).
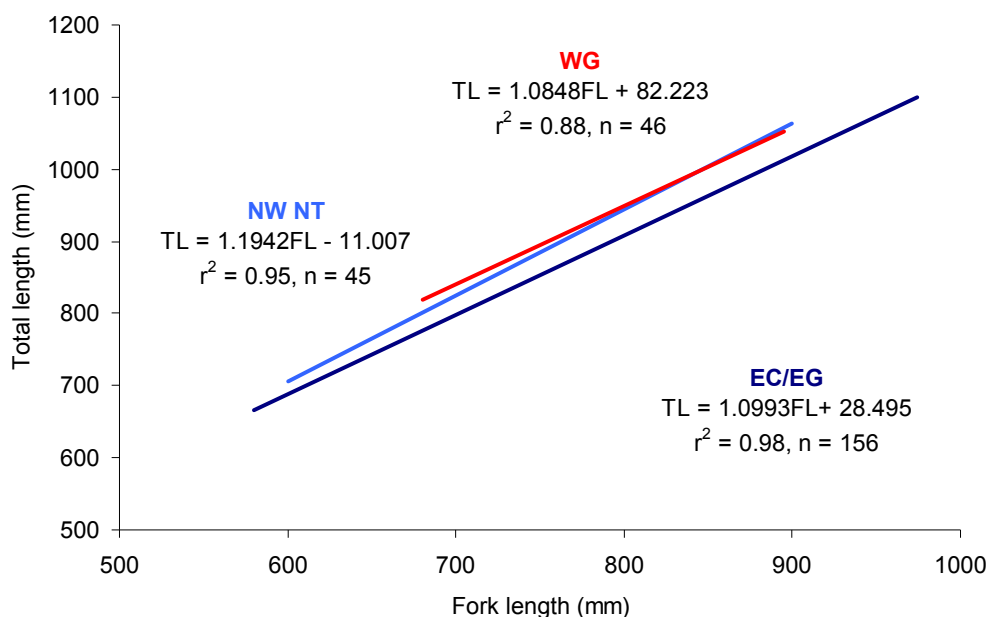
Figure 3.3. Total length (TL) – Fork length (FL) relationships among regions for female grey mackerel. The different regions are represented by the colours indicated (WG – red; NW NT – light blue; EC/EG – dark blue).

Fork Length-Upper Jaw Length relationship

A significant linear relationship between fork length and upper jaw length (UJL) was found for both male and female grey mackerel indicating reliability in predicting either FL or UJL from the other (Males: $F = 2222$, $df = 1,366$, $P < 0.001$; Females: $F = 2054$, $df = 1,314$, $P < 0.001$). Although similar regression slopes were found for each gender ($t = 0.128$, $df = 680$, $P = 0.899$), intercepts were different ($t = 5.365$, $df = 681$, $P < 0.001$) and so the linear relationships for males and females were subsequently dealt with separately (Figure 3.4).
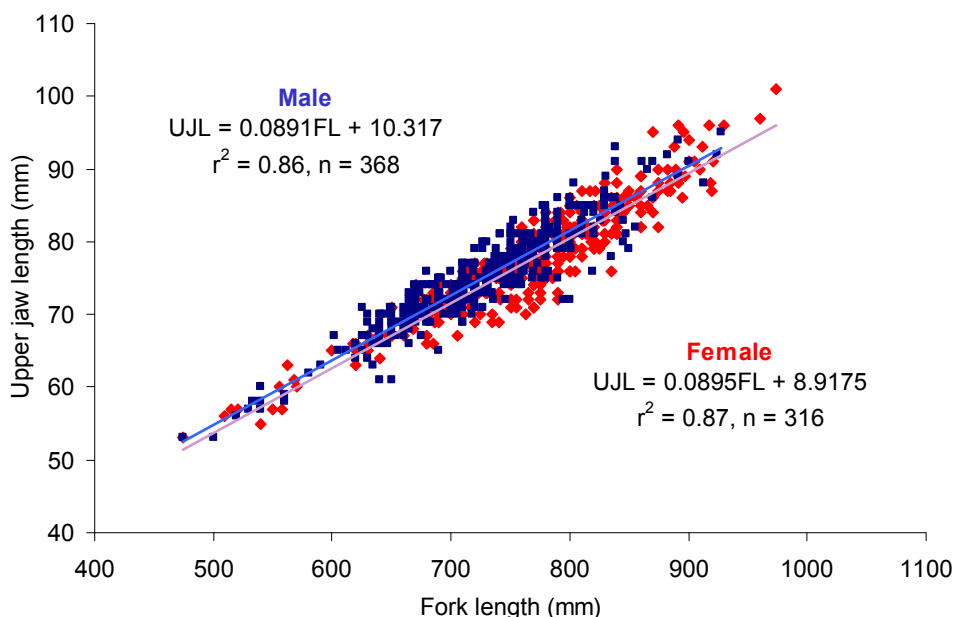
Figure 3.4. Fork length (FL) – Upper jaw length (UJL) relationships for male (blue line and data points) and female (red line and data points) grey mackerel (regions combined).

Regional comparisons of the FL-UJL regressions were only possible for 3 regions for both females and males (EC, WG, NW NT) due to the lack of some measurements taken from some regions. For females regression slopes were similar among regions ($F = 0.041$, df = 2,265, $P = 0.960$) while regression intercepts were estimated to be different among regions ($F = 42.11$, df= 2,266, $P < 0.001$). Multiple comparisons among intercepts revealed that the EC and NW NT regions were similar but both were different to the WG (Figure 3.5). For males regression slopes were different among regions ($F = 3.475$, df= 2,307, $P = 0.032$). Multiple comparisons among slopes showed that the WG was similar to both the EC and NW NT, while the EC and NW NT slopes were different (Figure 3.6).
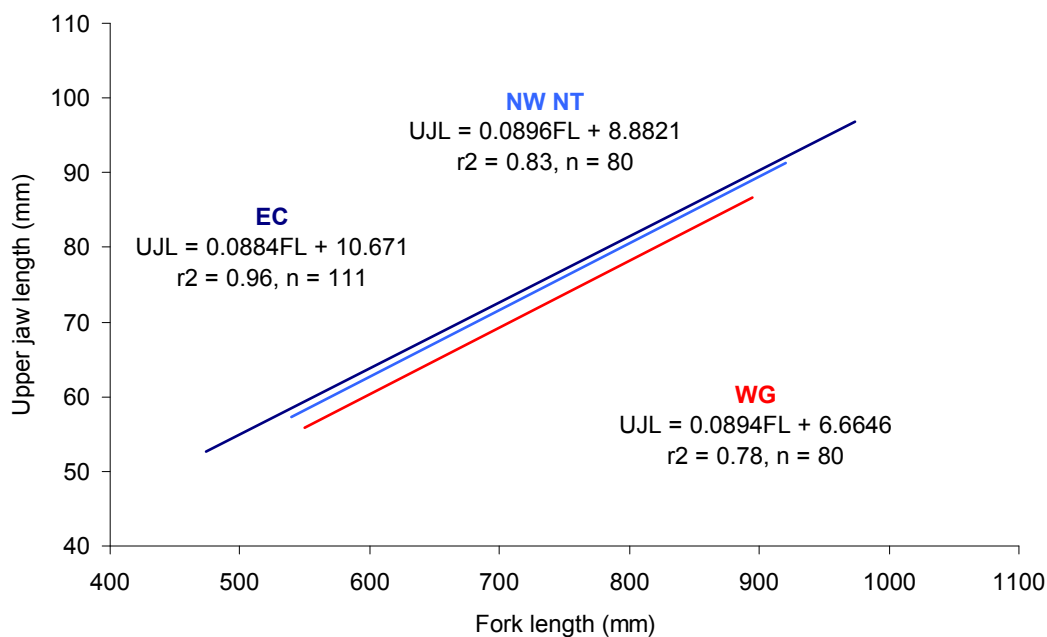
Figure 3.5. Fork length (FL) – Upper jaw length (UJL) relationships among regions for female grey mackerel. The different regions are represented by the colours indicated (WG – red; NW NT – light blue; EC – dark blue).
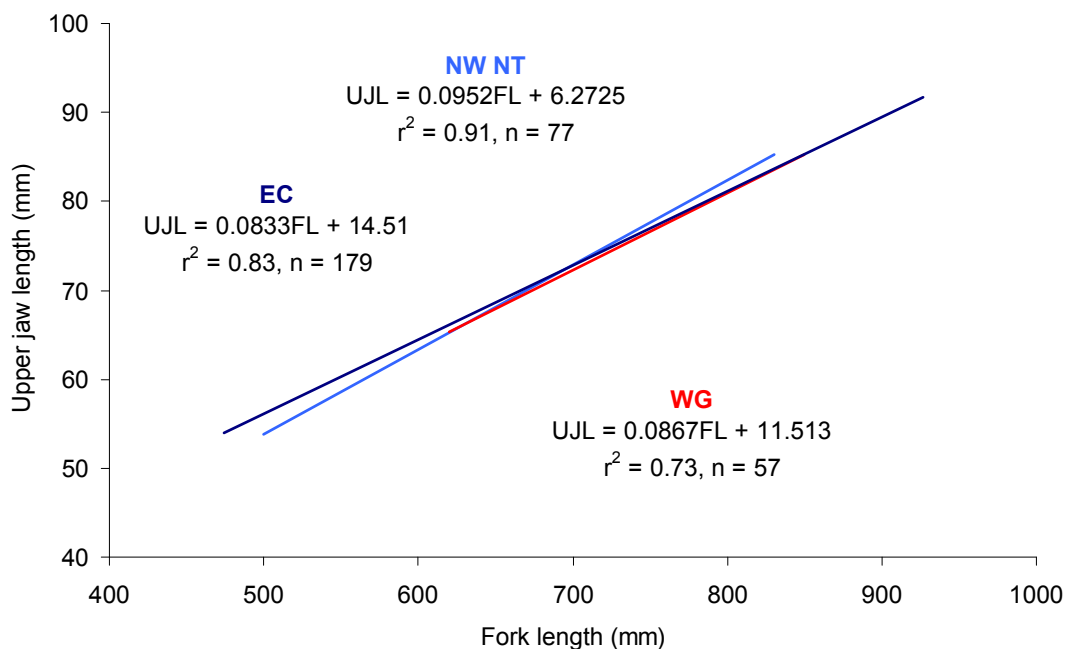
Figure 3.6. Fork length (FL) – Upper jaw length (UJL) relationships among regions for male grey mackerel. The different regions are represented by the colours indicated (WG – red; NW NT – light blue; EC – dark blue).

Fork Length-Weight relationship

A significant linear relationship between $\log_e$(Fork length) and $\log_e$(Weight) was found for both male and female grey mackerel (Males: F = 1338, df = 1,88, P < 0.001; Females: F = 1012, df = 1,67, P < 0.001). Similar regression slopes (t = 0.609, df = 155, P = 0.544) and elevations (t = 0.266, df = 156, P = 0.790) were found among genders and so male and female data were combined to describe the linear relationship (Figure 3.7). There were insufficient numbers of samples for comparing the FL-Weight relationship among regions.

$$\log_e(\text{Weight}) = 2.9968 \, \log_e(\text{FL}) - 5.0223$$
$$r^2 = 0.95, \, n = 159$$
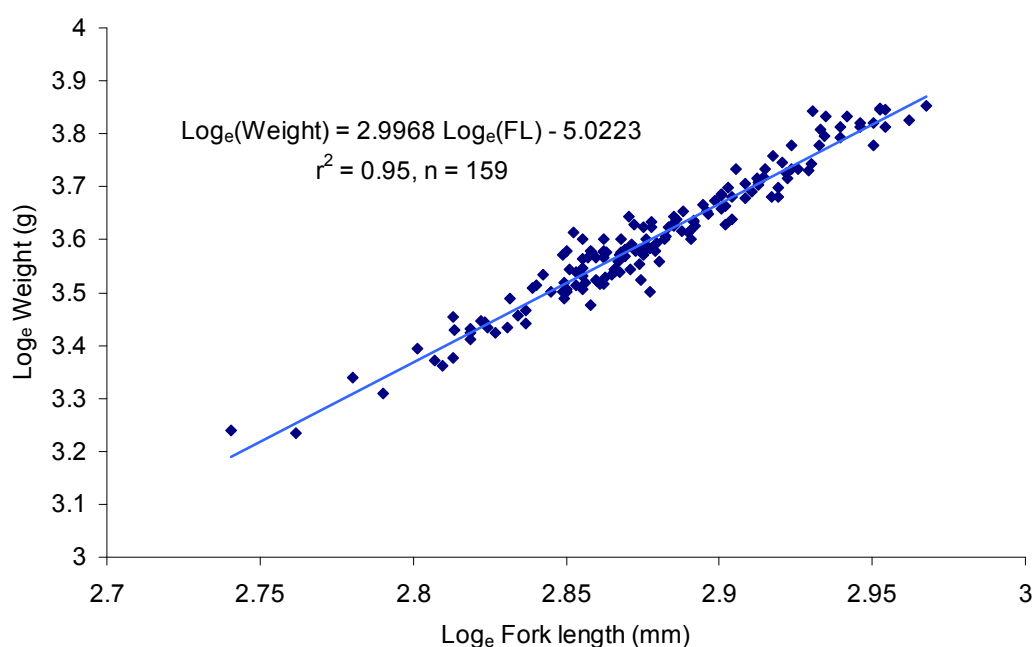
Figure 3.7. $\log_e$ transformed Weight (g) – $\log_e$ transformed Fork length (mm) relationship for grey mackerel (sex and regions combined).

3.3.2 Size, age and mortality

Otolith annulus formation

Analyses of monthly otolith margin categories required pooling of data among regions and years due to a lack of consistent temporal coverage across regions making regional comparisons invalid. Analysis indicates the formation of opaque annual increments

begins as early as August with completed opaque bands (annuli) generally appearing from November and December, although complete band formation is evident from as early as September (Figure 3.8). Monthly variation in mean marginal increments was only observed in the 2 (F = 5.906, df = 6,139, P < 0.001) and 4 year old fish (F = 3.131, df = 7,82, P = 0.006) (Figure 2.19). Tukey's HSD tests were used for post hoc pair-wise comparisons and in the 2 year olds found that the marginal increments for April and May were significantly larger than those for September and October (Figure 3.9), while for the 4 year olds the mean marginal increment for April was significantly larger than October (Figure 3.9). The margin category and marginal increment analyses are consistent with the annual formation of otolith bands (opaque regions) for 2 and 4 year old fish, with the formation of annuli occurring between September and November. Knowledge of this timing is important in helping to validate that the annuli are indeed formed annually, and in conjunction with information on the date of capture, can be very important in accurately interpreting age estimates.
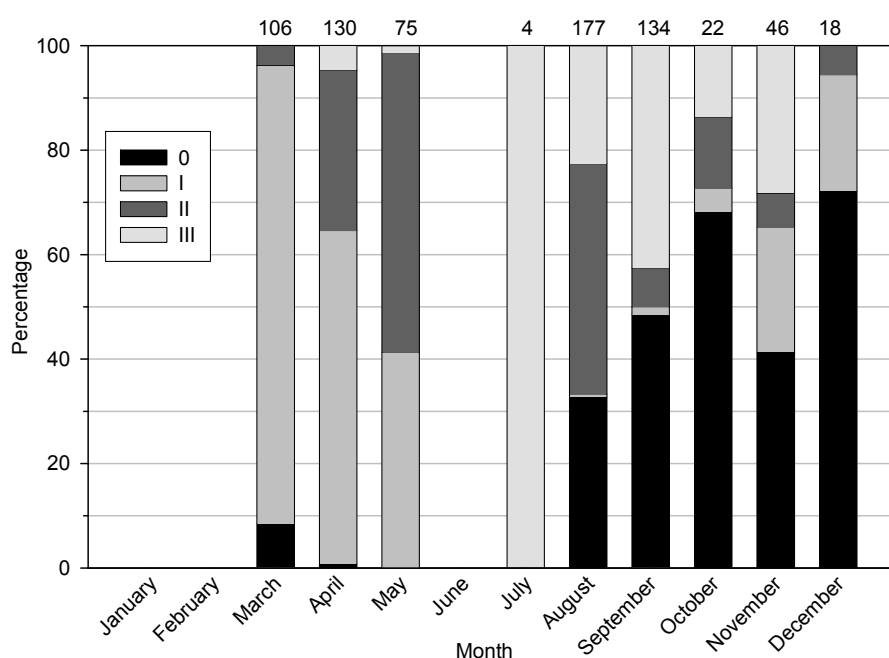
Figure 3.8. Monthly margin categories for grey mackerel otoliths (pooled among regions, years and gender). A description of the margin categories are given in Table 3.1. Sample numbers are given at the top of each months bar.
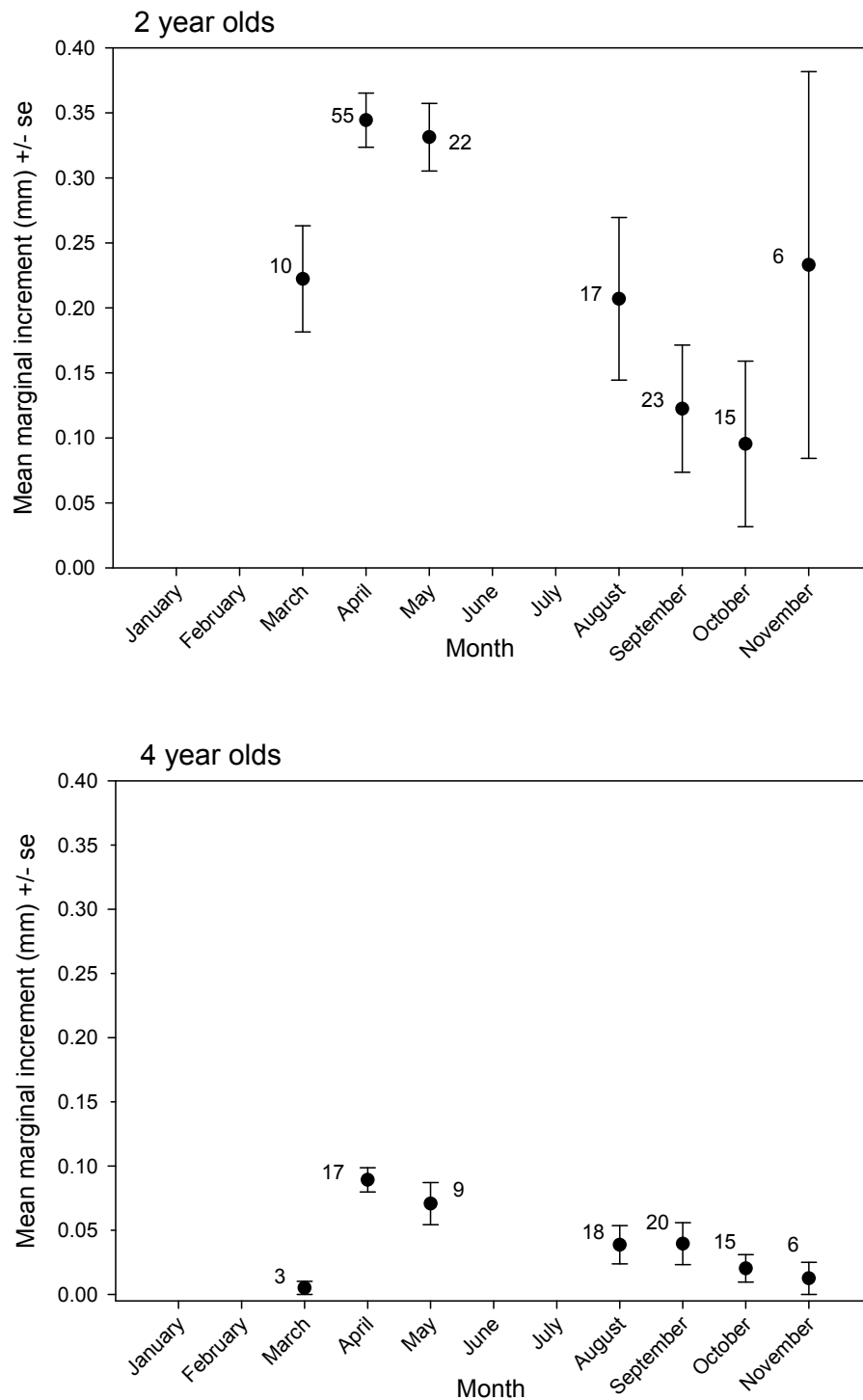
Figure 3.9. Monthly mean marginal increments (mm) for otoliths of 2 year old (top) and 4 year old (bottom) grey mackerel (pooled among regions, years and gender). Error bars represent standard error (se). Sample numbers are given for each mean.

Size and age structures

The size ranges of fish collected from each of the major areas were similar with the East Coast having the greatest range of sizes from 440mm FL – 1060mm FL. Average sizes were similar across regions with the exception of the West Coast which had larger fish on average than all other regions (Table 3.2). The youngest fish caught in each region was either 1 or 2 years old, and the oldest fish varied from 8 years to 12 years among regions with the East Coast having the greatest age range. The average regional age of fish sampled was highest in the Western Gulf and the West Coast, and lowest for the NW NT fish (Table 3.3).

Table 3.2. Size data summary of grey mackerel samples collected from each major region: Mean, minimum, and maximum sizes. All length measurements are in mm.

Region	Mean FL	Min FL	Max FL	n
Qld East Coast	742.04	440	1060	737
Qld Eastern GoC	740.69	540	928	548
NT Western GoC	758.12	550	895	147
NT NW Coast	742.58	500	920	207
WA Coast	790.39	550	900	64

Table 3.3. Age data summary of grey mackerel samples collected from each major region: Mean, minimum, and maximum ages. All ages are in years.

Region	Mean Age	Min Age	Max Age	n
Qld East Coast	3.22	1	12	221
Qld Eastern GoC	3.20	1	10	473
NT Western GoC	3.68	2	9	94
NT NW Coast	2.80	1	8	173
WA Coast	3.61	2	10	40

Mortality

We assumed that the age when fish were fully recruited to the fishing gear was 2 years based on that year class being the most common mode among the five regions. Instantaneous mortality rates among the regions ranged from 0.410 to 0.642 and were found to be similar (F = 0.492, df = 4,25, P = 0.742). The instantaneous total mortality rate (Z) for the regions pooled was 0.562 which corresponds to an annual survivorship of approximately 57% for grey mackerel (Figure 3.10).
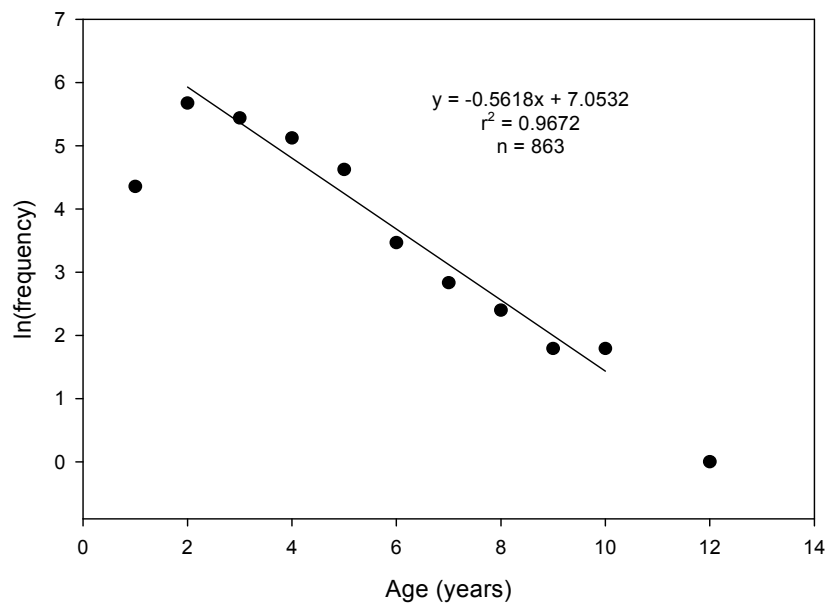
Figure 3.10. Catch curve for all data pooled across regions and gender. The instantaneous total mortality rate is estimated by the slope of the fitted curve (age range from 2 to 10 years).

3.3.3 Reproduction

Seasonality

An increase in gonad weight, represented by increasing GSI, would be expected with increasing reproductive macro-stages as the gonad develops (except for Stage 6 = spent). We were only able to test this for two regions (EC and EG) due to the limited gonad weight data from other regions. This test served to validate the macro-staging system used and to assess the interpretations of stages made by the respective agencies (F&FRC – EC; QDPI&F (NFC) – EG). For both regions there was an increase in GSI with increasing stage up to stage 5 validating the system used. Although the patterns of GSI increase with stage was similar among the two regions, GSI values for the EG were consistently higher than the EC (Figure 3.11).

Regional comparisons in spawning seasonality were limited by sample sizes of individual fish staged in each month and region, and also by the monthly numbers of fish for which gonad weight was measured for GSI determination. The EC, EG and the NW NT regions were used for examination of seasonality in spawning using monthly reproductive stages for both females (Figure 3.12) and males (Figure 3.13). Sampling across months varied

among these regions and was limited by targeting behaviour of the commercial fishers within each region and fish availability. Although these plots generally suggested a primary spawning season running between August and December, there was an indication also that some earlier spawning may be taking place in more northern regions on the EG coast and in NW NT. This was supported by the reproductive stages of both females and males with the possibility that NW NT fish have an extended spawning season beginning as early as May.

Regional comparison of monthly GSI estimates was only possible for the EC and the EG and both showed that significant gonad development occurs in September and spawning continues through until December (Figure 3.14). Despite different temporal coverage between these two regions, given the consistent timing of gonad development indicated we combined the data to more comprehensively represent the spawning season of grey mackerel at least for the EC and the EG (Figure 3.15). The identification of some spawning and spent females (Stages 5 & 6 respectively; see Appendix 3) during August in the EG was not supported by GSI estimates and may be due to subjectivity in the macro-staging system without histological examination. Unfortunately the estimation of GSI for the NW NT was not possible to corroborate the possibility of an extended spawning season.
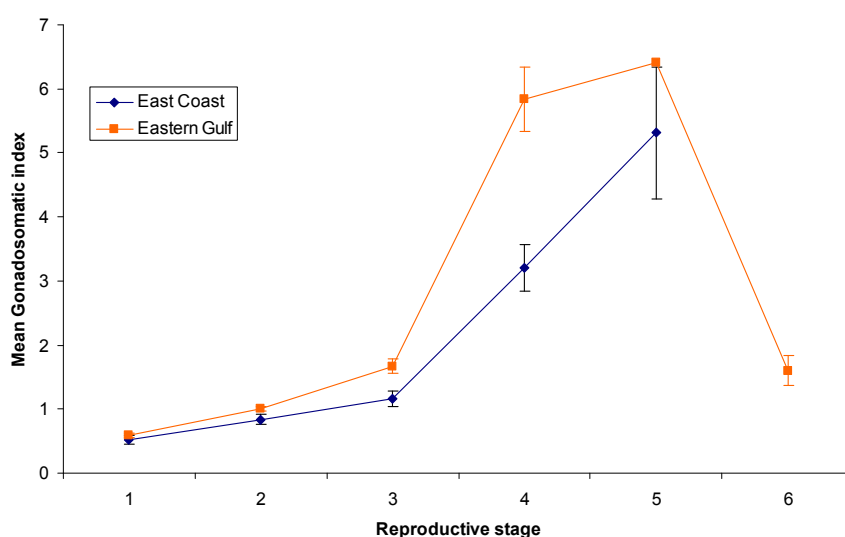
Figure 3.11. Reproductive macro-stages of female grey mackerel plotted against gonadosomatic index (GSI) for the Qld east coast and the eastern Gulf of Carpentaria. Standard error bars are shown.
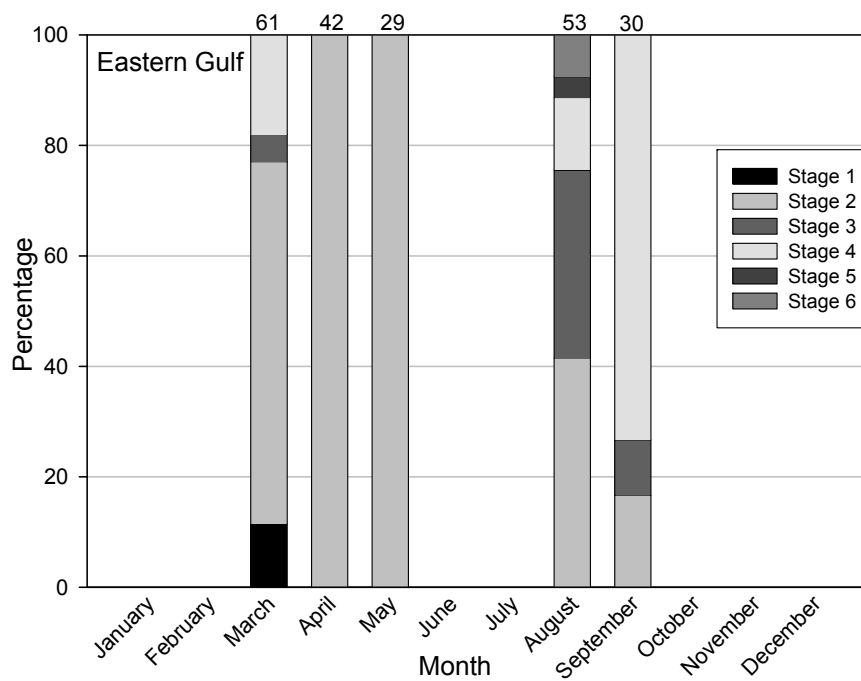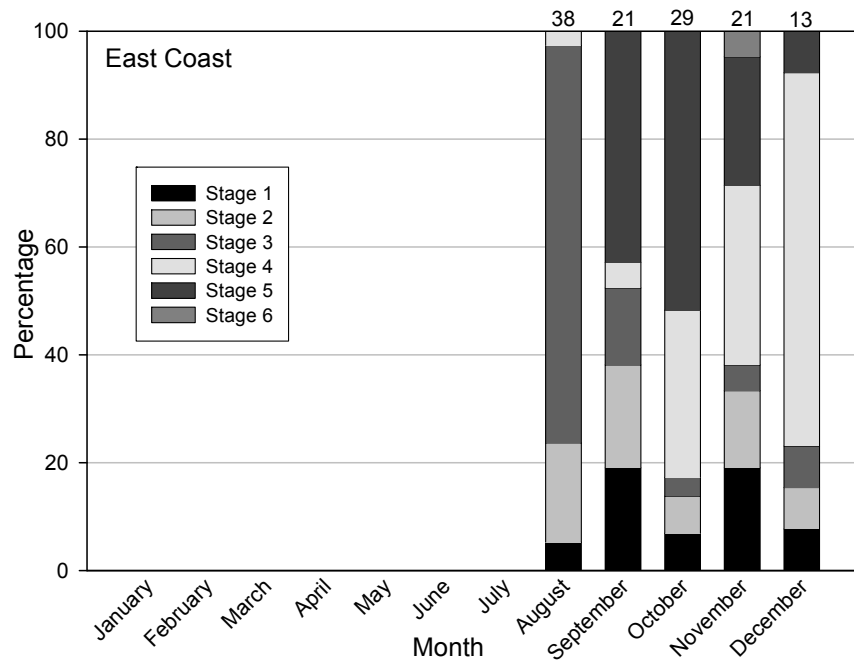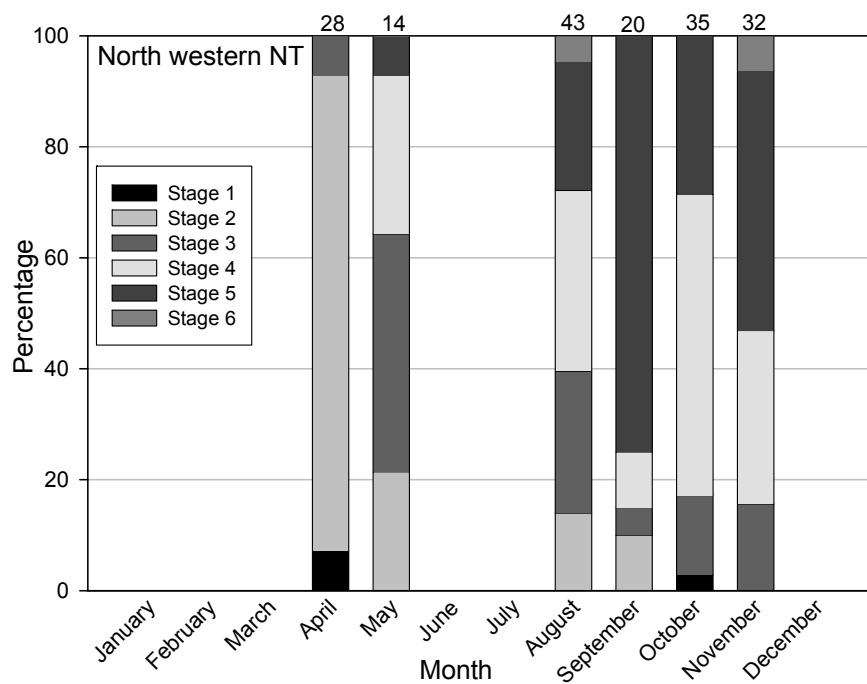
Figure 3.12. Monthly reproductive stages of female grey mackerel for the east coast, eastern Gulf of Carpentaria and the northwestern NT regions indicating seasonality in spawning. Numbers above monthly bars indicate sample sizes.
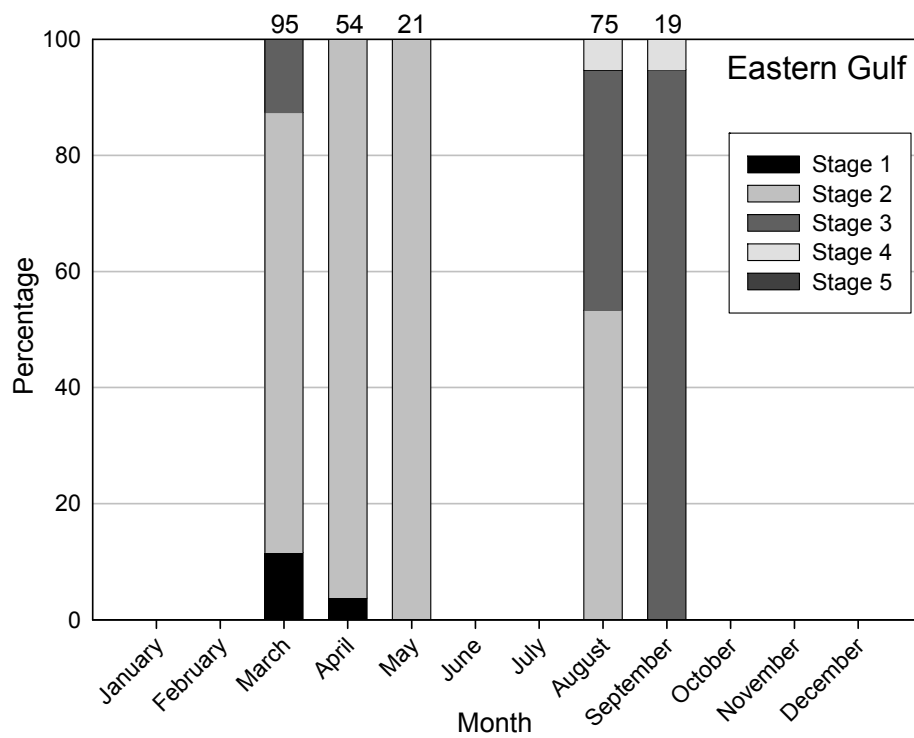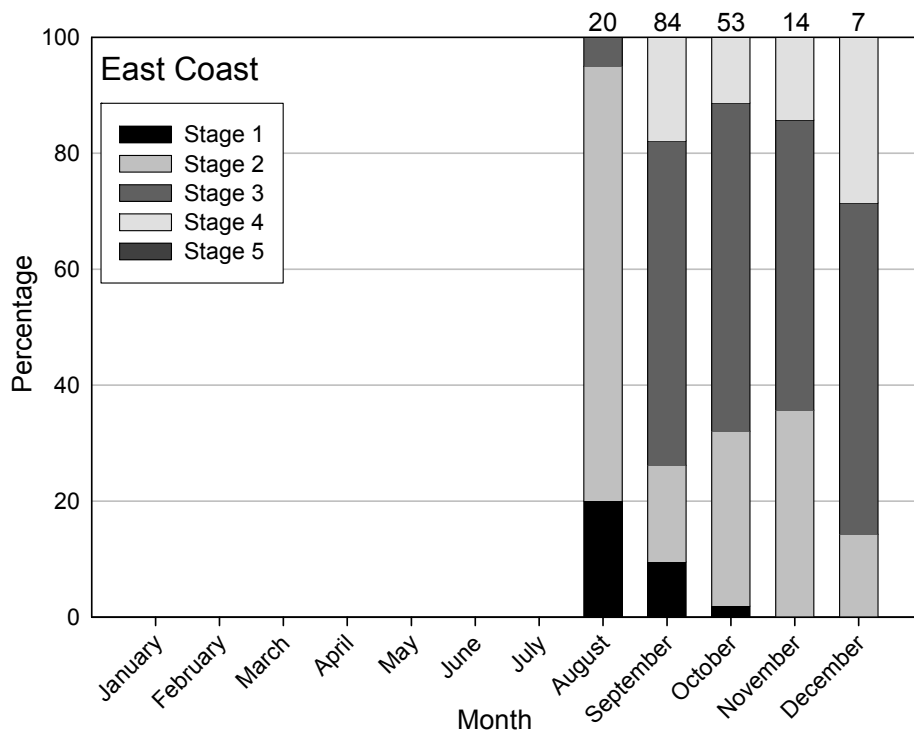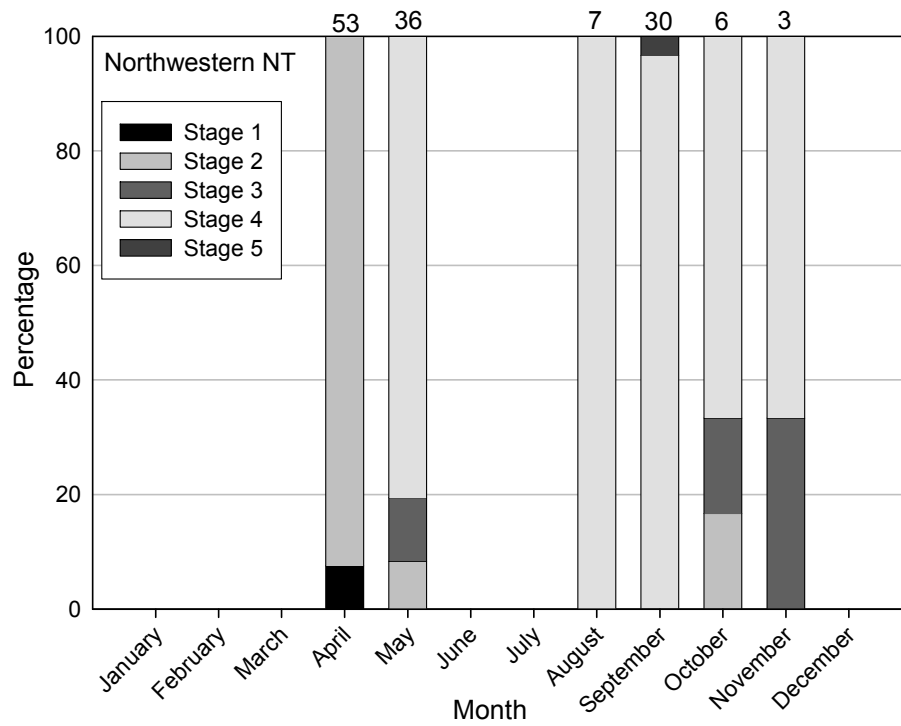
Figure 3.13. Monthly reproductive stages of male grey mackerel for the east coast, eastern Gulf of Carpentaria and the northwestern NT regions indicating seasonality in spawning. Numbers above monthly bars indicate sample sizes.
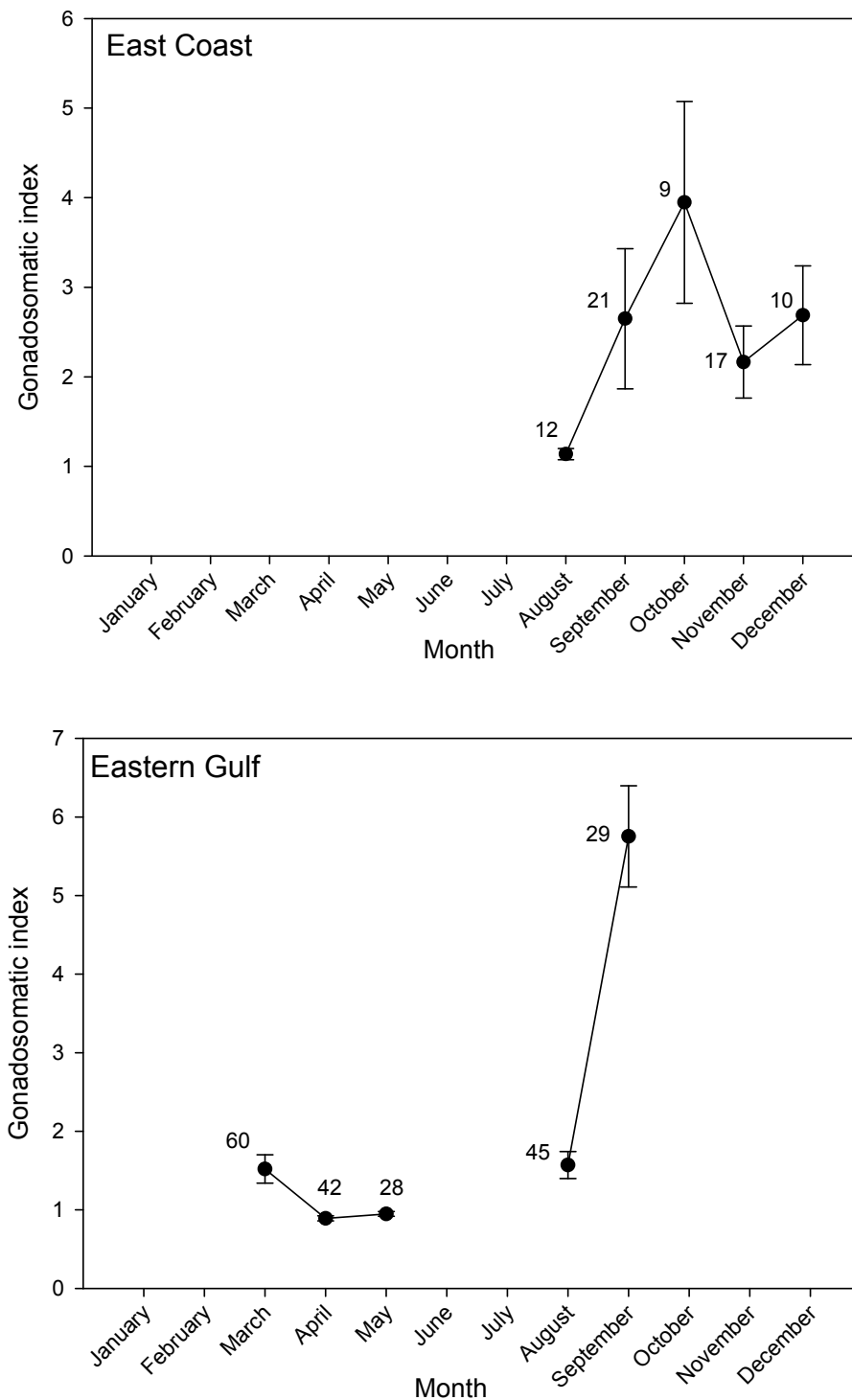
Figure 3.14. Monthly mean gonadosomatic indices (GSI) for female grey mackerel from the east coast and eastern Gulf of Carpentaria regions indicating seasonality in spawning. Numbers beside data points indicate sample sizes.
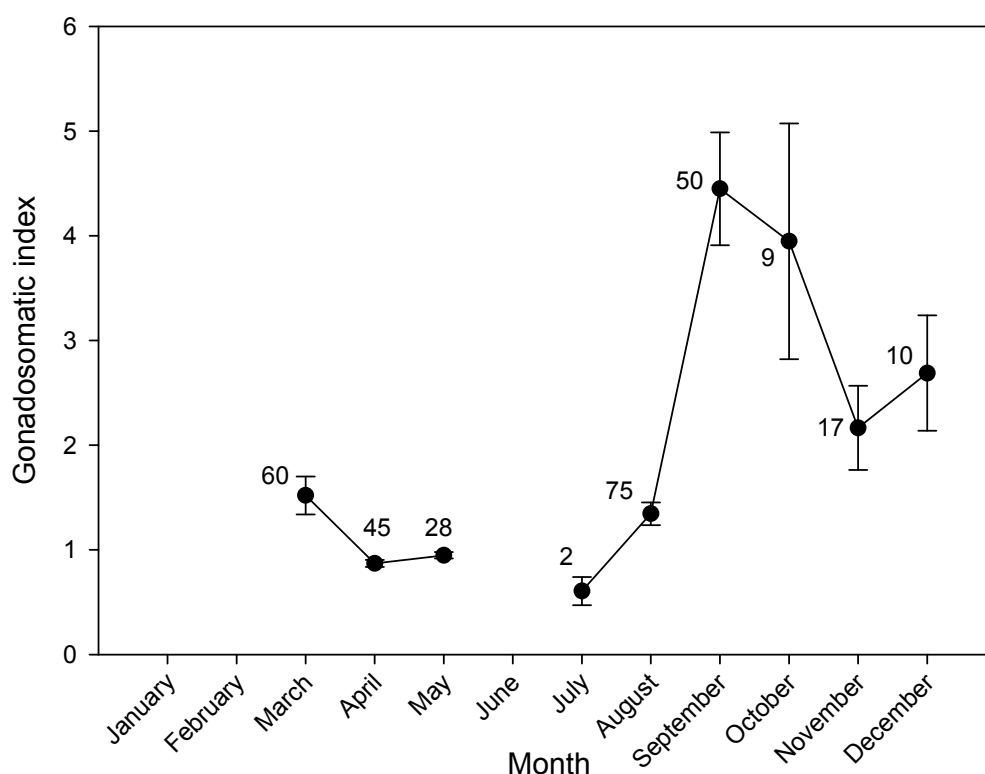
Figure 3.15. Monthly mean gonadosomatic index (GSI) for female grey mackerel from all regions pooled indicating seasonality in spawning. Numbers beside data points indicate sample sizes.

Maturity

Of the entire pooled samples from all regions, the smallest mature male and female were 474mm FL and 509mm FL respectively. The smallest ripe male and female was 540mm FL and 700mm FL respectively. Insufficient numbers of grey mackerel samples were obtained for each gender from each of the locations to carry out robust regional comparisons of both size and age at maturity estimates. This was likely due to the selectivity of nets used by commercial fishers from whom samples were sourced, as numbers were particularly lacking from the critical size and age classes across which first sexual maturity occurs. To more accurately estimate size and age at maturity we sourced 12 juvenile grey mackerel samples from trawl surveys conducted on the EC. These samples ranged from in size from 109 – 228 cm FL and were positively identified using DNA screening techniques. Logistic regressions were therefore fitted to maturity

data pooled across regions for each gender. The estimate of size at which 50% of the females were mature was 602 mm FL (697 mm TL; Figure 3.16a) while for males this estimate was 571 mm FL (666 mm TL; Figure 3.16b). The sizes at which 95% of the females and males were estimated to be mature was 694 mm FL (802 mm TL) and 697 mm FL (801 mm TL) respectively. The estimated age at maturity was A_{50} = 0.8 years for both females and males and A_{95} was 1.3 years for females and 1.4 years for males (Figure 3.17a and b respectively).
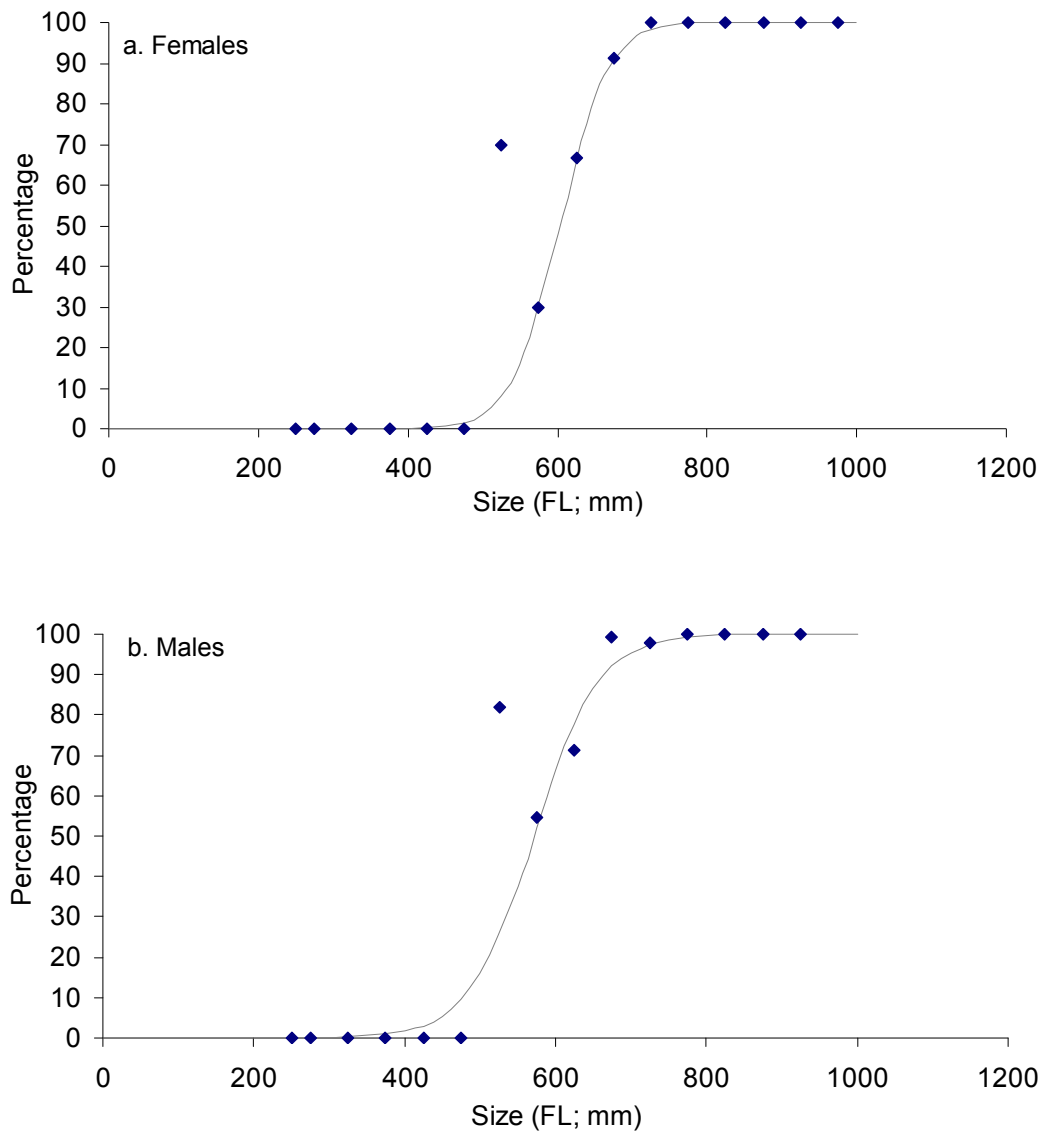
Figure 3.16. Logistic regression curves fitted to the proportion of mature fish (Reproductive stage > 1) per 50 mm size classes for a. females (above; n = 612) and b. males (below; n = 708). Data are pooled among regions.
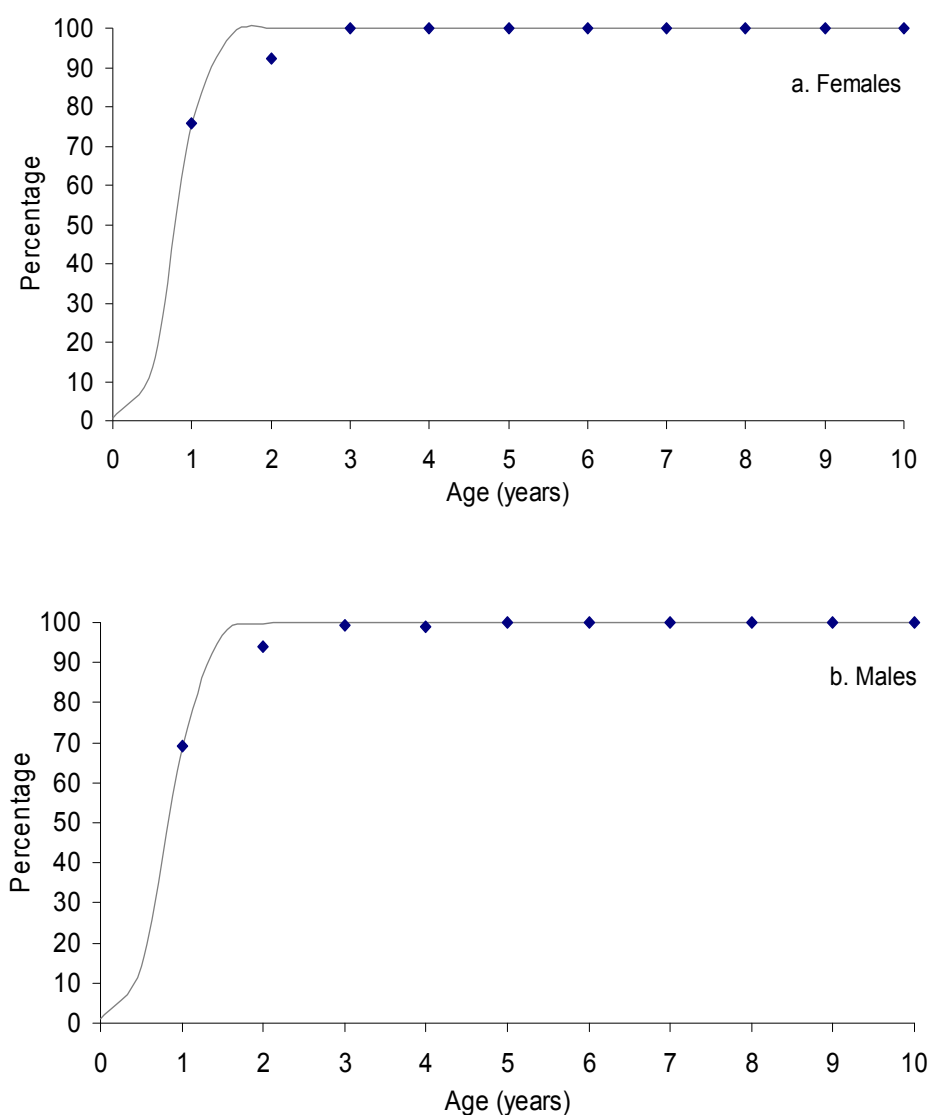
Figure 3.17. Logistic regression curves fitted to the proportion of mature fish (Reproductive stage > 1) per yearly age classes for a. females (above; n = 475) and b. males (below; n = 520). Data are pooled among regions.

3.4 Discussion

We were able to opportunistically estimate information about grey mackerel biology including morphometrics, otolith annuli formation, growth (see Chapter 4), mortality and reproduction. We did this even though the collection of this data was not part of the project objectives. Also, the objectives of this study did not include the use of biological

parameters in determining stock structure. Ideally, we would have also been able to carry out regional comparisons of all the different biological parameters and relationships to complement further the main techniques used during the project in determining stock structure, however the ability to do these comparisons was limited to only some of the metrics examined in this chapter. Despite this these metrics are valid methods for identifying different stocks and may be used in the future.

We were able to compare FL:TL and UJL:TL relationships among regions and, although we found consistency in the regional patterns of FL:TL for both males and females, as indicators of stock structure, the morphometric comparisons overall were variable and inconclusive. We also acknowledge that these regional morphometric comparisons were possibly confounded by measurement error associated with several different measurers used from the respective agencies in each region, as well as differences in sample sizes and the distribution in the sizes of fish measured. Fish were collected from different regions by local agencies involved in the project (WA Fisheries, NT DRDPIFR, QDPI&F and JCU) and frames were examined and dissected by the respective agencies. Although standardisation between measurers was discussed among project members and documents were prepared to assist training in measurements (see Appendix 3), it was not able to be tested and may have therefore influenced regional comparisons. This emphasises the importance of effective standardisation of morphometric measurements for robust comparisons in stock structure studies.

Morphometric relationships are important for being able to convert different direct measures commonly used in fish sampling to more useable data sets. Often with the collection of samples for fisheries research and monitoring it is only possible to obtain fish heads due to market product form preferences (eg. trunks) and limiting storage space on fishing vessels. This makes ageing possible but knowledge of the corresponding fish's length is vital information that is lacking. We found a significant relationship between the upper jaw length and the fork length of grey mackerel, enabling estimates of length to be easily and reliably derived from head samples. This makes sample collection in future a much simpler and more cost-effective process without compromising information.

We also detected differences between genders in some morphometric relationships (FL:TL, FL:UJL). Cameron and Begg (2002) did not appear to test for gender differences in the FL:TL relationship and had a relatively small sample size, however showed a significant difference in growth among gender. They did find no gender difference in the relationship of FL-Total weight, which is consistent with our findings in this study.

Significant monthly patterns in the formation of otolith bands from both margin category and marginal increment analyses supported annual formation. Both techniques also suggest that the formation of annuli occurs between the months of September and November. Monthly patterns were only found for 2- and 4-year old fish however. The inability to detect monthly patterns in annulus formation for 1 and 3 year old fish are possibly due to a combination of factors including low monthly sample numbers and few months sampled for some year classes. Independent validation of annual opaque increment formation was not possible during this study and has proven problematic for grey mackerel. Being a pelagic species they are difficult to maintain in captivity and they do not appear to respond well to capture, handling and tagging as they are more effectively caught by commercial gill nets compared to hook and line. As such past tagging efforts have been ineffective (Cameron and Begg 2002). Cameron and Begg (2002) concluded the timing of annuli formation was between November and February, 1 – 3 months later than that observed in the present study. Inter-annual variation in annuli formation has been observed in other fish species in the Australian tropics including *L. miniatus* (Williams et al 2005). The timing of opaque increment formation has been correlated with a number of factors including seasonal water temperature changes (eg. Schramm 1989; Pearson et al 1996; Smith and Deguara 2003), timing of spawning (Hostetter and Munroe 1993), location (Pearson et al 1996; Williams et al 2005), and also error in the measurement of marginal increments due to regional differences in otolith growth (Smith and Deguara 2003). In the present study the timing of annulus formation is consistent with the end of the cooler winter/spring water temperatures, and is also consistent with the timing of peak spawning. The differences in the timing of opaque increment formation between the studies may be due to differences in temperature during the years that samples were collected in since each study found spawning season to be relatively similar. All of the above factors, however, could contribute to the differences in timing of annuli formation observed between the two

studies as in each study samples were pooled across regions and years. Also, no samples were collected in the present study during the months of January and February.

Estimates of instantaneous mortality rates ranged among regions from 0.410 to 0.642 and were not different (pooled estimate = 0.562). These estimates are slightly higher to those found by Cameron & Begg (2002), which ranged from 0.297 to 0.499, and may reflect large increases in grey mackerel fishery catches in the intervening period between studies, particularly on the east coast. The current estimate however is still appreciably lower than that estimated for school mackerel (*S. queenslandicus*) and spotted mackerel (*S. munroi*) by Cameron and Begg (2002).

Comparisons of seasonality of spawning among different regions were limited due to the lack of samples across all months and regions. Evidence of spawning using the gonad staging system indicated that spawning occurred on the EC during the months from September to December, in the EG spawning females were only detected during August and males during August and September, while in NW NT spawning occurred throughout the period of August to November with evidence of spawning as early as May. Again this was consistent among males and females. Although the assessment of the validity of the staging system was positive (Figure 3.11), the macro-staging system is still subject to measurement error both within and among agencies, and the GSI values are regarded as being more reliable for assessing reproductive development. Unfortunately, sufficient numbers of gonad weight measurements for comparison were only taken for the EC and EG regions and so validation of the possible extended spawning in the NW NT wasn't possible. For this reason the result that suggests the possibility of spawning as early as May in the NW NT needs further validation. GSI values for the EC had good agreement with the staging system, while for the EG a sharp rise in GSI from low levels in August to very high levels in September indicated the onset of spawning during this period, at least for the period we sampled. This did not therefore agree with August spawning though it is possible that it was occurring though not widespread. All of the spawning or spent fish identified during August (n = 6) were caught from the same location and year, and in two separate catches only days apart. Cameron and Begg (2002) concluded that grey mackerel have peak spawning on the EC between October and January. Despite this, our results are relatively consistent for the EC results of Cameron and Begg (2002) with a protracted spawning period covering

September to December, though we did not obtain samples during January. Although we had limited temporal coverage across months for the EG, we were able to determine that spawning began in this region during at least September, and Cameron and Begg (2002) determined that the peak spawning was again from October to January. Inter-annual variability in the timing of spawning has been documented in many different fish species and is thought to be correlated with sea surface temperatures (eg. Scott and Pankhurst 1992, Sheaves 2006, Bani and Moltschaniwskyj 2008).

Similar to Cameron and Begg (2002), we were not able to carry out regional comparisons of maturity for grey mackerel. Our estimates of 50% size at maturity of 602mm FL (697mm TL) and 571mm FL (666mm TL) for females and males respectively were larger than those estimated by Cameron and Begg (2002). It was unfortunate that there were insufficient samples for comparisons of this parameter among regions as it is considered critical biological information for setting legal size limits. Currently legal size limits for grey mackerel in Queensland are 500 mm TL, significantly below the size at which it is estimated that 50% of either sex are mature. This suggests that an increase in the current minimum legal size limit is warranted. Cameron and Begg (2002) acknowledged this but urged that size limits not be increased unless education showed improved identification of the different mackerel species by the recreational line fishery. They also pointed out the low survival rate of net caught grey mackerel post release. However, it is likely that the recreational catch of grey mackerel is low relative to other mackerel species. Also, recent announcements of implementation of the East Coast Inshore Finfish Fishery Management Plan in early 2009 include an increase in the minimum size limit of grey mackerel to 600 mm TL along with changes to offshore net mesh sizes to minimise capture of undersize fish.

Sampling of grey mackerel for the Cameron and Begg (2002) study and sampling for this study were carried out 12-15 years apart. During this period targeting of grey mackerel in most regions has increased substantially, thus providing us in this chapter to assess for decadal changes in biological characteristics as a potential consequence of fishing. The major characters we were able to compare were timing of annuli formation, spawning seasonality, size at sexual maturity and mortality. The timing of annuli formation was found to be earlier in this study compared to Cameron and Begg (2002), however this more likely to be environmentally induced rather than a fishery effect. Despite

differences in the temporal coverage in sample collection between studies, spawning seasonality was broadly similar for each study period with an extended spawning season indicated. In this study size at maturity was found to be slightly larger than the earlier study. It might be expected that a fishery effect would result in the opposite observation and so it is likely that this difference in size at maturity is influenced by other factors. One might infer that the lack of apparent effects of fishing on biological characters simply means that fishing effort not sufficiently high enough to do so. This may be so however we would caution against this line of thought as the fishery has seen substantial increases in catches in recent years. Estimates of total mortality derived in this study are higher than those estimated by Cameron and Begg (2002), and support this increase in catch observed in commercial logbook entries.

Although not one of the key objectives for the current project, documenting biological attributes for fishery target species are vital for providing greater certainty in how these resources are managed. Through comparison with previous grey mackerel research by Cameron and Begg (2002) this chapter provides greater insight into the potential natural variability in grey mackerel life history, and although not conclusive, provides some evidence of stock structuring in grey mackerel across northern Australia. The biological information we provide here makes greater use of the samples collected and value-adds considerably to the project thereby further enhancing project outputs and management outcomes. Further, the estimates for various biological parameters so obtained will be of very great use as inputs to stock assessment investigations of grey mackerel at regional and jurisdictional fishery scales.

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4. STOCK STRUCTURE OF GREY MACKEREL (*SCOMBEROMORUS SEMIFASCIATUS*) INFERRED FROM BACK-CALCULATED GROWTH ESTIMATES

AC Ballagh, N Marton, DJ Welch and I Lawler

4.1 Introduction

Identification of the stock structure for harvested fish species is critical for fisheries management. Stock structure provides the basis for the determination of appropriate spatial management units and for developing optimal harvest and monitoring strategies (Ricker, 1981). There are several different methods that have been used to discriminate different fish stocks (Ihssen et al 1981) including genetic techniques, parasites, morphology and mark-recapture. Each of these methods is useful in stock determination, depending on the spatial and temporal scale of interest. Life history parameters have also been utilised in stock studies as they reflect both the genotypic and environmental influences on the stock; differences in these parameters are therefore likely to reflect geographically and/or reproductively isolated populations (eg. Begg & Sellin 1998). Hilborn and Walters (1992) in fact, define stocks as self-reproducing groups of fish, each with similar life history characteristics.

Measures of the growth of fish also determine important biological attributes of each stock, such as their productivity and responses to fishing (eg. Bianchi *et al.* 2000). Parameters of growth therefore also inform the selection of appropriate management strategies. As well as being affected by genetic differences (Sheehan et al. 2005), growth is strongly environment-dependent, with the influence of temperature and food availability of the greatest influence (Shoji & Tanaka 2003). It is likely that fish residing in isolated regions will be exposed to different environmental conditions during their life and variation in their growth will reflect these differences.

There are several methods available to determine growth in fishes. These include: observed size at age (Berg & Pedersen 2001), mark-recapture (Faragher 1992), length frequency analysis (Morales-Nin 1992), and back-calculation (De Vries et al. 1990,

Ballagh et al. 2006). There are problems associated with all of these methods of determining fish growth rate. For example, it is considered difficult to collect the random representative samples needed for observed size at age and length frequency analysis (Morales-Nin 1992), while mark-recapture requires very large initial sample sizes. In a previous study on movement of school and spotted mackerel off the Queensland east coast, 4427 and 2106 fish were tagged respectively, while only 93 and 38 were recaptured respectively (Begg et al. 1997). Back-calculation similarly has problems associated with it however these are generally once-off problems relating to initial set-up costs and validation of the periodicity of band formation. Other methods include rounding an age up or down depending on the amount of material on the otolith beyond the most recently formed band (eg. Tobin & Mapleston 2004), and assigning a birthday so age can be used in months instead of whole years (eg. Pilling et al. 2003). These methods are collectively termed '*adjusted methods*' and like the methods mentioned above, they also have problems associated with them.

Of all the methods currently available, observed size at age is the most commonly used for age and growth studies, largely due to its relative cheapness and the potentially accurate results provided. However due care must be taken to ensure the samples used are independent and representative of the wild population (Morales-Nin 1992). While observed size at age is the most commonly used method, back-calculation is considered to be a more accurate method as gear selectivity, a significant potential bias, can be removed from the sample (Campana et al. 1990). Slower growing fish aren't susceptible to many gear types until later in life, and thus are often under-represented in data sets using observed size at age. Once the fish are caught, if their previous size(s) can be calculated they can be added back into the sample population, reducing the bias present in observed size at age methods.

In this study stock structure of grey mackerel across northern Australian regions was inferred by comparing back-calculated estimates of growth parameters. Growth parameters were also estimated using observed size-at-age data and compared with the back-calculations to assess bias from gear selectivity associated with using commercially caught fish for determining growth rates.

4.2 Materials and Methods

4.2.1 Sample collection

Samples were collected by commercial fishing operations from several locations around the northern coast of Australia. Fork length and sex were recorded and sagittal otoliths were removed. Sample locations (regions) for this study included the Queensland east coast (EC), Queensland north eastern Gulf of Carpentaria (EG N), Queensland mid-eastern Gulf of Carpentaria (EG mid), Northern Territory western Gulf of Carpentaria (WG) and Northern Territory north-west coast (NW NT) (Figure 4.1). Several juvenile individuals were also collected from trawl surveys from the Qld EC region and were positively identified as *S. semifasciatus* from genetic analysis. Table 4.1 shows the regional sample numbers used in this study.

Figure 4.1. Sample locations used for estimating growth for comparison among regions.

Table 4.1. Number of samples used for back-calculation of growth, mean fork length (FL) and age of Grey mackerel from each of the sample locations (se = standard error).

Sample location	Sample numbers			Mean FL (mm) ±se	Mean age (yrs) ±se	Range	
	Females	Males	Juveniles			FL (mm)	Age (yrs)
NW NT	72	73		739 ± 7	2.8 ± 0.1	520 - 920	0^+ - 8
WG	63	31		768 ± 6	3.7 ± 0.2	620 - 895	2 - 9
EC	45	74	4	719 ± 12	3.3 ± 0.2	109 - 974	0^+ - 8
EG mid	63	76		747 ± 6	3.7 ± 0.1	578 - 928	1 - 9
EG N	69	72		766 ± 4	3.3 ± 0.1	630 - 880	1 - 10

4.2.2 Age estimation

Whole sagittal otoliths were immersed in mineral oil and viewed against a black background through a stereo-dissection microscope (10× - 40× magnification). Otoliths were aged whole rather than taking transverse sections as age estimates from whole otoliths has been found to be a more accurate method of aging for grey mackerel (Cameron & Begg 2002). Otoliths were aged through the 'read' area (Figure 4.2) from the posterior end on the proximal surface. Complete increments, annuli (one translucent and one opaque zone), were counted from the nucleus to the outer edge of the otolith. All otoliths were read at least twice and included annuli counts and estimates of the marginal increment category using the category system of Tobin & Mapleston (2004) (see Chapter 3). Annuli counts from the first two reads were compared. If the counts agreed, the count was accepted as the agreed age. If the ages from the first two reads did not match, the otolith was read a third time and the annuli counts compared across all three readings. If any two of the three counts matched, that count was accepted as the agreed age of the fish.

Reader accuracy for age estimates was determined by comparing ages assigned in this study with the ages of a subsample of fish determined by other experienced readers at the Fishing and Fisheries Research Centre (FFRC), James Cook University. This was done by assuming that the FFRC's assigned ages were accurate, and comparisons were then made using percent agreement and age bias plots. The precision (repeatability) of the aging method was also determined using percent agreement by comparing multiple age estimates.

4.2.3 Age adjustment

Age estimates were then adjusted based on the criteria outlined in Begg et al. (2006), where the age of each otolith was adjusted for the margin category. Samples with an agreed age were assigned a final margin category if any two of the margin reads for a single otolith agreed. For agreed margin categories of 0 or 1, no adjustment was deemed necessary and the agreed age was accepted as the final age. If the agreed margin category was 2, the agreed age was adjusted by adding 0.5. For samples with an agreed margin category of 3, the age was adjusted by adding 1 to the agreed age. For samples without agreed margin categories, the higher of the category estimates was accepted and the age adjusted accordingly.

For fish with no agreed age, it was still possible to assign an adjusted age in some cases based on the margin increment category and the age. If multiple age estimates were no more than one year apart and the margin category of the higher age was a 0 or 1 while the margin category for the lower age estimate was a 3, the adjusted age was accepted as the higher of the two age estimates. If there was still no agreement between ages and margin categories, the fish was removed from the sample and the otolith deemed to be unreadable.

4.2.4 Back-calculation

Digital still images of all otoliths were taken using an image analysis system (Diagnostic Instruments digital camera connected to an Olympus SZX9 stereo-dissection microscope and the Image Pro 6.2 digital analysis software). Images were checked against the age estimates to ensure that all the annuli were clearly distinguishable. Otoliths were measured through the 'read' area (Figure 4.2). Measurements were taken from the nucleus to the furthest point from the nucleus on the posterior end of the proximal side of the otolith (Figure 4.2). This provided a reference axis that was consistent across all otoliths. In cases where annuli along the reference axis were ambiguous, a second axis was measured from the nucleus to the edge of the otolith within the read area where the annuli were clearer (Figure 4.2). This second line was called the measurement axis. The length of the reference axis and measurement axis (where present) were then measured, as was the distance from the nucleus to the outer edge of the opaque band for each annuli. It was not always possible to accurately distinguish the outer edge of some annuli or differentiate between annuli, and so

measurements from the first annuli to the latest consecutively distinguishable annuli where taken.

Standardising the annuli distances taken on the measurement axis to the reference axis was done using a conversion ratio:

$$A_R = \frac{R_A}{M_A} \times A_M$$

Where R_A and M_A are the lengths of the reference and measurement axes respectively, A_R is the distance from the nucleus to the annulus measured along the reference axis and M_A is the distance from the nucleus to the annulus measured along the measurement axis.
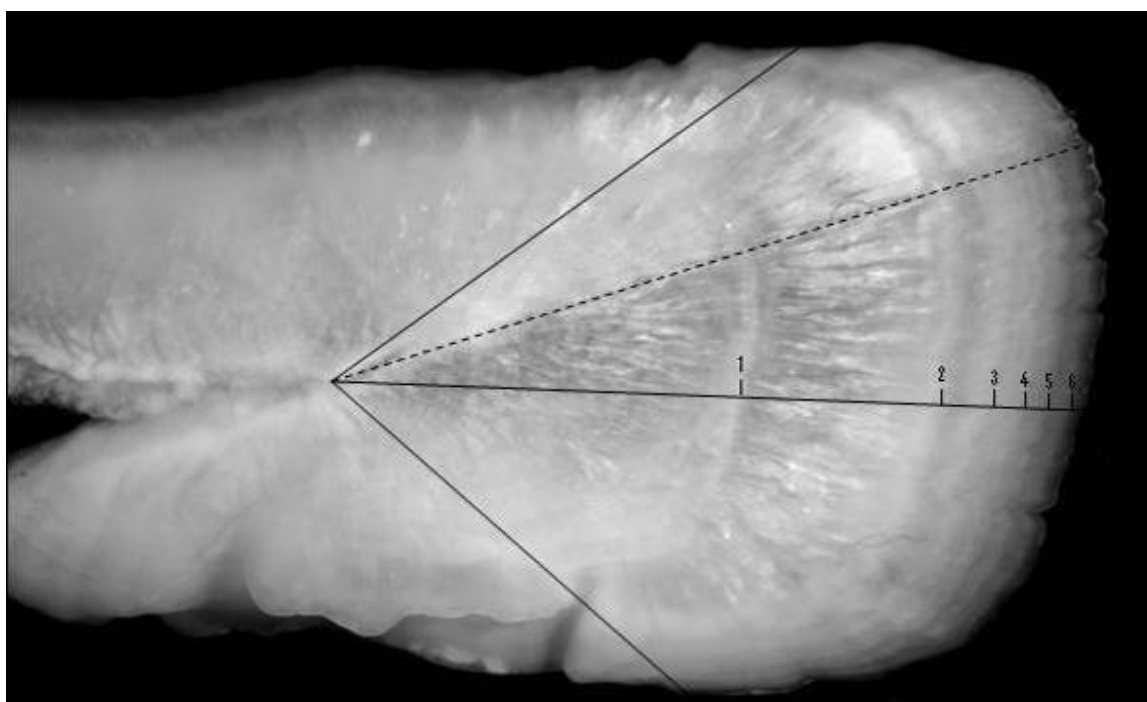
Figure 4.2. The read area on the posterior end of the proximal surface of a six-year-old Grey mackerel sagittal otolith, with the reference axis (dashed line) and the measurement axis with check marks at the outer edge of each annuli.

Ordinary least-squares regression analysis was used to determine the form of the relationship between otolith radius (reference axis) and fork length. This relationship was then determined using geometric mean regression (GMR) (Ricker 1992). Differences in the relationship between otolith radius and fork length for region and gender were tested

using Analysis of Covariance (ANCOVA) (Bartlett et al. 1984). Back-calculated length at age was determined using the body proportional hypothesis (BPH) of Francis (1990) combined with the GMR of otolith radius to fork length. The BPH assumes the ratio of average fish length to individual fish length is constant for any given otolith radius and is described by the equation:

$$L_t = ((c + dO_t)/(c + dO_c))L_c$$

where: *c* and *d* are the y-intercept and slope of the GMR, L_c is the length of the fish at capture, O_t is the length of the otolith at age *t* (the distance from the nucleus to annuli *t,* or A_R from equation 1) and O_c is the otolith radius (or R_A from equation 1).

The precision of back-calculated length at age was determined by measuring a random sample of 27 otoliths four times and comparing the back-calculated length for each annulus across the four readings using average percent error (APE) (Beamish and Fournier 1981). APE was calculated using the formula proposed by Ballagh *et al*. (2006):

$$APE_j = \left[\frac{1}{R} \sum_{i=1}^{R} \frac{|X_{ij} - X_j|}{X_j} \right] 100$$

where X_{ij} is the *i*th back-calculated length determined from the *j*th annulus, *R* is the number of times each annulus was measured and used to back-calculate length and X_j is the mean back-calculated length of the *j*th annulus from *R* measurements.

4.2.5 Data analysis

All analyses were done separately for male and female grey mackerel as previous studies have found growth to differ between the sexes (Cameron & Begg 2002). The von Bertalanffy growth function (Beverton and Holt 1957) was used to describe the growth of grey mackerel for both the back-calculated and adjusted length at age data. Likelihood ratio tests (Kimura 1980), which test for an overall difference in growth as well as differences in each of the individual parameters of the growth function, were used to test for differences in the growth of grey mackerel among regions and between growth estimates from back-calculated and adjusted length-at-age. All regions were tested in a

five-way likelihood ratio test to determine if any differences existed among the regions. Multiple comparisons using likelihood ratio tests were then performed on individual pairs of regions. A Bonferroni adjustment was used for the likelihood ratio test multiple comparisons by adjusting the significance level:

$$\alpha_{Adj} = \frac{\alpha}{n}$$

where: α is the significance level, α_{Adj} is the adjusted significance level and n is the number of multiple comparisons.

Analysis of variance (ANOVA) and Bonferroni adjusted multiple comparisons were also used to test for regional differences in back-calculated length-at-age. A full factorial ANOVA using back-calculated length as the dependent variable and age and region as fixed factors was initially done to test for differences among regions. One-way ANOVA and multiple comparisons were then used to test for differences among regions for ages 1 – 6. ANOVA was also used to test for differences between back-calculated and adjusted length-at-age data. The Student's *t*-test was then used to compare back-calculated and adjusted lengths for ages 1 – 6.

Mean back-calculated length-at-age from all annuli were compared to mean back-calculated length-at-age from the last annulus only to infer if there were any selectivity effects on the growth estimates, or the presence of Lee's Phenomenon. Lee's Phenomenon, whereby lengths at early ages back-calculated from younger fish are greater than lengths at the same age estimated from older fish, has been showed to bias estimates of growth from back-calculation of length-at-age using all annuli (Vaughan and Burton 1994). Differences in the mean back-calculated lengths-at-age from all annuli and the last annulus can also be used to infer any gear selectivity effects on sampling (Ballagh et al. 2006). The likelihood ratio test was used to test for differences in the growth estimates between back-calculated length-at-age from all annuli and back-calculated length-at-age from the last annulus only. ANOVA and the Student's *t*-test were also used to compare mean back-calculated length-at-age from all annuli to mean back-calculated length-at-age from the last annulus only for ages 1 – 6.

A novel approach to integrating and synthesising the results of the different tests for differences in regional growth was developed and is proposed here. Each significant multiple comparison test result was assigned a value of one and added to the results of other significant tests for each regional combination for males and females separately. For the likelihood ratio tests, each significant multiple comparison result (overall test and individual parameters) was assigned a value of one. For the ANOVA, each significantly different multiple comparison result for each age class was assigned a value of one. The scores for males and females were then combined to produce a matrix of difference indices for each region combination.

Estimates of growth from back-calculated data for grey mackerel on the Queensland east coast from this study were compared to back-calculated growth estimates from a previous study on the Queensland east coast (Cameron and Begg 2002). The von Bertalanffy growth curves from this study and Cameron and Begg (2002) were plotted together, as was mean back-calculated length-at-age.

4.3 Results

4.3.1 Otolith radius to fork length relationship

Ordinary least squares regression was used to determine that the relationship between otolith radius and fork length was linear (R^2 = 0.76). The relationship was determined to be consistent between regions and sex based on ANCOVA ($F_{10, 614}$ = 1.176, P = 0.304). Geometric mean regression was then used to determine the parameters of the relationship for the back-calculation model (Figure 4.3).
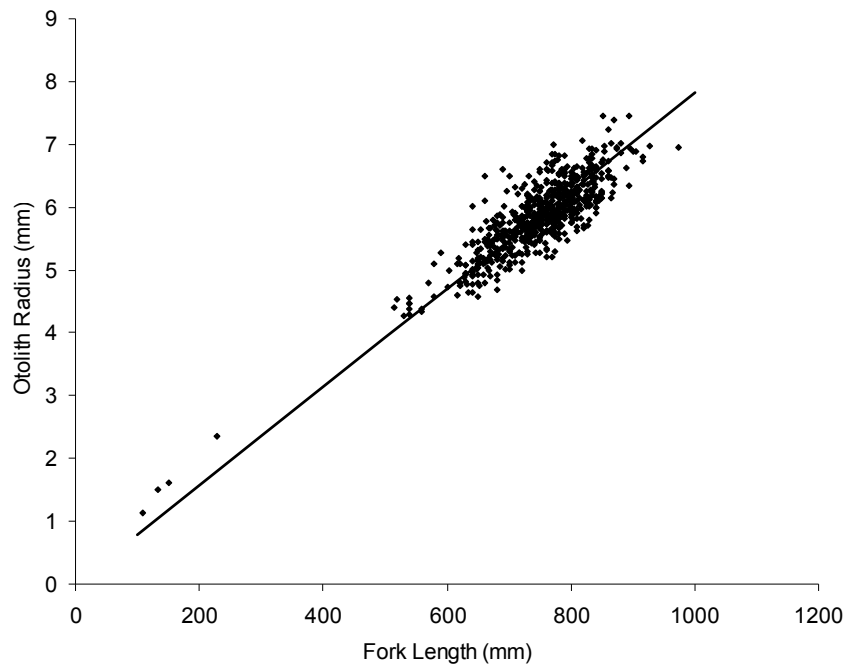
Figure 4.3. Plot of otolith radius on fork length, and the linear relationship (y = 0.0078x + 0.0028) estimated by geometric mean regression (n = 640).

4.3.2 Accuracy and precision

Assessment of the accuracy of the aging technique showed that there was 84% exact agreement between the two readers, and 99% of ages assigned were within one year of each other with no significant bias with age (Figure 4.4). Precision was also assessed, with 83% exact agreement between reads, and 97% of ages assigned between reads within one year of each other and no significant bias between reads. The precision of the back-calculation technique was assessed by comparing back-calculated length-at-age from four separate measurements of the same subset of otoliths using APE. Back-calculation was found to be less precise from the first annulus, however after the first annulus; there was a dramatic decrease in error for back-calculated length-at-age (Figure 4.5).
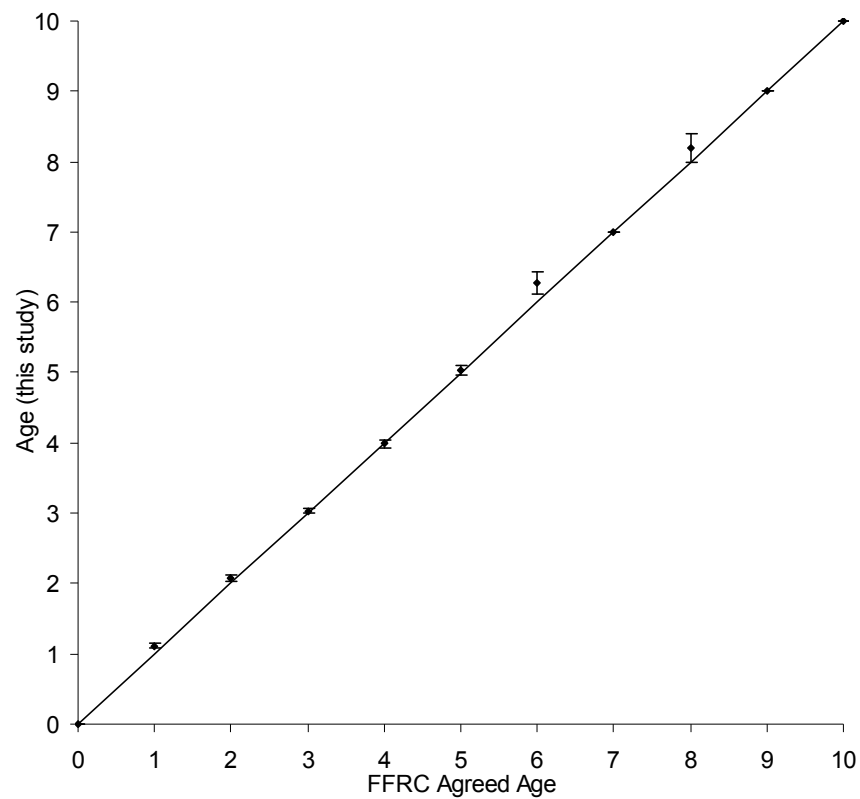
Figure 4.4. Age bias plot of age estimates from this study plotted against the Fishing and Fisheries Research Centre's (FFRC) agreed age to assess ageing accuracy. Solid line indicates 1:1 age estimates, error bars represent standard error.
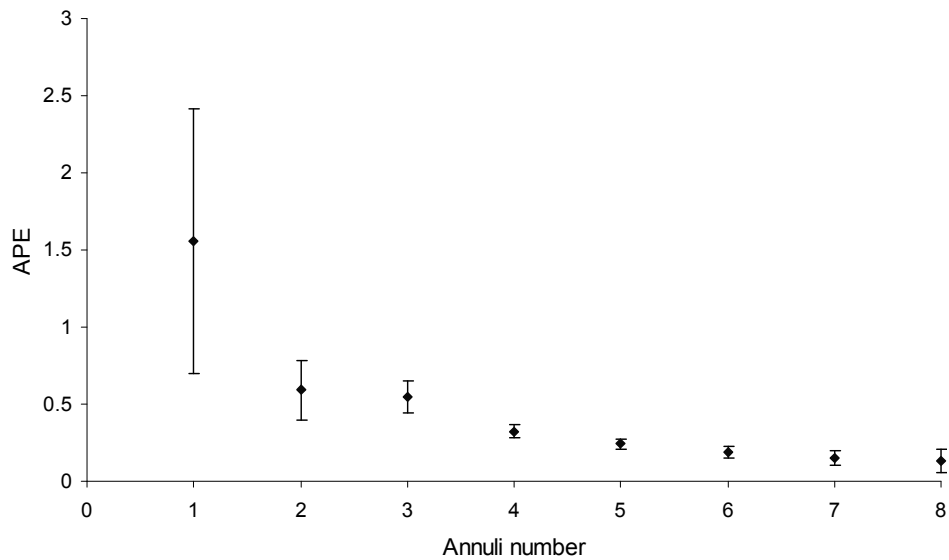
Figure 4.5. Average percent error (APE) of back-calculated length-at-age (± s.e.).

4.3.3 Growth

Growth in female and male grey mackerel was found to be different with female growth characterised by faster growth (K) and a larger average maximum asymptotic length (L_∞) (Figure 4.6). Significant differences in the growth of grey mackerel were found between back-calculated and adjusted length-at-age data using the likelihood ratio test for both females (χ^2 = 172.92, *P* < 0.000) and males (χ^2 = 169.15, *P* < 0.000) (Figure 4.6). ANOVA and *t*-tests of mean length-at-age also showed differences between back-calculated and adjusted length-at-age data for males and females with differences in mean lengths of one-year-olds for both males and females and two-year-old females (*P* < 0.05).

Back-calculated estimates of growth and length-at-age from all annuli and the last annuli did not differ significantly using likelihood ratio tests (Females: χ^2 = 0.55, *P* = 0.91, Males: χ^2 = 0.37, *P* = 0.95), ANOVA or *t*-tests (*P* > 0.05).

4.3.4 Regional Growth

Differences were found in the regional growth of grey mackerel (Figure 4.7, Table 4.2). The five-way likelihood ratio test for differences in regional growth showed that significant differences exist between the regional growth estimates for grey mackerel (Table 4.3). Multiple comparisons of regional growth using likelihood ratio tests revealed that significant overall differences in growth existed between 60% of the regional combinations for both males and females (Table 4.4). Within the regional combinations exhibiting significant differences in overall growth, only four female and two male regional combinations had significant differences in individual growth parameter estimates (Table 4.5).
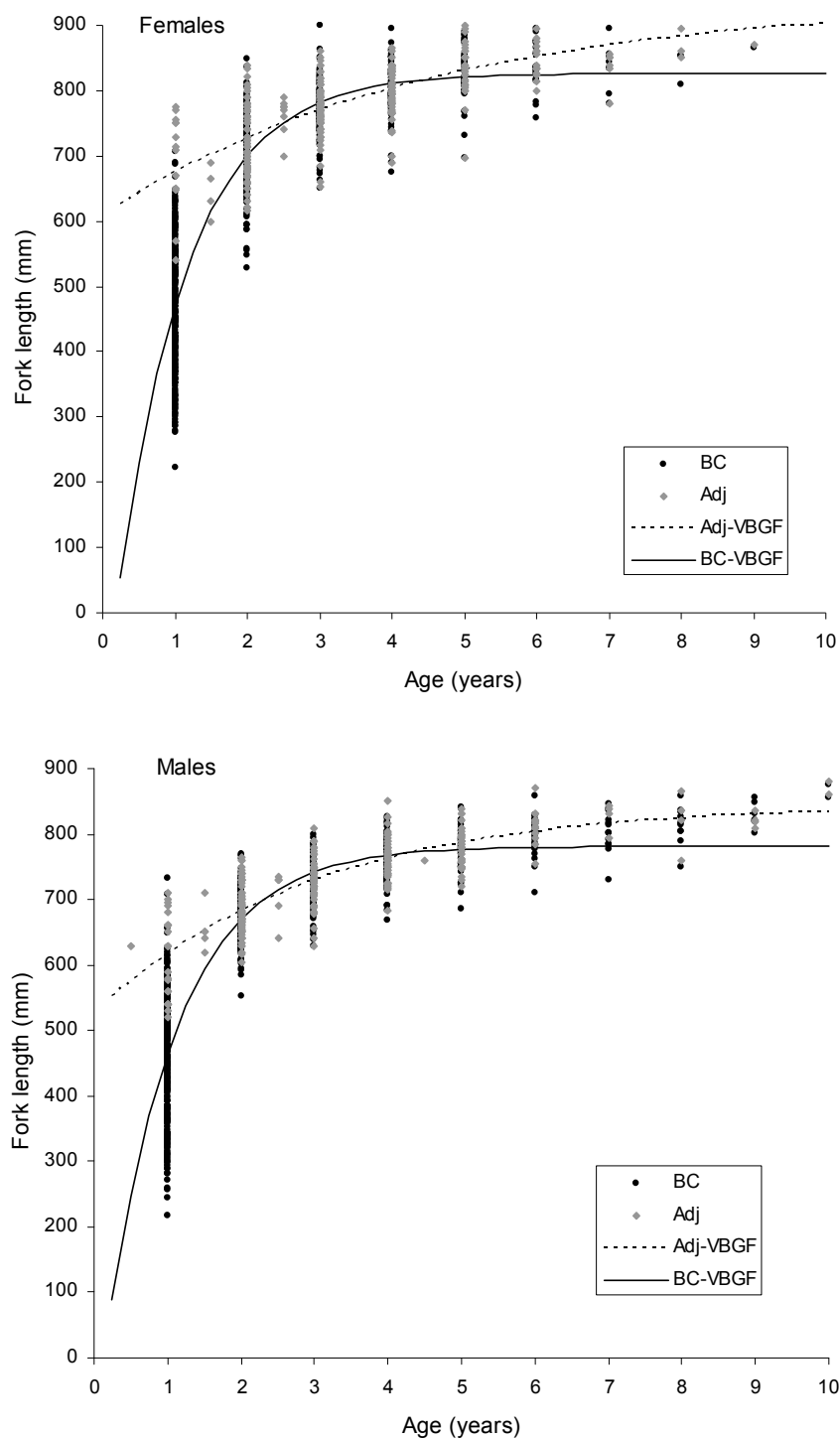
Figure 4.6. =Back-calculated (BC) and adjusted (Adj) length-at-age and the fitted von Bertalanffy growth curves (VBGF) for female (Above; Back-calculated: L_∞ = 827, K = 1.03, t_0 = 0.18, n = 917, Adjusted: L_∞ = 938, K = 0.23, t_0 = -4.64, n = 310) and male (Below; Back-calculated: L_∞ = 781, K = 1.05, t_0 = 0.15, n = 968, Adjusted: L_∞ = 845, K = 0.34, t_0 = −2.87, n = 325) grey mackerel.
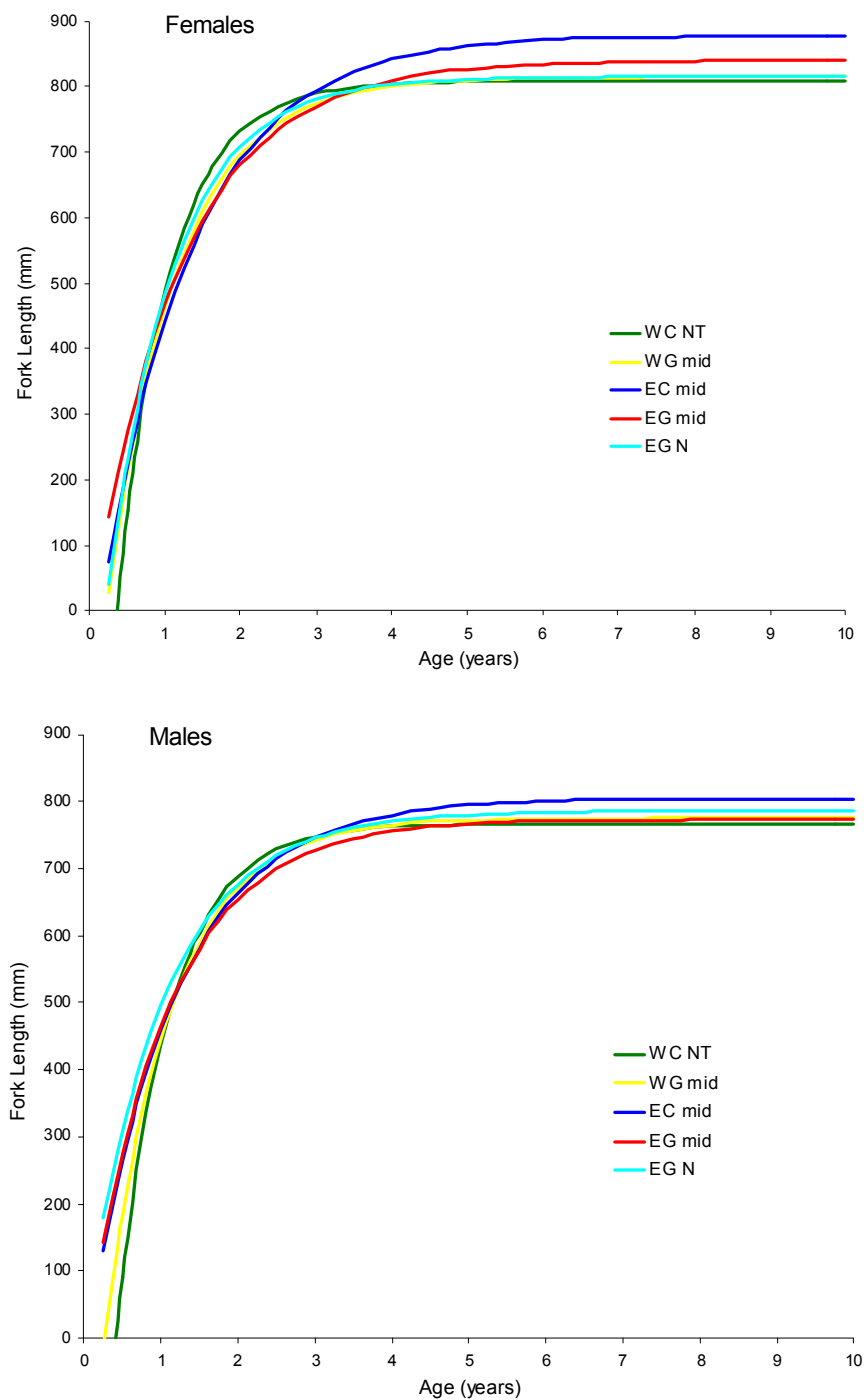
Figure 4.7. Regional von Bertalanffy growth curves for female (top) and male (bottom) grey mackerel.

Table 4.2. Regional von Bertalanffy growth function parameters for grey mackerel.

Sex	Region	K	L_∞	t_0	n
Female	NW NT	1.43	767	0.41	147
	WG	1.16	775	0.27	97
	EC	0.89	804	0.05	229
	EG mid	0.95	774	0.04	264
	EG N	0.99	786	-0.01	231
Male	NW NT	1.44	808	0.36	172
	WG	1.08	815	0.22	224
	EC	0.82	878	0.15	132
	EG mid	0.84	840	0.03	195
	EG N	1.14	815	0.21	194

Table 4.3. Five-way likelihood ratio test of regional back-calculated growth of grey mackerel.

Sex	Test parameter	Sum of Squares	χ^2	df	n	P
Female	Overall	3765199	49.56	12	911	0.000*
	K	3624372	14.83	4	911	0.005*
	L_∞	3622077	14.25	4	911	0.007*
	t_0	3597236	7.98	4	911	0.092
Male	Overall	3140441	64.51	12	960	0.000*
	K	2973647	12.12	4	960	0.017*
	L_∞	2965441	9.47	4	960	0.050
	t_0	2991851	17.98	4	960	0.001*

Table 4.4. Multiple comparisons of regional back-calculated growth from likelihood ratio tests (* indicates significant difference, Bonferonni Adjusted α = 0.005)

Sex	Region	NW NT	WG	EG mid	EG N
Female	EC	0.0001*	0.0008*	0.0235	0.0001*
	NW NT		0.0034*	0.0007*	0.0001*
	WG			0.2544	0.0898
	EG mid				0.0266
Male	EC	0.0155	0.1943	0.0024*	0.0000*
	NW NT		0.7639	0.0011*	0.0000*
	WG			0.0845	0.0001*
	EG mid				0.0000*

There were also significant differences in regional back-calculated length-at-age. Full factorial ANOVA showed the effect of region to be significant for females ($F_{(4)}$ = 2.555, P = 0.038), while One-way ANOVA demonstrated significant differences in regional back-calculated length-at-age for males (ages: 1, 2, 3 and 5) and females (ages: 2, 3 and 6) (Table 4.6). Multiple comparisons revealed which regional combinations differed for each age (Table 4.7).

Table 4.5. Likelihood ratio tests for regional differences in von Bertalanffy growth function parameters (* indicates significant difference, Bonferonni adjusted α = 0.005)

Sex	Region 1	Region 2	L_∞	K	t_0
Female	EC	NW NT	0.0050*	0.0076	0.1574
	EC	WG	0.0010*	0.0753	0.5864
	EC	EG N	0.0054	0.0755	0.7935
	NW NT	WG	0.7258	0.0892	0.2241
	NW NT	EG mid	0.0976	0.0032*	0.0144
	NW NT	EG N	0.0050*	0.0076	0.1574
Male	EC	EG mid	0.0077	0.4738	0.9990
	EC	EG N	0.0238	0.1318	0.9294
	NW NT	EG mid	0.7953	0.0163	0.0015*
	NW NT	EG N	0.6149	0.1140	0.0041*
	EG mid	EG N	0.7271	0.3922	0.9280

Table 4.6. One-way likelihood ratio test of regional growth of grey mackerel

Sex	Age	Sum of Squares	*df*	*Mean Square*	*F*	*P*
Female	1	73743.6	4	18435.9	2.21	0.0682
	2	53823.7	4	13455.9	6.07	0.0001*
	3	14259.7	4	3564.9	2.77	0.0289*
	4	4983.9	4	1246.0	0.81	0.5200
	5	13818.7	4	3454.7	2.38	0.0654
	6	16094.5	4	4023.6	3.20	0.0324*
Male	1	123910.3	4	30977.6	4.17	0.0026*
	2	26809.8	4	6702.5	5.73	0.0002*
	3	13251.2	4	3312.8	3.91	0.0045*
	4	6664.6	4	1666.1	1.97	0.1043
	5	10785.2	4	2696.3	3.55	0.0117*
	6	6320.6	4	1580.1	2.02	0.1301

Table 4.7. Bonferonni adjusted multiple comparisons of regional back-calculated length-at-age (* indicates significant difference).

Sex	Age	Region	NW NT	WG	EG mid	EG N
Female	2	EC	0.0244*	1.0000	1.0000	1.0000
	2	NW NT		0.0060*	0.0000*	0.1629
	2	WG			1.0000	1.0000
	2	EG mid				0.1172
	3	EC	1.0000	0.6570	0.3298	1.0000
	3	NW NT		0.1571	0.0720	1.0000
	3	WG			1.0000	1.0000
	3	EG mid				1.0000
	6	EC	0.0259*	0.3128	1.0000	1.0000
	6	NW NT		1.0000	0.2386	1.0000
	6	WG			1.0000	1.0000
	6	EG mid				1.0000
Male	1	EC	1.0000	1.0000	1.0000	0.1116
	1	NW NT		1.0000	0.8049	0.0017*
	1	WG			1.0000	0.0676
	1	EG mid				0.2938
	2	EC	0.0888	1.0000	1.0000	0.4813
	2	NW NT		1.0000	0.0004*	1.0000
	2	WG			0.7648	1.0000
	2	EG mid				0.0025*
	3	EC	1.0000	1.0000	0.0437*	1.0000
	3	NW NT		1.0000	0.0458*	1.0000
	3	WG			0.2644	1.0000
	3	EG mid				0.0140*
	5	EC	1.0000	0.3174	0.0107*	0.0923
	5	NW NT		1.0000	1.0000	1.0000
	5	WG			1.0000	1.0000
	5	EG mid				1.0000

The integration technique combining the results of the different regional comparisons revealed the NW NT region to be the most different from all other regions, while the WG region was the least different (Figure 4.8). The biggest difference in growth occurred between the NW NT and EG mid regions, while no differences were detected between the WG and EG mid regions (Figure 4.8).
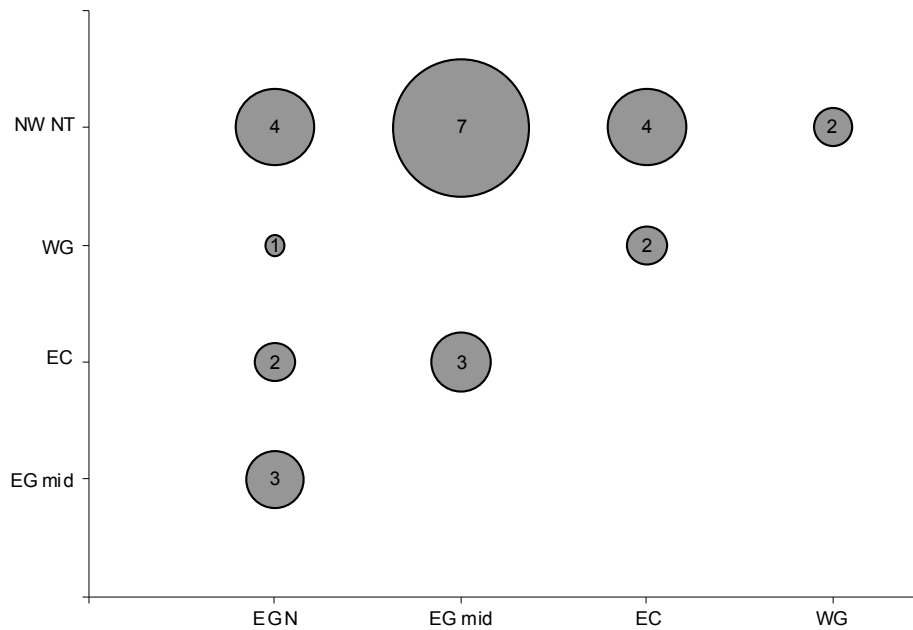
Figure 4.8. Relative differences among pairwise regional comparisons of growth as indicated by the difference indices. Larger indices (circles) represent greater differences.

4.3.5 Comparison to previous growth estimates

Differences in the estimates of growth for grey mackerel on the Queensland east coast were found between this study and Cameron and Begg (2002) (Figure 4.9). Their study produced estimates of maximum asymptotic length that were higher for both females and males, while their estimates of growth rate (K) were lower. Differences in mean back-calculated length-at-age between this study and those of Cameron and Begg (2002) estimates followed a similar pattern for both male and females whereby the mean length of one-year-olds were found to be higher in this study, while mean lengths from older ages (ages 4 – 7) were lower (Figure 4.10).
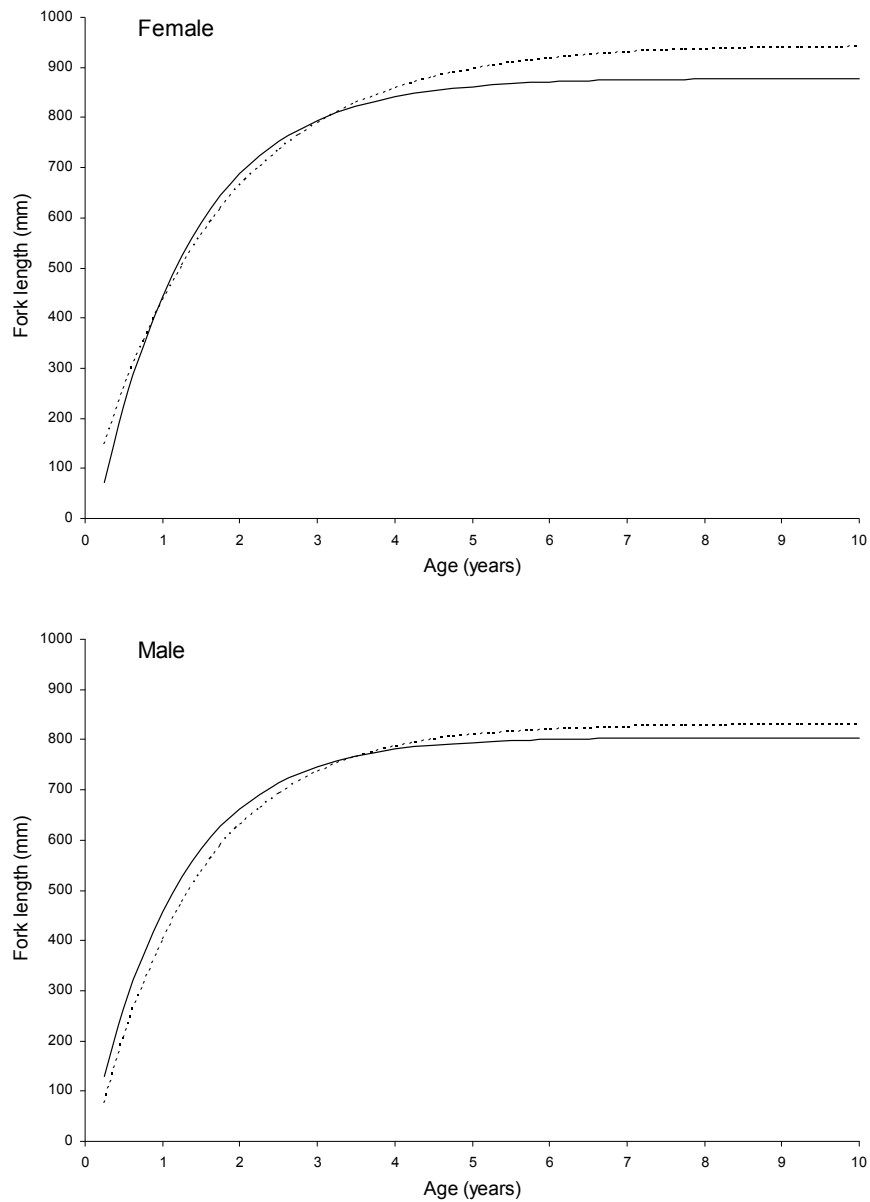
Figure 4.9. von Bertalanffy growth curves from back-calculated length-at-age for female (top) and male (bottom) grey mackerel on the Queensland east coast from this study (solid line) and Cameron and Begg (2002) (dashed line).
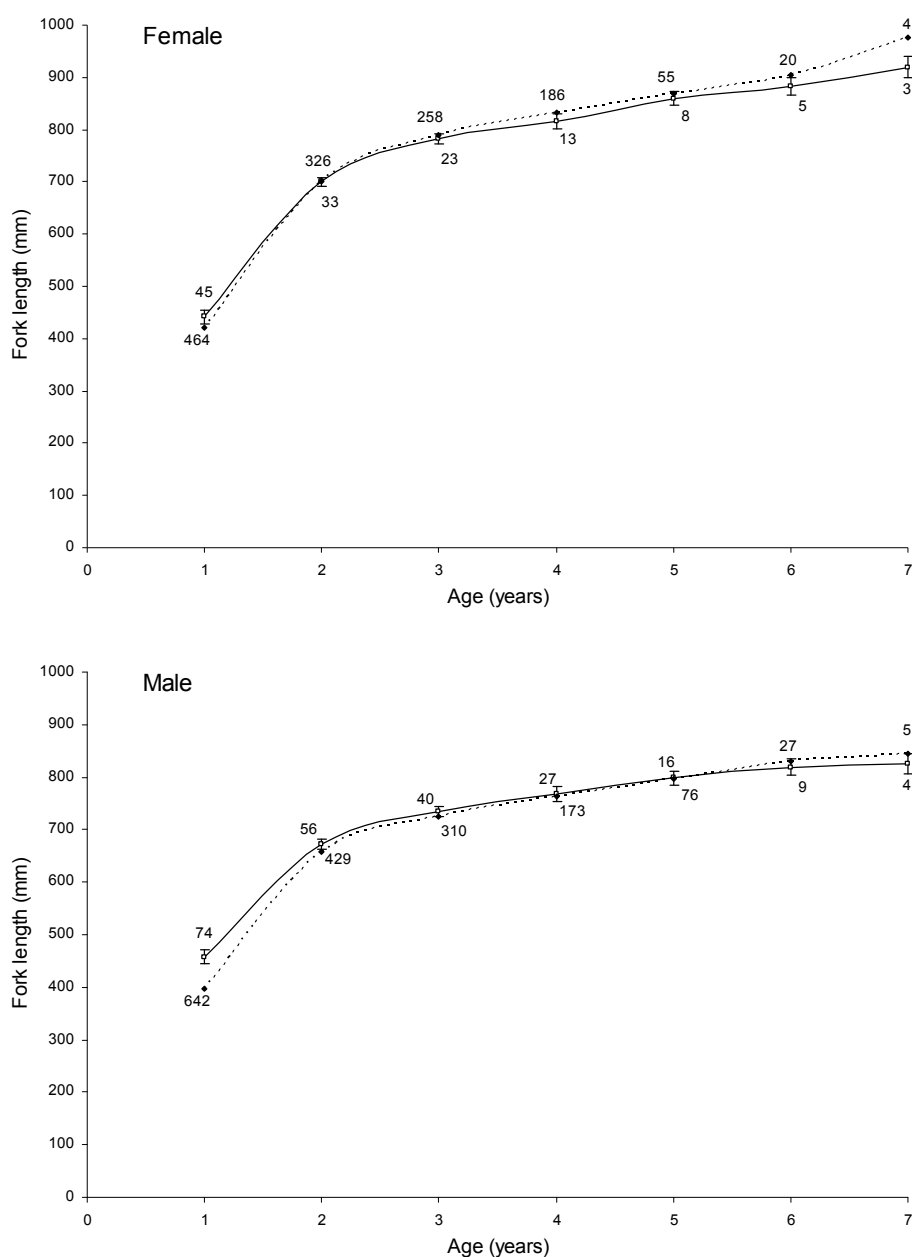
Figure 4.10. Mean back-calculated length-at-age for female (top) and male (bottom) grey mackerel on the Queensland east coast from this study (solid line, ± s.e.) and Cameron and Begg (2002) (dashed line). Numbers indicate sample size.

4.4 Discussion

The results of this study indicate the possibility that a number of different stocks of grey mackerel exist across the northern coast of Australia based on differences in growth among regions. The growth characteristics of fish from the NW NT location were

distinctly different from most other regions, as were those of fish from the EC. Within the Gulf of Carpentaria, results were ambiguous and it is possible that this area could comprise a single or multiple stocks. The findings of this study are supported by a previous study on the stock structure of grey mackerel in north-eastern Australia, which found fish from the east coast to be distinct from fish in the Arafura Sea using life-history parameters, otolith chemistry and genetic analysis (Cameron and Begg 2002). Cameron and Begg (2002) did not, however, statistically compare their estimates of growth from the different locations, as was done in this study.

One possible explanation for the apparent stock structure of grey mackerel across northern Australia is the historical land barrier between the tip of Cape York and southern Papua New Guinea (Chivas et al. 2001). This barrier was present until around 9700 years ago and has commonly been cited as a likely reason for isolation of stocks from the east coast of Australia, and those in the Gulf of Carpentaria, for a range of species including the Australian barramundi (Chenoweth et al. 1998a, b), mud crab (Gopurenko & Hughes 2002) and school and spotted mackerel (Begg et al. 1998). A similar, albeit deeper barrier; the Arafura Sill, also exists on the western rim of the Gulf of Carpentaria and runs north towards West Papua (Chivas et al. 2001). The Arafura Sill may have previously had a similar effect to the historical land barrier between Cape York and Papua New Guinea. During periods of low sea level, the Gulf is effectively blocked off from the Arafura and Coral Seas and therefore could isolate stocks from the east and west. As the Sill is not as shallow as the Torres Strait, this western barrier forms less often, and doesn't last for as long. Another possible explanation for stock separation on the east coast is the current patterns in the Torres Strait, which are thought to be unfavourable for the exchange of larvae (Chenoweth et al. 1998a, Gopurenko & Hughes 2002). This theory however, is partially dependent on the swimming abilities of grey mackerel larvae and may be unlikely as many species of fish larvae have been shown to have well developed swimming abilities (Leis & Carson-Ewart 1999, Bellwood & Fisher 2001, Leis & Carson-Ewart 2003).

It is during these periods of population isolation and separation that differences in environmental conditions can influence growth characteristics. In this study grey mackerel from the east coast were found to grow larger than all other regions. This is likely to be due at least in part to the cooler sea temperatures on the east coast of

Queensland compared to the Gulf of Carpentaria and the northwest coast (Anon 2007). Comparison of growth is a useful method for stock discrimination, however, it should be noted that where there are no differences in growth between different locations, it does not necessarily mean fish from these locations comprise one stock. Since growth can be influenced by genetic and environmental factors, where differences in growth are not found it may be a result of separate stocks of fish inhabiting similar environments and so exhibiting similar growth characteristics. There is no single stock identification method that can be used to emphatically discriminate separate populations and so it has been proposed that a number of complementary techniques be used (Begg and Waldman 1999).

Although this chapter reports on the results of one technique for stock identification (growth), we did utilise a number of statistical analysis methods for making inferences about stock structure based on growth. This was because for each statistical method for comparing growth, on its own, did not give a complete overview of differences in regional growth as differences were not entirely consistent between methods. In isolation, growth comparison methods may provide somewhat unclear results, due in part to the assumptions and sensitivities of the analyses and the variable nature of growth. The integration of several methods for comparing growth among regions however, produced difference indices that encapsulated all the differences in regional growth. This proved to be a useful technique for summarising the results of the different methods in a manner which is readily interpretable.

Significant differences were found between estimates of growth from back-calculated and adjusted length-at-age data, particularly for young fish. It is presumed that back-calculated length-at-age is more accurate than observed length-at-age when estimating growth for young fish as it incorporates the early growth history of fish from all ages thus avoiding potential issues with size-selective mortality (Ballagh *et al.* 2006). We used age adjustment in this study as a means of improving the resolution of length-at-age data (De Vries and Grimes 1997; Mackie et al. 2003; Begg et al. 2006). Despite the use of age adjustment, differences in adjusted and back-calculated length-at-age for young fish were still evident, suggesting the presence of size-selective mortality. Contrary to this, no difference was found between back-calculated length-at-age data from all annuli and the

last annuli only, suggesting neither size-selective mortality nor statistical bias (Lee's Phenomenon) was influencing back-calculated growth estimates.

Our estimates for the growth of grey mackerel on the Queensland east coast from back-calculation were similar to previous back-calculated estimates of Cameron and Begg (2002). Fishing effort and catch has increased substantially for grey mackerel on the east coast since the earlier study and this study (see Chapter 2). This increase in fishing mortality, at this stage at least, appeared to have had no influence on the population growth of grey mackerel.

Although this study provides evidence and reasoning for a review of management regimes for grey mackerel across north-eastern Australia, the ambiguous results within the Gulf of Carpentaria suggests further research is required to resolve whether there exists multiple stocks or a single stock. The Gulf is even more critical as it encompasses different jurisdictions for management of grey mackerel. Importantly, this study provides accurate regional estimates of grey mackerel growth parameters as input parameters for stock assessments, and stock structure results from this study will be an important guide as to the spatial scale at which these assessments are applied.

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5. GENETIC POPULATION STRUCTURE OF GREY MACKEREL (*SCOMBEROMORUS SEMIFASCIATUS*) IN NORTHERN AUSTRALIA

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5.1 Introduction

The identification of stocks and the capacity to discriminate among them are integral elements of fishery management (Waldman 1999). While the definition of a stock is often contextual, it generally refers to populations that are self sustaining and demographically independent (Dizon *et al.*, 1992, Moritz 1994, Begg and Waldman 1999). Stocks are important to fisheries management because fishing mortality needs to be offset against each stock's capacity to replenish itself as recruitment from adjacent stocks is often limited. Molecular techniques are commonly used to investigate stock structuring and have been deployed in a wide array of marine organisms (e.g. turtles, Dethmers *et al.* 2006; finfish, Ovenden *et al*. 2002; sharks, Dudgeon *et al*. in press; prawns, Ward *et al.* 2006; mud crabs, Gopurenko and Hughes 2002). Populations typically need to be isolated for extended periods (100's of generations) before genetic differences become apparent. Consequently, populations that are genetically differentiated will also be demographically independent and managing these populations separately will be key for successful fisheries management.

Similar issues faced the management of Spanish mackerel across northern Australia but were resolved by taking a multi-disciplinary approach to elucidate stock structure and determine the appropriate scale of management (Buckworth et al. 2006). Genetic, otolith microchemistry (Newman *et al.* in review) and parasite data (Moore *et al*. 2003) were combined to offer unique insights into the organization of fish assemblages over different temporal and spatial scales. In general, genetic data provides resolution over broad spatial and temporal scales while parasite and microchemistry data are typically more sensitive to environmental influences accumulated over a fish's lifetime. The current study of grey mackerel followed the same multi-disciplinary approach used to identify

stock structure in the Spanish mackerel fishery but here we report only the genetic findings.

Existing genetic studies using allozyme data (Cameron and Begg 2002) indicated that grey mackerel populations along the central Queensland coast were genetically distinct from more westerly populations in the Gulf of Carpentaria (GOC) and Arafura Sea. This allozyme study yielded important information about broad scale population structure but lacked the power to elucidate structure on a finer spatial scale. Our study expands on this earlier study by using more powerful genetic markers (mtDNA sequence and microsatellites) applied at major fishing grounds from the eastern coast of central Queensland (QLD) through to northwestern Northern Territory (NT) and Western Australia (WA).

5.2 Methods

5.2.1 Sampling

Grey mackerel were sampled from commercial catches from several populations across northern Australia (WA west coast, NT NW coast, GoC and QLD east coast; Figure 5.1). Biological information was linked to samples taken for genetics, otolith microchemistry and parasitology on standardized datasheets. Approximately 200mg of muscle tissue was dissected and preserved in 1ml of NaCl saturated solution with 20% dimethyl-sulphoxide. Sample vials were later air-freighted to the Molecular Fisheries Laboratory for DNA extraction and storage at -20^{0}C.

5.2.2 Laboratory

Total genomic DNA extraction

Total genomic DNA was extracted from small amounts of tissue (0.1g) by overnight digestion at 56°C in 500ul of extraction buffer containing 10mM NaCl, 10mM TRIS, 25mM EDTA, 0.1 mg/ml Proteinase K and 0.5% SDS (pH 8.0). Digested proteins and cellular material were precipitated by addition of a ½ volume of 7.5M Ammonium acetate and centrifugation (13 000 rpm for 20 min at 4°C). The supernatant was transferred to a new tube and DNA was subsequently precipitated by adding 1 volume of cold EtOH and centrifugation (13 000 rpm for 20 min at 4°C). Residual salts were removed by rinsing

the DNA pellet with 80% and 100% EtOH washes. The DNA was resuspended in 1x TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0) and concentration estimated by UV spectrometry using a Biotech Powerwave XS plate reader.

Microsatellite genotyping and analysis

Microsatellites developed from Spanish mackerel (Broderick *et al.* in prep), king mackerel (Broughton *et al.* 2002; Gold *et al*. 2002) and others that are known to amplify across a range of species (eg. Herwerden *et al*. 2000) were assessed for their utility in grey mackerel. Five of the seven loci (Sca30, Sm37, 90RTE, Sca8 & Sm30) used in the Spanish mackerel Genetag project (FRDC 2002/011), together with four additional loci (Sca44, Sca37, Bst6 & Sm31), were suitable for grey mackerel genotyping. Microsatellite PCR amplifications were performed in 96-well plates using Perkin Elmer 9600 & 9700 series thermocyclers. PCR reactions using a Qiagen Multiplex PCR Kit (12μl) contained 6μl of 2xMaster Mix, 1.2 u1 of 5xQ solution, 0.2μM forward and 2μM reverse primer, 2μM fluoro-labeled M13 primer and approximately 20ng of genomic DNA template. Forward primers had an M13 extension (GAGCGGATAACAATTTCACACAG) at the 5' end, enabling fluorescent labeling with the M13 primer (Broderick and Ovenden, MS; Schuelke, 2000). The DNA template and enzyme were denatured at 94°C for 15 min, followed by 35 cycles consisting of 94°C for 30 sec, 58°C for 45 sec and 72°C for 90 sec. A final extension at 72°C for 45 min was used to ensure complete addition of adenine to the PCR product, essential for consistent allele calling during genotyping. Compatible loci were amplified in multiplexed PCR reactions and all products were combined for gel separation on an ABI3130xl sequencer located in the Molecular Fisheries Laboratory. Positive and negative extraction and PCR controls were used throughout and genotypes were scored and binned using ABI Genemapper 3.7 software.
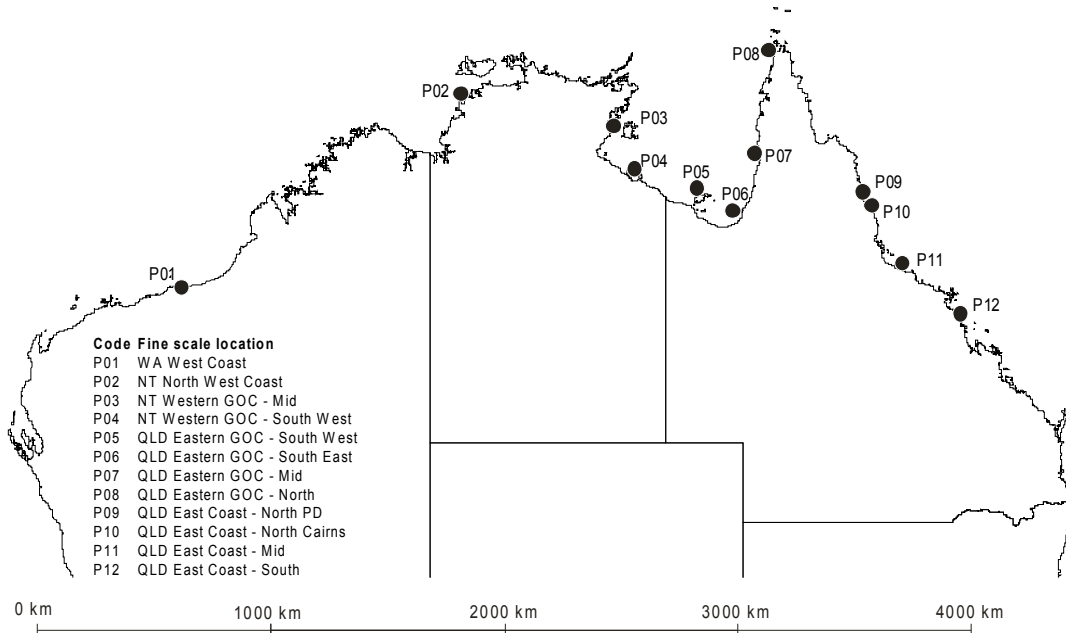
Figure 5.1. Collection locations for twelve grey mackerel populations across northern Australia.

GenAlEx (Peakall and Smouse 2006) was used to calculate a range of population genetic statistics including the number of alleles per locus, expected heterozygosity (H_E), observed heterozygosity (H_O), genotype probabilities (GP) and to investigate population structure using standard F_{ST} (Weir and Cockerham, 1984) genetic distance measures in an AMOVA framework. Additional tests for isolation by distance (IBD), Hardy Weinberg equilibrium, linkage disequilibrium and population differentiation were computed using Genepop 4.0.7 (Rousset 2007).

Mitochondrial DNA sequencing and analysis

MtDNA haplotypes from 4 genes were determined using direct sequencing of PCR amplicons. For D-loop we amplified the 5′ end of the control region (or D-loop) using primers Pro889U20 (CCW CTA ACT CCC AAA GCT AG) and TDKD1291L21 (CCT GAA ATA GGA ACC AAA TGC T; Ovenden *et al.* 2002), ATPase was amplified using primers ATP8.2 (AAA GCR TYR GCC TTT CAA GC) and COIII.2 (GTT AGT GGT CAK GGG CTT GGR TC; Hurwood and Hughes 1998), ND4 was amplified using primers H122293-LUE (TTG CAC CAA GAG TTT TTG GTT CCT AAG ACC; Inoue *et al.* 2001) and ND4r (CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC; Arevalo *et al.* 1994), and CO1 was amplified using primers FishF1 (TCA ACC AAC CAC AAA GAC ATT GGC AC) and

FishR1 (TAG ACT TCT GGG TGG CCA AAG AAT CA; Ward *et al.* 2005). Polymerase chain reaction amplifications were carried out in 25μl volumes using the following reagent concentrations: dNTP's, 100mM each; primers, 0.5 μM each and 3 mM $MgCl_2$. Each reaction contained 0.5 Units of Taq DNA polymerase and the reaction buffer supplied by the manufacturer (®Qiagen P/L). Thermal cycling conditions consisted of an initial denaturation, 94°C for 1 min 30 secs followed by 35 cycles of 94°C for 5 seconds, 55°C for 30 seconds and 72°C for 30 secs, with a final extension step of 72°C for 5 minutes using Perkin Elmer 9600 & 9700 series thermocyclers. PCR products were cleaned up prior to sequencing using a Qiagen QIAquick PCR cleanup kit protocol. Approximately 20ng of DNA was used in standard ABI Dye Terminator sequencing reactions and capillary gel separated on an ABI3130XL sequencer.

Sequence data was edited and aligned with Staden (Staden 2005). Phylogenetic trees were constructed from mtDNA sequence data using Tamura and Nei distances (Tamura and Nei 1993) with a gamma correction of 0.25 in MEGA 3.0 (Kumar *et al.* 2004). Arlequin (Excoffier *et al.* 2005) was used to calculate pairwise F_{ST} statistics and conduct Fisher's exact tests for population differentiation (Raymond and Rousset, 1995). Tests for isolation by distance were performed in GenAlEx (Peakall and Smouse 2006) from distance matrices generated in Arlequin.

5.3 Results

5.3.1 Species Identification

In large datasets, species misidentification can lead to erroneous patterns of population genetic structuring. Moreover, given the potentially ubiquitous nature of hybridization in mackerel (Srinivasa Rao and Lakshmi, 1993; Banford *et al*. 1999), and the prevalence of cross species amplification among related taxa (Broderick *et al.* in prep), we took a conservative approach to data analysis. Individuals with low genotype probabilities were removed, as were individuals that were genotyped with less than nine loci. With less than nine loci, there was insufficient statistical power for the genotype probability method to identify species misidentifications and possible hybrids. Animals can be outliers for either mtDNA or microsatellite genotypes, or both. Animals that are outliers for either mtDNA or microsatellites, but not both, can occur due to interspecies hybridization

followed by uni-directional backcrossing and as a consequence of the different modes of inheritance for mtDNA and microsatellites.

To identify individuals with unusual microsatellite genotypes, we plotted genotype probabilities (GP, Paetkau *et al.* 1994) in rank order and assessed the distribution for outliers (Figure 5.2). Three individuals had very low microsatellite GP's and were outside the continuous sinusoidal curve (e.g. GP $< 10^{-15}$) indicating that they are unlikely to have been drawn from the northern Australian grey mackerel genetic pool. Mitochondrial DNA sequence confirmed that one of these individuals was a school mackerel (*S. queenslandicus*). The other two had grey mackerel mtDNA sequences. All three individuals were removed from the dataset. More suspect individuals were identified among the partially genotyped dataset. MtDNA sequence data also indicated the existence of 'recombinant' mitochondrial genomes consistent with interspecies hybridization. This unusual finding will be reported elsewhere (Ovenden and Broderick in prep).
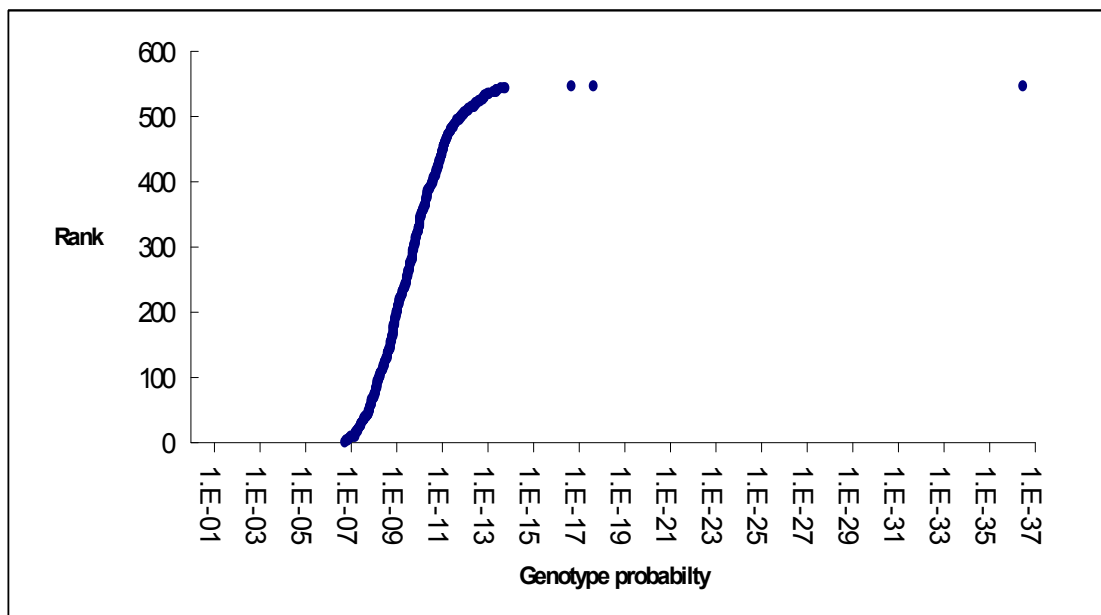
Figure 5.2: Plot of ranked genotyped probabilities of 547 grey mackerel based on allele frequencies at seven microsatellite loci. Individuals with very low GP's and which are outliers from the continuous sinusoidal curve are unlikely to have been drawn from a grey mackerel genetic pool.

5.3.2 Microsatellites

Microsatellite summary statistics from 544 grey mackerel genotyped at 9 loci are shown in Table 5.1. Average heterozygosity across all 9 loci was 0.623 and ranged from 0.356 at the SM31 locus to 0.903 at SCA8. Overall deviations from Hardy-Weinberg equilibrium were detected at a single locus SCA44 ($p < 0.036$), somewhat surprising given the close approximation of H_O and H_E (0.428 *c.f.* 0.449), and 8/108 population by loci tests were significant. Overall linkage disequilibrium was detected between SCA30 and SM3 loci (highly significant) however when we considered all population by loci pairwise combinations, linkage disequilibrium was detected in only 3% (12/404) of those combinations. Moreover, linkage disequilibrium between SCA30 and SM3 was only detected in one population, despite this combination of loci being highly significant overall. We judged both departures from Hardy-Weinberg equilibrium and levels of linkage disequilibrium to be slight and the data suitable for subsequent population genetic analysis.

Table 5.1: Summary statistics across 9 microsatellite loci. Reported are the number of individuals genotyped at each locus (N), the number of alleles observed (Na), effective number of alleles observed (Ne), observed heterozygosity (Ho), expected heterozygosity (He) and the p-value of no significant difference in observed compared to expected genotype proportions (Hardy Weinberg equilibrium (p(HWE))).

	SCA30	SM3	SM37	90RTE	SCA8	SM31	SCA44	BST6	SCA37
N	544	544	544	544	544	544	544	544	544
Na	24	9	30	6	33	7	4	9	4
Ne	9.994	3.893	6.535	2.016	10.258	1.553	1.815	1.628	2.510
Ho	0.906	0.748	0.827	0.487	0.884	0.344	0.428	0.375	0.608
He	0.900	0.743	0.847	0.504	0.903	0.356	0.449	0.386	0.602
p(HWE)	0.662	0.780	0.791	0.860	0.796	0.504	0.036	0.422	0.520

A strong east – west pattern of population genetic structuring is evident from the low (range: 0.033 – 0.0624, mean: 0.051) but significant pairwise F_{ST} measures that clearly differentiate PO1 - WA West Coast from all other locations surveyed in this study (Table 5.2a). By contrast, the remaining 11 locations are largely undifferentiated with only 2/55 comparisons significant; both involving NT North West Coast. As the vast majority of F_{ST}'s are near zero, the lack of significant structuring is more likely due to near homogeneity of gene pools rather than a lack of sampling intensity failing to detect underlying population genetic structuring. To explore potential subtle population genetic structuring further we took an iterative approach and pooled adjacent non-significant

populations and recalculated the pairwise F_{ST} statistics. This approach is supported by the linear, coastal distribution of Grey mackerel along northern Australia. In cases of ambiguous structuring patterns, where locations were differentiated from more distant but not adjacent locations (e.g. PO2 - NT North West Coast, Table 5.1), they were left ungrouped until an unambiguous pattern of structuring emerged in later rounds of pooling. In the first round we combined PO3-4, PO5-7, PO9-10 and P11-P12 and left PO1, PO2 and PO8 ungrouped. PO1 remained differentiated from all groups but the pooling strategy did not resolve the ambiguous position of PO2. In the second round we combined PO3-7 and P09-12 and left PO1, PO2 and PO8 ungrouped. Again, PO1 remained differentiated from all groups but the pooling strategy did not resolve the ambiguous position of PO2. In the third round we combined PO3-8 and P09-12 and left PO1 and PO2 ungrouped. In this round an unambiguous pattern of population genetic structuring emerged (Table 2b). PO1 and PO2 were significantly differentiated from all other groups but we could not differentiate between east coast QLD (PO9-12) and Gulf of Carpentaria (PO3-8) groups. The identification of three genetic groups (stocks) should be viewed with caution given the number of insignificant population comparisons in the first instance; nonetheless, this strategy is informative to indicate the spatial scale over which separate stocks may exist.

While the pattern of genetic distinctiveness of PO1 remains strong in the pooled data, there is a hint of isolation by distance (IBD) as F_{ST} and geographic distance appear correlated. This opens up the possibility that IBD, rather than discrete stock boundaries, may be a more appropriate way to describe relationships among grey mackerel populations across northern Australia. However tests for IBD among the 11 NT and QLD locations show that the slight positive relationship is insignificant (Figure 5.3, p < 0.7804).

Table 5.2: Pairwise F_{ST} distances measures from microsatellite data are reported below the diagonal and their significance are reported above the diagonal (significant comparisons are in bold) among a) 12 regions and b) 4 groups of pooled regions.

a) Pairwise F_{ST} 12 regions

Region	n	P01	P02	P03	P04	P05	P06	P07	P08	P09	P10	P11	P12
P01 W/A West Coast	15		**0.0000**	**0.0000**	**0.0004**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0340**	**0.0000**	**0.0000**
P02 NT North West Coast	144	**0.0406**		0.4348	0.7037	0.0625	0.1664	**0.0011**	0.2165	0.2889	0.9070	0.2129	**0.0333**
P03 NT Western GOC - Mid	43	**0.0591**	0.0013		0.6808	0.6179	0.8099	0.1005	0.1851	0.6714	0.8531	0.5846	0.5802
P04 NT Western GOC - South West	9	**0.0589**	-0.0095	-0.0041		0.7214	0.7237	0.5593	0.4788	0.8695	0.7682	0.7668	0.5250
P05 QLD Eastern GOC - South West	23	**0.0643**	0.0035	-0.0015	0.0025		0.7893	0.1052	0.5907	0.8204	0.9062	0.5567	0.2802
P06 QLD Eastern GOC - South East	23	**0.0478**	0.0019	-0.0016	-0.0023	-0.0016		0.6015	0.2941	0.9573	0.5460	0.8273	0.5870
P07 QLD Eastern GOC - Mid	122	**0.0468**	**0.0010**	0.0012	-0.0047	0.0038	0.0005		0.2979	0.6431	0.9176	0.1220	0.7162
P08 QLD Eastern GOC - North	54	**0.0519**	0.0001	0.0027	-0.0074	-0.0014	0.0001	0.0002		0.1847	0.2882	0.2405	0.2770
P09 QLD East Coast - North PD	29	**0.0557**	-0.0020	-0.0011	-0.0116	-0.0036	-0.0035	-0.0004	-0.0039		0.7049	0.9897	0.9001
P10 QLD East Coast - North cairns	7	**0.0332**	-0.0060	-0.0027	0.0001	-0.0063	-0.0007	-0.0055	-0.0017	-0.0057		0.9932	0.7839
P11 QLD East Coast - Mid	27	**0.0624**	0.0030	0.0003	-0.0027	0.0000	0.0008	0.0025	0.0027	-0.0062	-0.0124		0.7558
P12 QLD East Coast - South	48	**0.0480**	**0.0003**	-0.0013	-0.0077	0.0028	-0.0020	-0.0021	-0.0011	-0.0035	-0.0111	-0.0031	

b) Pairwise F_{ST} 4 groups of pooled regions

Group	Regions	n	Group 1	Group 2	Group 3	Group 4
Group 1	P01	15		**0.0000**	**0.0000**	**0.0000**
Group 2	P02	144	**0.0406**		**0.0003**	**0.0003**
Group 3	P03 - P08	274	**0.0514**	**0.0009**		0.0738
Group 4	P09 - P12	111	**0.0536**	**0.0017**	0.0005	
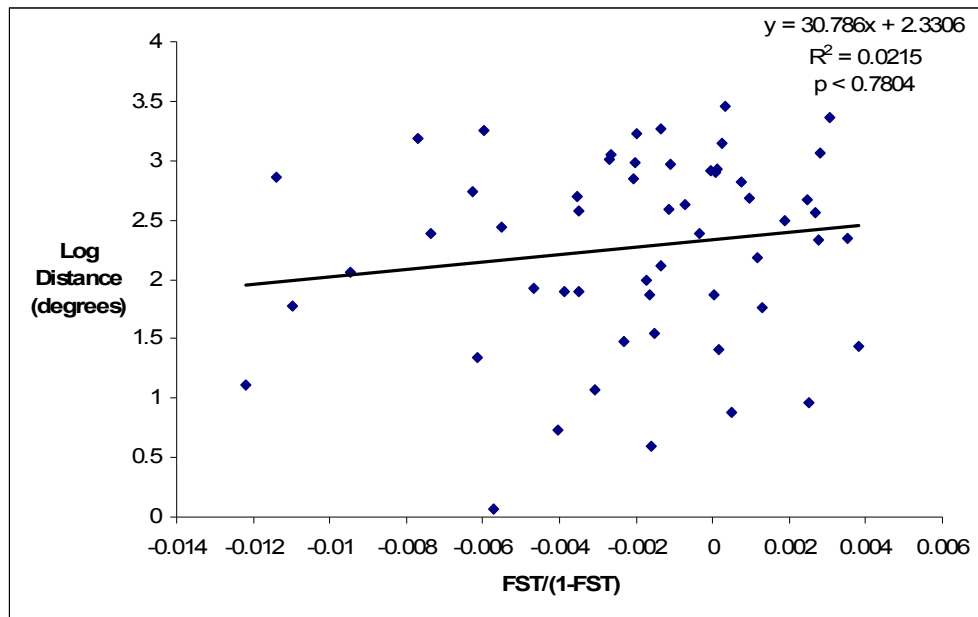
Figure 5.3: The relationship between genetic and geographic distance among NT and QLD grey mackerel populations. A mantel test revealed that the slight positive correlation is insignificant.

4.3.3 Mitochondrial DNA sequencing

An initial survey of sequence polymorphism at four mtDNA gene regions from 8 individuals sampled across northern Australia revealed that D-loop was the most polymorphic (π = 0.0113, 348bp), followed by ATP (π = 0.0030, 800bp), ND4 (π = 0.0014, 733bp) and CO1 (π = 0.0008, 626bp). We chose to expand our survey of the ATP and D-loop fragments as we judged them to be more informative for stock discrimination.

Nucleotide polymorphism in 348bp of d-loop sequence revealed 55 transitions and 13 transversions defining 95 haplotypes from 364 individuals surveyed. The three most common D-loop haplotypes (CR01, CR02 & CR03) were widespread and represented 67% of all individuals surveyed (Table 5.3). Nucleotide polymorphism in 800bp of ATP sequence revealed 34 transitions and 7 transversions defining 45 haplotypes from 357 individuals surveyed. The two most common ATP haplotypes (AT01 & AT02) were widespread and represented 78% of all individuals surveyed (Table 5.3). As expected, both nucleotide and haplotype diversity was higher in the D-loop (π = 0.0095, ĥ= 0.7919) compared to ATP (π = 0.0016, ĥ= 0.6019) fragment.

The phylogenetic relationships among d-loop and ATP haplotypes are represented by unrooted neighbour-joining radiation trees (Figures 5.4 & 5.5 respectively). Three star like clades are evident for the d-loop fragment and two clades are evident for ATP fragment. All clades are dominated by a high frequency central haplotype (assumed to be ancestral) with numerous closely related haplotypes in low frequency. Typically, divergent lineages are more likely to arise among populations that have been historically isolated and star like phylogenies are indicative of past population expansion because drift is ineffective at removing both ancestral and recently derived haplotypes due to large effective populations. The grey mackerel phylogenies have characteristics of both historical processes yet little phylogeographic structuring is evident in either fragment indicating that contemporary levels of gene flow are sufficiently high to mask any signal of historical population genetic structuring.

We used pairwise F_{ST} measures and haplotype frequency variation on data from each mtDNA gene region to test for contemporary population genetic structuring among the 12 locations. Comparisons were considered to be significant if they either had F_{ST}'s greater than expected under the null distribution of no subdivision or the Fisher's exact test indicated that their haplotype frequencies were different. As with the microsatellite dataset, we took an iterative approach and pooled adjacent non-significant populations, left ambiguous populations ungrouped and recalculated the pairwise F_{ST} statistics and exact-test until an unambiguous pattern of population genetic structuring emerged. Pairwise F_{ST} measures using the D-loop fragment revealed a strong pattern of differentiation between PO1 - WA West Coast and most other locations (9/11 significant comparisons; Table 5.4a). The remaining 11 locations were largely undifferentiated with 7/55 comparisons significant. After several iterations of pooling and recalculating F_{ST}'s, PO1, PO2, east coast QLD (PO9-12) and Gulf of Carpentaria (PO3-8) were all significantly differentiated from each other (Table 5.4b). Pairwise F_{ST} measures using the ATP fragment revealed a strong pattern of differentiation between PO1 - WA West Coast and most other locations (9/11 significant comparisons; Table 5.4c). The remaining 11 locations are largely undifferentiated with 8/55 comparisons significant. After several iterations of pooling and recalculating F_{ST}'s, PO1 and PO2 were significantly differentiated from all other groups but we could not differentiate between east coast QLD (PO9-12) and Gulf of Carpentaria (PO3-8) groups (Table 5.4d). Discrepancies

between the two tests of significance can arise because the F_{ST}'s incorporate a measure of genetic distance among haplotypes while Fisher's exact test is based of haplotype frequency alone. Discrepancies can also occur when populations are weakly differentiated and are at the margins of statistical significance. The pooled ATP data is a good example of this where F_{ST}'s are near zero and insignificant but the Fisher's exact test indicate that they have different haplotype frequencies.

As expected, the patterns of population genetic structuring revealed by the two mtDNA fragments are concordant and support the distinctiveness of the PO1 - WA West Coast location that was clear from the microsatellite dataset. Both fragments clearly indicated that most of remaining sampled locations were weakly differentiated requiring several rounds of pooling before any significant population structuring was revealed. This pattern is also concordant with that revealed by the microsatellite data. The difference between these markers appears to be one primarily of resolution with the mtDNA D-loop fragment being the only marker that can distinguish between east coast QLD (PO9-12) and Gulf of Carpentaria (PO3-8) groups (Table 5.4d).

Like the microsatellite data there was a hint of isolation by distance (IBD) as F_{ST} and geographic distance appear correlated in the pooled datasets of both mtDNA fragments. Tests for IBD that included PO1 - WA West Coast were significant (D-loop, $p < 0.01$; ATP, $p < 0.014$) but there was no evidence for IBD when P01 was removed from the analysis (D-loop, $p < 0.076$; ATP, $p < 0.229$) indicating that IBD was not a significant driver of population differentiation among northern and eastern Australian grey mackerel populations as a group.

Table 5.3: Frequency distribution of a) 95 d-loop and b) 45 ATP mtDNA haplotypes observed throughout the 12 regions surveyed.
a) D-loop mtDNA haplotypes

Haplotypes

Region	n	ĥ	π	CR01	CR02	CR03	CR04	CR05	CR06	CR07	CR08	CR09	CR10	CR11	CR12	CR13	CR14	CR15	CR16	CR17	CR18	CR19	CR20	CR21	CR22	CR23	CR24	CR25	CR26	CR27	CR28	CR29	CR30	CR31	CR32	CR33	CR34	CR35	CR36	CR37	CR38	CR39	CR40	CR41	CR42	CR43	CR44	CR45	CR46
P01 WA West Coast	19			4	3	1														1	1	1	1	1	1	1	1	1	1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
P02 NT North West Coast	58			18	2	2	1	1	4	6		1	2	2																		1	2	1	1														
P03 NT Western GOC - Mid	26			13	3	1	1	1							1																																			
P04 NT Western GOC - South West	21			6	2	2		2		1	1																																						
P05 QLD Eastern GOC - South West	25			12	2	2	1	2	1					1	1	1																																	
P06 QLD Eastern GOC - South East	22			9	3	3	1		1							1																1																	
P07 QLD Eastern GOC - Mid	45			24	1	1			1	2	2	1	1				1			1																													
P08 QLD Eastern GOC - North	22			10	1	3			1	1	1						1		1	1																													
P09 QLD East Coast - North PD	45			27	2	2						2	1					1	2																														
P10 QLD East Coast - North cairns	17			7	4													1	1																														
P11 QLD East Coast - Mid	37			20	2	1	1		1			1	1			1	1	1	1																														
P12 QLD East Coast - South	27			12	4	3	2	2										1	1	1													2															1	1
	364			162	29	18	8	7	7	6	4	4	4	3	3	3	5	4	4	3	1	1	1	1	1	1	1	1	1	1	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Haplotypes

Region (continued)	CR47	CR48	CR49	CR50	CR51	CR52	CR53	CR54	CR55	CR56	CR57	CR58	CR59	CR60	CR61	CR62	CR63	CR64	CR65	CR66	CR67	CR68	CR69	CR70	CR71	CR72	CR73	CR74	CR75	CR76	CR77	CR78	CR79	CR80	CR81	CR82	CR83	CR84	CR85	CR86	CR87	CR88	CR89	CR90	CR91	CR92	CR93	CR94	CR95	CR96
P01 WA West Coast	1	1	1	1	1	1	1	1																																										
P02 NT North West Coast																																																		
P03 NT Western GOC - Mid												1	1	1																																				
P04 NT Western GOC - South West										1	1	1	1	1	1	1	1																																	
P05 QLD Eastern GOC - South West																		1	1	1	1	1	1	1	1	1	1	1																						
P06 QLD Eastern GOC - South East	1								1																				2	1	1	1																		
P07 QLD Eastern GOC - Mid																																		1	1	1	1	1												
P08 QLD Eastern GOC - North																																								1										
P09 QLD East Coast - North PD																														2	1	1	1	1	1	1	1	1			1	1	1							
P10 QLD East Coast - North cairns																																									1	1		1						
P11 QLD East Coast - Mid																																			1					1		1	1	1	1	1	1		1	
P12 QLD East Coast - South			1																																2	2	1	1	1	2	2	1	1	1	1	1	1	1	1	1
	2	1	1	1	1	1	1	1	2	1	1	1	1	1	1	2	1	2	1	1	1	1	2	1	1	1	1	1	1	2	1	1	1	1	2	2	1	1	1	2	2	1	1	1	1	1	1	1	1	1

b) ATP mtDNA haplotypes

Region	n	ĥ	π	AT001	AT002	AT003	AT004	AT005
P01 WA West Coast	19			7	6	1	4	1
P02 NT North West Coast	58			29	8	6	2	
P03 NT Western GOC - Mid	29			20	4	4		
P04 NT Western GOC - South West	21			10	9			
P05 QLD Eastern GOC - South West	19			12	3	2		
P06 QLD Eastern GOC - South East	22			12	3	3		
P07 QLD Eastern GOC - Mid	45			25	9			
P08 QLD Eastern GOC - North	22			16	3	2		
P09 QLD East Coast - North PD	43			36	4			
P10 QLD East Coast - North cairns	17			9	4			
P11 QLD East Coast - Mid	36			24	5			
P12 QLD East Coast - South	27			16	4			
Total	**358**			**216**	**62**	**18**	**6**	**1**

Haplotype column totals (AT001–AT047): 216, 62, 18, 6, 1, 3, 2, 2, 2, 1, 1, 1, 1, 1, 1, 1, 1, 2, 1, 4, 1, 2, 1, 1, 3, 2, 2, 1, 1, 1, 1, 1, 1, 1, 1, 1, 2, 1, 1, 1, 1, 1, 1, 1, 1

Table 5.4: Pairwise F$_{ST}$ distances measures using D-loop and ATP mtDNA fragments are reported below the diagonal and p values above the diagonal with significance comparisons in bold. Significant Fisher's exact test comparisons are shaded.

a) Pairwise F$_{ST}$ D-loop 12 regions

Region	n	P01	P02	P03	P04	P05	P06	P07	P08	P09	P10	P11	P12
P01 WA West Coast	19		**0.0127**	**0.0313**	0.3272	**0.0000**	**0.0244**	**0.0029**	**0.0000**	**0.0000**	0.1065	**0.0000**	**0.0137**
P02 NT North West Coast	58	**0.0704**		0.2393	0.4063	0.1006	0.3359	0.0684	**0.0234**	**0.0020**	0.0586	**0.0176**	0.0986
P03 NT Western GOC - Mid	26	**0.0726**	0.0066		0.5801	0.6455	0.9688	0.7383	0.2266	0.0840	0.4063	0.2109	0.8818
P04 NT Western GOC - South West	21	0.0040	-0.0023	-0.0126		0.2148	0.6260	0.1865	**0.0430**	**0.0078**	0.3311	**0.0156**	0.3184
P05 QLD Eastern GOC - South West	25	**0.1299**	0.0269	-0.0129	0.0167		0.7236	0.7529	0.4541	0.2627	0.1240	0.5664	0.7959
P06 QLD Eastern GOC - South East	22	**0.0854**	0.0013	-0.0305	-0.0165	-0.0176		0.9883	0.3740	0.2363	0.2881	0.3565	0.9102
P07 QLD Eastern GOC - Mid	45	**0.1368**	0.0228	-0.0146	0.0150	-0.0139	-0.0252		0.4873	0.3135	0.1436	0.4404	0.8936
P08 QLD Eastern GOC - North	22	**0.1873**	**0.0545**	0.0121	**0.0628**	-0.0042	-0.0010	-0.0054		0.6260	0.0918	0.8604	0.3672
P09 QLD East Coast - North PD	45	**0.2479**	**0.0706**	0.0280	**0.0912**	0.0078	0.0107	0.0028	-0.0100		**0.0166**	0.9697	0.2207
P10 QLD East Coast - North cairns	17	0.0420	0.0544	-0.0028	0.0056	0.0358	0.0126	0.0324	0.0496	**0.0986**		0.0811	0.3057
P11 QLD East Coast - Mid	37	**0.2037**	**0.0603**	0.0114	**0.0656**	-0.0073	0.0001	-0.0038	-0.0186	-0.0172	0.0561		0.4307
P12 QLD East Coast - South	27	**0.1040**	0.0223	-0.0249	0.0036	-0.0201	-0.0279	-0.0186	-0.0008	0.0110	0.0039	-0.0041	

b) Pairwise F_{ST} D-loop 4 groups of pooled regions

Group	Regions	n	Group 1	Group 2	Group 3	Group 4
Group 1	P01	19		**0.0244**	**0.0000**	**0.0000**
Group 2	P02	58	**0.0701**		**0.0059**	**0.0000**
Group 3	P03-08	161	**0.1312**	**0.0273**		0.0043
Group 4	P09-12	126	**0.2032**	**0.0645**	0.1436	

c) Pairwise F_{ST} ATP 12 regions

Region	n	P01	P02	P03	P04	P05	P06	P07	P08	P09	P10	P11	P12
P01 WA West Coast	19		**0.0078**	**0.0088**	0.1191	**0.0244**	**0.0078**	**0.0020**	**0.0020**	**0.0000**	0.0879	**0.0000**	**0.0039**
P02 NT North West Coast	58	**0.1146**		0.9619	0.3428	0.9736	0.8447	0.2852	0.1690	**0.0469**	0.6924	0.0820	0.4424
P03 NT Western GOC - Mid	29	**0.1728**	-0.0201		0.1875	0.9180	0.9102	0.4307	0.3301	0.1621	0.4902	0.2207	0.4815
P04 NT Western GOC - South West	21	0.0616	0.0006	0.0192		0.2656	0.2568	0.2090	0.0957	**0.0098**	0.7227	**0.0518**	0.2256
P05 QLD Eastern GOC - South West	19	**0.1543**	-0.0287	-0.0424	0.0030		0.9902	0.6611	0.4053	0.1982	0.5928	0.3330	0.6504
P06 QLD Eastern GOC - South East	22	**0.1413**	-0.0199	-0.0322	0.0082	-0.0420		0.4512	0.3232	0.1035	0.5586	0.2412	0.4678
P07 QLD Eastern GOC - Mid	45	**0.1906**	0.0038	-0.0064	0.0199	-0.0214	-0.0081		0.6260	0.1504	0.3242	0.4932	0.7461
P08 QLD Eastern GOC - North	22	**0.2586**	0.0146	0.0026	0.0733	-0.0113	-0.0026	-0.0160		0.5127	0.1611	0.9316	0.4971
P09 QLD East Coast - North PD	43	**0.3619**	**0.0428**	0.0270	**0.1504**	0.0189	**0.0301**	0.0143	-0.0173		**0.0332**	0.5508	0.1436
P10 QLD East Coast - North cairns	17	0.0738	-0.0198	-0.0134	-0.0362	-0.0277	-0.0197	-0.0004	0.0337	**0.0997**		0.0772	0.3623
P11 QLD East Coast - Mid	36	**0.2810**	0.0253	0.0114	**0.0852**	-0.0022	0.0101	-0.0075	-0.0255	-0.0119	0.0477		0.3818
P12 QLD East Coast - South	27	**0.1786**	-0.0052	-0.0136	0.0165	-0.0245	-0.0113	-0.0167	-0.0146	0.0167	-0.0084	-0.0057	

d) Pairwise F_{ST} ATP 4 groups of pooled regions

Group	Regions	n	Group 1	Group 2	Group 3	Group 4
Group 1	P01	19		**0.0098**	**0.0010**	**0.0000**
Group 2	P02	58	**0.1154**		0.3574	0.0654
Group 3	P03-08	158	**0.1896**	-0.0011		0.1816
Group 4	P09-12	123	**0.2755**	0.0198	0.0040	
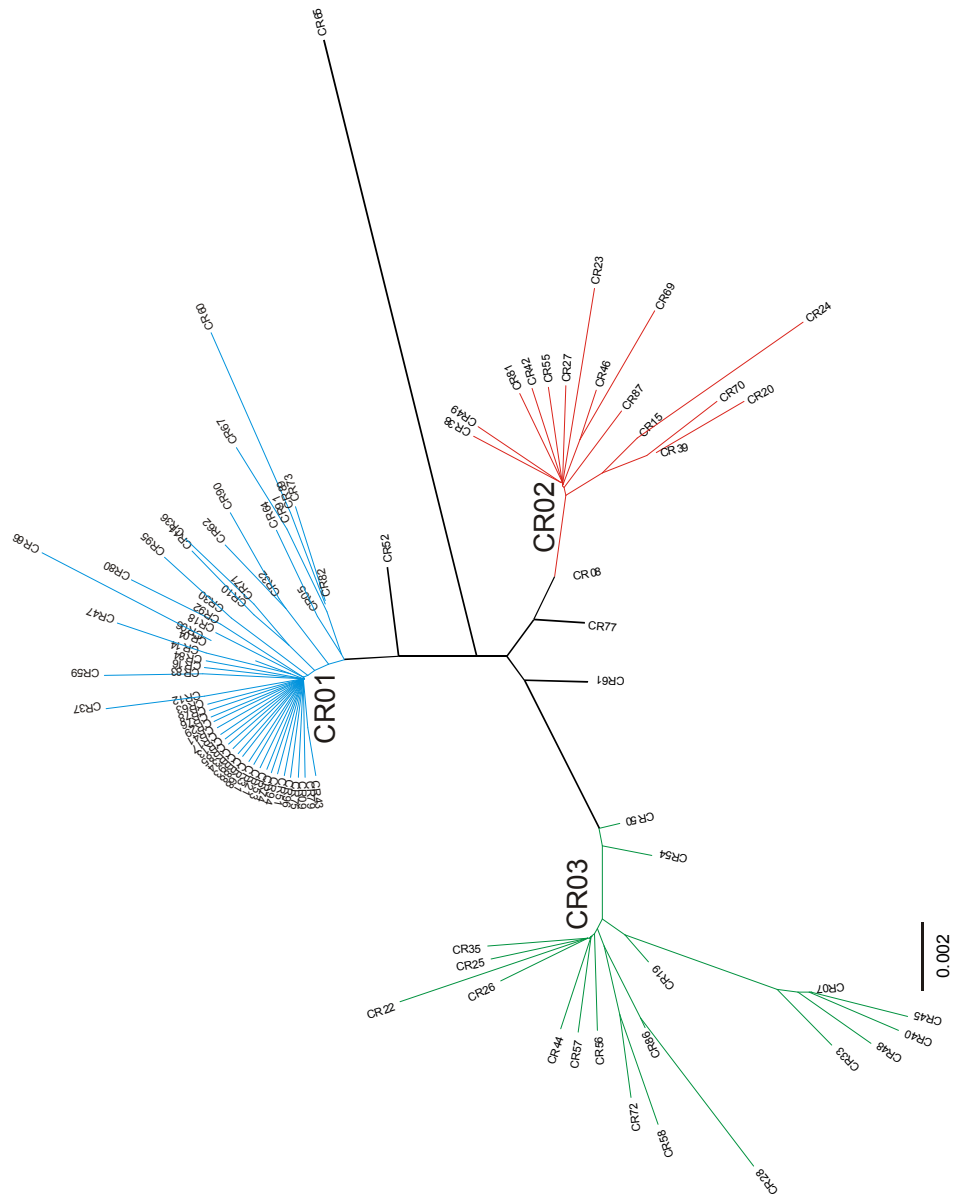
Figure 5.4: An unrooted neighborhood joining radiation tree describing the phylogenetic relationships among 95 mtDNA d-loop haplotypes in grey mackerel. Presumed ancestral haplotypes (e.g. CR01, CR02 & CR03) are at the origins of each radiation whereas derived haplotypes are on the tips.

Grey mackerel management units in northern Australia
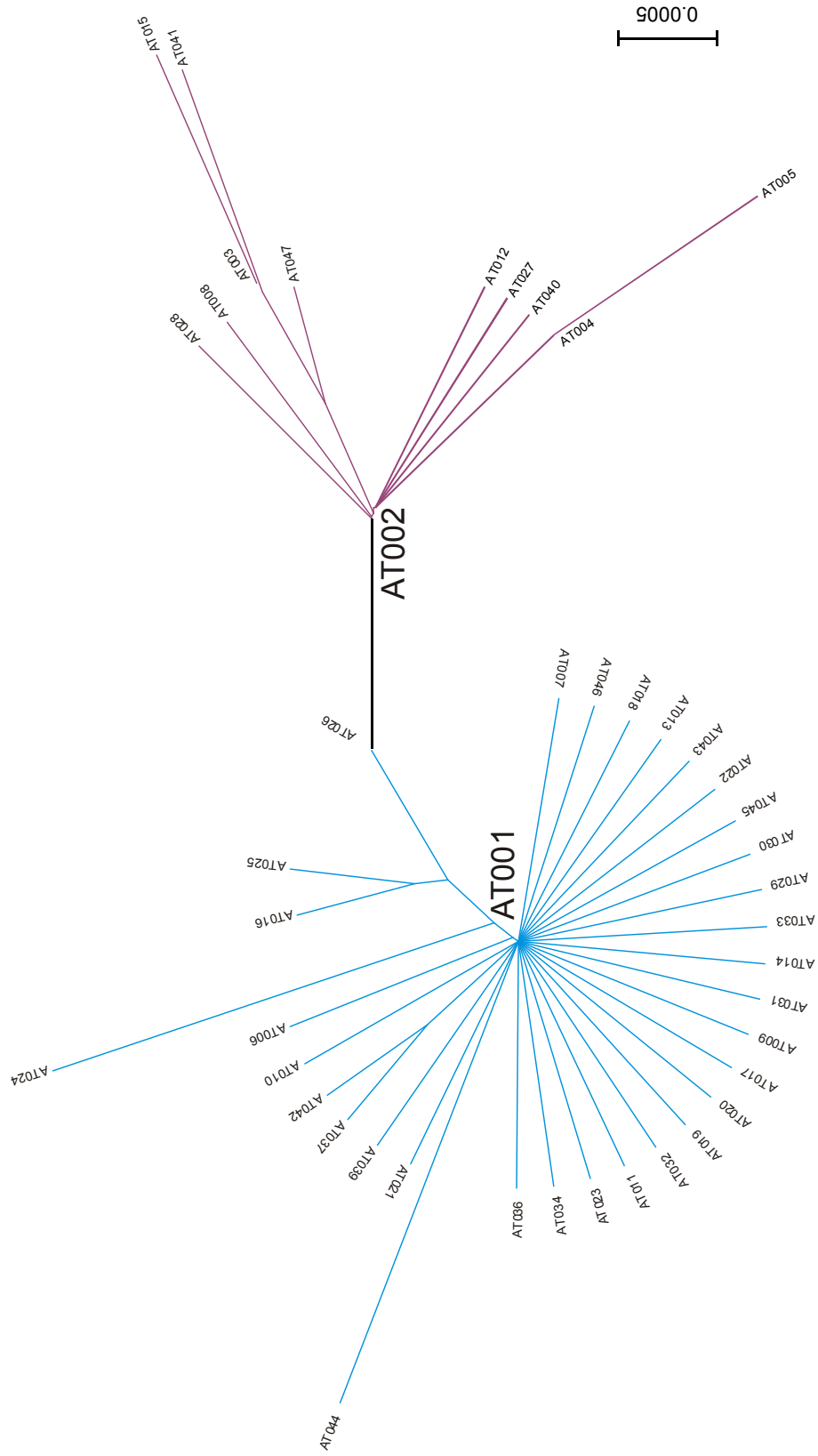
Figure 5.5: An unrooted neighborhood joining radiation tree describing the phylogenetic relationships among 45 mtDNA dATP haplotypes in grey mackerel. Presumed ancestral haplotypes (e.g. AT01 &AT02) are at the origins of each radiation whereas derived haplotypes are on the tips.

5.4 Discussion

5.4.1 Species identification

Accurate species identification is of critical importance in *Scomberomorus* as they are speciose (four in tropical Australia, but many more in Indo-pacific), morphologically similar and hybridization is known to occur (Banford et al. 1999, Rao and Lakshmi 1993 but see Collette 1994). We went to great lengths to identify and exclude non grey mackerel individuals from our dataset by considering only fully genotyped individuals and excluding those whose genetic profiles did not conform to expectations. This was necessary because hybrids among *Scomberomorus* are likely to be morphologically cryptic. Indeed we did find some misidentified school mackerel and also uncovered evidence of hybridization of grey mackerel with both school and spotted mackerel. Further work is clearly required to determine the prevalence and nature of hybridization among *Scomberomorus* in Australian waters as it may have implications for fisheries management. Fishing patterns altering species densities could be linked to the prevalence of hybridization where spawning aggregations are co-located. Persistent hybrid forms could pose challenges for fisheries management as they may not be covered by regulation.

5.4.2 Stock identification

We have compelling evidence for stock structuring in grey mackerel populations using both microsatellite and mtDNA markers. All markers revealed concordant patterns showing WA and NW NT to be clearly divergent stocks. The mtDNA D-loop fragment appeared to have more power to resolve stock boundaries because it was able to show that the GOC and east coast QLD stocks were genetically differentiated. We interpret this as clear evidence for broad scale stock structuring in grey mackerel and is consistent with previous allozyme data (Cameron and Begg 2002).

Geographic distance can only partly explain the genetic distinctiveness of the WA stock as the genetic break here also coincides with a species distribution break along the Kimberly's coastline. Grey mackerel are an inshore species and have preference for shallow turbid waters. Not only are grey mackerel uncommon in this region but the genetic evidence indicates that migration across the Kimberly's is also rare. Extrapolating from the observation of limited genetic differentiation over continuous habitat in northern and eastern Australia, we would expect similar low levels of genetic differentiation of grey mackerel throughout their WA distribution.

Patterns of stock structure on a finer scale, or where stock boundaries are located, are less clear. For both mtDNA and microsatellite data significant structure was observed between some locations and in the process of pooling adjacent insignificant populations some of these differences were not evident. Stock structuring only became apparent after several rounds of pooling adjacent, genetically homogenous locations. From an initial 12 locations we identified four stocks, two stocks comprised of single sampling localities (WA and NW NT) while the other two comprised of six (GOC) and four (EC QLD) pooled sampling localities. It is apparent that grey mackerel stock boundaries in the GOC and EC QLD do not have hard edges, rather they have soft edges. For example, PO8 may have clustered with the other GOC locations due to our pooling rules but it is genetically very similar to locations on the EC QLD and would not look out of place there. This is to be expected given grey mackerels potential for movement and that there is no apparent physical isolating mechanisms between populations.

5.4.3 Historical processes

The northern Australian coastline with its vast shallow waters encompassing the Gulf of Carpentaria and the Arafura Sea is profoundly affected by sea level change each glacial cycle. Most of this region was dry land during the Last Glacial Maximum 18,000 years ago and at its peak when sea levels were 120-150 m below present (Chappell & Shackleton 1986, Voris 2000), the Torres Strait formed a land bridge between the Australian and Papua New Guinean mainland effectively isolating tropical species from east to west. The patterns of molecular diversity in many marine species still have signatures reflecting these historical geological processes of isolation and recolonisation (Spanish mackerel, Buckworth *et al.* 2006; Barramundi, Chenoweth *et al.* 1998; Marine turtles, Dethmers *et al.* 2006; sea snakes, Lukoshek et al. 2007; mud crabs, Gopurenko and Hughes 2002). The most recent grey mackerel colonization of northern Australia occurred in the last 6-10,000 years when sea levels were comparable to contemporary levels. The star shaped phylogenies of grey mackerel mtDNA are characteristic of past population colonization and expansion most likely as a response to historical sea level change opening up new habitat. The divergent haplotypes that occur in high frequency throughout Australia were probably at the origins of independent expansions and radiations. Subsequent gene flow has been so pervasive that it is no longer possible, at least with this dataset, to reconstruct historical colonization pathways. Nor is it possible to tease apart whether this mixing is ongoing in some locations or whether gene flow is restricted in contemporary populations and our failure to detect this is due to a slow return to genetic equilibrium. The observation that we were able to elucidate

some stock structure across this species range indicates that contemporary levels of gene flow are more restricted compared to the past.

5.4.4 Implications for fisheries management

While the preference of fisheries managers is for hard stock boundaries to be defined, or even more preferably, for stock boundaries to be concordant with pre-existing fisheries jurisdictions, rarely is this achievable for wild fish populations. Fisheries managers are likely to be comforted, at least from a genetic perspective, that stock boundaries largely conform to state jurisdictions. The exception being in the Gulf of Carpentaria that straddles both Queensland and Northern Territory waters where we could find no evidence for population subdivisions. While this finding indicates that movement of fish through this habitat is considerable over ecological timescales, it does not preclude a scenario where movement is limited over generational timescales; a timescale more relevant to the management of fish stocks. That said, evidence gathered from other stock discrimination techniques (growth parameters, Chapter 4; otolith microchemistry, Chapter 6; parasites, Chapter 7) is consistent with considerable movement of grey mackerel in the Gulf of Carpentaria. Joint management is well within the capabilities of the state based authorities as several precedents exist to manage GOC fisheries cooperatively.

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6. STOCK STRUCTURE OF GREY MACKEREL, *SCOMBEROMORUS SEMIFASCIATUS,* ACROSS NORTHERN AUSTRALIA BASED ON OTOLITH ISOTOPE CHEMISTRY

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6.1 Introduction

An important concern for the rational management of fisheries is the ability to discern the stock structure of the targeted fish species so that each stock or management unit can be managed in an optimal manner (Begg et al., 1999; Newman et al., 2000). Information on stock structure is particularly important for fish species that are highly targeted and heavily exploited and/or are being managed as a single homogeneous stock or management unit. Knowledge of the stock structure of fish populations aids in the development of rational management plans that facilitate ecologically sustainable development by defining the appropriate spatial scale of fisheries management (Newman et al., 2000).

Previous studies have used stable isotopes within the otolith carbonate of fish to delineate separate stocks or management units and thus the degree of mixing of fish populations among different areas (Edmonds and Fletcher, 1997; Kennedy et al., 1997; Schwarcz et al., 1998; Edmonds et al., 1999; Newman et al., 2000; in review). Stable isotopes are neutral, non-radioactive variants of an element and as a result of their slightly different atomic mass; their relative incorporation into fish otoliths can be modified by environmental conditions or biological activity (Campana, 1999). The stable isotopes of oxygen within fish otolith carbonate are deposited in equilibrium, or very close to equilibrium, with the ambient seawater. Therefore, if there is no significant variation in the isotopic composition of the seawater across the area under study, differences in the average sea temperature should be reflected in the oxygen isotope ratios (Kalish, 1991; Thorrold et al., 1997; Campana, 1999). In contrast, the stable isotopes of carbon are deposited in otoliths under non-equilibrium conditions unrelated to ambient seawater (Mulcahy et al., 1979; Kalish, 1991; Campana, 1999). The disequilibria observed in carbon stable isotopes in fish otolith carbonates have been attributed to metabolic effects, habitat changes and nutrient sources for fish (Kalish, 1991; Thorrold et al., 1997, Schwarcz et al., 1998).

The grey mackerel, *Scomberomorus semifasciatus* (Macleay), has a restricted distribution and is confined to the waters of southern Papua New Guinea and around northern Australia from Shark Bay in Western Australia to northern New South Wales on the east coast (Collette, 2001). *S. semifasciatus* is known as an epipelagic, neritic, coastal species that can attain a maximum size of at least 1.2 m and 10 kg (Collette, 2001). In Australian waters, *S. semifasciatus* is an important commercial and recreational species that is targeted from as far south as the Houtman Abrolhos Islands area on the west coast (23°30'S), across northern Australia and along the east coast to the waters of northern New South Wales (Kailola et al., 1993).

This study aimed to determine whether it is appropriate to manage stocks of *S. semifasciatus* independently in the various management zones across northern Australia. Populations of *S. semifasciatus* are currently managed either as separate fisheries or as part of mixed mackerel fisheries by state-based management agencies in Western Australia, the Northern Territory and Queensland (Gulf of Carpentaria and East Coast. The effectiveness of managing the stocks separately depends on the degree of mixing between management areas. Stable isotope analysis of sagittal otolith carbonate is a quick and relatively inexpensive technique to study the degree of mixing between fish populations. Previous studies have indicated that over a wide latitudinal (and temperature) range, significant differences between areas should be detectable, if the fish populations in the various areas remain separate from each other.

6.2 Materials and Methods

6.2.1 Sampling design

Samples of *S. semifasciatus* were sourced from commercial fishers at each location across northern Australia. Otoliths were collected from fish at ten locations extending from Western Australia across northern Australian waters, throughout the Gulf of Carpentaria (GoC) to Queensland on the east coast of Australia, covering a coastline length of approximately 9000 km (~6500 km in straight line distance). Locations sampled were Western Australia west coast (WA, Port Hedland), Northern Territory north west coast (NT – nwc, Fog Bay, Bathurst Island), Northern Territory western GoC mid (NT – wGoC – mid, Cape Shields), Queensland eastern GoC south west (QLD – eGoC – sw, Mornington Island), Queensland eastern GoC south east (QLD – eGoC – se, Karumba), Queensland eastern GoC mid (QLD – eGoC – mid, Holroyd, Nassau and Coleman Rivers), Queensland eastern GoC north (QLD – eGoC – north, Crab Island), Queensland east coast north (QLD – EC – n, Port Douglas), Queensland

east coast mid (QLD – EC – mid, Townsville), Queensland east coast south (QLD – EC – s, Mackay). Where possible, a target of 40 pairs, of otolith samples (20 male, 20 female), were collected from each location on two separate occasions, a minimum of six months apart.

6.2.2 Otolith preparation

Sagittae were rinsed in water, allowed to dry and stored in vials prior to processing. One sagitta from each fish was selected at random and cleaned by scrubbing with a nylon brush under ultrapure water, air-dried (50°C) and crushed to powder in an agate mortar and pestle. Powdered sagittae were cleaned of organic matter (contaminants) by treatment with hydrogen peroxide and deproteinated by dissolving and extracting protein using a centrifuge. Powdered sagittae were then analysed for $^{18}O/^{16}O$ and $^{13}C/^{12}C$ ratios by standard mass spectrometric techniques after the carbonate was decomposed to CO_2 with 100% phosphoric acid. A laboratory reference sample was run every thirty samples. The laboratory reference sample, consisting of a batch solution of digested otolith reference material, was used to monitor measurement precision across sample batches, and was subsequently used to normalise sample batches to a constant reference value. Stable isotope ratios are reported using the international standard delta (δ) notation relative to the PDB-1 standard for carbonates (i.e. $\delta^{18}O$ and $\delta^{13}C$).

6.2.3 Statistical analysis

In order to identify relevant homogeneous groups of the locations an agglomerative, hierarchical cluster analysis on the mean values of the stable isotope ratios of $\delta^{18}O$ and $\delta^{13}C$, supplemented by MANCOVA and post –hoc analyses, was performed using all sites (a total of 10 locations). The cluster analysis is based on linkage analysis (closeness) between different subsets using euclidean distances and is an objective method to determine minimum variance clustering. In order to remove any confounding effects likely to be associated with a bias of young fish sampled at a particular location, and to improve homogeneity and normality and to make treatment effects additive, only mature fish were included in the analysis. Post-hoc tests from these analyses allowed the identification of DIGC (disjoint irreducible geographic clusters).

As a tool to quantify the statistical separation between main clusters, discriminant function analysis was undertaken using the stable isotope ratios of $\delta^{18}O$ and $\delta^{13}C$ as predictors of membership to each of the clusters. The observed misclassification rate then provides a measure of their separation. We first consider only the discrimination between the nearest neighbour regions (as a simplifying step), producing one discriminating function for each

comparison. In constructing the linear discriminant functions, each cluster was given equal prior weighting. In order to provide a measure of the misclassification probability between the clusters we present an analysis of classification errors of each of the discrimination functions. These statistics then provide an alternative measure of the similarity and separation between the subregion clusters.

6.3 Results

The dendogram resulting from the cluster analysis of δ^{18}O and δ^{13}C values for the 10 different locations across northern Australia illustrates six natural location groupings (Figure 6.1). Thus, the aggregation of some of these locations into their own natural clusters at the lowest level provided the following cluster sizes for further analysis; Western Australia (n=40), Queensland east coast south (n=40), Queensland north (n=91), eastern GoC (n = 101), western and southern GoC (n=324) and the Northern Territory west coast (n=122). The dendrogram resulting from the cluster analysis of δ^{18}O and δ^{13}C values for the six natural clusters is illustrated in Figure 2. Examination of the dendrogram (Figure 6.2) of these 6 natural clusters reveals no significant differences among the first 3 clusters and thus they are combined to form a GoC cluster that represents all GoC sites in addition to those on the Northern Territory west coast. These 4 significant clusters represent disjoint irreducible geographic clusters whose closeness can be investigated analytically.

The extent of the separation of these 4 natural clusters above was investigated using MANCOVA analysis of δ^{18}O and δ^{13}C values as dependent variables and geographic location as an independent variable for all data points simultaneously. The resulting homogeneous subsets identified from the post-hoc analysis are aggregated to form 4 contiguous areas. MANCOVA of the δ^{18}O and δ^{13}C ratios from all *S. semifasciatus* showed that the location effect was highly significant (Table 6.1).

In order to identify the pattern of location differences for the bivariate (δ^{18}O and δ^{13}C ratios) response variable, or rather the degree of statistical separation between sub-clusters of the dendrogram, a bivariate post-hoc test procedure, similar in operation to the univariate response situation would be extremely useful, but does not appear to be available in the statistical methodology literature. In this less than ideal world, the approach we take below represents the best we can get from existing methodology. Since the δ^{18}O ratio provides much more discriminatory power than the δ^{13}C ratio, evidenced by the much lower *p*-values associated with location main effect in the respective analyses of variance, homogeneous

subsets based on Duncans Test were determined for the $\delta^{18}O$ ratio within the clusters and are shown in Tables 6.2 – 6.3. The various mutually exclusive subsets of the various clusters are of considerable interest, and we designate them as DIGC, and see them as the base units of the population.

Examination of the homogenous subsets of $\delta^{18}O$ in Table 2 showed WA and QLD east coast south to be very similar, and significantly different from the other locations. Examination of the homogenous subsets of $\delta^{13}C$ in Table 3 showed WA and QLD east coast south to be significantly different, with QLD east coast south and QLD north very similar. Thus all 4 main clusters are significantly different and provide conclusive evidence of population subdivision. These represent the DIGC across northern Australia.

The discriminant function analyses between the identified groups are summarised in Table 4. For the discrimination between the GoC and the QLD north clusters, 425 of the 547 GoC values are correctly classified and 75 of the 91 QLD north values are correctly classified. In the discrimination between the QLD north and QLD EC south clusters, 74 of the 91 QLD north values are correctly classified and 32 of the 40 QLD EC south values are correctly classified. These discriminant functions clearly discriminate among clusters.
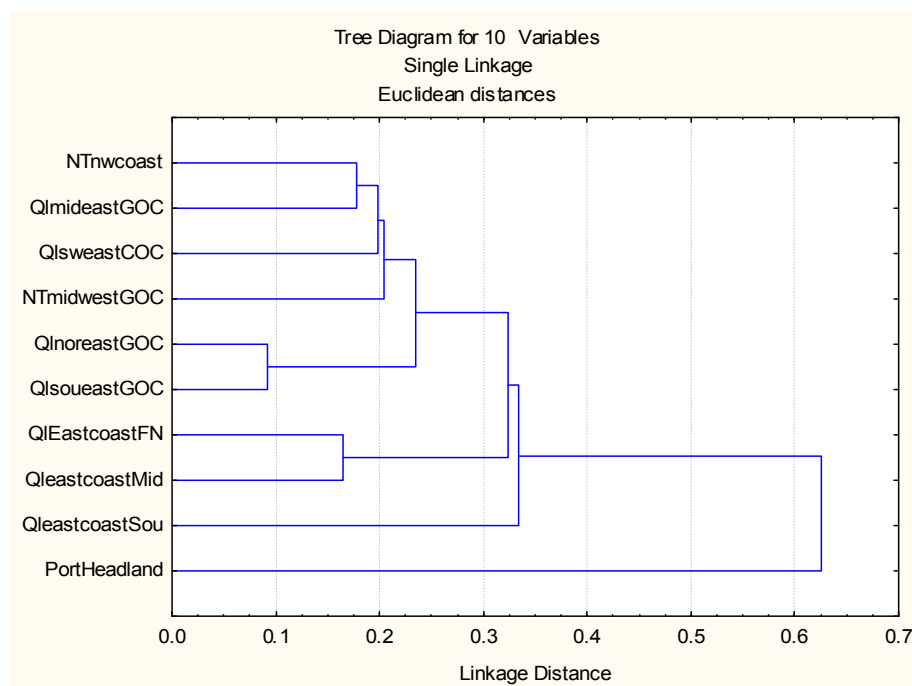
Figure 6.1: Dendogram summarising cluster analysis of mean $\delta^{18}O$ and mean $\delta^{13}C$ values for *S. semifasciatus* sagittal otolith carbonate for all locations across northern Australia.

Figure 6.2: Dendogram summarising cluster analysis of mean δ^{18}O and mean δ^{13}C values for *S. semifasciatus* sagittal otolith carbonate for the six natural clusters derived from Fig 1.

Table 6.1: MANCOVA results of δ^{18}O and δ^{13}C values from the sagittal otolith carbonate of *Scomberomorus semifasciatus* from the four significant natural clusters.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	carbonrat	43.656[a]	3	14.552	79.643	.000
	oxyrat	83.536[b]	3	27.845	222.441	.000
Intercept	carbonrat	4133.698	1	4133.698	22623.447	.000
	oxyrat	373.106	1	373.106	2980.515	.000
loccode02	carbonrat	43.656	3	14.552	79.643	.000
	oxyrat	83.536	3	27.845	222.441	.000
Error	carbonrat	130.460	714	.183		
	oxyrat	89.380	714	.125		
Total	carbonrat	13623.759	718			
	oxyrat	2157.697	718			
Corrected Total	carbonrat	174.117	717			
	oxyrat	172.916	717			

[a]. R Squared = .251 (Adjusted R Squared = .248)

[b]. R Squared = .483 (Adjusted R Squared = .481)

Table 6.2: Analyses of homogeneous subsets based on Duncans Test were determined for the $\delta^{18}O$ ratio from the four significant natural clusters.

Location	N	Subset		
		1	2	3
GoC	547	-1.846		
QLD north	91		-1.264	
WA	40			-0.876
QLD south	40			-0.855
Sig.		1.000	1.000	.737

Means for groups in homogeneous subsets are displayed, based on Type III Sum of Squares. The error term is Mean Square (Error) = .125 (a = uses Harmonic Mean Sample Size = 63.677, b = alpha = 0.05).

Table 6.3: Analyses of homogeneous subsets based on Duncans Test were determined for the $\delta^{13}C$ ratio from the four significant natural clusters.

Location	N	Subset		
		1	2	3
GoC	547	-4.445		
QLD north	91		-4.105	
QLD south	40		-4.094	
WA	40			-3.470
Sig.		1.000	0.885	1.000

Means for groups in homogeneous subsets are displayed, based on Type III Sum of Squares. The error term is Mean Square (Error) = .183 (a = uses Harmonic Mean Sample Size = 63.677, b = alpha = 0.05).

Table 6.4. Discriminant function analysis results from comparison of clusters (the structure matrix consists of pooled within-group correlations between discriminating variables and standardized canonical discriminant functions with the variables ordered by the absolute size of the correlation within functions, all tests are significant p < 0.001).

Discriminant analysis – GoC vs QLD north			
Crosstabulation			
Cluster	Predicted Group for Analysis		
	GoC	QLD north	Total
GoC	425	122	547
QLD north	16	75	91
Total	441	197	638
Discriminant analysis – QLD north vs QLD EC south			
Crosstabulation			
Cluster	Predicted Group for Analysis		
	Q north	Q EC south	Total
QLD north	74	17	91
QLD EC south	8	32	40
Total	82	49	131

6.4 Discussion

In this study, significant and consistent differences in the isotopic composition of otoliths among populations of *S. semifasciatus* from a number of locations were established. Four major groups of *S. semifasciatus* were identified: Western Australia, North Australia/Gulf of Carpentaria, northern Queensland east coast and central Queensland east coast. Differences in the isotopic composition of whole otoliths indicate that these groups must have spent their life history in different locations. The magnitude of the difference between the groups suggests a prolonged separation period at least equal to the fish's life span.

The greatest difference in otolith isotopic signatures was found between *S. semifasciatus* between Western Australia and all other groups. This indicates that the Ria coast of the Kimberley region of Western Australia may be an effective barrier to adult movement.

Smaller but significant differences were found among the other groups. If *S. semifasciatus* freely migrated among groups the difference in isotopic signatures between the groups would not be present, as they would inhabit similar environments. The differences in isotopic signatures indicate that population separation exists and that these groups remain largely separate and non-mixing. The Gulf of Carpentaria locations sampled were not discriminated from each other and neither was the northern Australia site near Darwin suggesting that the Gulf of Carpentaria and northern Australia is widely mixed.

The results from this study provide support for the view that adult populations of *S. semifasciatus* do not represent a single homogeneous stock. Populations of *S. semifasciatus* are currently managed separately by state-based management agencies in Western Australia, the Northern Territory and Queensland (Gulf of Carpentaria and East Coast) either separately or as part of mixed mackerel fisheries. Little spatial structure is currently assumed in those states with formal management arrangements in place. Commercial fisheries management is generally based upon controlling effort within the fisheries rather than its output; however this is not consistent among the different jurisdictions. Within each state management is based on the implicit assumption that each population represents a single homogeneous stock and that demographic parameters do not vary substantially. The presence of spatial subdivision among adult assemblages of *S. semifasciatus* and thus the formation of distinct management units suggest that the spatial scale of management needs to be reviewed. For example, there is a shared stock within the Gulf of Carpentaria and the results of this study strongly suggest that the management of this stock needs to be redesigned taking into consideration the existence of these different groups or management units.

The present study demonstrated that the stable isotope composition of sagittal otoliths of *S. semifasciatus* can be used effectively to delineate assemblages of fish where there is sufficient water temperature variation to enable differences in $\delta^{18}O$ to be detected. The method provides strong evidence of stock separation at the juvenile and adult stage when there are differences in stable isotope ratios between fish assemblages. However, this method does not provide any information regarding mixing or movement during the pelagic larval stage.

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7. THE STOCK STRUCTURE OF GREY MACKEREL *SCOMBEROMORUS SEMIFASCIATUS* IN AUSTRALIA AS INFERRED FROM ITS PARASITE FAUNA

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7.1 Introduction

Parasites are ubiquitous (Torchin *et al*., 2002) and it has been suggested that almost every fish caught commercially has at least one parasite (Lester, 1990). The high occurrence of parasites in fish species presents an opportunity to use them as biological markers to explore stock structure (MacKenzie, 2002). One of the advantages of using biological markers over other more-intrusive methods, such as tagging, is that information about the history of the animal is available from one sampling event. This means that there is no effect of catching and releasing an animal that may result in a reduction in growth or change in behaviour that may bias results.

Parasites have been used as biological markers to discern the stock structure of a number of fish species (Lester, 1990; Melendy *et al.,* 2005; MacKenzie and Abaunza, 2008; Mackenzie *et al*., 2008). In theory, if the number and type of parasites of two groups of fish are similar they are likely to have had some common history. In contrast, if the parasitic load of one group of fish is significantly different from that of another group of fish they are likely to have had a separate history. The length of time the parasite is visible in the fish determines for how long parasite data can be informative about fish movement.

A stock can be considered to be a group of fish, possibly a breeding population, which exhibits no significant mixing with neighbouring individuals for a temporal period defined by the longevity of the parasite fauna in the host. Therefore, to develop a long-term understanding of the history of the fish, permanent (long-lived) parasites are preferable to temporary (short-lived) ones (Lester, 1990).

The grey mackerel is an important commercial and recreational fish species across northern Australia. Despite its importance little is known about its stock structure and movement, knowledge is now required to ensure the sustainable harvest and management of the

resource. This study aims to elucidate both the spatial and temporal stock structure of grey mackerel across northern Australia using its parasite fauna.

7.2 Materials and Methods

Data from 593 fish between 1 and 9 years old were analysed from 21 samples collected from 11 locations around the coasts of the Northern Territory and Queensland by commercial shark and mackerel fishing operators during 2005 – 2007 (Table 7.1). The samples collected from Western Australia were damaged by heat so were not analysed for parasites. A pilot study of the parasite fauna of whole fresh grey mackerel (Charters, 2006, unpublished honours thesis), identified four parasites considered suitable for stock identification analyses, *Pterobothrium pearsoni*, *Callitetraryhnchus gracilis, Anisakis simplex* and *Terranova* sp. Fresh samples to accurately identify these parasites were collected from the eastern Gulf of Carpentaria and the east coast of Queensland. Live trypanorhynchs were placed in fresh water for approximately 30 minutes to encourage their tentacles to evert. When the tentacles had everted, specimens were fixed in 3% gluteraldehyde in 0.2 M sodium cacodylate buffer (Gestal and Azevedo, 2005). Samples were dehydrated using critical point drying (Sarmiento, 2006), coated in platinum and examined under a scanning electron microscope (SEM) using standard techniques (Gerrity and Forbes, 2003; Giberson *et al*. 1997; Giberson *et al*. 2003).

Gills and viscera for parasitological examination were frozen after measuring the fish. Dissections were carried out using the methods of Lester *et al.* (2001) with the exception that the whole stomach wall was examined. The four parasites selected were well attached and easily observed making counts reliable and relatively easy to do, thus improving the dependability of the analyses and reducing time spent processing samples.

All fish were between 1 and 9 years old (3 fish older than 9 years were excluded from the analyses). For the analyses the parasite counts were transformed using log (counts + 0.05). Second order polynomial contrast analysis was conducted to evaluate the effect of age on parasite numbers in GenStat, v 1.1 using the ANOVA procedure. The parasite abundances in different samples were compared using univariate (ANOVA) and multivariate (MANOVA, canonical discriminant analysis) analyses. A log(x+0.05) transformation of the parasite numbers was applied in both cases. Multivariate analyses used eigen values generated in Minitab 14.

7.3 Results.

Parasites identified in the preliminary study and selected for analysis were the trypanorhynchs *Pterobothrium pearsoni* and *Callitetrarhynchus gracilis* (Figure 7.1)*,* and juvenile nematodes identified as *Anisakis simplex* and *Terranova* Type II (after Cannon 1977). These parasites were mainly encysted in the liver and stomach wall. The parasites selected were considered to be long lived based on their suspected life cycle (see Discussion) and because few were found dead in the host.

The four parasites being long-lived and acquired through the diet were expected to accumulate in fish as the fish age. A positive linear trend in parasite counts and fish age was significant in *A. simplex* though the coefficient of explained variation was very small (R^2=3.5%) and when the site effect was taken into account the age correlation was no longer significant. *Pterobothrium pearsoni* showed a negative correlation with age even after the site effect was taken into account though the coefficient of determination was again very small (< 5%). For *C. gracilis* and *Terranova* sp. there was no correlation with age. As the correlations were weak or absent, parasite abundances were not adjusted to a fish of a standard age prior to the multivariate analyses. Most frozen trypanorhynchs had died without everting their tentacles. However, once the species had been identified from fresh samples, the morphology of the scolex, bothridia and blastocyst were considered adequate for diagnostic purposes. *C. gracilis* had a double-walled cyst noticeably different to the smaller single walled cysts of *P. pearsoni*. *Terranova* spp. differed from *A. simplex* in their smaller size and the presence of an intestinal caecum

A   B

Figure 7.1. SEM images of the bothridia and metabasal armatures A. *Pterobothrium pearsoni.* Scale bar = 100µm. B. *Callitetrarhynchus gracilis.* Scale bar = 1mm.

Summary statistics for each of the samples including sample size, mean length and age of the fish, and mean abundance for each of the parasites from each location are given in Table 7.1. In four cases samples were in two parts (A+B, E+F, H+I, and Q+R). These were combined in later analyses unless otherwise stated. The varied range of ages among samples was unknown until after the samples had been dissected. Given the poor relationships between fish age and parasite abundance, data from all 9 age classes were combined in the analyses.

7.3.1 Univariate analysis

The Tukey-Kramer pair-wise comparisons (Table 7.2) indicated which pairs of samples had one or more parasites that were significantly different at a 95% level of confidence. Thus by chance, significant differences in one parasite may arise every 20 comparisons. If two or more parasites differ for the same comparison it is an indication that the differences may be biologically significant. Table 7.2 shows that fish from the east coast, sites A to G, show few differences among themselves but are generally different from elsewhere. Fish on the eastern side of the Gulf of Carpentaria generally have similar parasite faunas except for the northern location of Crabb Island (L) which appears more similar to fish on the east coast. Locations sampled within the western Gulf are similar and also tend to be similar with the rest of the locations within the entire Gulf. Within the NT northwest coast locations (O, P and Q) samples were very similar but were notably different from the east coast and the eastern Gulf, but not the western Gulf. Temporal (between-year) comparisons among the locations EC S (E v G), EG mid (H v J), WG mid (S v T) and NW NT (O v Q) all showed no differences.

Table 7.1. Collection details for the 17 sample and 4 subsample locations with means for the fish lengths, ages, and abundance of the four parasite species (untransformed data).

Location		Sample date	# fish	Mean FL	Mean age	*P. pears.*	*C. gracilis*	*Terra-nova*	*A. simplex*
A	EC mid	Oct 2005	22	776	3.5	2.1	1.5	79	0.3
B	EC mid	Sep 2005	49	716	3.1	1.3	1.9	17	0.2
C	EC N	Nov 2006	13	718	3.1	2.4	1.5	56	0.4
D	EC PD	Aug 2007	41	788	3.9	0.9	0.9	22	0.9
E	EC S	Nov 2005	14	619	1.5	0.4	0.8	19	0.1
F	EC S	Dec 2005	23	693	2.6	0.7	0.2	20	0.9
G	EC S	Sep 2006	24	796	3.0	0.4	1.3	39	0.1
H	EG mid (Coleman R.)	Aug 2005	29	723	3.0	3.9	4.9	7	0
I	EG mid (Nassau R.)	Aug 2005	28	776	4.2	4.9	4.6	13	0
J	EG mid	Sep 2006	32	766	3.7	4.0	4.3	9	0.1
K	EG mid	Mar 2007	25	738	3.4	4.2	4.9	11	0
L	EG N	Apr 2007	27	746	2.8	1.1	0.9	11	0.1
M	EG SE	Mar 2007	29	722	3.0	5.3	15.8	9	0.1
N	EG SW	Mar 2007	19	651	1.4	2.2	6.7	10	0
O	NW NT	May 2005	29	689	2.4	7.4	12.6	15	0
P	NW NT	Aug 2005	50	789	3.5	7.7	10.1	30	0.2
Q	NW NT (Fog Bay)	Apr 2007	15	714	2.1	6.2	1.7	15	0
R	NW NT (N Bynoe)	Apr 2007	25	742	2.8	4.4	3.1	16	0.1
S	WG mid	Sep 2005	50	750	3.5	8.8	7.8	25	0.6
T	WG mid	Oct 2006	15	791	3.7	10.9	4.1	23	0.3
U	WG SW	Nov 2006	34	776	4.0	5.5	1.8	14	0

Table 7.2. Tukey-Kramer significant differences in mean parasite abundance in *Scomberomorus semifasciatus* between sites. A space indicates no significant difference, the numbers 1-4 indicate that mean parasite abundance was significantly different between the two sites (p < 0.05) for the respective parasites: 1. *P. pearsoni*, 2.*C. gracilis*, 3. *Terranova* Type II larvae, and 4. *A. simplex*. Sub samples have been combined (A+B, E+F, H+I, and Q+R).

	A	C	D	E	G	H	J	K	L	M	N	O	P	Q	S	T	U
A																	
C																	
D																	
E		3															
G				3													
H	1,2,3	3	1,2,3	1,2,3	1,2,3												
J	1,3	3	1,2,3	1,2	1,3												
K	1	3	1,2,4	1,2	1,3												
L		3			3	1,2	1,2	1,2									
M	1,2	2,3	1,2	1,2	1,2,3	2	2	2	1,2								
N	2	2,3	2	1,2	1,2				2								
O	1,2	1,2,3	1,2	1,2	1,2,3	2	2	2	1,2		1						
P	1,2	1,2	1,2	1,2	1,2	1,2,3	1,2,3	1,2,3	1,2	3	1						
Q	1	3	1,4	1,2	1	3			1	2	2	2	2				
S	1,2	1,2	1,2	1,2	1,2	1,3	1,3	1	1,2		1			2			
T	1	1	1	1,2	1	1,3	1	1	1	2	1	2					
U	1	1,3	1,4	1	1,3	2			1	2	2	2	2,3		2		

7.3.2 Multivariate analysis

We were unable to discriminate between any samples or areas using the Random Forests analytical method. However, the multivariate method clearly distinguished EC fish from the Gulf of Carpentaria and NT fish (Figure 7.2). *Anisakis* data had little discriminating ability and their numbers varied widely. The high mean in one of the EC S samples (F), for example, was from one fish with 20 *A. simplex* (CRC0425) while virtually the rest of that sample was free of the parasite. The variability suggests that one fish may have a very different history from the other 23 fish in the sample. Figures 7.2 to 7.7 use data from the other three parasites only.
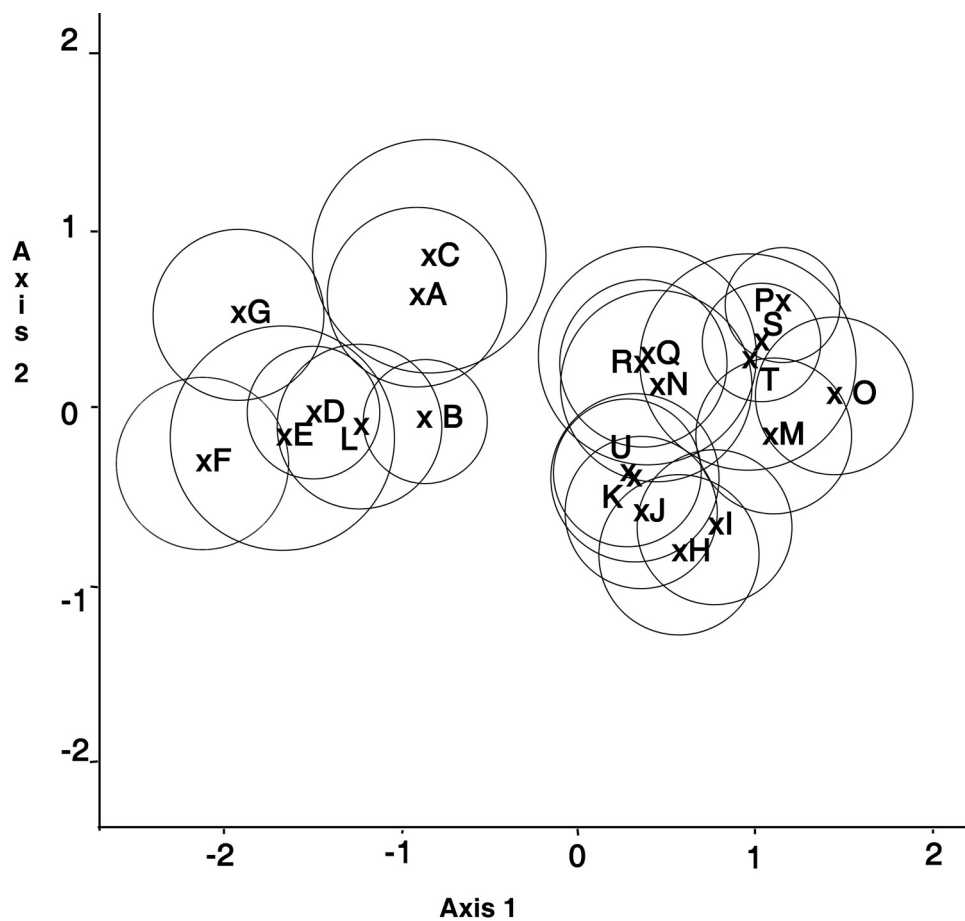
Figure 7.2. Canonical variate analysis of the abundances of three parasites in the 17 samples and 4 subsamples using transformed data. First two axes are shown. Circles represent approximate 95% rings of confidence.
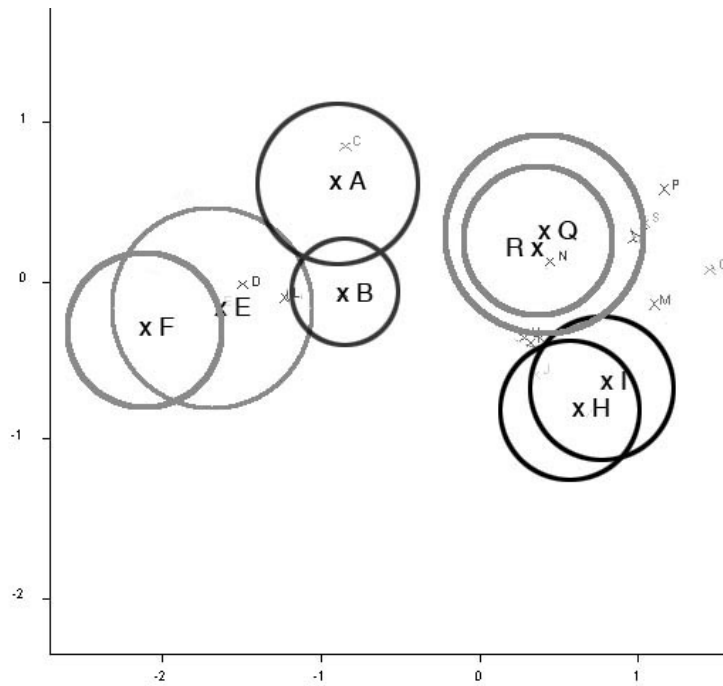
Figure 7.3. Four pairs of subsamples with their 95% confidence rings. Two pairs in black and two pairs in grey. Note the strong overlaps within the pairs. These pairs are combined in the later figures.

To check the reliability of the multivariate technique, sub samples that were taken from the same area within a few weeks of one another were analysed separately in the preliminary analysis. Four pairs are shown in Figure 3 with their approximate 95% confidence limits (derived from the sample sizes). Samples A and B were from EC mid location, samples E and F from EC S location, samples H and I (EG mid) from adjacent rivers in the Gulf of Carpentaria, and samples Q and R were from the NW NT region. The pairs of points fall relatively close to each other on the graph indicating that the multivariate technique is surprisingly reliable for these relatively small and somewhat heterogeneous (e.g. in age) samples of grey mackerel. The pairs have been combined in the other analyses.
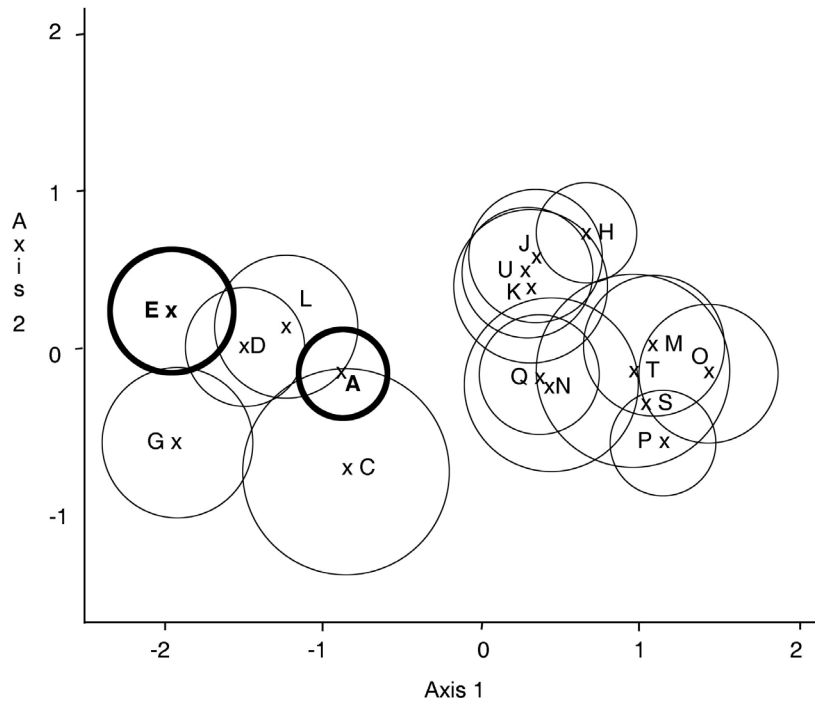
Figure 7.4. Samples from EC mid 2005 (A) and EC S 2005 (E) are highlighted in bold for comparison. (Subsamples combined).

The EC region samples fell in a group separate from those from elsewhere (Figure 7.4). Within this group the parasite fauna from the EC S sample (E) differed greatly from that of the EC mid sample (A) suggesting little or no mixing. The EC S (G) also is largely distinct from the EC mid samples. The EC N sample (C), though small, overlaps with EC mid but appears distinct from the EC PD sample (D). In the comparisons, the most significant parasite appears to be *Terranova* sp. (Table 7.2).
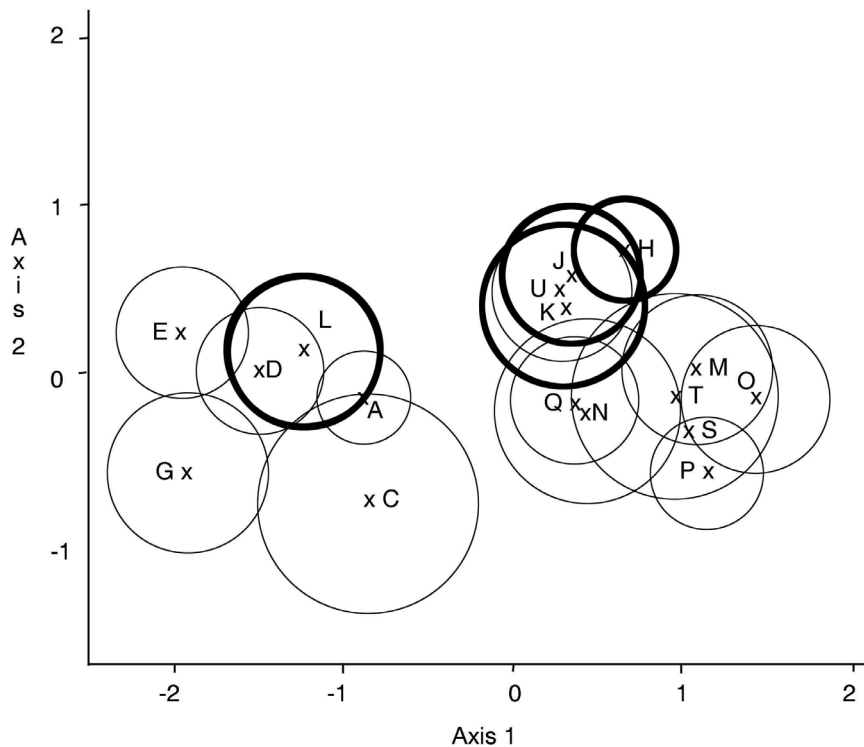
Figure 7.5. Eastern Gulf samples, in bold.

From the eastern side of the Gulf of Carpentaria there are five samples (Figure 7.5): EG mid (H, J & K), the EG N (L) and EG SE (M). Though H, J and K were taken in different years the parasite faunas are very similar suggesting temporal consistency along the eastern side of the Gulf. In the south, the EG SE sample (M) only overlaps with one of the EG mid samples. The EG N sample (L) is very different from all other locations sampled in the EG and may represent a different subpopulation of fish (Figure 7.5).

Samples from the southern and western Gulf of Carpentaria appear to be more variable and distinctions and similarities less clear (Figure 7.6). Certainly there is overlap between EG SE (M) and EG SW (N) but they also show strong similarities to the WG mid locations (S and T). The only area that seems a little different is WG S (U) which is more similar to the eastern Gulf samples of J and K than to any others. Thus, in the Gulf of Carpentaria the parasite samples suggest there could be at least two groups of fish; an eastern group (H, J, K), a southern and western group (M,N,S,T) and an EC N group (L). This result is somewhat ambiguous however due to the similarity between the eastern group (EG mid) and the WG SW (U) location.

On the northwest coast of the Northern Territory, samples O and P show some similarities, while Q differs (Figure 7.7). All three are not reliably distinguished from the WG mid samples or the EG SE sample.
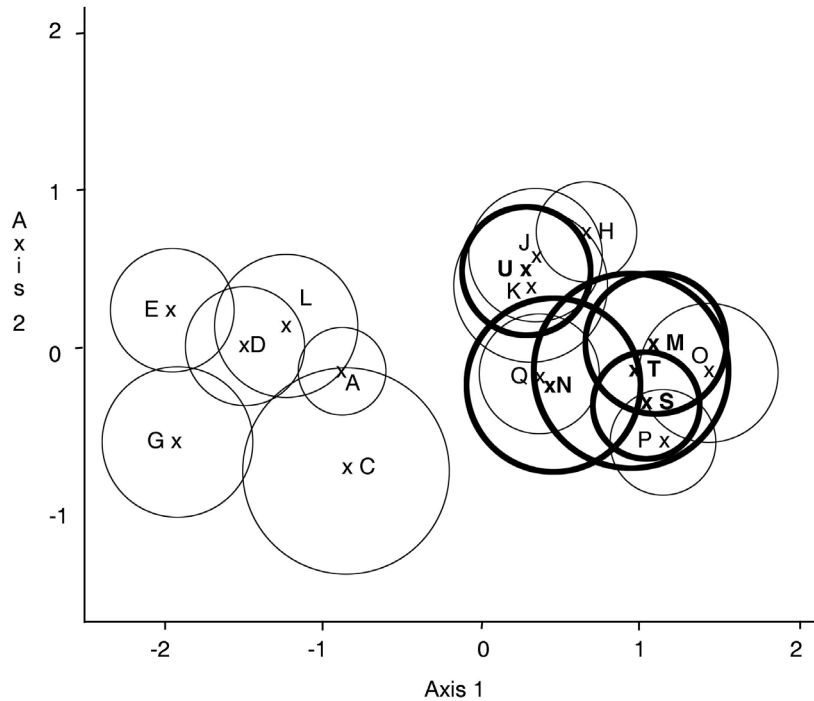
Figure 7.6. Samples from southern and western Gulf of Carpentaria (in bold).
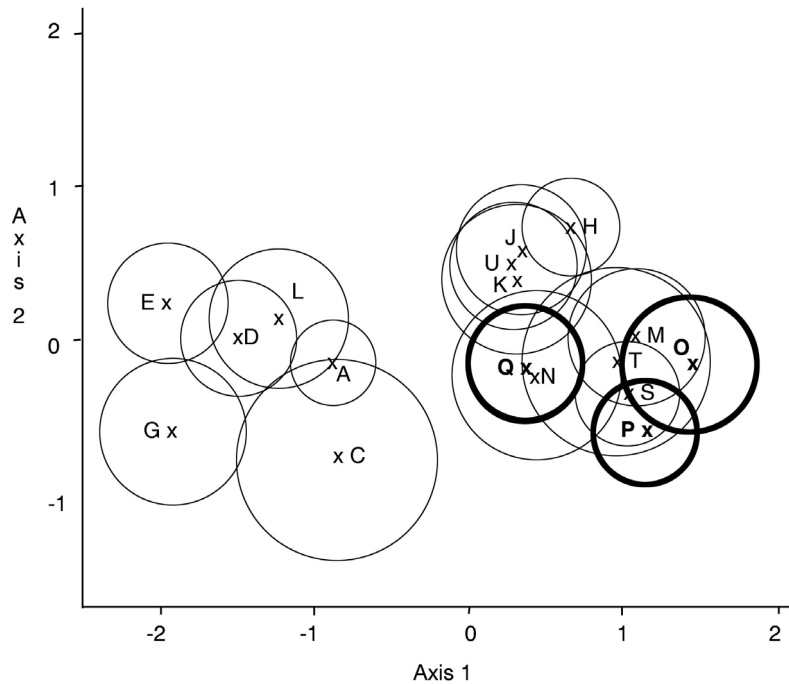
Figure 7.7. Samples from the north coast of the Northern Territory in bold.

In four cases, samples were taken from the same areas but in different years. On the EC of Australia, samples were taken from EC S in 2005 and again in 2006 (Figure 7.4; E and G). These show little overlap suggesting a different group of fish may have been sampled in the second year. Similarly on the north coast of the Northern Territory, samples taken in 2005 and 2007 appear to have different parasite faunas (Figure 7.7; O and Q). On the other hand, samples from the EG taken in 2005 and 2006 (Figure 7.5; H and J) strongly overlap suggesting the same subgroup of fish were sampled in the second year. Samples from the WG taken in different years overlap (Figure 7.6; S and T). The fish could still be moving about but there is insufficient difference between the parasite faunas of these areas to detect any movement. Thus the parasite data give conflicting results. In two cases the fish in subsequent years apparently belong to a different group from that sampled in the first year, and in two cases there is no detectable difference between the years.

7.4 Discussion

The definitive host of the trypanorhynch *Pterobothrium pearsoni* is unknown but adults in this genus have been found in carcharinids and other elasmobranchs. The larval parasite evidently remains alive in the grey mackerel until the fish dies or is ingested by the definitive host. The trypanorhynch *Callitetrarhynchus gracilis* has a similar life cycle, having been found as a larva in over 140 teleost species, and as an adult in 17 species of carcharinids worldwide (Palm, 2004). The anisakid nematodes *Terranova* spp. have been found in many species of marine fish that serve as secondary or paratenic hosts (Doupe *et al*., 2003). Those in the grey mackerel probably remain alive until they are ingested by a definitive host, which is likely to be an elasmobranch or piscivorous bird. The nematode *Anisakis simplex* infects cetaceans as definitive hosts (Klimpel *et al*., 2004). The larvae use grey mackerel as an intermediate or paratenic host and probably remain alive until they are ingested by the definitive host.

Combining data from fish of several ages possibly increased variability and decreased sensitivity though the close similarity of the 4 pairs of subsamples suggested that the method was reasonably reliable. This was likely to be especially true when two or more samples from the same region fall together on the canonical graphs.

In a similar study on the closely related *S. commerson*, Moore et al. (2003) concluded using canonical analysis found that there were at least 6 parasitologically distinct stocks of *S. commerson* across northern Australia, a finding that was supported by otolith isotope

analysis on the same fish species (Newman et al., 2007). In *S. semifasciatus* the differences across northern Australia were less marked though differences did exist. This may have been partly due to the smaller sizes of some of the grey mackerel samples. Had the samples been added together into larger areas, between-area variability may have decreased but we would have lost the indication of within-area variability. The *S. commerson* analysis also probably benefited from the high abundance of two of its parasite species, *Grillotia branchi* and *Otobothrium cysticum*, which were more prevalent than any of the parasites in *S. semifasciatus*.

The parasite faunas of *S. semifasciatus* were almost indistinguishable over large areas, such as from NW NT to WG mid. The parasite faunas could be interpreted as evidence that the fish all belonged to the same stock or alternatively that the probability of infection was similar over a wide area.

The EG N sample (L) from the near the tip of the York Peninsula had a fauna most similar to fish from the EC mid region. Again this may indicate that similar parasite faunas occur in the areas rather than the fish belonging to a common stock. The strong differences between the EG N sample and the other Gulf samples suggest that Gulf fish are not a homogeneous group, a conclusion that has already been drawn from comparison of samples from the east and west sides of the Gulf. However, ambiguities in the fine scale regional comparisons lead us to conclude the existence of a single Gulf of Carpentaria stock, with the possibility of localised adult populations.

On the east coast of Queensland the samples come from a heterogeneous population with samples A and E showing significant differences (Figure 7.4). In a comparative study on *S. commerson*, east coast fish from several areas showed great similarity in parasite fauna, even to the rate at which the parasites were accumulating. That and the seasonal nature of the southern fishery for *S. commerson* suggested that part of a relatively homogeneous population of this species were moving south each summer (Williams and Lester, 2006). The *S. semifasciatus* results are quite different; the parasite fauna suggesting these fish are less mixed and may form sub-populations along the coast.

The parasite data suggest the existence of several stocks of grey mackerel in northern Australia. Queensland east coast grey mackerel possess a parasite fauna that suggests at least two east coast stocks are evident: a northern and a southern stock with a separation apparent somewhere between Townsville and Mackay. The NW NT region also appears to comprise a separate stock while within the Gulf of Carpentaria there exists a high degree of

variability in parasite faunas among the regions sampled. This may be due to 1. natural variation within the Gulf and there is one grey mackerel stock, or 2. the existence of multiple localised adult sub-stocks (metapopulations) within the Gulf of Carpentaria. These results suggest that localised depletions may warrant concern for the future sustainability of the grey mackerel resource. It is recommended that for management purposes, fisheries managers consider these stocks in determining spatial scales for grey mackerel management, monitoring and stock assessment.

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8. INTEGRATION OF RESULTS: DEFINITION OF GREY MACKEREL SPATIAL MANAGEMENT UNITS

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8.1 Introduction

Identification of different fish stocks forms the basis of fisheries management, monitoring and assessment, as well as the study of populations (Secor 2005). Many techniques exist that can be used in discriminating fish stocks (Ihssen et al 1981a). These techniques can range from the very simple and qualitative to more technical and quantitative and include analyses of fisheries catch data, mark-recapture experiments, molecular approaches (see Cadrin et al 2005), parasite incidence (Mackenzie and Abaunza 2005), scale and otolith characteristics such as microchemistry, shape, microstructure (Friedland and Cadrin 2005; Campana 2005), and life history characteristics (Begg 2005). In each of these techniques different spatial and temporal scales that are method dependent, will influence the attributes being measured. Genetic analyses typically identify differences on large spatial and temporal scales, where gene flow is minimal. In contrast, otolith microchemistry and parasite incidence reflect the residence and movements of fish through different environments during its lifetime but are influenced by different factors, and may be used to resolve a genetically homogeneous population into discrete units of adult fish that may be more appropriate for management (Buckworth et al 2007). Because each method of assessing stock structure addresses different aspects of the population, the choice of method is a very important one and depends on the specific research and/or management questions under consideration (Begg and Waldman 1999).

The most powerful way to reliably identify whether different stocks exist is through a holistic approach that utilises different techniques concurrently (Begg and Waldman 1999). Despite some unplanned comparisons of different techniques to identify stocks (Todd 1981; Graves et al 1992), and some very early integrated technique approaches (Ihssen et al 1981b; Claytor and MacCrimmon 1988; Safford and Booke 1991), it is only relatively recently that multi-technique approaches have been advocated as the preferred approach for stock identification studies. Hancock (1998) concluded that an analysis of stock structure is most effective if several techniques are used because of the different population scales addressed by each method. Utilising different techniques that are complementary allows for greater

power in the ability to detect different stocks, providing a more comprehensive overview of species stock structure. This greatly reduces the inherent uncertainty in the results obtained. One of the limitations of single-technique studies is that when no differences are detected between fish from different locales, does not necessarily mean they are not different stocks. It may merely be a reflection of the discriminating power of the particular method. Consistency among methods in failing to identify different stocks gives much greater certainty (yet not proof) that the fish from the regions in question do actually comprise one stock. By 'weight of evidence', a multi-technique approach therefore provides greater power in identifying separate stocks, and gives greater certainty where single stocks are detected.

In this study we utilised multiple techniques (genetics – microsatellites, mitochondrial DNA; parasites; otolith isotope ratios - OIR; and growth characteristics) to test the null hypothesis of a single stock of grey mackerel across northern Australia. Further, we applied these techniques to the same grey mackerel specimens, facilitating a holistic and integrated interpretation of the results (Abaunza et al 2008). In this chapter we bring together the results from the respective techniques and present a simple tool to assist in integrating and interpreting these results to determine the stock structure and appropriate spatial management units for grey mackerel in Australia.

8.2 Methods

The details of the methods used for the respective techniques, including sample treatment and data analyses, are provided in the respective chapters. Results from the respective techniques produce heterogeneous data types that make combining them into a single quantitative analysis problematic. Differences in the spatial and temporal scales at which each of the techniques are informative also make interpretation of combined data challenging. This is a persistent issue now facing stock identification studies such as this one that utilise multiple techniques that require integration of results.

In this study, analyses of data from the different techniques necessarily used different statistical methodologies. Where possible a similar iterative analysis approach was adopted whereby adjacent non-significant regions were pooled and re-analysed in an attempt to determine the most parsimonious description of grey mackerel stock structure. To integrate the results of the respective analyses and assist in their interpretation we developed a matrix demonstrating where significant differences were found in pairwise comparisons among different regions. In doing this we presented only regions that best represented the broadest spatial scale determined by any of the different analyses. This matrix gave us an overview of

the respective results, thereby providing an indication of the strength of the differences detected (or not) among the different regions. To further assist in interpreting the spatial structure of stocks through integration we quantified the pairwise results using the mean number of tests carried out for each technique that were significant and called this the Stock Difference Index (*SDI*) given by:

$$SDI = \frac{\sum SV}{Count(SV)}$$

where *SV* is the significance value (sig. = 1; non-sig. = 0). The mean was used since analyses among all combinations of techniques and regions were not possible due to sampling issues. This method merely provides a value that indicates the relative differences among regions, with regions showing the maximum difference having a *SDI* of 1 and regions with no differences having a *SDI* equal to zero. The full interpretation of these values can still only be accurately carried out by considering the individual results of each technique, taking into account the time scales by which each of them are defined. It is also best applied when the same samples are analysed across a suite of methods. Nevertheless it provides a useful illustrative tool in identifying likely stock boundaries.

8.3 Results

The results from the genetic analyses incorporated both the microsatellite and mitochondrial data (see Chapter 5). These analyses concluded that there were at least four discrete genetic stocks across northern Australia for grey mackerel comprising WA, NW NT, the GoC and the EC (Table 8.1). The results of the OIR analyses (see Chapter 6) also separated WA from all other regions, but couldn't separate among the regions sampled across the very top of northern Australia (NW NT, WG and EG). However, on the east coast OIR analyses found a clear separation between fish sampled from the south (Mackay, EC S) with those sampled farther north (EC Nth: EC mid, N, PD). For both the genetics and OIR analyses, WA showed the strongest signal of separation from all other regions.

Due to inadequate sample sizes and some samples being heat damaged, WA samples were not available for parasite and growth comparisons. Further, due to a different approach undertaken in the analysis of the parasite data, separate steps were carried out in order to make inferences of stock structure to the broadest possible level, which made integration of results with the other techniques as consistent as possible. The separate steps involved

overlaying both the univariate and multivariate analyses results based on significant pairwise comparisons, and inferring overall broad spatial scale differences. The spatial scale at which this was conducted was consistent with that determined by the genetic and OIR techniques (Table 8.1). As a guide to inferring differences from the parasite results we estimated a Parasite Difference Index (*PDI*) according to the following:

$$PDI = \frac{UV + 1.5 \times MV}{2.5}$$

where *UV* is the univariate value (proportion of the total pairwise comparisons for that broad region that were significant from univariate analyses), and *MV* is the equivalent value from the multivariate analyses. Since the multivariate analyses were considered to be a more reliable test as it takes into account all parasites concurrently, we weighted the value from the multivariate results higher by applying a multiplier of 1.5. The denominator is derived by summing the weighting values (ie. 1.0 + 1.5 = 2.5).

In the parasite analyses (see Chapter 7), the east coast locations showed the strongest and most consistent differences from all other regions as reflected in the higher *PDI* values (Table 8.2). Within the EC there was evidence of stock separation between northern (EC Nth: EC PD, N, mid) and southern (EC S) locations with the strongest difference evident between the adjacent EC S (Mackay) and EC mid (Townsville) locations from the multivariate analysis. The parasite fauna of NW NT fish were not dissimilar to the western Gulf fish, but showed stronger differences to the eastern Gulf. Within the Gulf of Carpentaria there initially appeared to be differences between the eastern and western fish (Table 8.2), however, after further exploration of the data it was apparent that this difference was explained mostly by a single location (EG N, Crabb Island) (see Figure 7.5), which was more similar to the east coast fish. Among all other regions within the Gulf of Carpentaria there was a high degree of variability and although the GoC was concluded to comprise a single stock for management purposes, it was acknowledged that there was the possibility of the existence of localised adult sub-stocks.

Results from the analyses of growth characteristics among regions (see Chapter 3) were strongest between the NW NT and most other regions, with the western Gulf, the nearest region, being most similar (Table 8.1). Although not as strong, the east coast also tended to be different from other regions. However, the EG N (Crabb Island) sample of fish was more similar to the east coast than the other locations within the Gulf (EG mid & WG), which exhibited similar growth.

Table 8.1. Results matrix of the finest regional scale pairwise comparisons among the four techniques used in the study. Significant results for each pairwise comparison are indicated by the capital letters: G – genetics, P – parasites, M – otolith stable isotopes, V – growth characteristics. Non-significant results are indicated by "n", and where the analysis was not carried out is given by "-".

Regions	WA	NW NT	WG	EG	EC Nth
WA					
NW NT	G - M -				
WG	G - M -	G n n n			
EG	G - M -	G P n V	n P n n		
EC Nth[2]	G - M -	G P M V	G P M n	G P M V	
EC S	G - M -	G P M -	G P M -	G P M -	n P M -

Table 8.2. Parasite Difference Indices (*PDI*) for the main regional pairwise comparisons estimated from the parasite data analyses.

Regions	NW NT	WG	EG	EC Nth	EC S
NW NT					
WG	0.18				
EG	0.44	0.32			
EC Nth	0.91	0.82	0.70		
EC S	0.93	0.87	0.88	0.30	

From the integration of all methods into the one matrix of Stock Difference Indices general patterns begin to emerge for stock identification. The WA region is clearly differentiated from all other regions as belonging to a separate stock of fish, while the east coast can also be clearly identified as a separate stock to the Gulf of Carpentaria and NT fish (Table 8.3). Within the Gulf of Carpentaria and the NW Northern Territory region the identification of stocks was less obvious. A strong difference between the NW NT and the eastern Gulf was indicated by a large *SDI*. The lowest differences were within the Gulf itself between the eastern and western sides, and between the NW NT region and the western Gulf.

Table 8.3. Stock Difference Indices (*SDI*) for the main regional pairwise comparisons estimated from the integration of the results of data analyses from all techniques.

Regions	WA	NW NT	WG	EG	EC Nth	EC S
WA						
NW NT	1.00					
WG	1.00	0.25				
EG	1.00	0.75	0.25			
EC Nth	1.00	1.00	0.75	1.00		
EC S	1.00	1.00	1.00	1.00	0.67	

---

[2] Growth was estimated for the EC mid location only but is grouped as EC Nth in Table 8.1 due to clear EC S and EC Nth separation indicated by other methods.

8.4 Discussion

In this holistic stock identification study we have been able to clearly demonstrate the existence of separate stocks of grey mackerel throughout their major fishery regions across northern Australia, thereby identifying the appropriate spatial management units for grey mackerel fisheries. The integration of different but complementary stock identification techniques in the "weight of evidence" approach used here, and employing the *SDI* approach as an aid in synthesising that evidence, usefully gives greater confidence in stock identification. Although we have not identified a critical *SDI* value, intuitively, any *SDI* > 0 is evidence for separate stocks and the actual value indicates the level of corroboration among the different stock identification methods used (higher values = higher level of corroboration). Therefore, this study has indicated the existence of at least five stocks of grey mackerel across northern Australia, with the possibility of additional stock structuring within the Gulf of Carpentaria (Figure 8.1).

That there was a high level of corroboration among the techniques imparts confidence in the stock structure identified. The *SDI* approach presented here does not, however, take into account the different intrinsic time scales of the techniques employed (Table 8.4; Buckworth et al 2007) or the mechanisms behind them. These are critical factors in the interpretation of such data. Buckworth et al (2007) defined the intrinsic time scale as "the approximate time span to which most information derived from that method applies". Genetic data tends to be informative about population differences over a range of temporal scales and generally large spatial scales (Table 8.4). Given this, it was quite surprising that the genetics results corroborated very well with the results from other techniques that have much shorter intrinsic time scales. The greater the degree of isolation of fish between adjacent regions, the greater the strength in stock division. For example, the genetics data showed that the sample of fish from WA were very strongly differentiated from samples collected further east, and the OIR data also demonstrated this clear separation. The genetic data also showed the east coast was genetically different from regions further west, with very strong corroboration from all other techniques. However in several areas, despite genetic homogeneity, the techniques with shorter intrinsic time scales were able to provide greater resolution. The QLD east coast was determined to represent one genetic stock; however the OIR results indicated a clear separation showing at least a northern and a southern stock. This was also corroborated by the parasite results. Homogeneous genetic characters suggest larval dispersal mechanisms or low levels of adult mixing between adjacent stocks (possibly sex-biased). The latter mechanism was determined to be occurring for Spanish mackerel in Australia (see Buckworth et al. 2007).
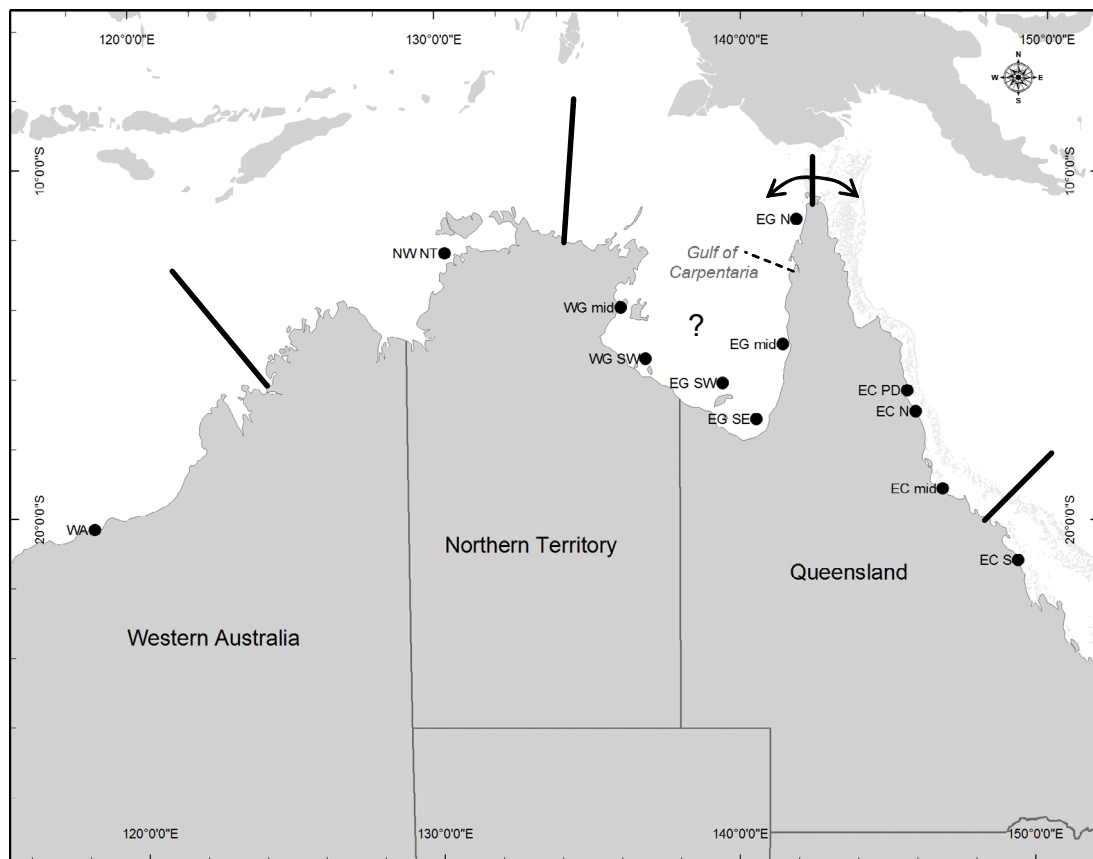
Figure 8.1. Map of northern Australia showing the study sample locations and the approximate boundaries separating grey mackerel stocks. These stock boundaries are approximate due to the inability to sample at finer spatial scales during this study; however sampling locations are indicative of major regions of grey mackerel fisheries. Dotted lines within the Gulf of Carpentaria show where stock division was evident and indicate the possibility of more localised stocks.

In the Gulf of Carpentaria only the parasite data indicated that there may be different grey mackerel stocks present. The differences detected between fish within the GoC, however, were ambiguous, suggesting some limited movement of adults between these vast regions. An interesting result was the clear difference in parasites of fish sampled from the Eastern Gulf north location (Crabb Island). Fish from this location had a different parasite fauna than all other regions in the GoC but were similar to fish from the northern east coast locations. Interestingly the genetics data also suggested a high degree of genetic similarity between Crabb Island and the east coast, and growth data were more similar between Crabb Island and the EC compared to the adjacent EG mid region. Crabb Island lies at the very north-eastern tip of the GoC just to the west of the Torres Strait. This is less simple to explain and provides some evidence of the possibility of fine scale subdivision of stocks, at least in the GoC.

Table 8.4. Intrinsic time scales of the different stock identification techniques used in this study. Table has been adapted from Buckworth et al (2007).

Method	Intrinsic time scale	Origin of information
Genetic spatial analyses	10,000 – 500,000 yrs	Rate of evolution of genetic markers
Genetic temporal analyses	5 – 50 yrs	Comparison of genetic markers over time
Whole isotope ratios	5 – 10 yrs	Average ambient chemical environment over fishes life span
Parasite abundance	Seasonal – 10 yrs	Parasite and fishes life span, biology
Population parameters	5 – 10+ yrs	Fishes life span and longer. Mediated by the environment, genetic influences, generation times and density-dependent mechanisms

Despite the genetic differences between the GoC and the NW NT, results of the OIR, parasite and growth analyses could not separate these regions. This provides evidence for little or no reproductive mixing of fish between these regions and may represent evidence for philopatry to spawning grounds with dispersal during non-breeding periods. This would require samples used in the genetic analyses to have been collected during the spawning season. The vast majority of samples, though not all, were collected during the spawning periods identified in Chapter 3. The possibility of philopatry occurring remains, however, this hypothesis would need to be further tested. The lack of corroboration among methods is not surprising as the OIR analyses are strongly influenced by temperature and these broad regions are spread across similar latitudes with correspondingly similar temperature regimes. Similarly, parasites and growth characteristics probably reflects a homogeneity of environmental conditions experienced among these locations.

Detailed interpretation of the spatio-temporal patterns in movement of grey mackerel stocks from the results of this study is not possible from the methods used here. The results in the study show discrete stocks of adult fish with evidence of some connectivity between adjacent stocks that is likely to be due to larval dispersal or limited adult movement. This appears to be occurring on the east coast and in the GoC. Within the GoC there may also be some mixing among adult fish deriving from adjacent stocks. Movement of these stocks may vary seasonally and not necessarily in a uniform manner for all stocks. Strong seasonality in catches is evident for the EC, WA and the GoC with catches peaking during winter and spring, whereas in the NT (Timor and Arafura) catches are fairly uniform throughout the year. Clearly grey mackerel are present and available for capture in these waters throughout the year. In contrast, on the east coast there is a strong seasonality in catches. This is due

largely to a large increase in availability to capture during the winter/spring months, when they appear to aggregate in large schools in inshore waters. Whether these schools move to deeper waters further offshore or whether the schools simply disaggregate and the fish disperse is unknown. As discussed in Chapter 2 interpretation of movement patterns from the regional catch data is not straightforward due to other factors influencing targeting of grey mackerel. Tagging studies would be beneficial in gaining further insight into movement patterns; however, species such as grey mackerel are not readily tagged effectively using traditional tagging methods (Cameron and Begg 2002) and information in recapture patterns would be limited by the distribution of fishing effort.

Multi-technique stock structure studies have relied on qualitative appraisals of the collective results from the respective techniques (e.g. Abaunza et al. 2008), and final interpretation of stock structure and the appropriate spatial management scales have not been based on any consistent framework for integration. Although there are no clear set of objective decision rules available to guide researchers and provide a clear and consistent basis for analysis and interpretation, it is the intrinsic scales of information provided by the different techniques that dictate their interpretation. Nevertheless, the *SDI* approach we adopted in this study enabled us to better identify stock divisions and provide confidence in these observations.

It is important to note that no difference detected among samples does not prove the existence of a single stock but merely fails to falsify the null hypothesis of a single stock. The use of multiple techniques with different intrinsic time scales and mechanisms behind them, gives greater resolving power in identifying stocks. That is, one technique may identify differences between populations where others have failed to do so. The use of multiple techniques in this study has therefore been its strength and enabled stocks to be identified in some instances by only one or two of the techniques and not others.

The management implications of these results indicate the need for management of grey mackerel fisheries in Australia to be carried out on regional scales finer than are currently in place. In some regions the spatial scales of management might continue as is currently (e.g. WA), while in other regions, such as the GoC and the EC, management arrangments may need to be adjusted. On the east coast, managers should at least monitor grey mackerel fisheries at more local scale and assess accordingly. In the GoC, there is a need for joint management, and potentially further investigation of stock structuring. Stock assessments should consider the stock divisions identified and use life history parameters particular to each stock.

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9. CONCLUSIONS

9.1 Benefits and adoption

The results of this project will benefit the commercial and recreational fishing sectors where they target grey mackerel, through improved management of the resource. Identification of the appropriate spatial scales and units for management helps inform management in ensuring sustainability of grey mackerel fisheries in Western Australia, the Northern Territory and Queensland, particularly for the commercial sector. Results of this project have already been incorporated into the development of a monitoring program for Queensland east coast grey mackerel where spatial coverage of catch monitoring has ensured northern and southern regions are taken into account and considered explicitly. The results have also indirectly influenced management changes proposed for the Qld east coast grey mackerel fishery including an increase in the minimum legal size, a reduction in the recreational bag limit, and changes in commercial net mesh sizes that limit catch of smaller fish.

9.2 Further development

Recommendations for further grey mackerel research and development include:
HIGH IMPORTANCE

- Management strategy evaluation should be employed to examine the implications and alternative management responses to the conclusions of this work.
- The status of each grey mackerel stock should be assessed, or steps taken to facilitate these assessments. This includes the development of an appropriate age-structured model.
- Catch monitoring and assessment should be carried out at spatial scales consistent with the findings here as a minimum spatial scale, and the potential for localised depletions investigated. Historic management changes should be taken into account when interpreting these data sets.
- Develop a reliable estimator of grey mackerel stock abundance and/or harvest rates for the respective stocks.
- These R & D recommendations are of highest priority for the Gulf of Carpentaria due to substantial recent growth in catches, and due to the possibility of more localised adult populations. The east coast is the second highest priority region due to substantial growth in catches in recent years.

MEDIUM IMPORTANCE

- Further research should establish the stock structure of grey mackerel south of Mackay on the Queensland east coast, and throughout the Western Australian fishery regions.

- Tagging of grey mackerel using strict fish capture and handling protocols to maximise survival of released fish, should be developed and carried out to better establish seasonal movement patterns and interchange of adult fish among stocks. Such a program should be designed to facilitate the estimation of harvest rates from the respective stocks.

- Methods such as laser ablation inductively coupled plasma mass spectrometry of grey mackerel otoliths should be investigated to examine fine scale life history patterns to complement tagging.

- Larval dispersal/retention mechanisms should be investigated to explore the connectivity potential among stocks, and establish mechanisms that isolate stocks.

LOW IMPORTANCE

- The reliability of the use of upper jaw length as a predictor of fish length should be tested.

- Determine the prevalence and nature of hybridization among *Scomberomorus* in Australian waters as it may have implications for fisheries management. Fishing patterns altering species densities could be linked to the prevalence of hybridization where spawning aggregations are co-located. Persistent hybrid forms would pose challenges for fisheries management as they are not covered by regulation.

9.3 Planned outcomes

The major planned outcome from the project was to determine the stock structure, and therefore appropriate management units, for grey mackerel fisheries across Queensland, the Northern Territory, and additionally, for Western Australia. This was achieved by combining the results of analyses of several independent stock identification methods to provide robust conclusions about stocks in relation to existing fisheries effort, and to ensure the resolving power of the study in achieving this goal was maximised.

The planned outcomes achieved to date include:
The project has indicated that the appropriate spatial scale at which grey mackerel fisheries be managed is by state/territory, and by regions within these jurisdictions. The project identified at least five separate stocks of grey mackerel throughout northern Australia for

management purposes: a Western Australian stock, a NW Northern Territory stock, northern and southern east coast stocks, and a Gulf of Carpentaria stock. This will provide the basis for reliable and robust assessment of the status of grey mackerel stocks and help deliver sustainable harvest and profitable utilisation of grey mackerel resources in northern Australian waters. This will also assist with compliance of the Commonwealth EPBC Act for northern Australian grey mackerel.

The project has provided the ability for more accurate stock assessment of grey mackerel fisheries. Regional growth parameter estimates are critical input parameters for stock assessments of the respective stocks identified during the study. Samples collected have been utilized to provide these growth estimates, as well as estimates of mortality, spawning seasonality, and maturity.

This project addresses some of the major strategic research recommendations of the FRDC report of Ward and Rogers (2003). This review of northern mackerel research recommended stock structure determination and fisheries biology of grey mackerel as high priority research needs.

The project results have influenced the development of monitoring strategies for grey mackerel fisheries on the east coast, and in the stock assessments for the Gulf of Carpentaria. The QDPI&F Long Term Monitoring Program has developed their monitoring program for grey mackerel based on the spatial dynamics identified during this project.

The project provided further evidence for the utility of holistic approaches in stock structure studies. Using the template provided by FRDC Project No. 1998/159, the use of multiple concurrent techniques has resulted in greater certainty and resolution in the identification of grey mackerel stocks. Further, to enhance interpretation in stock structure studies this project has developed a more standardised and quantitative approach for presenting integrated study results.

The project helped develop relationships between community groups, research and management across northern Australia. The project helped to inform emerging local community concerns of grey mackerel localised depletions on the Queensland east coast through regular and direct communication of results, and also involved the inclusion of extra project sampling and analyses to better inform these concerns.

The project further enhanced the link between research and management. Due to the inter-jurisdictional nature of the project and the possibility for the need for co-operative management approaches depending on results, fisheries managers from each jurisdiction were key partners throughout the project, including milestone reporting requirements (see Appendix 4).

The project provided significant human capital development opportunities. The project provided material for two BSc (Hons) projects (James Cook University, Nic Marton; University of Queensland, Robbie Charters). These projects made significant contributions and formed the basis for Chapters 3 and 6 respectively.

9.4 Conclusions

The conclusions from this project are:

- Grey mackerel fisheries across northern Australia are comprised of multiple stocks.
- For the purposes of management of grey mackerel fisheries at least five management units were identified: Western Australia, north western Northern Territory (Arafura/Timor region), the Gulf of Carpentaria, the Queensland northern east coast, and the Queensland southern east coast.
- There are at least four genetic stocks of grey mackerel: Western Australia, north western Northern Territory (Arafura/Timor region), the Gulf of Carpentaria, and the Queensland east coast.
- The use of different but complementary techniques to identify stocks proved invaluable in providing greater resolution of stock structure due to the different scales of information provided.

10. APPENDICES

Appendix 1: Intellectual Property

No patentable or marketable products or processes have arisen from this research. All results will be published in scientific and non-technical literature. The raw data from compulsory fishing logbooks remains the intellectual property of the Queensland Department of Primary Industries and Fisheries. Raw catch data provided by individual fishers to project staff remains the intellectual property of the fishers. Intellectual property accruing from the analysis and interpretation of raw data vests jointly with James Cook University, Queensland Department of Primary Industries & Fisheries, Fisheries Western Australia, Northern Territory Department of Primary Industries, Fisheries & Mines, University of Queensland and the Principle Investigator.

Appendix 2: Staff

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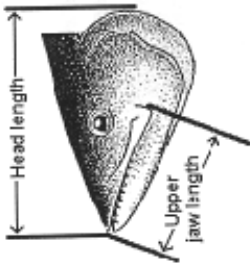
Appendix 3: Copies of data sheets and accompanying guides to standardise data collection

Copy of field/lab data collection sheet.

Grey Mackerel Catch Data Sheet		Observer:	Catch Date:	Source:	Page#: of
State:	Sample Region:	Sample Location:	Site:	Grid ref:	Lat:	Long:	Gear details (mesh,drop, length):

Tag Number	Fork Length mm	Total length mm	Upper Jaw Length mm	Head Length mm	Whole weight (g)	Sex	Stage	Lobe 1or2	Gonad weight (g)	Otoliths 1or2	Gonads Y/N	Genetics Y/N	Gut & Gill Y/N	Comments

Grey mackerel management units in northern Australia

Table 1. Data collection field descriptions for Grey mackerel

Field	Description	Values
Tag Number	Unique ID tag number given to each individual fish	Format: CRC#### (eg. CRC0123) (see file Tag DPI#### (eg. DPI0123) Numbers.xls for NT#### (eg. NT0123) current numbers)
Fork Length	Length of fish from tip of head to the fork in the tail	Record to the nearest mm
Total Length	Length of fish from tip of head to the end of the tail	Record to the nearest mm
Head Length		Record to the nearest mm
Upper Jaw Length		Record to the nearest mm
Total Weight	The total Weight of the whole fish	Record to the nearest 10/100 grams if possible
Sex	Sex of the fish from macroscopic gonad observation	M = Male, F = Female, J = Juvenile and U = Unknown / not recorded
Stage	Macroscopic stage of gonad using Mackie and Lewis (2001) macroscopic staging system	Females: 1 = virgin, 2-3 = mature resting, 4 = developed, 5 = spawning, 6 = spent Males: 1 = virgin, 2 = mature resting, 3 = developed, 4 = spawning (see Table 2)
Lobe	Record which gonad lobe has been weighed	1 or 2 lobes weighed
Gonad Weight	The weight of one or both gonad lobes (only complete whole gonad lobes)	Record to the nearest gram
Otoliths	Otoliths removed from fish	0 = no otoliths, 1 = only one otolith, 2 = both otoliths, 3 = otoliths removed but broken
Gonads	Gonads removed, stored and fixed for histology / fecundity	Yes/No
Genetics	Genetic sample taken from fish	Yes/No
Gut/Gill	Gut and gills removed for parasite research	Yes/No

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Biological Data Collection (See Table 1 for data field descriptions and data details):

1. Tag Numbering

   For consistency use the same tag format across all samples (i.e. otoliths, genetics, gonads, guts etc…). The tag format consists of the prefix for the organization (eg. CRC, DPI or NT) followed by four digits. Numbering can follow on from the last number used from last years sampling (see attached file 'Tag Numbers.xls' for list of previous tag numbers)

2. Morphological Measurements

   If time permits, all morphological measurements should be recorded for each fish, however as a rough guide the following should be recorded:
   - Fork length (FL) and/or total length (TL) should be recorded for each sample if possible.

   - In the absence of FL or TL, upper jaw length and/or head length should be recorded.

   - Total weight of the whole fish if possible

   Remember the more measurements the merrier, however, I understand the constraints in the field and this should hopefully provide a guide as to what measurements to record.

3. Reproductive Data

   Macroscopic sex and stage should be recorded for each fish if possible. The staging system we are using comes from Mackie and Lewis (2001) (see Table 2) with an additional stage for spent females (stage 6). Gonad weight should also be recorded if possible and the lobe(s) weighed (i.e. both lobes weighed or one lobe weighed).

4. Samples collected

   Record what biological samples are collected from each fish (i.e. otoliths, gonads, gut/gills and/or genetic sample). Sample numbers required from each region:
   - At least 50 gut/gills and genetic samples,

   - All otoliths (100+) to look at age and growth by region,

   - Gonad collection will be opportunistic as no allowance is built into the budget for collection of gonads. However, if the logistic opportunity exists and the individual agencies budget permits, collection of gonads would be valuable for estimating region specific fecundity. Gonads from spawning (stage 5) females only should be collected across as broad a range of lengths as possible. Gonads should be snap frozen in zip lock bags with the tag number. At least 25 gonads need to be collected per region to make the exercise worth while.

Location Information:

It is important to define the sampling location for the samples as accurately and consistently as possible. To do this I have added another level of location detail. The highest level of location information is **State**, which is implicit from our current regions but may be useful for future sampling locations that may be near state borders. In future, **Sampling Region** will refer to the broad and fine scale sampling regions (see Figure 1, Table 3). Obviously additional Sampling regions will be sampled in the next phase of the project, and we will have to add names for these new regions. The next level of location information will be **Location**, which is a slightly finer scale than sample region. The next level of location detail will be **Site**, which will be a specific reference to a more localized site within a sampling location. The final location fields are **Grid**, which is the grid cell reference that fishers report to, and **Latitude/Longitude** in degrees minutes seconds (see Table 3 for current location data from the database)**.**
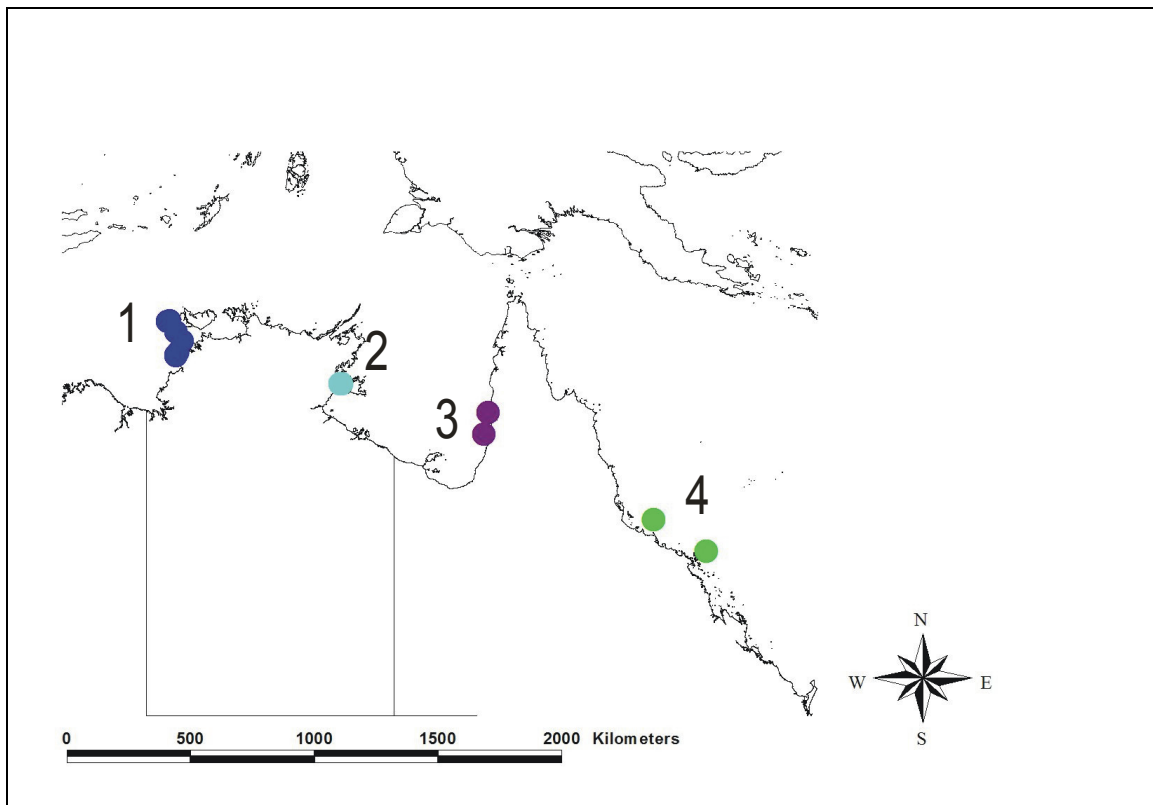

Figure 1: Sample regions for grey mackerel in North West Coast NT (1), Mid Western Gulf of Carpentaria NT (2), Mid Eastern Gulf of Carpentaria QLD (3) and from the Mid East Coast QLD (4).

Grey mackerel management units in northern Australia

Table 3 Current location fields in the Grey Mackerel Database

State	Sample region	Location	Site	Grid	Latitude	Longitude
QLD	Mid East Coast	Townsville	Offshore from Bohle River mouth			
QLD	Mid East Coast	Townsville	Offshore from Bohle River mouth			
QLD	Mid East Coast	Townsville	Offshore from Bohle River mouth			
QLD	Mid East Coast	Townsville	Paluma shoal			
QLD	Mid East Coast	Townsville	Albino Rock			
QLD	Mid East Coast	Townsville	Albino Rock			
QLD	Mid East Coast	Townsville	Havannah Island			
NT	North West Coast				12.7754	130.1300
NT	North West Coast				15.9135	130.0702
NT	North West Coast				12.8746	130.0513
NT	North West Coast				12.0426	130.0529
NT	North West Coast				12.3652	130.3043
NT	North West Coast	West of Bathurst Island			11.6379	129.8458
NT	North West Coast	West of Bathurst Island			11.6619	129.7647
NT	Mid Western GoC				13.9183	136.0616
NT	Mid Western GoC				13.9230	136.0702
NT	Mid Western GoC				13.9408	136.0184
QLD	Mid Eastern GoC	15.3 WNW Coleman River		AC12	14.9736	141.4072
QLD	Mid Eastern GoC	11.1 NW of Nassau River		AC14	15.7721	141.2616
QLD	Mid Eastern GoC	11.9 NW Nassau River		AC14	15.7533	141.2627
QLD	Mid Eastern GoC	11.2 NW Nassau River		AC14	15.7716	141.2605
QLD	Mid Eastern GoC	9 W Nassau River		AC14	15.9090	151.2193
QLD	Mid Eastern GoC	8.5 sw Pompurae		AC12	14.9913	141.4836
QLD	Mid Eastern GoC	11.2 sw Pompurae		AC12	14.9801	141.4340
QLD	Mid East Coast	Mackay	Offshore Mackay			
QLD	Mid East Coast	Townsville	Offshore from Bohle River mouth			
QLD	Mid East Coast	Townsville	Offshore from Bohle River mouth			
QLD	Mid East Coast	Townsville	Chilcott Rocks			
QLD	Mid East Coast	Mackay	Offshore Mackay			

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Table 2. Simplified macroscopic staging system for *S. commerson* gonads. F = female, M = male (Mackie and Lewis 2001).

J (Juvenile)	Gonad is a small, translucent pink ribbon lying imperceptibly along the dorsal wall of the peritoneal cavity. Sex of the fish cannot be determined. Refer to Plate 1A.
F1 (Virgin)	Ovaries are small and usually translucent pink, apricot or ivory in colour (more opaque and red in unbled fish). In smaller females, the ovaries are flattened, flaccid, and relatively inconspicuous, but they become rounded and firmer with a distinct lumen as the fish approaches maturity. The oocytes are microscopic resulting in a smooth, uniform appearance to the ovarian tissue. Yellow-brown bodies are uncommon. Refer to Plates 1B, 2 and 3.
F2-3 (Mature resting)	Soon after completion of spawning activity, the resting ovaries appear flaccid with prominent exterior blood vessels. Internally, the lumen is large. Few, if any, oocytes can be seen, whilst yellow-brown bodies are distinct (sometimes very common) and blood clots may also be present. As time since spawning increases, the ovaries become progressively rounder and firmer as the gonad wall contracts and thickens and the ovarian tissue develops. Yellow-brown bodies may be evident for sometime and are the main feature used to distinguish mature resting from virgin ovaries. Colour is typically semi-translucent rose, pink or ivory, although in unbled fish the ovaries are often red. Refer to Plates 4 – 9.
F4 (Developed)	Early in this stage, the ovaries appear semi-translucent and speckled because of the many pre-vitellogenic oocytes. As more oocytes develop and turn opaque, the ovaries become large, rotund and opaque with prominent blood vessels. The opaque oocytes are visible through the thin gonad wall and the colour is typically pale yellow or apricot. Towards the end of the reproductive period, the ovaries become more bloodied and flaccid as oocyte reserves are depleted during spawning, and yellow-brown bodies may become more common and the lumen larger. Refer to Plates 10 – 16A.
F5 (Spawning)	Ovaries are very large and swollen, although towards the end of the reproductive season they may become somewhat flaccid. Colour is apricot to peach with a prominent network of external blood vessels. The presence of translucent hydrated oocytes gives the ovaries a distinctive speckled or granular appearance through the thin gonad wall. Eggs may also be released from the gonoduct when pressure is applied to the abdomen and may be present within the ovarian lumen. Refer to Plates 16B, 17, 20 – 22, 24.
M1 (Virgin)	Testes are small and straplike with a smooth appearance and opaque, ivory or bone colour (red if unbled). No milt is present in the transverse section. It is difficult to distinguish testes early in this stage from juvenile gonads, and testes late in this stage from mature resting (M2) testes.
M2 (Mature resting)	Testes are small, opaque and straplike. Little or no milt is extruded from the transverse section when squeezed (unless the sample has been frozen). The section is quite angular in shape, with the central tissue often browner than the bone or ivory coloured peripheral tissue. Sometimes the testes may also be tinged in red.
M3 (Developed)	Testes are large, opaque, and ivory or bone in colour. The exterior dorsal blood vessel is large and small blood vessels are usually present. Internally, white sperm (milt) can usually be squeezed from the central sperm sinus. In some cases this may not be possible, although milt should be visible in the outer areas of the transverse section.
M4 (Spawning)	Running ripe. Similar to the ripe testis but more swollen and with larger exterior blood vessels. Milt is released with little or no pressure on the abdomen or when the testis is cut.

Appendix 4: Fisheries manager's responses to project results.

Western Australia

Rachel Green
Regional Fisheries Management Officer - North
Department of Fisheries, Government of Western Australia

The FRDC-funded project titled '*Determination of management units for grey mackerel fisheries in Queensland and the Northern Territory*' has provided definitive information for grey mackerel that will assist fishery managers across northern Australia by definition of population boundaries. Further, it will assist fisheries management agencies in developing appropriate harvest strategies for grey mackerel across northern Australia.

In regard to Western Australia, the results from each aspect of this project indicate that the grey mackerel population in Western Australia is distinct from those in each other state and territory both in terms of gene flow and also in terms of adult population separation. This indicates that fisheries management approaches in Western Australia can be undertaken independently of all other jurisdictions.

However, as this project has been instrumental in bringing together fisheries managers and researchers from a number of jurisdictions to discuss the management of grey mackerel in northern Australia, it has raised the need for complementary management as well as collaborative research and monitoring arrangements across jurisdictions into the future.

Discussions regarding complementary management arrangements for grey mackerel across northern Australia will be facilitated through the Northern Australia Fisheries Management Forum.

Queensland

Mark Lightowler
Queensland Department of Primary Industries & Fisheries
Brisbane

Grey Mackerel occurs throughout Queensland East Coast waters and in the Gulf of Carpentaria. Grey Mackerel are a hyperstable species which heightens the need for appropriate management. A major consideration when managing a fishery is whether the fish within the area being managed is a single or multiple stock. The FRDC-funded project titled '*Determination of management units for grey mackerel fisheries in Queensland and the Northern Territory*' was designed to provide that information for northern grey mackerel stocks from Western Australia to Mackay on Queensland's East Coast.

Queensland East Coast
The catch of grey mackerel taken in Queensland East Coast has increased over the past few years to levels that are concerning. While new management arrangements are being put in place to minimize the impact of the increased catch (increased size limit, mesh standardization and reduction in netting through a range of mechanisms) a harvest strategy has not been implemented because of information paucity.

The FRDC-funded project titled '*Determination of management units for grey mackerel fisheries in Queensland and the Northern Territory*' has been timely as it will assist in the development of a robust stock assessment following the successful implementation of a long term monitoring program.

The project has also provided comfort that, in all likelihood, there are only two major stocks on the Queensland East Coast between Port Douglas and Mackay. Having said that it would have been useful had the project extended its sampling further south than Mackay to ascertain whether the Mackay stock extended to the Queensland/New South Wales border. Given the concerns raised in

the Port Douglas Region about increased netting and the views raised there that it was an isolated stock the project has been valuable to back up the previous view that the Port Douglas stock was likely to be part of a much larger stock.

<u>Gulf of Carpentaria</u>
New management arrangements were considered for grey mackerel within the Gulf of Carpentaria several years ago. At that time it was considered inappropriate to develop new arrangements until it was known whether the grey mackerel found in the Gulf of Carpentaria were from a single or multiple stock. The results of the project have indicated that one stock occur within the Gulf of Carpentaria. This information will be the key for developing new management arrangements for grey mackerel in the Gulf of Carpentaria. In particular, the QDPI&F will need to work closely with NT Fisheries in how to best manage the single stock identified across the two different jurisdictions.

Northern Territory

Tricia Beatty
Northern Territory Department of Primary Industries, Fisheries & Mines
Darwin
Results from this project have provided robust information on the grey mackerel stock structure boundaries across northern Australia. This information is invaluable in ensuring appropriate fisheries management is undertaken for these shared stocks.

The results have indicated two separate stocks of grey mackerel found within the Northern Territory's waters. One stock has been identified as being situated over the Gulf of Carpentaria area (crossing the Northern Territory / Queensland border). It is unknown to what extent this Gulf of Carpentaria stock extends westwards into Territory waters. In addition, it is currently unknown whether the North West stocks crosses over the Western Australian border. To address these questions, the project requires additional sampling across the Northern Territory western border and the Arnhem area to identify the location of the boundary for the North Western stock and the western boundary of the Gulf of Carpentaria stock.

Future discussions regarding management arrangements for grey mackerel will be addressed through the Northern Australia Fisheries Management Forum. In recognition of the outcomes of this project, any future stock assessments conducted for grey mackerel by the Northern Territory will involve Queensland and Western Australian fishery agencies.

Appendix 5: Extension

Numerous extension activities took place during the course of the project and included:
- Fishing & Fisheries Research Centre Newsletter articles
- The Queensland Fisherman article
- FRDC 'Fish' News articles
- Recreational fishing magazine articles
- Newspaper articles
- Radio interviews
- Fishing & Fisheries Research Centre website

Presentations:
- Management Advisory Committees
- Local Marine Advisory Committees
- Australian Society for Fish Biology conference 2006 (Hobart), 2009 (Fremantle)
- ASFB/AFS 'Advances in fish tagging and marking technology' International Symposium, February 2008, New Zealand
- Northern Australian Fisheries Management Forum (NAFMF), Darwin, 2007
- Annual workshops with stakeholders (see Figure 2 below)
- James Cook University seminars & lectures

Figure 2. Project research staff and fisheries managers from each of the three northern Australian jurisdictions discuss the results and management implications at the final project workshop held in Darwin on May 21, 2008. Photo: J. Ovenden.