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THE STOCK STRUCTURE OF NORTHERN AND WESTERN AUSTRALIAN Spanish Mackerel

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FRDC Project No. 1998/159

The University Of Queensland AUSTRALIA

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The Stock Structure of Northern and Western Australian Spanish Mackerel

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OBJECTIVES

1. Establish the degree of stock structure in the northern Australian *Scomberomorus commerson* stock, over a wide geographic range;
2. Having demonstrated structural differences within the northern stock on the large scale, describe finer scale spatial structure;
3. Provide advice to the fishery administrations on the appropriate geographic scale of assessment and management actions.
4. To include the analysis of otolith and genetic material collected from Kupang (Indonesia); and,
5. To collect and analyse parasite samples from Spanish mackerel.

Objectives 4 and 5 were in addition to the project's original objectives, and were included as a variation decided in October 1998.

OUTCOMES ACHIEVED TO DATE

Close international management, between Australia, central Indonesia and Timor-Leste, of the north/western Australia stock of Spanish mackerel was demonstrated to be unnecessary. The project identified three strongly distinct Australian genetic stocks: a northern/western (N/W) Australia stock, a Torres Strait stock, and an east coast Australian stock. These were all distinct from a West Timor (Indonesia) stock. All methods used indicated that these Australian/ Indonesian stocks were distinct for Australian management purposes.

The project indicated that the appropriate scale of management responsibility for northern Australia Spanish mackerel was the level of state/ territory, or region within those instrumentalities. Although there is a single north western genetic stock, movements within this stock are fine-scaled. This does not preclude the need for cross-border collaboration that may be required to accommodate the effects of several poorly known aspects of fine scale dynamics (eg linkages between life history stages).

Partly in response to the results of this project, conservative management regimes are now in place in the Spanish mackerel fisheries of the Northern Territory, Western Australia and Queensland. This project has alerted fishery managers that the probable Spanish mackerel stock structure in the waters of northern Australia is a metapopulation: although relatively homogeneous genetically, the northern/western Australia stock at least consists of a mosaic of small assemblages (substocks or demes) that, during the adult phase at least, show little mixing. The extent to which these are self-replenishing is not known, nor is the mechanism or rate at which such stock units would recover from local depletion. This is in contrast to a previous understanding for this stock of *Scomberomorus commerson*, and shows that an apparently strongly-mixing, large pelagic species may exhibit contrasting fine scale dynamics. It is important to match the scales of management questions and activities and knowledge, and to be aware that cross-scale effects may occur. It may be a critical error to assume that lack of detected genetic differences indicates no spatial dynamic structuring or fine scale implications of management actions.

The project indicated the need to develop management approaches that are resilient to fine scale complexity. This implies that fisheries for Spanish mackerel, as well as fisheries for other species, for which definitive stock structure analysis has not been undertaken, may also need to accommodate strategies that are resilient to a variety of stock structures.

The project provided a blueprint for studies addressing stock structure. The project emphasized that various methods provide information relevant to different spatial and time scales and life history stages. Questions addressed, and methods applied, must match the time and spatial scales relative to the management and assessment goals. The project simultaneously employed three methods based on otolith isotope ratios, genetics and parasite abundance to examine attributes of stock structure and population spatial dynamics at different time and spatial scales. The methods and information developed by this project have been applied in subsequent studies of biology and stock structure (e.g. FRDC 2001/19, "Stock discrimination of east coast Spanish mackerel"; FRDC 2005/010 "Determination of management units for grey mackerel fisheries in Queensland and the Northern Territory") and monitoring methods (FRDC 2002/011, "Genetag: Real time harvest rate monitoring using genetic tagging: pilot studies in northern Australia Spanish mackerel fisheries").

The project provided further evidence that northern Australia's Spanish mackerel were over fished by the Taiwanese-Australia joint venture fishery of the 1970s and 1980s. This was indicated by a reduction in genetic diversity of samples from the late 1990s compared to those taken in the early 1980s. This reinforces the need to include this information in stock assessments of Spanish mackerel in Australia's north. Given uncertainties in the quality of the log data from the joint-venture fishery, it is important to seek methods for assessment that are independent of catch history through time.

The project stimulated placement into the public domain of valuable information that was previously unavailable or difficult to obtain. It prompted the analysis and extensive review (McPherson, chapter 2) and submission for publication (Shaklee Appendix 4) of relevant stock structure-related works funded by FRDC's predecessors. As a consequence, and as our methods of allozyme analysis were carefully matched to Shaklee et al. (1990), we were able to undertake comparison of genetic diversity in the northern Australia stock over a roughly 15 year timespan.

The project stimulated significant human capital development. It provided material for the completion of three BSc (Hons) projects (University of Queensland, Moss 1999, Thompson 1999 and Moore 2002). These made important contributions to the results of this project (Chapter 6), and led to two peer-reviewed published articles (Lester et al. 2001; Moore et al. 2003), with further publications planned. The spatial complexity of Spanish mackerel populations revealed in this project also directed the questions examined extensively in the PhD project (University of British Columbia) by Buckworth (2004).

Keywords: narrow-barred Spanish mackerel, *Scomberomorus commerson*, stock structure, spatial dynamics, metapopulation, otolith isotope ratios, population genetics, allozymes, mitochondrial DNA, microsatellites, parasite abundance, mark-recapture, tagging.

SUMMARY

Decisions about the allocation of management responsibilities for fisheries for narrow-barred Spanish mackerel, *Scomberomorus commerson*, as well as on-going stock assessments, require an understanding of the spatial relationships of the species. We used a suite of methods, isotope ratios in otoliths (earbones), parasite abundances, and genetic analyses (allozyme, mitochondrial DNA (mtDNA), and microsatellite DNA (msDNA) methods), to examine the spatial stock structure of Spanish mackerel. Fish were sampled from across northern Australia, and from Kupang (Indonesia). Project objectives included describing stock structure of the northern Australian stock of Spanish mackerel, advising on the appropriate scale of assessment and management for the stock, and comparison with material from Kupang (West Timor, Indonesia).

In order to efficiently manage the project and to design sampling approaches, we proceeded by firstly erecting a series of contrasting, basic hypotheses about Spanish mackerel spatial dynamic structures. These ranged between: 1). panmixia hypotheses, in which fish mixed substantially over the extent of the large scale genetic stocks defined by previous research, through 2). complex, alternative population structures in which population units might periodically separate but with substantial seasonal mixing (e.g. for feeding or spawning), to 3). structures in which populations units might be small and fish substantially sedentary, with minimal movement occurring between most units.

We additionally recognized that different methods provide information at differing temporal scales (and consequently, at correspondingly different spatial scales). These intrinsic scales mean that combinations of methods might enable us to test the general stock structure hypotheses. Although there could be many feasible hypotheses that would vary in detail from our basic hypotheses, we anticipated that a program designed to resolve between the basic hypotheses would capture the important attributes of Spanish mackerel stock structure.

We adopted a phased approach, in which we first tested samples from widely spaced primary locations, across the geographic range of the study, from southern Western Australia to the Queensland east coast. A panmixia hypothesis was considered to be a strong candidate for *S commerson* stock structure, and could be tested with limited sampling, and by the otolith isotope ratio method alone. If panmixia were supported, then the study could be terminated at that point and further sampling and analyses would be unnecessary, potentially conferring substantial cost savings.

We showed that over a fish's adult life span, groups of adult narrow-barred Spanish mackerel mix little on scales of 100-300 km and as large as 1,000 km. This was evidenced with oxygen and carbon isotope ratios from whole otoliths, and with parasite abundances.

Isotope ratios in whole otoliths are a signature of the average physical conditions encountered by a fish through its life, which for mackerel in the northern Australian fisheries would be a period of about five years. In the initial tests, discrete location-specific stable isotope signatures were evident from *S. commerson* across northern and western Australia, indicating that there is unlikely to be any substantial movement of fish among spatially

discrete groups. The analyses provided no evidence of a latitudinal cline. The panmixia hypotheses were consequently rejected, and hence further sampling undertaken.

More sampling was conducted at the primary and additional (secondary) locations, to provide for tests of temporal stability and finer spatial resolution. With the replicate sampling, it was demonstrated that the location-specific stable isotope signatures were persistent through time, at least over the course of the study. At spatial scales of 100 – 300 km, the population units sampled therefore comprise functionally distinct independent management units or separate 'stocks' for many of the purposes of fisheries management.

Of further interest is that a predictive relationship between oxygen isotope concentration, sea surface temperature and internal body temperatures was developed. This indicated that *S commerson* may maintain an elevated internal temperature relative to ambient conditions. This phenomenon is well known in the related tunas (Tribe Thunini of Family Scombridae), and was predicted for Spanish mackerels (Tribe Scomberomorini) on the basis of anatomy by Kishinyoue (1923). However it has not previously been experimentally observed for Spanish mackerels.

Parasite faunas of subsamples of the mackerel were evaluated for evidence of movement and migration. Parasite faunas have intrinsic time scales varying from about one year or less, to the average life span of the animal, similar to otolith isotope analyses. Parasite abundance rates were thus used to corroborate and add to the information provided by otolith isotope analyses. Parasites were particularly common in fish from the NT and northern WA and almost absent from fish from Kupang. We concluded that there had been no movement of adult fish from Australia into the population from which our Kupang samples had come, but we could not ascertain whether Kupang fish moved to Australia from the parasite data.

In northern areas, the fish had distinct parasite faunas, indicating little post-recruitment movement. In fact, some spatially-separated samples for which there were similar otolith isotope ratios were distinct by parasitological analyses. This might arise where non-mixing subpopulations experience comparable ambient conditions. In contrast, the west coast parasite fauna was similar from several adjacent areas which would be consistent with some fish, especially males, moving between these sites. A detailed study of long-lived parasites from six sites in the Gulf of Carpentaria suggested some exchange between locations near the Sir Edward Pellew Group and Groote Eylandt, but no mixing between these areas and Mornington Island, Weipa, Torres Strait and the Cape Wessel areas, all of which had distinct parasite faunas.

Parasite analysis also provided examination of spatial dynamics on a sub-annual time scale. Analysis of parasite data from fish caught in the same area on different half-days indicated that variability was the same for fish within half days as it was between half days. Hence there was no evidence that fish formed into semi-permanent schools.

Although the otolith isotope and parasite data provided strong evidence for spatially discrete groups, allozyme and mitochondrial DNA (mtDNA) population genetics showed that there is genetic interchange between these groups, so that Spanish mackerel in northern Australia

form just three distinct genetic stocks: an east coast stock, a Torres Strait stock, and a single stock across the north and west coasts of the continent. With a longer intrinsic time scale (typically centuries or millennia), the genetic analyses reflect the past isolation of the east coast from the northern populations during a period of lower sea levels, and subsequent re-colonization of the Torres Strait region.

Matching of allozyme analytic protocols with those of a previous study (Shaklee, Appendix 4) as well as replicate sampling in 1998 and 1999 meant that both short-term (one year) and long-term (15-20 years) tests of the temporal stability of allozyme genic and genotypic frequencies could be incorporated. Kupang mackerel were genetically distinctive, as were, to a lesser degree, fish from the east coast of Queensland. There was no evidence of genetically distinct stocks among populations sampled from Shark Bay in the west to the Gulf of Carpentaria.

Additional genetic analyses, of msDNA (funded by Qld DPIF), in addition to the mtDNA studies, were used to further examine gene flow in Spanish mackerel in the Torres Strait area. Although the mtDNA analyses would alone indicate a separate genetic stock in Torres Strait, this was not supported by the analysis of microsatellites. There was evidence that this lack of agreement between the results of differing methods was due to greater dispersal by males than females. This reflects the relative lack of movement by females, as mtDNA is maternally inherited. This interpretation is independently supported by the parasite analyses on the west coast where it was concluded that male and female *S commerson* exhibit slightly different dispersal dynamics.

Spanish mackerel thus form a fine-scale mosaic of population units through Australia's north; this may best be considered as a metapopulation structure. There were additionally between-sex differences in mixing, with females being more sedentary than males (shown with both parasite and genetic methods). However, there is genetic flow between the sub-populations so that, reflecting larger time and spatial scales, Spanish mackerel in northern Australia form just three distinct genetic stocks: an east coast stock, a Torres Strait stock, and a single stock across the north and west coasts of the continent. The northern Australian and west Timor samples were distinct by most analyses – otolith isotope, parasite abundance and genetics – so that joint international management across the Timor Sea is not recommended. All methods indicated that movement of adult fish from Australia to Kupang did not occur. Nevertheless, phylogenetic analyses indicated possible movement of mackerel from Kupang to Australia, while parasite analyses did not preclude movement in this direction. We thus met project objectives of demonstrating both the large and fine scale structure of *S. commerson*, and of indicating the scale of administrative responsibility.

We concluded that Spanish mackerel stocks should be regarded as metapopulations for management purposes. Observed seasonal variations in availability of Spanish mackerel probably reflect cycles of seasonal aggregation and dispersal. Large movements by east coast Spanish mackerel are accommodated in this model as responses to seasonal expansion and contraction in habitat along latitudinal gradients (for example, preferred temperature ranges). A variation of the metapopulation hypothesis is that fish form

contingents, groups of fish with differing instinctive or learned migratory behaviour, so that in some areas, some groups of fish join migratory groups (contingents) while others do not.

Comprehensive comparison of allozyme analyses of the samples in this study, to Shaklee's results (Appendix 4) suggested a shift away from genetic equilibrium (Hardy-Weinberg) in the intervening 15-20 years. This may be further evidence that the northern Australia stock of Spanish mackerel was overfished by the Taiwanese-Australia joint venture fishery of the 1970s and 1980s, or indicates that the stock has since been overfished. This supports the conclusion that the metapopulation structure might render Spanish mackerel vulnerable to serial depletion, in which Spanish mackerel numbers are repeatedly depleted on local scales, progressively over larger spatial scales. The metapopulation structure may have been a contributing factor to observed overfishing of this species in northern Australia and Oman. At the same time, the metapopulation structure might be to advantage in the design of management approaches that capitalise on spatial complexity, such as a rotating harvest area approach.

The metapopulation structure means that management responsibility may reside at the state or regional level. We emphasise that this precludes neither the desirability of cross-border collaboration nor the need for finer scale investigation and control, to accommodate effects of fine scale dynamics. Aspects of spatial dynamics such as connectivity between life history stages, and population responses to depletion or extirpation of population elements were not addressed by the project and are unknown. Management actions must be robust to these unknowns.

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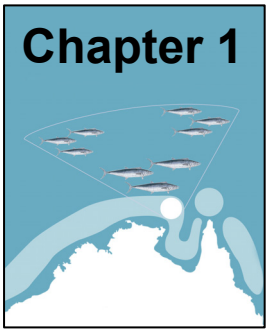

INTRODUCTION

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OVERVIEW

Prior to this study, the spatial dynamics and relationships of the Spanish mackerel fisheries of the northern Australian region were poorly understood. The general consensus on narrow-barred Spanish mackerel, *Scomberomorus commerson* (Lacepède) stock structure was that this species was known to undertake extensive longshore migrations (Munro 1943; McPherson 1981a,b; Lewis 1981; Govender 1994). Such migrations were observed for some fish in east coast mark recapture studies conducted during the 1970s (Chapter 2), supporting the longstanding belief of seasonal southern movement down both the east (McPherson 1982) and west (Donohue et al. 1982) coasts, and on the basis of temporal catch rate patterns were predicted to exist for the Gulf of Carpentaria (GoC; Kailola et al. 1993). At the same time, permanent resident populations were thought to exist as well (Colette and Nauen 1983, Chapter 2). This understanding was not adequate to resolve questions about management responsibility, or to address stock assessment, or development of optimum monitoring and management controls.

Given the observed long distance movements in the eastern Australian and other Spanish mackerel stocks (Chapter 2; Lewis 1981; Govender 1994), and the apparent lack of genetic diversity in the northern/western Australia stock (Shaklee et al. 1990), the latter might be regarded as one large well-mixed population, and hence require a joint management approach. Indeed, an assessment by Walters and Buckworth (1997), which observed a low frequency of older fish in catches, suggested that offshore movement and capture, in international waters, of larger fish might indicate an urgent management problem for the northern fisheries. There were, however, some indications that spatial dynamics of the species might be more complex. These hints included occasional anecdotes of local depletions, that typical movements of tagged Spanish mackerel were limited (see Chapter 2) and that there were some observations of differences in ectoparasite loadings (Chapter 2). The incidence of ciguatera poisoning ascribed to *Scomberomorus commerson* has been so localized that it is managed in Queensland by closing small areas to fishing. Together these observations imply that many Spanish mackerel are effectively resident on a small spatial scale. In this case management responsibility and many actions might be appropriate at a fine scale.

This project set out, therefore, to resolve some of the stock structure issues for Spanish mackerel and its fisheries. Using a variety of methods as well as summarizing existing information, the project has produced a substantial amount of new information. It is organized here into a series of chapters that are essentially stand-alone, detailed articles that describe the outputs of each of the separate project activities. While this chapter provides an overview, a summary of the prior state of knowledge about Spanish mackerel stock structure is presented as Chapter 2. This makes available information from studies supported by the Fishing Industry Research and Development Trust Account (FIRTA, a pre-cursor of the FRDC), and the Queensland Fisheries Service, that was hitherto not generally available. Chapter 2 concentrates upon analysis of mark-recapture studies but also summarizes the history of the east coast fishery for Spanish mackerel, provides analysis of catch information, and includes historical anecdotal information.

Subsequent chapters provide detailed descriptions of new investigations using three broad methods applied to samples of *S commerson* collected across the west, north and east of Australia. Supplying differing types of information, the methods were essentially complementary. Whole otolith isotope ratios were used as the 'core' method in this project (Chapter 3). This analysis was carried out for some samples collected at all sites. Genetic analyses - allozyme and mitochondrial DNA (mtDNA) studies described in Chapter 4 - used a subset of samples to address specific hypotheses, firstly establishing whether studies undertaken in northern Australia Spanish mackerel populations in the 1980s were repeatable, and enabling comparisons over a near-two decade time span. Microsatellite DNA (msDNA) and mtDNA analyses (Chapter 5) were used to examine genetic flow in the Torres Strait. By simultaneously using two methods, differing dispersal rates between males and females, were confirmed. This attribute was also demonstrated by parasite analyses of subsets of samples (Chapter 6). These analyses provided independent tests of the interpretation of otolith isotope ratio analyses, and demonstrated the power of parasite abundance analyses at extracting information with fine temporal scale (1 year or less, to average lifespan of the fish). Based upon three BSc. (Honours) thesis projects, these analyses were informative, cost effective, and developed the skills of three junior scientists in parasitology of fishes, and additionally led to wider application of the method in other fisheries. It is also worthy of note that, as a consequence of negotiations undertaken for the standardization with past studies necessary to undertake the allozyme studies of Chapter 4, material gathered under FIRTA support was analysed and is for the first time generally available (Shaklee Appendix 4).

In this study, we showed that over the adult life span, groups of adult narrow-barred Spanish mackerel are distinct over as little as 100-300 km. There was some indication that this scale increased with latitude. This was supported with oxygen and carbon isotope ratios from whole otoliths and parasite abundances. Between-sex differences in this mixing were evident, with females being more sedentary than males (shown with both parasite and genetics methods). Spanish mackerel thus form a fine-scale mosaic of population units through Australia's north. However, there is genetic flow between these sub-populations so that, reflecting larger time and spatial scales, Spanish mackerel in northern Australia form

three distinct genetic stocks: an east coast stock, a Torres Strait stock, and a single stock across the north and west coasts of the continent.

We conclude that Spanish mackerel stocks probably form a metapopulation structure (a "population of populations"). Observed seasonal variations in availability of Spanish mackerel probably reflect cycles of seasonal aggregation and dispersal. Large movements by east coast Spanish mackerel are accommodated in this model as responses to seasonal expansion and contraction in habitat along latitudinal gradients. A variation of the metapopulation hypothesis is that fish form contingents, so that in some areas, some groups of fish join migratory groups (contingents) while others do not. Aspects of spatial dynamics such as connectivity between life history stages, and population responses to depletion of population elements were not addressed by the project and are unknown.

The results have important implications for management of Spanish mackerel fisheries. Because fish are aggregative, relatively high catch rates might be maintained even as population levels decline. The metapopulation structure may render Spanish mackerel vulnerable to serial depletion, in which high densities are fished down in turn. Indeed, the study provided evidence of overfishing that has occurred some time since the mid 1970s.

BACKGROUND

One of the fundamental needs of effective fishery assessment and management is an understanding of the spatial dynamics in a fishery – the way that parts of the population may respond to changes in abundance or demographic processes in other spatially distant parts of that population. In northern Australia's fisheries for narrow-barred Spanish mackerel, *Scomberomorus commerson*, these relationships have been poorly understood. Assessments of these fisheries are thus uncertain even in their basic assumptions. In the face of such uncertainty about fishery status, Western Australia, the Northern Territory and Queensland have instituted or are moving toward conservative management regimes for their Spanish mackerel fisheries. But the results of management actions cannot really be predicted, and it is even possible that fishing has been too tightly constrained by management measures. This project was designed to reveal, by using a suite of complementary methods, the spatial structure of Australian *S. commerson* populations at several spatial and temporal scales.

Spanish mackerel supports commercial fisheries across Australia's north, with 1997 ex-vessel sales estimated at more than $7 million. Conservative interpretation of logbook data estimates the catch at a minimum 1340 tonnes annually; however, catches and value could in reality have been be much larger, when recreational catches are considered – Buckworth (2004) estimated the northern Australia total catch to be 2000-3000 tonnes annually. Although most of the landings have traditionally been sold into the fish-and-chip trade, the excellent quality of this species (see for example Hay et al. 1996, and Slattery 1998) has been driving steady price rises, with the penetration of new markets. Spanish mackerel is

also a highly prized light game fish in the rapidly-growing angling and fishing tourism sectors. Recreational catches of this species were roughly estimated to be at least 20% of the current commercial catch, but may be considerably higher (Henry et al. 2003).

The biology of *S. commerson* in Australia is well-known (e.g. McPherson 1981a,b, 1987, 1992, 1993; Buckworth 1998, 2004; Mackie et al. 2003; Tobin and Mapleston 2004). Further research into the fisheries is now driven by management needs, particularly the requirements for on-going stock assessments, and decisions about management structures and responsibilities. Studies into the stock structure are seen as an important step that will not only improve the basis for management, but also as a necessary precursor of studies into international stock relationships and finer scale spatial dynamics.

The potential sustainable yield of each of the northern Spanish mackerel fisheries within each state is uncertain and may be quite limited. Stock assessments for the species, for example those conducted in Darwin in July 1997 (Walters and Buckworth 1997) and subsequent assessments for the Queensland east coast fishery (Welch et al. 2002; Hoyle 2003) and the NT fishery (Buckworth 2004) have mostly been very uncertain but indicated that Spanish mackerel in northern Australia may be a relatively limited resource. Movement of fish across state and international boundaries is highly probable but before the current study, little could be inferred about rates of this movement, or its significance. In each State/Territory jurisdiction, Spanish mackerel fisheries are subject to management regimes designed to contain fishing mortality. At the inception of this project, commercial and recreational fishing effort was growing rapidly, pressure on stocks building. But what were the “stocks” to be managed?

Although Spanish mackerel are usually regarded as a highly migratory fish, large scale stock differences certainly exist: allozyme electrophoresis indicated that east coast Spanish mackerel are probably distinct from populations in Torres Strait and further west (Shaklee et al., 1990.). However, electrophoresis of blood (McPherson, 1981b) and muscle (CSIRO, unpublished; Shaklee et al., 1990) did not indicate any subdivision of Australian stocks west of Torres Strait. Tagging studies on the Australian east coast and eastern Gulf of Carpentaria (GoC) show that some individuals move large distances, yet most recaptured fish had moved very little; there were no confirmed tag returns from the GoC or Top End, although some movement of fish from the north-western GoC to Torres Strait was suggested (McPherson 1985, 1992). Anecdotal information on local depletions and observation of differing parasite abundances suggested the possibility of semi-resident sub-stocks, even in the east coast population. The population structure of the north and west coasts was not hitherto investigated.

Stock assessment workshops in Darwin led by Dr. Carl Walters (UBC), in July 1997 identified putative stocks from the distribution of Taiwanese gillnet catches (Stevens and Davenport, 1991), with additional anecdotal information. In these assessments, five functional stocks were suggested: The Kimberleys; Joseph Bonaparte Gulf / western Top End; Arnhem Land/western GoC; eastern GoC/ Torres Strait, and Qld East Coast. As relationships among WA populations of Spanish mackerel had never been examined, we additionally included the Perth/Gascoyne and Pilbara “stocks” in this project.

An initial analysis of the mitochondrial DNA (mtDNA) of Spanish mackerel from the waters of the NT (Lavery and Buckworth, unpubl. results) indicated that variation in certain regions of the genome was sufficient to test hypotheses about genetic structuring of the populations. Several hypotheses could thus be erected and tested about the structure of Australia's *S. commerson* stocks. These ranged through three basic genetic models: the panmixia model, in which all populations can be part of a single random mating unit; the stepping stone or island model, in which the species consists of a number of sub-populations which have limited genetic exchange; and lastly, the isolation-by-distance model (Baverstock and Moritz 1996), in which individuals mate only with nearby conspecifics. A spectrum of possible spatial dynamics models were consistent with the genetic models, and could provide alternate management strategies depending on the population structure. Alternative examples of a panmictic population, for example, are a population with a single spawning location, to which all spawners migrate, or a population in which there is extensive movement, and little fidelity to several spawning sites. Appropriate management strategies are patently different. Where stocks may be shared between jurisdictions, effective and cooperative management can only be built on an understanding of stock structure.

The tactical approach in this project was to first test the hypothesis of panmixia for Australian *S. commerson*. Subsequently, other boundaries indicated by the distribution of fishing, or finer scale structuring, were tested. Use of established methodologies, allozyme and mtDNA genetic analyses, otolith isotope analysis, and parasite abundance, could provide a robust approach to resolving questions about the northern Australia *S commerson* stock structure. It was made very clear at the FRDC-funded workshop of July 1997, "Taking Stock: Defining and Managing Shared Resources", that an analysis of stock structure would be most effective if several methods were used. The genetic analyses typically identify differences on the large spatial and temporal scales, where gene flow is minimal. Parasite species composition and loadings, and otolith chemistry, each 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. Resolution of such methods can even be fine enough to detect integrity of individual schools (Lester et al., 1985). The methods are fully complementary: a population of fish defined as having similar otolith and parasite characteristics may also be composed of several genetically defined sub-populations.

The results from each methodology were consecutively synthesized during the project in a series of six monthly meetings of co-investigators. Expert external advice was sought as needed.

NEED

This project provides an understanding of Spanish mackerel populations, a national shared resource, with a view to the development of complementary management approaches. It sought to describe the stock structure of *S. commerson*, as a basis for assessment and sustainable, optimal harvest of Australia's Spanish mackerel resources.

The NT, WA, Qld, Torres Strait and NSW have separate management regimes for the mackerel fisheries in their waters. Although these northern Australian jurisdictions are moving toward joint or complementary management of these resources, lack of information on stock structure meant that the appropriate scale of management units was unknown; nevertheless it was considered unlikely to coincide with current administrative boundaries. Basic questions such as whether management actions in one state might impinge on the fisheries of others could not be answered. Would declines in one area reflect interception during migration, or over-fishing of spawners in another area? Different responses to such questions may require fundamentally different management approaches. Hence the Northern Australia Fisheries Management meeting of May 1997 recognized that stock definition was required for effective assessment and management of this species.

Most fishery assessments assume a randomly distributed unit stock; an alternative is to explicitly include spatial dynamics. Possibly with the exception of the east coast, there has hitherto been no real basis for defining Spanish mackerel unit stocks; in none of the Australian fisheries have spatial relationships been sufficiently understood to be addressed in assessments. The research is requisite for basic stock assessment, and the first step in developing spatially structured models and management.

Spanish mackerel are also taken across our northern boundaries, in Indonesia, Papua New Guinea, and Pacific Island states. The research we describe is the basic work necessary to develop the methodology and information base for future research into these shared resources, and for future studies into fine-scale spatial dynamics.

OBJECTIVES

1. Establish the degree of stock structure in the northern Australian *Scomberomorus commerson* stock, over a wide geographic range;
2. Having demonstrated structural differences within the northern stock on the large scale, describe finer scale spatial structure;
3. Provide advice to the fishery administrations on the appropriate geographic scale of assessment and management actions;
4. To include the analysis of otolith and genetic material collected from Kupang (Indonesia); and,
5. To collect and analyse parasite samples from Spanish mackerel.

All project objectives were achieved.

Note that Objectives 4 and 5 were additional to the original project agreement.

METHODS

Development of sampling approach using intrinsic time scales and stock structure hypotheses

This study sought to define the "stock structure" of Spanish mackerel for two distinct reasons: to assist in decisions about responsibility for management, and to enable management actions, and contributing activities such as stock assessments, to refer to spatial scales that matched the spatial dynamics of the fish populations. Studies of "stock structure" are frequently thought of as drawing lines on maps – assigning management responsibilities or boundaries for stock assessment. However, stock structure really is dynamic and must include temporal scales of the rates at which animals move between areas, or interbreed, and so on. Most studies of stock structure recognize that definition of a "stock" is contextual – what questions are asked, what management needs are addressed dictate the working definition of a stock Begg and Waldman (1999), for example, considered "stocks" simply as semi-discrete groups of fish with some definable attributes of interest to managers.

We began this project recognising that various questions that might be asked during the management of a fishery entail differing spatial and temporal scales. Moreover, the different analytic methods available each have their own "intrinsic time scale" (Table 1.1), which we define as the approximate time span to which most information derived from that method applies. Consequently, different methods similarly apply at different spatial scales. Alternate hypotheses about spatial stock structure also entail different time scales, which might be resolved by applying different methods.

To structure our approach to sampling, we erected a series of general hypotheses about stock structure of Spanish mackerel (Table 1.2). Our intention was simply to establish whether available methods and fishery-based sampling would be likely to discriminate between broad groups of possible life histories and thereby provide general management policy guidance. Thus a panmictic population (Item 1 in Table 1.2), entailing extensive adult movements and opportunistic location of reproduction would be homogenous in both genetic and OIR analyses from adult or juvenile samples. We would expect that, in contrast, relatively sedentary adults (Items 2 and 3), would tend to produce large differences in OIR between distant samples. However, genetic differences would depend upon the extent of larval dispersal: if this were extensive, as in Item 2, we would expect minimal differences among juvenile samples but limited dispersal would produce heterogeneity among juveniles, as in Item 2. Residence, movement and reproductive strategies would all contribute to observed patterns. Samples from philopatric populations (Item 4; e.g. Pacific salmon species, where there extensive high seas mixing as fish grow and mature, but a high degree of fidelity to natal spawning sites, might produce samples from spawning fish that were genetically distinct between different locations, yet homogeneous in OIR analyses. Additional information to that provided by genetic or OIR analyses might be required to indicate more complex life histories. For example, in Item 5, we considered a species which forms contingents, groups of fish that may be distinguished by their differing learned migratory

behaviour (eg Atlantic herring, McQuinn, 1997; Hudson River striped bass, Secor 1999), even though they are genetically homogeneous. Such homogeneity might result from spawning behaviour that leads to outbreeding or larval dispersal. Such a species, in which there might be both sedentary and migratory groups of adults, would produce small genetic differences in fish sampled at different localities but OIR sampled from sedentary groups would be distinct at different localites. Samples, however, might be from migratory contingents (possibly leading to smaller between group differences in OIR) or be an admixture of migratory and sedentary fish (OIR analyses would have a large variance). Clearly ancillary information produced by other techniques at finer spatial and temporal scales would be valuable.

The information that would be expected with genetic and OIR analyses with sampling from different life history stages indicated that most general hypotheses might be separated using sampling from mature adults, the major constituent of commercial catches. In terms of benefit relative to cost, this enabled us to focus our study just on the adult life history stage.

We recognised from Table 1.1 that parasite abundance data should test interpretation of the otolith isotope data. As the two forms of data have similar intrinsic scales, parasite data should provide similar predictions to those of the otolith isotope ratios in Table 1.2. Additionally, parasite data might provide information at finer scales, depending upon parasite life history. As parasites could be collected and analysed at small marginal cost, we broadened the project to include the parasite abundance method. Additionally the opportunity arose to collect material from Kupang, also at small marginal cost.

Table 1.1. Intrinsic time scales of various methods providing stock structure information

Method	Intrinsic time scale	Origin of information
Genetic spatial analyses	10 000 – 500,000 y	Rate of evolution of genetic markers
Genetic temporal analyses	5 – 50 y	Comparison of genetic composition over time
Whole otolith isotope ratios	5 y	Averaged ambient chemical environment over life span
Adaptive management	Years – decades	Rates of population replenishment at different scales, density-dependence
Parasite abundance	Seasonal – 5 y	Parasite life span, biology
Mark-recapture	Hours – years	Time at liberty and capture locations, reflecting feeding cycles, transient responses to capture and fishing distribution
Sonic/ archival tagging	Minutes, seasons, years	Continual polling of position and environment

Table 1.2. Predicted magnitude of between-site differences in genetic or otolith isotope analyses, for mature or juvenile fish sampled from hypothetical stocks of Spanish mackerel with differing spatial dynamics

Technique	Genetic		Otolith isotope ratios	
Life history hypothesis	**Juveniles**	**Spawners**	**Juveniles**	**Spawners**
1. Panmixia **move around to feed and breed anywhere**	Small	Small	Small	Small
2. Sedentary **live and breed in same locale *significant larval dispersal***	Small	Small (big variance)	Small (big variance)	Large
3. Sedentary **live and breed in same locale; *limited larval dispersal***	Large	Large	Large	Large
4. Philopatric **move between locales to feed, but briefly return to same locale to breed each year**	Small	Large	Large	Small
5. Split contingents - sedentary *or* migratory groups **Complex outbreeding?**	Small	Small	Small	Large (big variance)

Sampling

The basic approach adopted was to sample mature (spawning or near spawning) fish from a series of sites throughout the distribution of the N/W stock. All analyses of otoliths, genetics and parasites were to be performed on the same sample of fish. To potentially reduce the cost of the project, a phased approach was used. This recognized that some of the

contending hypotheses that were considered consistent with existing observation (eg panmixia), might be tested using this limited sampling and analysis. If supported, then this would define further research and management direction. The study would have achieved the basis of most objectives, and sampling could be terminated. Fish were sampled at a series of widely-spaced primary sites covering the geographic range of the N/W stock, as well as one site on the east coast (detailed locations of sampling are provided in Chapter 3), in 1998 and 1999. Secondary sites, located between the primary sites and thus reducing the spatial scale, were sampled in 1999 and 2000. The east coast site was included as a methodological control, as previous studies (Shaklee et al. 1990, Appendix 4) had indicated that the east and N/W stocks were distinct.

Material was collected in 1998 for all analyses, but was initially examined for OIR only. Given the previous conclusion of Shaklee et al. (1990), minimal difference between the OIR for different sites would have supported a panmictic stock hypothesis (Table 1.2). This was not so, however, and the full sampling program was undertaken.

Sampling typically involved collection of material and information from a target 50 fish of each sex. Length, sex and visual reproductive stage were recorded. Details of sample treatment and analysis are included in Chapters 3 to 6.

Isotope ratios in whole otoliths are a signature of the average physical conditions encountered by a fish through its life. For mackerel in the northern Australian fisheries this would be a period of about five years. In the initial tests, discrete location-specific stable isotope signatures were evident from *S. commerson* across northern and western Australia, indicating that there is unlikely to be any substantial movement of fish among spatially discrete groups. The analyses provided no evidence of a latitudinal cline. The panmixia hypotheses were consequently rejected. More sampling was conducted at the primary and additional (secondary) locations, to provide for tests of temporal stability and finer spatial resolution.

With the replicate sampling it was demonstrated that the location-specific stable isotope signatures were persistent through time, at least over the course of the study. At spatial scales of 100 – 300 km, the population units sampled therefore comprise functionally distinct independent management units or separate ‘stocks’ for many of the purposes of fisheries management.

Analyses

Analytical methods and statistical analyses are described in detail in Chapters 3 to 6. Ratios of isotopes of carbon and oxygen in whole otoliths were employed as the core method, and were established for all samples at all locations (Chapter 3). Three separate genetic analyses employed to address specific questions and hypotheses. Allozyme methods were included (Chapter 4) so that comparisons could be made between the current study and earlier genetic analysis from the study area (Shaklee 1990, Appendix 4). Mitochondrial DNA (mtDNA) (Chapters 4 and 5), and microsatellite DNA (msDNA) methods (Chapter 5) were also employed to examine specific questions. Analysis of parasite abundance (Chapter 6)

was used to provide independent corroboration of the otolith isotope and genetic analyses, as well as provide additional information on finer time scales.

RESULTS AND DISCUSSION

This project has sought to understand the spatial scale and details of the dynamics of the mackerel populations of northern Australia, so that assessment and management of the fishery might be conducted at spatial scales matching those of biological processes. This report reveals that the spatial stock structure of northern Australian Spanish mackerel is complex, and much more finely structured than hitherto suggested for this species. This section provides an overview of the results from the project components and readers are referred to individual chapters for in depth analyses and discussion.

It is highly probable that the Australian Spanish mackerel stocks form metapopulations. This is strongly indicated by the results of the OIR analyses – nearly all sites were distinct, with discrete location-specific stable isotope signatures indicating that there is unlikely to be substantial Spanish mackerel movement between spatially distinct groups; these groups were temporally stable (Chapter 3). This was supported by parasite analyses, to the extent where, when sites were not distinct in OIR analyses, subsequent parasite analysis usually indicated a difference. This was the case for Cape Van Diemen and Cape Wessel samples. These locations are over 700 km apart but are similar in latitude and fish may encounter similar environmental conditions: samples were not distinct by OIR analyses (reflecting environments which were not distinct; Chapter 3) but were clearly so when parasites were examined (Chapter 6). The mtDNA analyses indicated a separate genetic stock in Torres Strait, a separation supported by parasite analyses but not by the analysis of microsatellites. There was evidence that some apparent lack of agreement between the results of differing methods was due to greater dispersal by males than females (mtDNA is maternally inherited and reflects relative lack of movement by females).

There was no evidence in location-specific stable isotope signatures that there was a latitudinal cline. However, the physical scale of spatial units was not constant: both OIR and parasite techniques indicated an increase in the distance at which samples were distinct with latitude (Chapters 3 and 6). Additionally, the finer temporal scale of some parasite analyses revealed seasonal movement that differed between the sexes (Chapter 6). As above, sex-biased dispersal, in which males are more likely than females to move and breed with adjacent populations, was also indicated by the comparison of mtDNA and microsatellite DNA analyses (Chapter 5).

The results indicate a metapopulation structure, a fine-scale mosaic of population units through Australia's north and west. Support for this interpretation of our results is provided by observed differences between locations in demographic parameters in *S commerson*. Mackie et al. (2003) documented variation in growth and mortality parameters through Western

Australia, and reviewed information from other Australian studies. McIlwain et al. (2005) demonstrated widespread variation in *S commerson* growth parameters in Omani waters.

A serendipitous output of the otolith isotope work was development of a predictive relationship between oxygen isotope concentration, sea surface temperature and internal body temperatures. This indicated that *S commerson* may maintain an elevated internal temperature relative to ambient conditions. While this phenomenon is well known in the related tunas (Tribe Thunini of Family Scombridae), and has been predicted on the basis of anatomy (Kishinouye 1923) it has not previously been observed in the Spanish mackerels (Tribe Scomberomorini). This result could stimulate further research.

Despite the fine scale of the adult assemblages, there is genetic flow between these population units, so that only three distinct genetic stocks are evident – an east coast stock, a Torres Strait stock and, to the west of that, a single stock across the north and west coasts of the continent. It is also important to note that the Torres Strait stock is newly identified in this work, a result of the greater resolution provided by mtDNA compared to the allozyme techniques. This indicates that there is genetic flow through some combination of straying of individuals into adjacent demes (population units), shown here by both genetic and parasite analyses, and subsequent participation in spawning. Using the combination of mtDNA and msDNA genetic techniques, as well as parasite abundance analysis, indicated the subtle difference between spatial dynamics of males and females of the species, in that straying between adjacent population units might be greater in males. Nevertheless, little is known of population reproductive dynamics, of connectivity between mackerel spawning and subsequent recruitment linkages to adult populations. It is feasible that spawning and mixing of larvae and juveniles may occur at larger scales to the adult assemblages, and could also ensure the contiguity of the genetic stocks.

Strong seasonality is a characteristic of Spanish mackerel fishery catches, and it has often been interpreted as a result of seasonal migration (eg Donohue et al. 1982; Kailola et al. 1993). However, little dispersal would be required to reduce local densities at any point, and moderate reductions in feeding intensity might make fishing commercially unattractive. It could be possible that in some parts of the *S commerson* population, fish might reside for much of the year in specific feeding grounds, and then return to distant spawning grounds over a short period of time.

The large movements observed for some *S commerson* (McPherson 1987; Chapter 2; Govender 1994) over latitudinal gradients are accommodated in this model by noting that in these areas, the habitat available to *S commerson*, defined for example by ambient temperatures or baitfish abundance, is subject to strong seasonal expansion and contraction – so that observed movement is just the fish exploiting that habitat. Not all tagged fish tagged on the east coast moved large distances (McPherson 1987; Chapter 2). The larger movements might just represent the extremes of a continuum of dispersal/ migration rates within the stock, or a group with different instinctive migratory behaviour. An alternative interpretation is that populations of mackerel may form contingents –groups of fish may be distinguished by their differing learned migratory behaviour (eg Atlantic herring, McQuinn,

1997; Hudson River striped bass, Secor 1999), even though genetically homogeneous. Secor (1999) suggested that early life decisions regarding energy allocation (the maintenance of ``retentive'' and ``exploratory'' behaviours) lead to later divergence in habitat shifts (migration). It may be possible that the east coast stock consists of relatively sedentary groups of fish, contingents of fish that migrate latitudinally, and also, perhaps, fish that annually disperse offshore (but we note that gamefishing and longline catches of mackerel offshore are very small). It may be inappropriate to assume that the stock structure details observed in one *S commerson* stock necessarily apply to another.

It is not known what physical factors might define demes, what limits their spatial extent, or how these might vary between stocks. The spatial separation between adult assemblages, demes, increased with latitude in this study. In the east coast stock, no subdivision was apparent in parasite studies (Williams and Lester 2006) but was evident in OIR for the small number of sites we examined (Chapter 3). It is feasible that there may be a lack of differentiation in parasite faunas even when there is separation of assemblages (in which case the null hypothesis is not rejected, but is not true), even as the results of OIR analyses for Cape Van Diemen and the Wessels were not significantly different, yet the parasite analyses were.

A metapopulation structure has several fishery management implications. The first is that the responsibility for management of most Spanish mackerel fisheries is appropriate at state or regional scales. The strong difference between the Kupang samples and the Australian samples, by all analyses, indicates that international management across the Timor Sea boundaries is probably not necessary.

The second is that the metapopulation structure might be vulnerable to serial depletion: as they are highly aggregative, it may be possible to severely deplete Spanish mackerel numbers on local scales; this could occur repeatedly, over progressively larger spatial scales. Catch rates (catch per unit of effort) would tend to remain high (hyperstable – Hilborn and Walters 1987), reflecting fishers' concentration upon aggregations (high densities at fine scales), even though population abundance was severely declining.

This project presented evidence that northern Australia's Spanish mackerel have been overfished. The metapopulation structure may have been a contributing factor, making the Spanish mackerel stock susceptible to targeted fishing. Comprehensive comparison of allozyme analyses to Shaklee's results from the early 1980s (Appendix 4) suggested a shift away from genetic equilibrium (Hardy-Weinberg) conditions in the intervening 15-20 years, implying transitory and recent population declines. If harvesting has led to significantly fewer spawners since the 1980s, and fish have to travel larger distances to form spawning aggregations, samples taken from current spawning aggregations may represent a greater range of populations. This may be further evidence that the northern Australia stock of Spanish mackerel was overfished by the Taiwanese-Australia joint venture fishery of the 1970s and 1980s (Stevens and Davenport 1991) from catch rates and size composition, and Buckworth 2004 (from catch per unit effort data and age composition) both indicated that the joint venture fishery led to depletion of the *S. commerson* stocks. Overfishing has also been

observed in the Middle East fisheries for the species (Dudley et al. 1992; Siddeek and Al-Hosni 1998). It was evident from the assessment of stocks in the Middle East that overfishing had been a consequence of intense fishing on fish at sizes below maturity. The extent to which spatial dynamic effects were also responsible was not examined in studies on these stocks.

Fisheries monitoring needs to be tailored to detect signals of serial over fishing (for example, logbooks must be fine spatial and temporal scale; age and length sampling need to accommodate spatial variation, mark-recapture analyses must be adjusted for the distribution of fishing effort). Management approaches should be sought that are robust to metapopulation structures. In this context,a metapopulation structure might also confer some management advantages. For example, spatially rotating management regimes or regimes which rely on replenishment of stocks by straying from protected areas might become part of the set of management options. An advantage is that heavy fishing at one location in the stock may have little impact on abundance at other localities.

Spatial management approaches depend upon the density dependent mechanisms of connection between demes (eg straying of mature adults or recruitment of juveniles) and rates by which depleted or extirpated demes are re-populated are not known. It is possible that there is useful information on the depletion and subsequent recovery of population units contained in logbook systems or fishers' individual records and observations. In the absence of information accumulated during such past experience, experimental management, entailing the deliberate depletion in some localities, would be the only way to establish replenishment periods. This may not, of course, be desirable.

We found the approach of using a suite of hypotheses to be a useful tool for developing this project. Although a metapopulation structure was not considered likely at the inception of the project, consideration of the need to falsify metapopulation-type hypotheses meant that an appropriate experimental approach was developed. Use of a concept of intrinsic time scales made explicit the importance of matching time and spatial scales of the information produced by the project to the scales of the management questions being addressed. The management questions addressed, of course dictate which methods should be used. Each method produced sets of results that revealed different aspects of the stock structure and biology of Spanish mackerel. Genetic methods thus revealed larger-scale population histories, otolith isotope ratios and parasite methods emphasised lifespan-scale dynamics. Where their scales overlapped, the methods in combination generally provided not only corroboration but also provided greater resolution. The general fine spatial scale revealed by the isotope ratio method was generally corroborated by parasite abundances; each method occasionally provided greater illumination than the other. The parasite methods and a combination of genetic methods indicated subtle differences between the sex's spatial dynamics. A great strength of the project, then, was in emphasising the utility of using a combination of methods, each with their own complementary, intrinsic scales.

BENEFITS

- The major benefit of this study has been the increase in understanding of the stock structures and spatial dynamics of Spanish mackerel stocks in northern Australia. The results have already been adopted by management agencies of northern Australia who are instituting or planning management approaches that accommodate the metapopulation structure described here. Assessment and management can be based upon stock structure knowledge rather than on administrative boundaries. The work also has application in other countries, for Spanish mackerel fisheries, and for other species.
- The methods and results from this project have had direct application in subsequent studies e.g. FRDC 2001/19, "Stock discrimination of east coast Spanish mackerel"; FRDC 2005/010 "Determination of management units for grey mackerel fisheries in Queensland and the Northern Territory" and monitoring methods (FRDC 2002/011, "Genetag: Real time harvest rate monitoring using genetic tagging: pilot studies in northern Australia Spanish mackerel fisheries") as broader application in ACIAR projects (for red snappers, and sharks and rays) and other FRDC projects (for sharks).
- The work provides new direction to stock structure investigation, emphasising that it is really about dynamics at several time and spatial scales (much more than drawing boundaries on maps.) We showed that an apparently widely distributed, migratory species may in fact have a much more complex spatial structure. The study demonstrated that a combination of methods is effective, and that these methods should be chosen by matching the intrinsic temporal and spatial scales of methods to the questions to be addressed.
- The project prompted significant skilling, with three BSc (honours) theses produced (Moss 1999, Thompson 1999 and Moore 2002), and the approaches developed stimulating a further BSc (honours) study of the east coast Spanish mackerel stock (Williams 2002).
- The project brought into the public domain information that was otherwise difficult to obtain. Both McPherson's (Chapter 2) and Shaklee's (Appendix 4) studies were funded by predecessors of FRDC.

FURTHER DEVELOPMENT

It is rare that more than one method has been employed to examine stock structure – yet it is clear that a single method certainly cannot be informative about all possible spatial dynamic attributes, "stock structure", that might have fishery management implications or be of scientific interest. This study used a set of three main methods to indicate the approximate spatial scale of Spanish mackerel populations. Each of these methods has subsequently been applied to other fisheries for Spanish mackerel and in fisheries for other species.

These applications have included parasitological examination of mackerel on the east coast FRDC 2001/19 ('Stock discrimination of east coast Spanish mackerel'). A second student, Stephanie Rawlinson, used parasites to investigate movement of mullet *Mugil cephalus* along the east coast, while Mark Robertson, honours student with Steve Barker (UQ) and Jenny Ovenden (QDPI) examined genetic markers for mackerel species in Australasia.

This project used combinations of different methods to ensure that the intrinsic scales of information provided by the project were similar to those required by management questions. This approach of using the suite of methods was applied in development of a project on the stock structure of southern Australian blue mackerel (*Scomber australicus*) and FRDC 2005/001 "Determination of management units for grey mackerel fisheries in Queensland and the Northern Territory".

Our project did not include mark-recapture work, or sonic telemetry, which might indicate seasonal or shorter term movements of the species. Combined with adaptive management experiments, these could also be used to measure straying, replenishment and recovery rates for demes that have been depleted. Information may already exist in current logbook systems, or in fishers' records, about depletion and recovery of Spanish mackerel populations at different sites. It may be at this scale that some of the responses to fishing of Spanish mackerel stocks can be measured.

Archival tagging and genetic mark-recapture might be used to examine fidelity to spawning grounds, and studies of larval behaviour and hydrodynamic modelling might be employed to examine the connectivity, describing links between spawning and ultimate recruitment. A range of developments might therefore be adopted which would be pertinent to the management of fisheries for Spanish mackerel, and in demonstrating the spatial complexity.

The above approaches would all add to our set of knowledge about Spanish mackerel, and about spatial dynamics of fish populations is general. It is unlikely, however, that many administrations could support the definition and mapping of the functional groups we have described for Spanish mackerel, let alone develop a suite of suitable management arrangements to ensure that each such group was managed optimally. The alternative is to develop management approaches that are resilient to the kinds of spatial complexity that we have described. As a result of this project, Buckworth (2004) initiated the process, simulating monitoring and management over a wide range of spatial structures. He concluded that

mark-recapture approaches for tracking harvest rates and catchability provided effective monitoring for most spatial structures considered. This work was necessarily general and did not address many of the questions that a manager might apply to a specific fishery, that there is need for a substantial amount of simulation work to address the questions pertinent to each of these fisheries. Nevertheless, Buckworth (2004) did indicate that mark-recapture would be a good approach for monitoring a spatially complex Spanish mackerel fishery. This led in turn to the development of the genetag approach and Project 2002/011 *Genetag: Genetic mark-recapture for real-time harvest rate monitoring. Pilot Studies in northern Australia Spanish mackerel fisheries*, which seeks to develop a genetic tagging approach in line with Buckworth (2004). But there would be substantial merit in further management strategy evaluation (MSE) under a wide variety of spatial stock structure assumptions, even given a constraint of a meta-population structure. We suggest that genetic tagging and MSE are the basic future directions for research for northern Australia's Spanish mackerel fisheries.

PLANNED OUTCOMES

- The project defined the scale at which Spanish mackerel stocks should be managed, i.e. it was demonstrated that international management of the N/W stock was not necessary, and that management is probably appropriate at state or regional levels.
- Recognition by management agencies that Spanish mackerel populations are fine scale complex stocks, and this entails fine scale data requirements (e.g. logbooks) and robust management approaches.
- The project provided bench-marked methodology for other stock structure studies.

CONCLUSION

Objective 1. **Establish the degree of stock structure in the northern Australian *Scomberomorus commerson* stock, over a wide geographic range.**

This objective was achieved. Spanish mackerel samples taken at widely-separated primary sites across northern Australia were initially subjected to otolith isotope ratio analyses. Marked differences in otolith isotope ratios were observed, indicating that Spanish mackerel stocks were unlikely to mix at the scale of sampling.

Objective 2. **Having demonstrated structural differences within the northern stock on the large scale, describe finer scale spatial structure.**

This objective was achieved. Samples from a large number of secondary sites, placed between primary sites to afford finer spatial scale, as well as samples from primary sites, were examined with otolith isotope ratio analyses. Subsets were examined with parasite analyses and genetic analyses. The otolith isotope and parasite analyses established that *S commerson* have a complex spatial structure in which adult assemblages are largely distinct, with little movement between these groups on scales of 100-300 km. There were between-sex differences in this mixing, with females being more sedentary than males (shown with both parasite and genetic methods). Spanish mackerel form a fine-scale mosaic of population units through Australia's north; this may best be considered as a metapopulation structure. At the same time there is genetic flow between these units on the large scale, so that just three distinct genetic stocks were defined in Australian waters: an east coast stock, a Torres Strait stock, and a single stock across the north and west coasts of the continent. This work showed that a highly migratory species may nevertheless be structured as a metapopulation.

***Objective 3*. Provide advice to the fishery administrations on the appropriate geographic scale of assessment and management actions.**

This objective was achieved. Given the fine scale of the metapopulation structure, the appropriate scale of assessment and management actions is at state or regional levels. Management and assessment must be robust to the complex, fine scale structures.

***Objective 4*. To include the analysis of otolith and genetic material collected from Kupang (Indonesia).**

This objective was achieved. Otolith and genetic material, as well as parasite material were collected from Kupang (Indonesia). Analysis of this material indicated that the northern Australian and west Timor samples were distinct, so that close international management is not necessary across the Timor Sea.

***Objective 5*. To collect and analyse parasite samples from Spanish mackerel.**

This objective was achieved. Parasite material was collected, suitable parasites were identified, and frequencies analysed from a subset of the sample sites. Parasite analyses were important in demonstrating the fine scale of Spanish mackerel stock structure, corroborating and adding to the interpretation of otolith isotope ratio analyses - they have a similar intrinsic time scale, but different parasite types provide information on time scales from seasonal to the life span of the fish. Parasite analyses first indicated the difference in dispersal between male and female Spanish mackerel.

Objectives 4 and 5 were in addition to the project's original objectives, and were included as a variation decided in October 1998.

REFERENCES

Baverstock, P.R. and Mortiz C. (1996). Project Design. **In:** *Molecular Systematics*, D. M. Hillis, C. Moritz , and B. K. Mable (eds). Sinauer, Sunderland.

Begg, G.A., Friedland, K.D., and Pearce, J. B. (1999). Stock identication and its role in stock assessment and fisheries management: an overview. *Fisheries Research* **43**:1-8.

Begg, G.A. and Waldman, J.R. (1999). An holistic approach to fish stock identification. *Fisheries Research* **43**:35-44.

Buckworth, R.C. (1998). World fisheries are in crisis? We must respond! Chapter 1. **In**: *Reinventing Fisheries Management*, P.J.B. Hart, T. Pitcher, and D. Pauly (eds). Pp. 4-17. Kluwer Academic Publications, London.

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

Collette, B.B. and Nauen, C.E (1983). Fao Species Catalogue. Vol.2. Scombrids of the world. An annotated and illustrated catalogue of tunas, mackerels, bonitos and related species known to date. *FAO Fisheries Synopsis* **125**(2):137.

Donohue, K., Edsall, P., Robins, J., and Tregonning, R. (1982). Exploratory fishing for Spanish mackerel in waters off Western Australia during the period June 16 to October 16, 1981. *Dept of Fisheries and Wildlife. Western Australia. Report* **57**. Perth, WA, Australia. 45 p.

Dudley, R.G., Aghanashinakar, A.P., and Brothers, E.B. (1992). Management of the Indo-Pacific Spanish mackerel (*Scomberomorus commerson*) in Oman. *Fisheries Research* **15**:17-43.

Govender, A. (1994). Growth of king mackerel (*Scomberomorus commerson*) off the coast of Natal, South Africa - from length and age data. *Fisheries Research* **20**:2063-79.

Hay, T., Mikolajczyk, A., Julius, C. and Poole, S. (1996). Edibility characteristics of fish species from Northern Territory waters. Northern Territory Department of Primary Industry and Fisheries, Darwin, Australia. *Fishnote* **19**. 6 p.

Hilborn, R. and Walters, C.J. (1987). A general model for simulation of stock and fleet dynamics in spatially heterogeneous fisheries. *Canadian Journal of Fisheries & Aquatic Sciences* **44**:1366-1369.

Hilborn, R. and Walters, C.J. (1992). *Quantitative fisheries stock assessment. Choice, dynamics and uncertainty.* Chapman and Hall, New York.

Hoyle, S.D. (2003). *2003 update: Management strategy evaluation for the Queensland East Coast Spanish Mackerel Fishery.* Agency for Food and Fibre Sciences, Fisheries and Aquaculture, Southern Fisheries Centre, Deception Bay. Dept. of Primary Industry, Qld, Australia.

Kailola, P., Williams, M., Stewart, P., Reichelt, R., McNee, A., and Grieve, C. (1993). *Australian fisheries resources.* Bureau of Resource Sciences, Department of Primary Industries and Energy. Canberra, Australia.

Kishinouye, K. (1923). Contributions to the comparative study of the so-called scombroid fishes. *Journal of the College of Agriculture, Imperial University, Tokyo* **8**(3):293-475.

Lester, R. J.G., Barnes A., and Habib, G. (1985). Parasites of skipjack tuna, *Katsuwonus pelamis*; fishery implications. *Fishery Bulletin* **83**:343-356.

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

Lewis, A.D. Population studies of northern Australian pelagic species utilizing the electophoretic approach. **In**: Northern Pelagic Fish Seminar, C. J. Grant, and D. G. Walter (eds). Pp. 35-44.

1981. Canberra, Australia, Department of Primary Industry, Fisheries Division, Australian Government Publishing Service, Canberra, Australia.

Lyle, J.M., Henry, G.W., West, L.D., Campbell, D., Reid, D.D., and Murphy, J. J. (2003). National recreational fishing survey. **In**: *The National Recreational and Indigenous Fishing Survey*, G. W. Henry and J. M. Lyle (eds). Pp. 27-97. Fisheries Research & Development Corporation Project 99/158. NSW Fisheries, Sydney, Australia.

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

McIlwain, J.L., Claereboudt, M., Al-Oufi, H., Zaki, S., and Goddard, J. (2005). Spatial variation in age and growth of the kingfish (*Scomberomorus commerson*) in the coastal waters of the Sultanate of Oman. *Fisheries Research* **73**:283-298.

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

McPherson, G.R. (1981b). Preliminary Report: Investigations of Spanish mackerel *Scomberomorus commerson*in Queensland waters. **In**: *Northern Pelagic Fish Seminar*, C. J. Grant, and D. G. Walter (eds). Pp. 51-58. Fisheries Division, Department of Primary Industry, Canbera Australia. Australian Government Publishing Service, Canberra, Australia.

McPherson, G.R. (1985). Development of the northern Queensland mackerel fishery. *Australian Fisheries* **11**:15-17.

McPherson, G.R. (1987). Identification of stocks and migration routes of Torres Strait and Gulf of Carpentaria narrow-barred Spanish mackerel, *Scomberomorus commerson*. Final Report. Fishing Industry Research Trust Account, Project 83/12. 31 p.

McPherson, G.R. (1987). Identification of stocks and migration routes of Torres Strait and Gulf of Carpentaria narrow-barred Spanish mackerel, *Scomberomorus commerson.* Final Report. Fishing Industry Research Trust Account, Project 83/12. 31 p.

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

McPherson, G.R. (1993). Reproductive biology of the narrow-barred Spanish Mackerel (*Scomberomorus commerson* Lacepède, 1800) in Qld waters. *Asian Fisheries Science* **6**:169-182.

McQuinn, I.H. (1997). Metapopulations and the Atlantic herring. *Reviews in Fish Biology and Fisheries* **7**:297-329.

Moore, B.R. (2002). Parasites of narrow-barred Spanish mackerel (*Scomberomorus commerson)*: Distribution and significance in fisheries. BSc(Hons.) Thesis, Department of Parasitology, University of Queensland, Brisbane, Queensland, Australia.

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

Moss, H. (1999). Parasites on the stomach wall of *Scomberomorus commerson* and their use as indicators of Australian stocks. BSc(Hons.) Thesis, Department of Parasitology, University of Queensland. Brisbane, Queensland, Australia.

Munro, I.S.R. (1943). Revision of Australian species of *Scomberomorus*. *Memoirs of the Queensland Museum* **12**(2):65-95.

Secor, D.H. (1999). Specifying divergent migration in the concept of stock: the contingent hypothesis. *Fisheries Research* 43**:**13-34.

Shaklee, J.B., Phelps, S.R. and Salini, J. (1990). Analysis of fish stock structure and mixed-stock fisheries by the electrophoresis characterization of allelic isozymes. Chapter 8 **In**:

Electrophoresis and isoelectric focusing techniques in fisheries management, D.H. Whitmore (ed.). CRC Press, Inc. Florida, USA. pp. 173-196.

Siddeek, M.S.M. and Al-Hosni, A.H.S. (1998). Biological reference points for managing kingfish, *Scomberomorus commerson,* in Oman waters. *Naga* October- December, 33-37.

Slattery, S.L. (1998). Shelf-life of Spanish mackerel (*Scomberomorus commerson*) from northern Australian waters. *Journal of Aquatic Food Production Technology* **7**: 63-79.

Stephenson, R.L. (1999). Stock complexity in fisheries management: a perspective of emerging issues related to population sub-units. *Fisheries Research* 43, 247-249.

Stevens, J.D. and Davenport, S.R. (1991). Analysis of catch data from the Taiwanese Gill-net fishery off Northern Australia, 1979-1986. *CSIRO Marine Laboratories Report* 213.

Thompson, C. K. (1999). Gill parasites of Spanish mackerel *Scomberomorus commerson* for stock discrimination and host movements and virtual three-dimensional reconstruction of *Pricea multae* (Monogenea: Polyopisthocotylea). BSc(Hons.) Thesis, Department of Parasitology, University of Queensland.

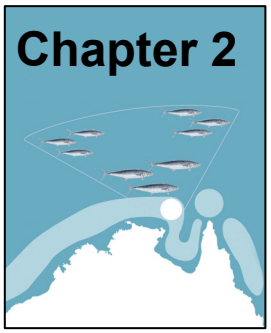
Tobin, A. and Mapleston, A. (2004). 'Exploitation dynamics and biological characteristics of the Queensland east coast Spanish mackerel (*Scomberomorus commerson*) fishery.' (CRC Reef Research Centre : Townsville, Australia.)

Walters, C. and Buckworth, R. (1997). Shark and Spanish Mackerel stocks assessed. *Northern Territory Fishing Industry Council Newsletter, July 1997.* 8, 14-15.

Welch, D. J., Hoyle, S.D., and McPherson, G.R. and Gribble N.A. (2002a). *Preliminary Assessment of the Queensland East Coast Spanish Mackerel Fishery.* Agency for Food and Fibre Sciences, Southern Fisheries Centre, Dept. of Primary Industry, Brisbane, Queensland, Australia.

Williams,R.E. (2002). Stock-structure of narrow-barred Spanish mackerel (*Scomberomorus commerson*) on the east coast of Australia as indicated by trypanorhynch parasites. B.Sc. Honours thesis, University of Queensland, Brisbane, Queensland, Australia. 52p.

Williams, R.E. and Lester, R.G.J. (2006). Stock structure of Spanish mackerel *Scomberomorus commerson* along the Australian east coast deduced from parasite data. *Journal of Fish Biology*. **68:**1707-1712.



HISTORICAL STOCK DEFINITION RESEARCH ON *SCOMBEROMORUS COMMERSON* IN QUEENSLAND WATERS

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HISTORICAL DEVELOPMENT

Historical industry understanding of narrow-barred Spanish mackerel life

In the 1920s and 1930s the main east coast fishing grounds were coastal headlands and near shore islands within a few hours steaming of major Queensland ports. Fishing operations were usually restricted within a day's steaming radius for the small (<10 m) petrol-powered, ice-based fishing vessels (McPherson 1985).

By the late 1930s an increase in localised fishing effort resulted in an apparent decline in fish availability on the near shore grounds that forced many vessels to move out to offshore islands (McPherson 1985, 1989). A fishery developed around a group of inner Great Barrier Reef reefs north of Townsville and just east of Lucinda, during what became known as the acknowledged major spawning season for the species in October and November each year.

During the Second World War many of the fishing vessels based in north Queensland were utilised for service in the Australian and American Small Ships services. Despite a number of the navigable reef entrances being mined (at least off Townsville and Cairns with some tragic results) a small amount of Spanish mackerel fishing effort was conducted for local, including Australian and United States, military consumption.

During the War years Mr Ian Munro of the Australian Army Medical Service developed a strong association with the members of the fishing community, including those fishermen working with the Australian and US Small Ships Services. He took every opportunity to go to sea on these vessels (Ian Munro *pers. com.*). It was during the 1941 October-November spawning season off Lucinda that he conducted his work on Spanish mackerel spawning and egg and larval development (Munro 1942). Munro had investigated an apparent decline of fish abundance on the offshore reefs reported by the fishermen in the 1930s although he was unable to establish overfishing as the main cause.

An artificial propagation procedure comparable to a trout hatchery was adopted within the industry as a method to supplement the natural Spanish mackerel stocks and thus hedge against possible overfishing. Earl (1883) had proposed this technique for US populations of *Scomberomorus maculatus* when they had demonstrated a decline.

Fish with ovaries in spawning condition, *i.e*. running ripe, were not regularly encountered by the fishermen cleaning their morning catch. Nevertheless, they were common enough (perhaps one every second night from catches of up to six dories) for mothership vessels to mount a 44-gallon drum on the fishing deck during the spawning season to permit ready fertilisation of eggs from occasional running ripe ovaries. After a brief mixing of oocytes and sperm in a small container, the fertilised eggs would be maintained in shade for 24 hours with running water (B.J. 'Snowy' and Neil Whitaker, *pers. com*). Following the observations of Munro that larvae would hatch at 24 hours and take a further 12 hours during the relative safety of darkness to resorb the egg mass and assume an upright orientation, the drum with developing larvae would be emptied at dusk to optimise larval survival and to prepare for another artificial fertilisation attempt.

Artificial fertilisation of eggs by fishery operators became common (Ian Munro*, pers. com.*). The last known time artificial fertilisation of Spanish mackerel to be conducted on a large scale was thought to be the early 1960s. However, small scale artificial fertilisation procedures occurred during the 1970s and 1980s (author*, pers. obs*.).

Munro was the first to enunciate the views of industry that Spanish mackerel populations demonstrated inner 'steamer track' (now referred to as the Barrier Reef lagoon) spawning locations throughout northern and central Queensland during October-November, with the major reef waters spawning locations off Lucinda. Smaller concentrations occurred between Gladstone and Cairns (Munro 1942). Immediately after the war several fishermen would follow fish concentrations up the coast landing catches at progressively more northern ports with the onset of winter, beginning at Bundaberg in April and finishing off Lucinda in November ('Snowy' Whittaker, *pers. com*.).

When a Spanish mackerel fishery developed in the Torres Strait following the discoveries of an Army Fishing Unit stationed in the Torres Strait during the War (McPherson 1985), the observations about the temporal aspects of spawning behaviour were passed on to Mr Munro who had joined CSIRO Fisheries after the War. Mr Munro kept up an interest in the biology of the species to the mid-1970s when the Fisheries Branch, Queensland Department of Primary Industries, conducted the Fisheries Torres Strait Survey in 1974 and analysed results in subsequent years. Mr Munro volunteered considerable insight into the development of an understanding of the movement pattern of the species.

During the 1970s, industry representatives with experience of one to two decades in the fishery recognised an apparent movement of fish northwards from southern Queensland during the winter months. This movement culminated in peak concentrations of fish east and north of Lucinda by October-November. Fish concentrations during the apparent northward movement were considered to be near shore coastal, although the small size and short operational duration of the vessels (due to ice-based refrigeration of catch) prevented more detailed indication of fish abundances in offshore reef waters (McPherson 1977). A summary of industry assessment of the life history aspects of Spanish mackerel, primarily reproductive and movement biology in Queensland by the 1970s, was provided by McPherson (1981).

Fishery landing data for the Queensland Fish Board (QFB) averaged over a 10 year period (1971-1980) indicated a seasonal change of abundance of Spanish mackerel throughout the year. Using landing data as an index of abundance close to QFB receiving depots in major coastal towns, changes in landings suggested a northward movement of fish abundance from southern to northern Queensland during the winter months (Figure 2.1).

Brisbane (approximately 28°S) monthly percentage landings were highest during the mid-summer to autumn period. With the further onset of autumn and winter landings at Bundaberg (mean latitude of fishing area east of Fraser Island and reef islands approximately 25°S) increased rapidly in May-July, after the decline in Brisbane landings. Landings from the Gladstone / Rockhampton (approximate area centred at 24°S) and Mackay / Bowen (approximate area centred at 21°S) regions rapidly increased shortly afterward during June-July, then decreased sharply during August-September. As relative abundance declined from central Queensland regions during August-September, a rapid increase was apparent for Townsville / Innisfail (approximate area centred at 18.5°) and Cairns regions from September (approximate area centred at 17°S).

When monthly proportional landings were declining in all northern waters, the percentage landings increased off Brisbane from November and particularly December.

A flourishing 'black market' fish trade existed to non-QFB organisations during this time. Some industry operators would sell a proportion of their landings to 'black market' buyers to avoid a 10% QFB surcharge on Spanish mackerel, while non-licensed seasonal sugar and meat industry workers would fish without full disclosure of landings during the October-November spawning season. Landing locations would also vary a little, as smaller landings outside designated QFB zones, would be attributed to landings made to the Brisbane (Colmslie) QFB.

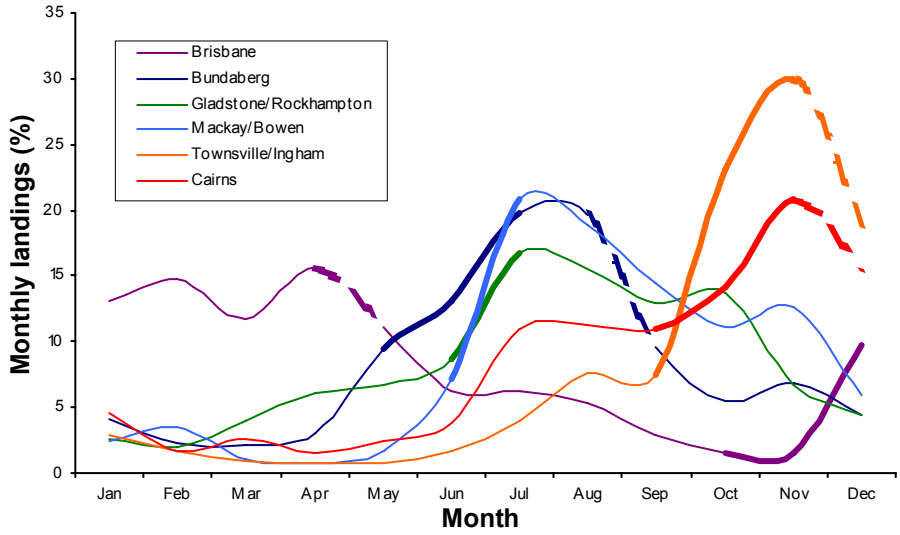


Figure 2.1. Monthly percentage landings (10 year average 1971-1980) from Queensland Fish Board regions
Periods of peak catches are highlighted – increasing as solid lines, decreasing as broken lines.

Sea surface temperature data, based on the point of intersection with the coast of the 24°C isotherm in 1980, are given in Figure 2.2. These data are indicative as no ground truth calibration data were available at the time. The peak landings at QFB depots coincided with the change in location of the 24°C isotherm through the general fishing regions. Spanish mackerel abundance generally appeared to be northward of the arbitrary sea surface isotherm.

Landings increased off Brisbane in December with the onset of summer and the southward movement of the arbitrary 24°C isotherm. Spanish mackerel availability occurred to at least Coffs Harbour (32ºS), and in some years just north of Newcastle, to where the arbitrary isotherm may extend in some years. Fish availability off Coffs Harbour usually decreased by April. During the 1960s and 1970s the last significant catches were made during a recreational fishing competition conducted over the ANZAC Day Weekend. Landings off Brisbane (namely Gold, Stradbroke / Moreton and Sunshine Coasts) declined rapidly during May.

Apparent fish abundance increased off Bundaberg during May-July when the 24°C isotherm was to the south of the region as did the landings in the central Queensland coast off Gladstone / Rockhampton and Mackay / Bowen. Landings at all three regions decreased when the 24°C moved through the areas and fish abundance increased to the north around Townsville / Innisfail and Cairns (Figure 2.2).

The annual change in relative abundance of Spanish mackerel along the Queensland coast over the 10 year period 1971-80 was interpreted as a winter induced movement of fish concentrations from higher latitude summer distributions to lower latitudes where spawning activity occurred during the spring months of October-November. A comparable movement

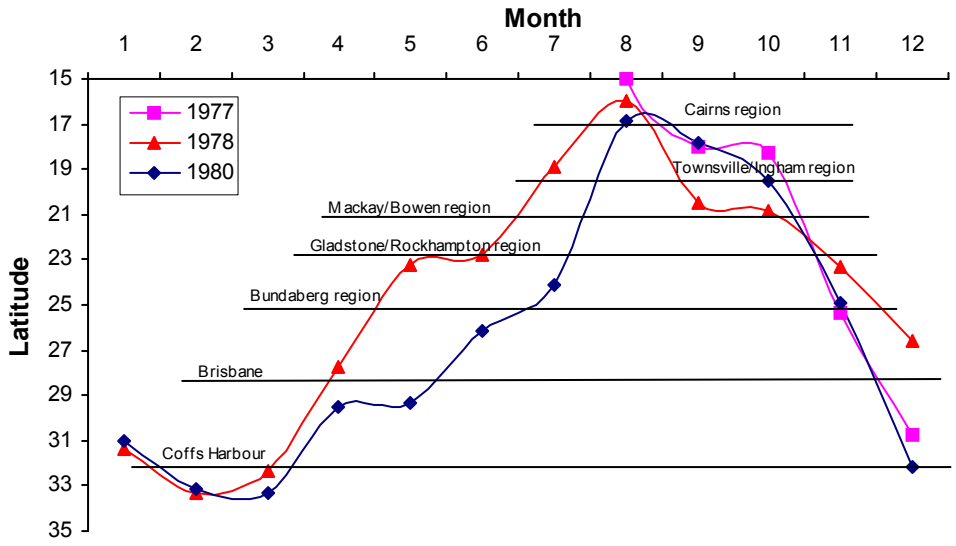


Figure 2.2. Position of the 24°C isotherm at the east Australian coastline recorded by NOAA GOSCOMP system for 1977, 1978 and 1980

pattern toward lower latitudes was reported for king Spanish mackerel along the Atlantic coastline between North Carolina and Florida (Williams and Finucane 1979). Other Australian east coast pelagic species such as the coastal scombrid species *Thunnus tonggol* (Marc Wilson, MSc manuscript) and the shark *Carcharhinus limbatus* (John Stevens, CSIRO, *pers. com*.) were also thought to demonstrate comparable winter-summer movements.

A Spanish mackerel fishery did not exist in Queensland Gulf waters until the development of fisheries infrastructure in association with the Gulf prawning industry in the early 1970s. This restricted the development of an understanding of life history aspects of the species in Queensland's Gulf waters until the 1970s. Spanish mackerel abundance at that time was associated with islands and headlands adjacent to safe vessel anchorages. Fishery operators envisaged a seasonal pattern of large fish concentrating around the northern rocky outcrops around Mornington Island in the southern Gulf during winter months, with observations of concentrations off headlands around Weipa later in the year.

Crews on coastal trading vessels made many anecdotal observations of large offshore schools of fish in the north-east Gulf up to the shallow shoals immediately west of Bamaga on the tip of Cape York. However these observations were made by vessels on tight steaming schedules with no capacity to remain in an area to maintain fishing effort on a school of fish. Fishing vessels at this time were not equipped to remain at sea in open waters and exploit these offshore schools during this period, nor to develop effective fishing practices for these offshore waters.

There was no clear understanding of what occurred to fish abundances after the spawning season in both east coast and Torres Strait waters. Fish abundances would reappear in southern east coast waters each year. However industry operators could not identify a specific southward movement of fish that was worth following to support fishing operations and many operators hypothesised an oceanic movement of fish on the basis that the species occurs in adjacent reef waters in New Caledonia (McPherson 1977). It was not till the Cairns game fishery developed in oceanic waters off north east Queensland after 1965, and the results of Japanese longline catches in the Coral Sea became known to fisheries agencies throughout the Pacific, that any offshore movement was discounted. In addition, Munro (1943) had described the species as being a neritic pelagic with a continental shelf distribution making such a movement pattern unlikely.

Department of Primary Industries Spanish mackerel life history research

The Queensland Department of Primary Industries (DPI) commenced Spanish mackerel research in Torres Strait during the 1974 Fisheries Branch Torres Strait Survey. The work was a component of a large fisheries resource survey of Torres Strait for the Queensland Government and was restricted to documenting current and historical catch and effort data with minimal collection of basic biological data.

The Torres Strait work included an expansion of Spanish mackerel work to the east coast between Cairns and Townsville where some catch and effort data were collected until the late 1970s. Industry had indicated a relative decline in availability of Spanish mackerel on the fishing grounds, particularly on the east coast. While some catch and effort data were available, trends could not be detected.

Major changes had begun to occur in the nature of fishing operations. From the late 1970s, echo sounders were increasingly being used on mother ships as well as dories. Travelling stabilisers were developed for mother ships, which permitted fishing in much higher winds and sea states than in previous decades, while freezers continued to improve, permitting vessels to remain at sea for longer periods.

The industry perception of reduced fish availability resulted in DPI sampling biological aspects of catches from the east coast (Lucinda to Lizard Island) and Torres Strait in 1976-1979. These samples resulted in an initial description of the growth rate of Spanish mackerel (McPherson 1981). This was not confirmed until critical aspects of early growth were determined by daily otolith growth assessment by Dr. Ed Brothers of Cornell University with the assistance of Dr. Antony Lewis (PNG Fisheries). Tag return data supported age validations using otolith marginal increments (McPherson 1992) and the growth rate of the species was described by a von Bertalanffy growth equation. Subsequent reanalysis of the same data demonstrates that alternate growth equations are more appropriate to describe the growth of fish older than 15 years and lengths in excess of 160 cm (fork length) (McPherson, *unpublished data*). These older fish had been recorded previously by industry and by DPI sampling.

The reproductive cycle of the species was tentatively described by macroscopic staging and a series of arbitrary ovary development stages were categorised. The stages were not fully described, however, until the descriptions of post-ovulatory follicle degeneration by Hunter and Macewicz (1985), and a later study of yellowfin tuna reproductive biology (McPherson 1991), permitted the analysis of preserved ovary specimens of Spanish mackerel samples. The reproductive development of Spanish mackerel closely mirrored that of yellowfin tuna, and was described by McPherson (1993).

Timing of spawning was usually associated with new moon periods of October and November off the east coast from Townsville to Cairns (Munro 1942) and often a month earlier in Torres Strait from industry observation (McPherson 1993). With spawning likely to occur over such broad regions of coastline the concept of restricted or isolated populations of Spanish mackerel based on restricted spawning regions seemed unlikely.

The association between spawning activity and moon phase was tenuous, as the triggering of spawning activity appeared to be linked with rising water temperature associated with calm weather conditions. The timing of fishing operations with moon phase was as much an arbitrary long term planner for fishing operations as it was a predictor of spawning activity as spawning occurred over a range of moon phases. Munro (1942) indicated that Spanish

mackerel spawned when water temperature exceeded a specific temperature, irrespective of moon state. The same was observed for yellowfin and bigeye tuna in the Coral Sea (Hisada 1973).

Unpublished data indicate that the onset of spawning over short time frames of one to several days off Lucinda and Cairns occurred on the same day that sea surface temperatures (SST) exceeded a specific (though un-calibrated) value. Figure 2.3 shows SST data demonstrating the localised increase in water temperature in the Lucinda (and Cairns to the north) area that coincided with an initiation of spawning. The spawning was confirmed by standardised industry at-sea observations. Throughout each spawning period for Queensland regions where Spanish mackerel would normally be present, the time at which SST reached specific temperatures provided some indication of the possible extent of spawning activity.

No *S. commerson* research was conducted off the east coast between the conclusion of the tagging work in the early 1980s and the commencement of the Queensland Fisheries Service (QFS) Long Term Monitoring Programme in the late 1990s. McPherson (1981) had indicated that the major challenges likely to face the east coast fishery were:

- The inshore life history of the species and the major environmental effects of El Nino etc. could well be responsible for variability in landings
- Increasing recreational effort would become an issue for management in the future
- Increasing use of rod-and-line gear (deployed by any sector) differentially targeting large (predominantly female) fish.

The relevance of these challenges was confirmed by the assessment of appropriate management strategies for the Spanish mackerel fishery in 2002 by Hoyle (2002). The assessment included an acknowledgment that recreational fishing (using rod-and-reel) had become a major source of fishing mortality for the species, and that available commercial fishing effort had been directed toward the fishery in responses to effort restrictions on other species.

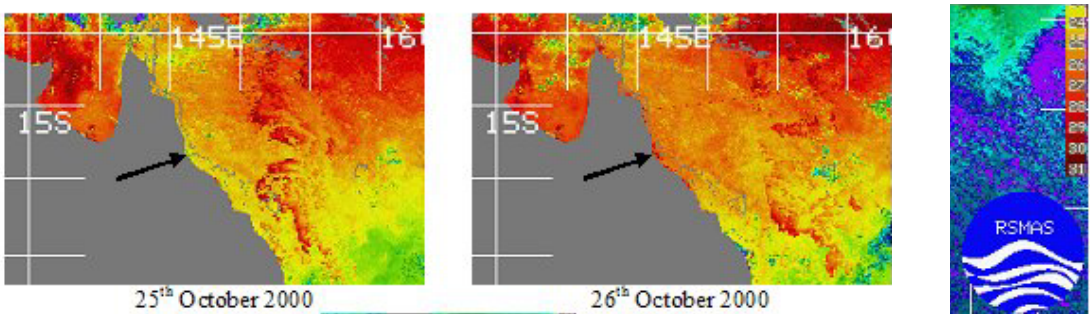


Figure 2.3. Low resolution RSMAS SST images for 25th and 26th October 2000 (temperature scale on right, °C)

Spawning activity commenced off Lucinda on 26th October (arrow).

FIRTA- and FRDC-funded stock definition research

From 1979 FIRTA (Fishing Industry Research Trust Account) and its successor, FRDC, funded four projects involved with aspects of stock definition of Spanish mackerel in Australian waters. The projects primarily involved the use of tagging and genetic analysis to examine the interaction between adjacent stocks.

The FIRTA/FRDC projects were:

- 1979–1980 QFS tagging in east coast and Torres Strait
- 1982–1983 QFS tagging in Torres Strait and Gulf
- 1983–1984 Northern Pelagic Program that opportunistically tagged in Gulf waters.
- 1982–1983 CSIRO genetic project.

Tagging

As an initial attempt to determine the stock integrity of *S. commerson* in northern waters between the east coast and Torres Strait, FIRTA funded a two season tagging study for primarily east coast waters in 1979-80 (Anon. 1978). The most appropriate tag colours and protocols for tagging had been developed during trials between Cairns and Torres Strait in 1976-1977. Only limited tagging was conducted in Torres Strait. By tuna tagging standards the project was modest with funds only sufficient for four staff (two QFS and two casual) over a seasonal basis.

A limited Spanish mackerel tagging program was conducted by the Papua New Guinea Department of Agriculture Stock and Fisheries in the northern Torres Strait in 1979 (Stewart Frusher, *pers. com.*). One tag recapture was returned by Queensland fishermen operating in Torres Strait. The fish had moved little from its tagging site in northern Torres Strait.

Following the success of the initial tagging study on the east coast and given the concerns about the impact of the northern Australian gillnet fishery conducted by Taiwanese vessels from Western Australia to western Torres Strait and into the Gulf of Papua, FRDC funded a second tagging study focussed on Gulf and Torres Strait waters for 1982-83. This project was again modest. All tagging was undertaken by a single QFS scientist conducting tagging from a single vessel, with additional tagging of small fish by industry. Tagging locations and recaptures are summarised in McPherson (1986).

The FRDC Spanish mackerel tagging project was supplemented by the FRDC-funded Northern Pelagic Program that conducted some opportunistic tagging work in the north-eastern Gulf during 1983. Locations of the releases and the single recapture are summarised in McPherson (1986). A summary of the numbers and lengths of releases and recaptures from all the FIRTA-funded studies is given in Table 2.1.

The study identified both long range seasonal movement and residence behaviour of individual fish on the east coast and in Torres Strait waters. The results were comparable to

the overall assessment of fish behaviour by industry and were consistent with results from the US king mackerel (*S. cavalla)* tagging programs of the 1970s (Williams and Finucane 1979).

Table 2.1. Results of *S. commerson* tagging projects by fisheries agency and fishing sector
QFS, Queensland Fisheries Service; **FIRTA**, Fishing Industry Research Trust Account;
FRDC, Fisheries Research and Development Corporation

Funding Agency	Locality and organisation	Approx. dates	Releases	Returns
QFS	Cairns	1976 – 78	96	7
	Cairns (fishermen)	1976 – 78	49	1
	Torres Strait (fishermen)	1978	19	0
FIRTA	Torres Strait (fishermen)	1979 – 81	81	0
	Lizard Is – Townsville	Aug 79 – Nov 80	1,602	64
	Lizard Is – Townsville (fishermen)	1979 – 81	104	2
	Bundaberg – Brisbane	Mar – Aug 1980	19	0
	Brisbane recreational fishermen	1980	2	2
FRDC	southern Gulf of Carpentaria	July – Aug 1983	204	0
	Torres Strait	Sep – Oct 1983	404	4
	Torres Strait (fishermen)	Jul – Oct 1982	141	1
FRDC	eastern Gulf of Carpentaria	Jul 1984	128	1
	western Gulf of Carpentaria	Jan – Feb 1984	26	0

Genetic stocks

The earliest genetic sampling was conducted by QFS in 1977 off the east coast and in the Torres Strait for the Inter-American Tropical Tuna Commission (IATTC) based in La Jolla, California. The analysis of the 288 samples was arranged by Dr Antony Lewis, Papua New Guinea Fisheries, as part of an identification of heterozygous enzymes in South Pacific scombrid fishes. No results were published from this study although Dr Gary D. Sharp from IATTC did confirm in 1996 (*pers. com*.) the presence of heterozygous enzymes in *S. commerson* blood samples.

Between 1977 and 1980 Dr Antony Lewis collected 570 blood samples from north east Queensland, Torres Strait, Gulf of Carpentaria, Monte Bello Island and northern Papua New Guinea with the assistance of QFS and PNG Fisheries. The northern Papua New Guinea and northern Australian populations were considered to be reproductively isolated. In northern Australian waters there was a sufficient level of interbreeding to distribute genetic material so that no stock definition was apparent from Torres Strait to Western Australia although the east coast stock was genetically isolated (Lewis 1981).

Genetic material was sampled between the east coast of Queensland and New Caledonia in 1983 for a later review of scombrid fishes in the Indo-Pacific region. The New Caledonia stocks were genetically distinct from the Australian east coast stocks (A.D. Lewis, *pers. com*.).

The 1982-83 FRDC tagging projects conducted by QFS also included additional sampling for genetic stock assessment work. Several hundred genetic samples were taken from the east coast, Torres Strait and Gulf of Carpentaria for Dr Jim Shaklee (CSIRO) from a total of 1,830 samples collected between northern Papua New Guinea to the east coast and to Northern Territory. The east coast stock was again confirmed as being distinct from the northern Australian stock. Northern Papua New Guinea stocks were distinct from southern Papua New Guinea although the latter stocks were not significantly different from Port Moresby, Torres Strait or Northern Territory fish.

Stock definition using parasite occurrences

In 1977 a whole *S. commerson* specimen displaying common skin marks or lesions, along with a photo of an external parasite thought to be causing the problem, was forwarded to Dr. Bruce Collette at the American Museum of Natural History. Dr. Collette was conducting a review of *Scomberomorus* and *Grammatorcynus* species.

The external parasite was identified as a parasitic copepod. The parasite would most commonly leave feeding sites that were small oval shaped rings up to 5 mm by 3 mm in size on the ventral / thoracic region of a fish (Figure 2.4). In cases of heavy infestation, the feeding sites would extend along the ventral surface and over the sides to approximately the midline.

An arbitrary category of parasite feeding sites was established for fish sampled from the east coast and Torres Strait in 1977 and 1978. The feeding sites were either not present, or were not noticed during low level sampling in 1976. Fish with ‘light’ infestation displayed less than 5 marks on either side of the fish. Fish with ‘medium’ and ‘heavy’ infestation displayed 5-15 and more than 15 marks, respectively.

No feeding sites were apparent on the fish sampled from Torres Strait during 1978, nor during the sampling in 1983. The fish with feeding sites were totally restricted to the east coast between Cairns and Townsville during 1977 and 1978. The feeding sites were evident on approximately 50% of fish sampled, especially from Townsville in 1977.

Figure 2.4. Evidence of heavy infestation of parasitic copepod feeding sites on an east coast *S. commerson*

The feeding sites were less evident from commercial samples during 1978 and not evident from east coast sampling during the 1979-1980 east coast tagging work conducted between Lizard Island and Townsville. The parasite appeared to be locally abundant on the major east coast spawning grounds at least during October and November 1977 and less so in 1978.

Comparable copepod feeding sites were known to occur on tuna-like species, while the occurrence had not been reported for *Scomberomorus* species (Bruce Collette *pers. com*.). The isolated, short term appearance of the parasite on east coast fish was taken as at least an indication that the east coast and Torres Strait fish were isolated. Evidence of the parasites disappeared so quickly they would not be as useful as a population marker though could give indications of movement over a shorter term (Dr Bob Lester*, pers. com*.).

FRDC-funded mackerel movement model of 1981-1988

Data sources and assumptions

The 1979-1980 FRDC tagging project report of 1981 is not readily available although it was summarised in McPherson (1981) and McPherson (1988). No tag recapture data were reported from this project following the recapture of a fish after 10 years at liberty in 1991. The result was used for otolith age validation (McPherson 1992).

The limited tag recapture data reported from the Torres Strait and Gulf several years after the tagging work were not sufficient, with a single exception, to modify the movement model developed for the 1979-80 FRDC report. The exception involved the recapture of a fish at Bramble Cay released off Weipa several weeks previously. The most that could be assumed from this movement was that it provided some confirmation of the industry belief that some

Gulf fish may move through Torres Strait to the spawning grounds in the north eastern Torres Strait.

The current east coast movement model was based on the 1981-1988 model, then reviewed in the light of discussions with operators within the fishery in 1998-99. Some of the statements are based on observations of tag recaptures, but others are based on hypotheses and conjectures derived from circumstantial evidence. Considerable insight was gained from conversations with fishermen and scientists engaged with scombrid fisheries research.

I would particularly acknowledge the assistance of Queensland mackerel fishermen 'Snowy' and Neil Whittaker, Vic Ugarte, Bob Dean and Jack Jarrett for sharing their long-time experiences with the Spanish mackerel fishery and to Lindsay and Norm Chapman, Peter Coombs, the Jarrett family (Jack, Lorna, John and Loraine), and Bruce and Brent Batch for years of tagging juvenile fish in Queensland waters.

Scientists Dr Antony Lewis, Dr Robert Kearney, Dr John Sibert and Dr Ray Hilborn (all for some time of the Tuna and Billfish Programme with the South Pacific Commission in New Caledonia), Dr Roy Williams (Florida Department of Natural Resources), Dr Chris Legault (National Marine Fisheries Service, Miami), and Dr. Rik Buckworth (Northern Territory Department of Primary Industy, Fisheries and Mines) provided considerable support and discussion for the development of the hypotheses based on their experience with *Scomberomorus* species and other scombroids.

The elements of the east coast movement model was based on several broad assumptions, namely,

- **Identification of major spawning reefs**. Groups of reefs are recognised by industry as being major spawning reefs. The recognition did not exclude spawning occurring at isolated reefs as far south as Bundaberg (Munro 1942, McPherson 1981). However, based on the observed and reported concentrations of fishermen conducting fishing operations on concentrations of fish periodically in spawning condition, the reefs were considered to be dominant spawning reefs. The topographic features of spawning reefs (generally on the inside reefs adjacent to the steamer track) were described by McPherson (1989).
    - The FRDC tagging study based its efforts at spawning reefs off Lucinda, and reef areas off Cairns and Lizard Island where spawning activity were observed. This was done partly to maximise releases, and partly to examine the pattern of movement subsequent to the spawning season.
    - Movements from spawning reefs were measured as movement from the mid-point of recognised fishing areas.
- **Time strata**
    - *Spawning period*. October and November are universally accepted to be the peak spawning periods on the east coast. Spawning activity has been reported

outside this period; however the reports are either isolated or fishing effort not documented in logbooks.

- *Post-spawning and Pre-spawning periods.* Each five month period was a simple distribution into two equal periods of the 10 month non-spawning period. It was coincidental that observed long range movement after the spawning period, and anecdotal observations of fish moving northward coincident with the onset of winter were in different five month periods.

- **Data used for movement**
  - *Distances travelled.* No fish were recorded moving any significant distance south from Torres Strait. Only fish released from Lizard Island (14º30'S) and areas to the south were used for the east coast model. Distances were assumed to be the most direct line of movement in multiple vectors to account for coastlines and reefs, usually within main open water areas. Short distance movements were grouped within 50 nautical mile regions; other movements were shown as vectors.
  - *Unidentified tags.* Ten percent of all tags reported could not be identified. For the majority of these tags, numbers had been removed by deep abrasion on the outside surface of the tag at the position of identification, or the majority of the tag was severed when the fish was small and the body had grown around the tag stub. A small proportion of this total featured discolouration of the external surface of the tag, apparently caused by body fluids, that prevented identification of the black writing on the yellow tag.
  - *Tag styles.* All tags were made by A.E. King of Sydney and coloured blue or yellow. Data for blue tags were not used, as preliminary trials with free-swimming blue trevally in Torres Strait in 1978 indicated that blue tags attached to fish were differentially attacked by other large predators.
- **Time and environmental frame.** The observations should be considered appropriate for the immediate tagging and post-tagging recapture years of 1976 - 1983 when >95% of returns were reported. Environmental effects, such as El Nino and La Nina patterns, that did not exist during the tagging and recapture periods may result in slightly different recapture patterns when these environmental patterns prevail.
- **Fishing effort.** No fishing effort data were available for any component or sector of the fishery.

The movement model was based on the possibility of both migratory and resident behavioural components to the east coast stock. The concept of resident and nomadic populations of scombroids was advanced by Sharp and Dotson (1977). They indicated that resident populations could remain in an area throughout the year with a seasonal influx of nomadic fish providing the bulk of the harvest. No specific identification parameter could be

attributed to either resident or nomadic populations. Tanaka (1979) felt there was evidence to suggest localised yet differentially moving groups of yellowtail kingfish (*Seriola quinqueradiata*) on the east coast of Japan.

Skipjack in northern Papua New Guinea waters and the South Pacific generally were shown to be comprised of resident and nomadic components (Lewis 1981, Kearney 1978), the presence of the latter being influenced by variations in the levels of primary productivity. Lewis (1981) also noted that local environmental characteristics favoured residents of particular size classes of skipjack.

The majority of tagging was conducted at reef locations off Lizard Island, Cairns and Ingham during the October-November spawning season when fish were concentrated and opportunities for maximising tag release were greatest. The remaining 10 months of the year were arbitrarily divided into a five month *Post-spawning season* (December-April) and a five month *Pre-spawning season* (May-September).

Hilborn and Sibert (1988) demonstrated that while some individual tagged skipjack moved long distances, these movements were the exception rather than the rule. In order to assess whether the apparently directed longer distance *S. commerson* movements were distractions to a more modest or diffuse pattern of movement (McPherson 1988), tagging data were examined using the technique of Hilborn and Sibert (1988) (John Sibert and Ray Hilborn, *pers. com*.).

General results

A frequency distribution of the distances travelled between tagging and recapture demonstrated that the vast majority were recaptured within 50 nautical miles (nm) of release (Figure 2.5). While this may provide some indication of the movements of some fish, other unexplained factors such as undefined fishing effort and short term tag loss present additional factors that complicate understanding tag returns in this species.

Short term tag loss may have occurred through conventional tag shedding processes. A rapid disproportionate loss of one coloured tag type was observed with tagged carangids adjacent to a fishing vessel in Torres Strait over a five day period (McPherson, *unpubl. data*). Tag loss also occurred through a form of ‘preening’ behaviour. Tag identification information was either rubbed off the tags where the tag identification faced outwards from the body, or the projecting tags were drastically shortened when the tags that projected substantially from the animal were severed by sharp toothed animals, presumably other *S. commerson*.

As the majority of tags were released in the spawning season when fish are in readily accessible concentrations, it was not surprising that the peak number of returns occurred during that same spawning season (Figure 2.6). There appeared to be a secondary peak in tag returns in northern waters during the following spawning season. Tag returns were recorded during the late summer to winter months in northern waters when fishing effort was dispersed throughout reef waters. However fish were recaptured in southern waters in the summer months after the initial tagging season, and the subsequent southern fishing season,

indicating that movement of at least some component of the population was a regular occurrence.

The distances moved by fish within successive migration model periods, after during-season period release, are given in Figure 2.8. Not surprisingly tag recaptures decline with increasing time. Most tag recaptures were made within 100 nautical miles of release. Nevertheless, the distances travelled by some fish after the initial tagging period confirmed that movement of at least some component of the population was a regular occurrence (Figure 2.7). Returns of fish that had been tagged between Lizard Island to Townsville that subsequently travelled 700 to 1,000 nautical miles were all recaptured within the south east Queensland to northern NSW region.

The longest time at liberty was 10 years. The fish was recaptured in northern waters at the time of the spawning season. The long period did not assist interpretation of movement patterns although as the animal was returned with the head for otolith removal, the recapture was extremely important to assist age validation (McPherson 1992).

Long range movements from northern to southern Queensland and NSW waters were made by both male and female *S. commerson* (Figure 2.8). Nine fish recorded movements >500 nm; however sex data were not reported from the primarily recreational recaptures made in southern Queensland or northern NSW. There was no evidence to suggest a differential movement by sex. However, there was a clear correlation (R=0.72) between fish size and distance moved into higher latitudes in southern Queensland and northern NSW. Larger fish are usually females (McPherson 1992), so it is likely that females being larger at older ages may move longer distances.

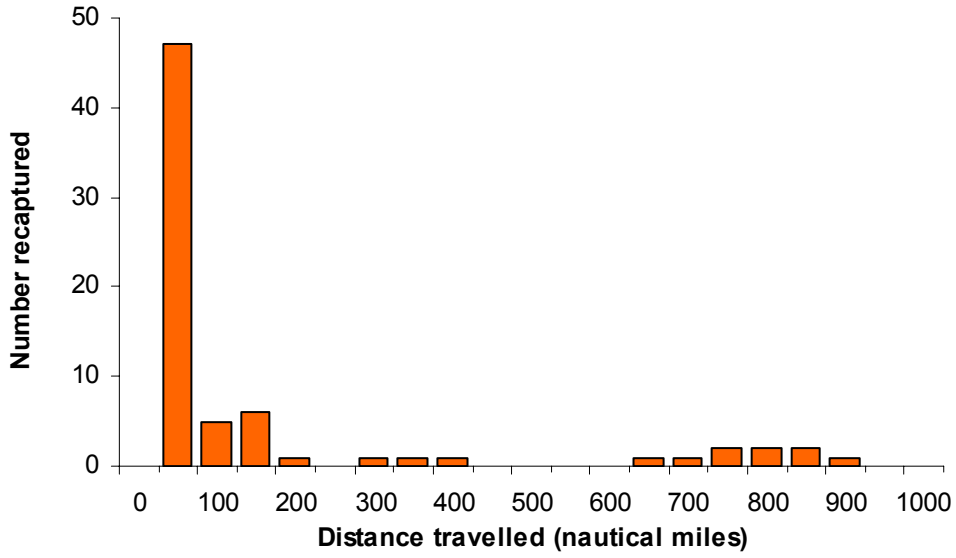


Figure 2.5. Distance between tag release and recapture

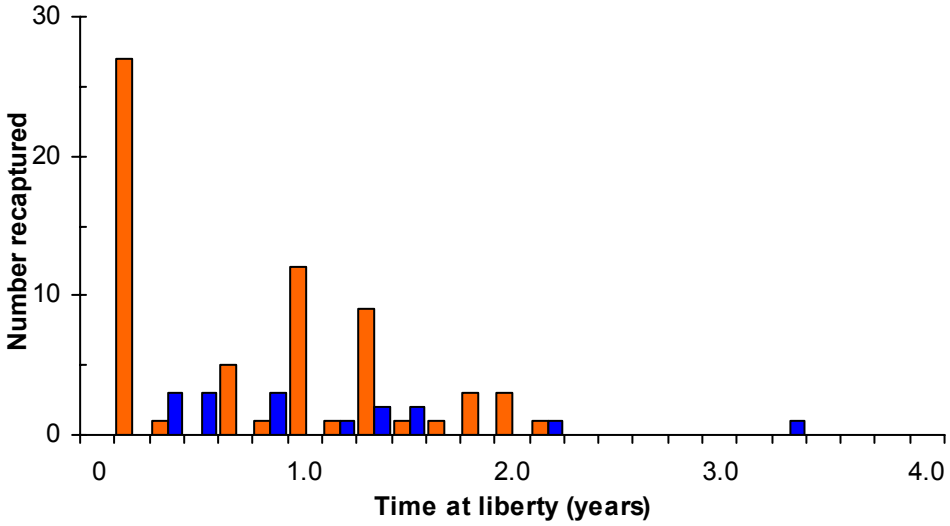


Figure 2.6. Time at liberty for tag returns in northern (orange) and southern (blue) waters

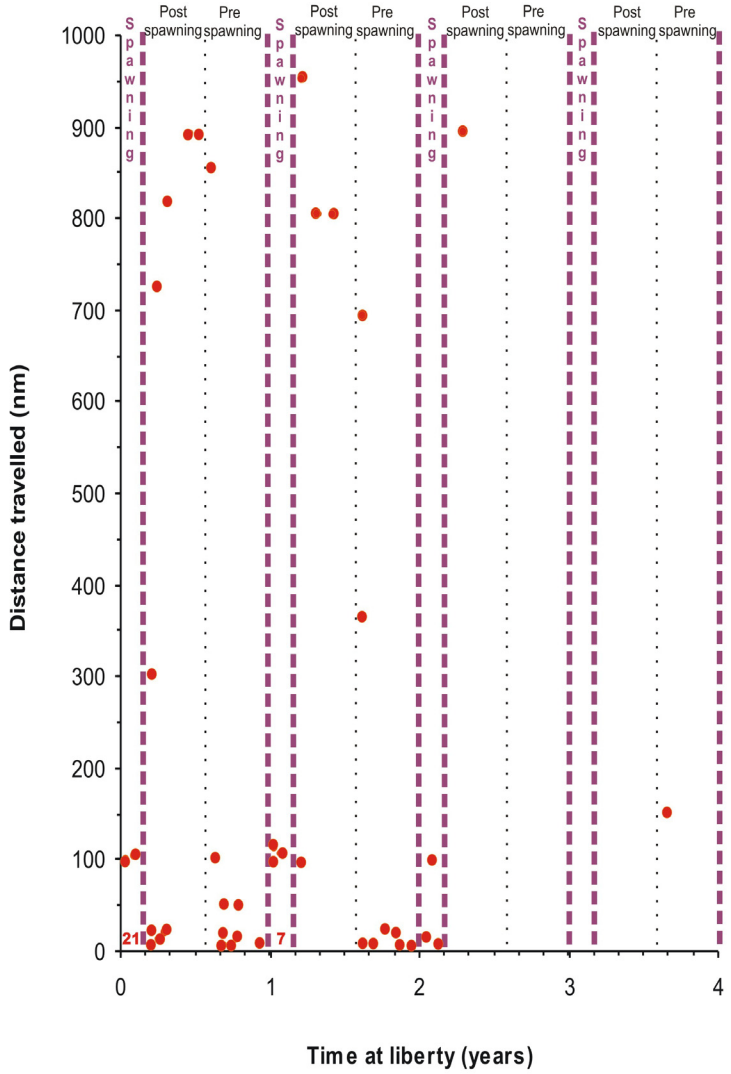


Figure 2.7. Distance moved by time of recapture for *S. commerson*
Observations are marked by dots or numbers that reflect the number of observations.

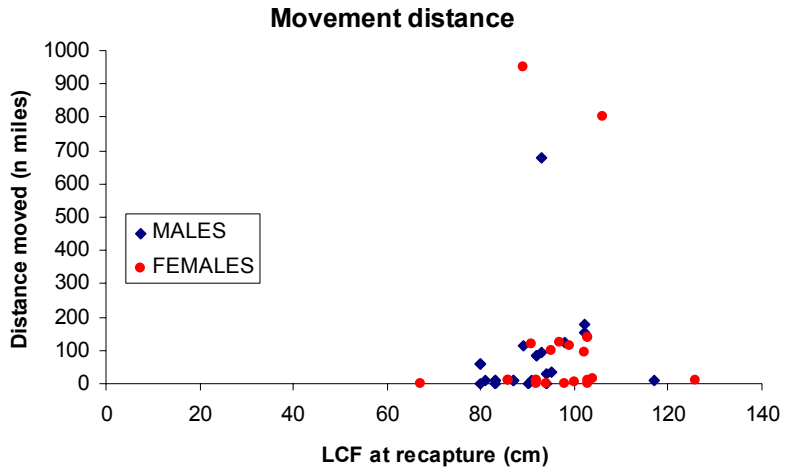


Figure 2.8. Distance moved by length and sex for S. *commerson*

Movement Model for *Scomberomorus commerson*

The model is based on a combined map of tag recaptures and a schematic figure to explain the recaptures for each of the three time periods with respect to the spawning season. The model is given in Appendices 1, 2 and 3.

Spawning season period — October – November. The Spawning-season model (October-November) depicts a concentration of fish within inshore reef areas referred to as spawning reefs (Appendix 1). They are described as inshore reefs adjacent to the 'steamer track' or Great Barrier Reef lagoon where concentrations of spawning fish are encountered in waters greater than 25 m deep along the north-western to western reef faces of spawning reefs. During the period of the study industry referred to limited spawning occurring in isolated areas in southern waters, and specifically around the coastal location of Bustard Head near Bundaberg (Appendix 1).

Observed movement of adult fish during the spawning season is limited. Movements during a spawning season may occur between adjacent reefs (<15 nm), and other adjacent recognised spawning reefs regions (<100 nm). The observed movements were all southward and associated with the late part of the season when spawning activity was decreasing and may well have been indicative of post-spawning season movement.

Movements during the season were between inshore spawning reefs. No movement was observed to outer Great Barrier Reef areas where extremely large fish (not often observed in commercial catches on spawning grounds) were encountered by game fishing vessels. Large fish (>140 cm) were present on the spawning grounds although they were only taken by commercial handline gear on 'wog' lures. The mean size of fish taken by baits and lures was significantly smaller than those taken on 'wogs', gear that does not appear to be used as frequently in the present day fishery (McPherson *unpubl. data*). These fish remote from the recognised fishing / spawning grounds were encountered in spawning condition, but due to the low numbers of fish encountered and the relative shortage of male fish (none larger than 120 cm), the contribution to the annual spawning effort was not estimated.

Restricted spawning potential of other large scombrid fishes has been encountered in the Australian Fishing Zone. In the east coast tuna fishery, for example, extremely large yellowfin tuna with no reproductive development were encountered in southern waters at a time when fish in the northern AFZ were in spawning condition (McPherson 1991).

Fish tagged during spawning were observed on the same spawning grounds in subsequent spawning periods. Given that substantial fishing effort occurred on the spawning grounds throughout the year and few returns were reported, the most parsimonious explanation available was that fish moved onto the spawning grounds on a seasonal basis. No conclusions could be drawn about individual fish returning to natal reefs or sites of previous spawning activity, but given the common short term tag movement between adjacent reefs and major spawning reef regions just prior to the spawning season (*e.g.* Cairns to Townsville and Townsville to Cairns), it is unlikely that the species displays a salmonid-like spawning region or reef affiliation.

The Queensland Fisheries Management Authority (QFMA) logbook data indicated that fishery landings were concentrated within a 1° latitude grid east of Ingham, most of the landings being reported from within the October-November spawning period (Williams 2002). This situation had not appreciably changed from industry perceptions since the 1960s and 1970s at least. However, there was a perception among fishermen based north of Ingham that the spawning reef catches around Cairns and Lizard Island, so important during the tagging studies, had ceased to be viable fisheries by 1998 (when this FRDC project commenced), or at least could not support fishing activities. This perception could well indicate that the observed movement of fish between adjacent spawning reefs may have been reduced. This would have implications for genetic diversity.

Post-spawning season period — December – April. The Post-spawning model (December to April) depicts two types of fish movement at the conclusion of the spawning season: long range movements usually >700 nm into southern Queensland and northern NSW waters; and shorter range movements usually <100 nm (Appendix 2). The 1981-88 developed model recognised migratory and resident categories within the east coast population.

Segregation into these categories occurred at the end of the spawning season when catch rates declined dramatically. Resident fish that remained in northern reef waters and the southward moving migratory fish captured in southern waters all had spent or resting gonads (McPherson 1993). The segregation into resident and migratory components of the stock occurred for two year old fish and older, *i.e*. those that had spawned for the first time at approximately 24 months of age.

Migratory component. The model indicates that larger fish have the potential to make longer range movements. This assertion came from the clear relationship (linear regression R^2 = 0.91) between length at recapture and latitude of recapture for n = 13 fish between Mooloolaba and Coffs Harbour / Nambucca Heads during the summer months of the post-spawning season. The vast majority of the fish were tagged between Ingham and Lizard Island. The model did not however predict that all large fish moved long range distances.

The basis for the long range movements was seen as means of large fish attaining favourable summer environmental or feeding conditions. This is consistent with other observations of a number of tuna species (yellowfin, Atlantic bluefin tuna, skipjack, albacore and king Spanish mackerel). Generally, larger scombrid fishes exhibit preferences for cooler water waters whether horizontally or vertically, as they increase in size, with the exception of shorter duration movements for feeding or spawning.

Complementary to the predominantly northern tagging efforts by QFS and commercial fishermen were two returns from recreational releases. The first was from the precursor to the current AUSFISH tagging program, and the second was from the current AUSFISH programme.

- The first recapture was a short term movement of a fish tagged off Brisbane at a time when schools were arriving in December, and its recapture 100 nm south within the

same time/area stratum in NSW waters associated with fish previously tagged in northern Queensland.

- The second recapture was a long range recapture of a fish, tagged on the spawning reefs off Townsville a couple of weeks prior to the spawning season, on reef areas off Mackay. The fish had been at liberty 1.5 years.

In the absence of effort data, the relative proportions of fish undertaking longer or shorter range movements could not be determined.

The location of the southward migration route is not known. No defined fisheries have existed between northern waters and the primarily rod and reel commercial and recreational fishery that commences off south-east Queensland in December. Multiple tag returns from vessels moving in open water suggest that the movement occurs in the 'steamer track' within Barrier Reef waters. All *Scomberomorus* species are known from continental shelf waters (Munro 1943, Collette and Russo 1979) suggesting that movements from the northern spawning grounds would not occur off the continental shelf. It was not until the development of the Cairns-based game fishing industry in 1965 and the Japanese longline industry in the Coral Sea that captured wahoo (*Acanthocybium solandri*) and not *S. commerson*, that an offshore movement for *S. commerson* was fully discounted.

The extent of the southward movement appears to be variable. Over the past decade industry has indicated considerable variability in landings off Brisbane and northern NSW. The temperature data presented in Figure 2.2 indicate variability of summer water temperatures that, in association with appropriate east coast food resources, may be sub-optimal for *S. commerson* resulting in variation in the extent of southerly movement. If the arbitrary 24°C isotherm is indicative of the lower limit of fish abundance, it is confirmed by observations from the 1979 northern Queensland tagging showing long range movements into southern waters as far as Coffs Harbour during the summer months of 1979-1980. These movements correlated with the 24°C isotherm extending south of Coffs Harbour in early 1980.

Movement off the east coast may resemble movement off Western Australia. Donohue et al. (1982) observed that the apparent pattern of migration of *S. commerson* in Western Australian waters closely parallels the southward and northward movement of the 22-24°C sea surface isotherm (Figure 2.2). The southern limit of the species in Western Australian waters was approximately 33°S, a latitude that coincides with the southern limit of the 22°C at approximately 34°S.

Seasonal movements of east coast Spanish mackerel matched observed movements along the roughly north-south oriented South African east coast (Rudy van der Elst, Oceanic Research Institute, Durban, *pers. com.*). The species demonstrated a summer distribution off Transkei and southern Natal during the summer months and a winter distribution in waters off Zululand (generally northern Natal and southern Mozambique) with an inshore movement of schools along rocky coastlines. Smaller scale seasonal movements around the Gulf of

Thailand were described by Tongyai (1976). Lewis et al. (1983) reported seasonal changes in distribution in Fiji waters.

Sutherland and Fable (1980) described long range tag movements of *S. cavalla* from Gulf of Mexico waters as far west as Texas to spawning grounds in southern Florida. A return movement was proposed from one tag return (moved from Florida to the north west Gulf) and perceived movement of fish concentrations (Roy Williams, Florida Dept. Natural Resources, *pers. comm*.). However, they recognised that some fish remained in Florida waters throughout the year. They also proposed, from observed movements of fish size/age concentrations rather than tag recaptures, that large fish may over-winter in Gulf waters (*i.e*. higher latitude areas of the Gulf of Mexico) and not make the move to southern Florida waters with the onset of winter, to spawn.

The *S. cavalla* tagging work in the U.S. proposed the possibility of migratory movements of some fish with non-movement of others, suggesting the existence of resident stocks. For a variety of reasons, the stock status of *S. cavalla* declined in U.S. waters after the tagging work of the late 1970s and early 1980s (Sutherland and Fable 1980). It was relevant that when the levels of spawning stocks approached their lowest levels based on NMFS biannual Stock Assessments, migratory fish were still evident within the fishery; however the year-around resident stocks in Florida waters were not as evident in commercial and recreational catches as they had been (King Mackerel Stock Assessment Panel, *pers. comm.*). Consideration was given to the possibility that the resident component of the stock had ceased to exist (Chris Legault, *pers. comm*.). The genetic implications for these possible changes could never be considered.

Rivas (1978) observed that the migratory ranges of younger and mid-size northern Atlantic bluefin were, respectively, 40 and 85% of that of giant fish. Giant fish demonstrated an ability to withstand lower temperatures for extended periods of time. This behaviour could have been due to an appropriate adaptation of the lateral heat exchanger mechanism that provided large fish with an ability to forage in a food rich environment. The heat exchanger mechanism also permitted larger fish to tolerate a much wider temperature range. All size classes appeared to move at least between adjacent areas in the north Atlantic while there was no evidence for resident stocks.

In the absence of any physiological data for the *Scomberomorus* genus I make the broad generalisation that as a tuna-like species, when *S. commerson* approaches its maximum size limits so too does it approach its physiological limits. As individuals approach the species von Bertalanffy growth parameter L_∞ (irrespective of the problems inherent with this expression as an indicator of growth) there is a tendency for larger fish to inhabit cooler waters than younger fish of the species and they occupy a wider habitat (Rivas 1978). This behavioural adaptation may result in a slower metabolic rate for larger *S. commerson* for at least part of the year, thereby decreasing the energy demands of the fish.

There may also be a phylogenetic explanation for latitudinal migration behaviour for the species. Scombroids generally evolved in tropical oceans and over time invaded more

temperate waters. Those species that did adapt to cooler waters as adults retained the requirement for adults to briefly move long distances back towards the equatorial regions to spawn, *e.g*. southern bluefin tuna and northern Atlantic bluefin tuna. Williams and Finucane (1979) noted a substantial movement of *S. cavalla* adults in these terms *i.e*. from temperate waters toward the tropics to spawn in southern Florida waters.

The movement rate of individual fish may be rapid. One return demonstrated a southward straight multi-vector line of 950 nm in 28 days, a daily average of 34 nm. Migrating Atlantic bluefin tuna travelled 7-8 nm per day over weekly periods (Mather et al. 1974) while two tagged albacore tuna maintained a daily movement of 30 nm for in excess of 100 days (Sharp and Dotson 1977). The energy demands would most likely be great for a rapid migration of *S. commerson*, a species far less adapted to faster or sustained swimming than tuna species (Kishinouye 1923).

Sharp and Dotson (1977) discussed the problem of energy availability and utilisation in migration and stated that unless pelagic fish were assisted by currents, the energy demands would be quite expensive. Sharp and Vlymen (1978) also predicted that the long-range migration of giant Atlantic bluefin would be lethal (due to muscle overheating) unless currents assisted for at least 50% of the overall observed distances.

The Australia Pilot (1928 and all subsequent issues) as well as navigation charts and industry experience, identify southward moving currents within the steamer track during the spring and early summer months (during and immediately after the spawning period) assuming strong south-east weather patterns did not occur. Further to the south and beyond the limits of the Barrier Reef and continental shelf waters, the Australia Pilot (1973) reported a southward current set of 21-24 nm per day at the time of the recorded migrations. These currents would assist the southward migration of *S. commerson*. One the basis of one recapture at least, the current could, perhaps, account for at least 70% of the distance travelled which would seem to be energetically very conservative.

Resident component. The model indicated that optimal habitats, such as those with favourable water temperature, salinity and food resources, during the non-spawning period could also be reached by individuals within relatively short distances of the spawning reefs. Deeper waters off the outer barrier reefs, and perhaps the relatively deep waters adjacent to some coastal headlands and islands, could provide appropriate conditions for *S. commerson* individuals from various size classes under specific wind and current conditions.

Restricted southerly movement may not be entirely limited to fish from northern spawning reef areas. The possibility that the (presumably) smaller concentrations of fish that spawn in some years in more southerly areas could move south (although with reduced amplitude perhaps dictated by environmental conditions) might well result in restricted distributions of fish on a variable basis and would be an issue for appropriate sampling design for a stock assessment study.

Pre-spawning season period — May – September

For the Pre-spawning season period the lack of long range tag recaptures from southern waters to northern waters represents a substantial shortfall for the east coast movement model. Northward movement has been inferred from the historical accounts of fishermen following apparently northward moving schools, with the resultant progression of QFB landings.

The model suggests a departure of larger fish from southern areas at the limit of the summer distribution with the onset of autumn (Appendix 3). This behaviour was most clearly demonstrated for fish in northern NSW waters.

Commercial fishermen in the Swains Reefs areas referred to a marked decline in *S. commerson* abundance from early winter. Presumably, these fish move northward. However with no fishery directed toward the species, the status of *S. commerson* abundance in the southern reef waters was not clear.

Seasonal movement of fish in the Bundaberg region (latitude approximately 25°S) demonstrate a combination of movement and residence over different years. Fishermen indicated that fish would not always leave the Bundaberg area. Catches would usually drop off in the eastern areas around Hervey Bay; however schools of large fish would sometimes remain throughout the winter in the Bustard Head area. If they remained until October-November they would demonstrate some spawning activity.

During the late 1970s and early 1980s, cases of ciguatera fish poisoning became common for *S. commerson* in the Bundaberg area. The source of the earliest outbreak was isolated and steps were taken to exclude consumption of fish from an area on the western side of Hervey Bay. Ciguatera poisoning has always been reported from a wide variety of reef species in northern Queensland, including *S. commerson*. A movement model that suggested fish left all southern distribution areas, and included the possibility of fish moving through ciguatera areas to northern areas, would have indicated annual rises in poisoning from *S. commerson* in northern areas. The absence of any increase in poisoning in *S. commerson,* compared to the major problem species such as large coral trout species, provides some evidence that movement throughout the southern areas does not necessarily involve longer range northerly movements. The observed residence of large fish around coastal headlands north of Bundaberg also provides some indication of this. No data were available regarding the relative incidences of ciguatera from these fish.

The movement model allowed for residence in, or limited movement of fish from, southern areas. Sutherland and Fable (1980) suggested the possibility of some large fish not joining the long range movement with the onset of winter toward lower latitude waters, and hence a movement to major recognised spawning grounds.

RE-ASSESSMENT OF THE MOVEMENT MODEL PRIOR TO AND DURING THE FRDC MACKEREL STOCK ASSESSMENT STUDY 1998-2002

Sampling for the FRDC study was conducted at a time of increasing management and industry concern for the status of the fishery within Queensland (Williams 2002) and from other states with developing *S. commerson* fisheries. The experiences being developed at this time by managers and researchers were only appropriate for recent time scales in the order of approximately 15 years, since QFS commenced commercial logbook returns in 1988.

QFMA landing figures and catch effort data were almost certainly more accurate than the QFB total landings of decades before. Since the commencement of logbook returns and up until the commencement of the FRDC project in 1998, landings had increased although they were substantially lower than the historical landings of decades before (Figure 2.9), closer to the time when the movement model was developed.

The early data from 1935 to 1975 are taken from QFB and Bureau of Statistics data derived from live weight conversions from fillet and headed/gutted product purchased by the QFB. The data represent total Queensland catches although Gulf catches were small prior to 1975 and Torres Strait landings rarely representing the annual catch. The later data 1988-2000 are from commercial logbooks obtained from the Commercial Fisheries Information System Database and represent east coast landings only.

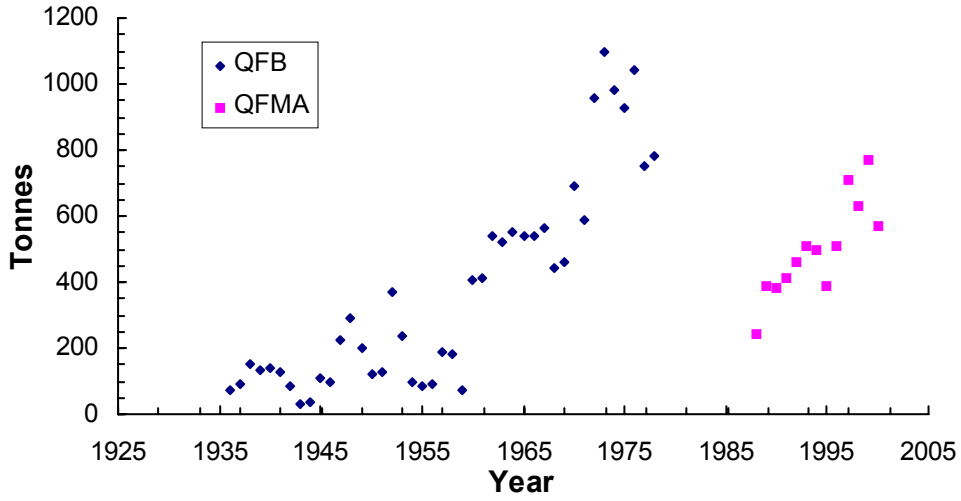


Figure 2.9. Landings of *S. commerson* from Queensland Fish Board annual reports and Queensland Fish Management Authority commercial logbook data (financial years 1935/36 to 2000)

Landing figures probably reflect a wide range of changes during the development of the fishery. World War II resulted in a short term decline in landings in 1943/44 and 1944/45 although no explanations are available for the extremely low landings between 1954 and 1959.

Vessels did not commence fitting freezers until the early 1960s. Their initial size and efficiency were extremely small compared to present day equipment.

Many other aspects of the fishery have changed, such as the advent of echo sounders in mother-ships, and even dories from the early 1980s. The widespread use of travelling stabilisers permitted mother-ships to continue fishing in weather that would have previously prevented vessels even steaming between reefs. These changes were probably minor compared to the effective effort increases brought about by the advent of GPS systems in mother-ships and dories.

The movement model was developed from data obtained between 1979 and 1983, a period for which no landing data are available (Figure 2.9). There has to be some uncertainty about the status of the stocks when the tag return data were obtained, or at least the comparability of stock status at that time and the stock status during the FRDC stock definition study. The FRDC study commenced in the late 1990s, in an environment of increasing catches when only less than a decade before concerns were being expressed about the status of the fishery. Given the prior history of rapidly increasing catches in the mid 1970s followed by an initial observed decline then, by what probably was an even greater decline in landings (although data were totally absent), there was concern in some areas that the fishery could be headed for another crash unless prevailing industry practice, management or environmental conditions changed.

The effect of these changes on the efficiency of the fishery, and indeed the impact on fish stocks has not been quantified. It is possible that the apparent changes in fish availability, hence density, may have an effect on fish behaviour, either reproductively, or in the nature of post and pre-spawning movements and even the nature of resident fish stocks.

Any modification of fish behaviour could, in turn, influence the reliability of sampling of fish populations for stock diversity, whether by genetic structure, parasite structure or otolith microchemistry. Major, or even fluctuating, changes to the east coast *S. commerson* stock had the potential to influence the results of the FRDC project.

The movement model indicated that larger fish possessed the potential to migrate the greatest distance to find environmentally suitable conditions at the conclusion of the spawning season, provided that favourable conditions could not be found in adjacent areas without the requirement to travel great distances. The model was based on models of other scombroid fish movement and presented what appeared to be long-range movements as a mechanism to save energy by utilising a positive transport mechanism to a region of optimal habitat.

No significant *S. commerson* tag return data became available prior to the FRDC study. There were however tag recapture observations from bigeye tuna in the Corals Sea that

provided support to a resident / migratory (with a cyclical nature) partitioning of a scombroid population. Clear et al. (2005 with approval) observed that Coral Sea bigeye tuna were largely composed of localised populations, a proportion of which were transitory either making cyclical large scale movements before returning to the Coral Sea or dispersing outside the Coral Sea. Of seventeen archival tags recovered 14 were recaptured within 250 nm of their release site. Two archival tags moved large distances away from their release site off Cairns, then returned to their release site.

REFERENCES

Anon. (1978). Major Queensland bid to track Spanish mackerel. *Australian Fisheries* **3**, 18-19.

Clear, N.P., Evans, K., Gunn, J.S., Hampton, J., Hartmann, K., Patterson, T. and Sibert, J. (2005). Movement of bigeye tuna (*Thunnus obesus*) determined from archival tag light-levels and sea surface temperatures. Working paper presented to the Scientific Committee of the Western and Central Pacific Fisheries Commission WCPFC-SCI, BI WP-6, 32p.

Collette, B.B. and Russo, J.L. (1979). An introduction to the Spanish mackerels, genus *Scomberomorus*. **In:** *Proceedings of the Colloquium on the Spanish and King mackerel resources of the Gulf of Mexico*, Nakamura, E.L. and Bullis, H.J. Jr. (eds). Publication of the Gulf States Marine Fisheries Commission **4**: 3–16.

Donohue, K., P. Edsall, J. Robins and R. Tregonning. (1982). Exploratory Fishing for Spanish Mackerel in Waters off Western Australia during the period June 16 to October 16, 1981. Dept. of Fisheries and Wildlife, WA. Rep. No. 57, 46 p.

Earl, R.E. (1883). *The Spanish mackerel Cybium maculatum (Mitchell), its natural history and artificial propagation with an account of the origin and development of the fishery*. Report of the U.S. Fisheries Commission 1880 VIII, 395–426.

Hilborn, R. and Sibert, J. (1988). Is international management of tuna necessary? *Marine Policy* **January**:31-39.

Hisada, K. (1973). Investigation of the tuna handline fishing grounds and some biological observations on yellowfin and bigeye tunas caught in the north-western Coral Sea. *Bulletin of the Far Seas Fisheries Research Laboratory* **8**:35–69.

Hoyle, S.D. (2002). *Management Study Evaluation for the Queensland East Coast Spanish mackerel Fishery*. Department of Primary Industries, Queensland. Information Series **QI**022111, 55p

Hunter, J.R. and B.J. Macewicz. (1985). Measurement of spawning frequency in multiple spawning marine fishes, p. 79-94. **In**: An egg production method for estimating spawning biomass of pelagic fish: Application to the northern anchovy *Engraulis mordax*, R. Lasker (ed.). *U.S. NMFS NOAA Technical Report* **36**, 99 pp.

Kearney, R.E. (1978). *Some hypotheses on skipjack (*Katsuwonus pelamis*) in the Pacific Ocean*. South Pacific Commission, Occasional Paper 7. 23p.

Kishinouye, K. (1923). Contributions to the comparative study of the so called scombroid fishes. *Journal of the College of Agriculture, Imperial University Tokyo* **8**(3):293–475.

Lewis, A.D. (1981). Population studies in northern Australian pelagic species utilising the electrophoretic approach. **In**: *Northern Pelagic Programmme*, C.J. Grant and D.G. Walter (eds). Australian Government Publishing Service, Canberra, Australia.

Lewis, A.D., L.B. Chapman and A. Sesewa. (1983). Biological notes on coastal pelagic fishes in Fiji. *Fisheries Division of Fiji, Technical Report* **4:**68 p.

Mather, F.J., Rothschild, B.J., Paulik, G.J. and Lenarz, W.H. (1974). Analysis of migrations and mortality of bluefin tuna, *Thunnus thynnus*, tagged in the northwestern Atlantic Ocean. *Fishery Bulletin U.S.* **72**(4):900–904.

McPherson, G.R. (1977). North Queensland Spanish mackerel fishery. Northern Fisheries Research Session, Brisbane July 12, 123–127 (unpubl. report).

McPherson, G.R. (1981). Preliminary report: Investigation of Spanish mackerel *S. commerson* in Queensland waters, p. 50-58. **In**: *Northern Pelagic Program*, C.J. Grant and D.G. Walter (eds). Australian Government Publishing Service, Canberra, Australia.

McPherson, G.R. (1981). Spanish mackerel migrations. Australian Society for Fish Biology, Hobart 1981, 4p.

McPherson, G.R. (1985). Development of the northern Queensland mackerel fishery. *Australian Fisheries* **44**(8): 15-17.

McPherson, G.R. (1986). The Torres Strait mackerel fishery: Review of Australian development, production and research. **In**: *Torres Strait Fisheries Seminar*, Haines, A.K., Williams, G.C. and Coates, D. (eds). Australian Government Publishing Service, Canberra, Australia. Pp. 151–164.

McPherson, G.R. (1987). Observations of food items of narrow barred Spanish mackerel *Scomberomorus commerson* (Lacepde) in north Queensland waters and their relevance to the commercial troll fishery. *Queensland Journal of Animal Science* **44**(1):69–73.

McPherson. G.R. (1988). A review of large coastal pelagic fishes in the south Pacific region, with special reference to *Scomberomorus commerson* in north-east Australian waters. Workshop on Pacific Inshore Fishery Resources Working Paper 15, 41p. South Pacific Commission Noumea, New Caledonia, 14–25 March.

McPherson, G.R. (1989). North-eastern Australian mackerel (Scomberomorus) fishery. **In**: Tropical Fin Fisheries Research and Management in Australia. Proceedings of the Workshop Australia-Mexico on Marine Sciences, Quintana Roo, Mexico. July 6–17, Chavez, E.A. (ed.).

McPherson, G.R. (1991). Reproductive biology of yellowfin tuna in the eastern Australian Fishing Zone with special reference to the north-western Coral Sea. *Australian Journal of Marine & Freshwater Research* **42**(6): 465–477.

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

McPherson, G.R. (1993). Reproductive biology of the narrow barred Spanish mackerel (*Scomberomorus commerson* Lacépède, 1800) in Queensland waters. *Asian Fisheries Science,* **6**:169-182.

Munro, I.S.R. (1942). The eggs and early larvae of the Australian barred Spanish mackerel, *Scomberomorus commersoni* (Lacépède) with preliminary notes on the spawning of that species. *Proceedings of the Royal Society of Queensland* **54**(4):33-48.

Munro, I.S.R. (1943). Revision of Australian species of *Scomberomorus*. *Memoirs of the Queensland Museum* **12**(2): 65-95.

Rivas, L.R. (1978). Preliminary models of annual life history cycles of the North Atlantic bluefin tuna. **In**: *The physiological ecology of tuna. Tuna Physiology Workshop, La Jolla, CA (USA), 10 Jan 1977,* G.D. Sharp and A.E. Dixon (eds). New York, Acedemic Press.

Sharp, G.D. and Dotson, R.C. (1977). Energy for migration in albacore, *Thunnus alalunga*. *Fishery Bulletin* **75**(2):447–450.

Sharp, G.D. and Vlymen, W.J. (1978). The relation between heat generation, conservation, and the swimming energetics of tunas. **In**: *The physiological ecology of tunas. Tuna Physiology Workshop, La Jolla, CA (USA), 10 Jan 1977,* G.D. Sharp and A.E. Dixon (eds). New York, Academic Press.

Sutherland, D.F. and Fable, W.A. (1980). Results of a king mackerel (Scomberomorus cavalla) and Atlantic Spanish mackerel *(Scomberomorus maculatus)* migration study 1975–79. NOAA Technical Report Memoir. NMFS-SEFC-12, 23p.

Tanaka, S. (1979). Migration model and population dynamics of large-sized yellowtails in the Pacific Ocean along the Japanese coast inferred from tagging experiments. *Bulletin of the Japanese Society for Fisheries* **45**(3):297–303.

Tonygai, M.L.P. (1976). Plah In-See, *Scomberomorus* spp. in Thailand, 1967. **In**: *The Kuroshio – A Symposium on the Japan Current*, Marr, J.C. (ed.). Pp. 557-564. East-West Centre Press, Honolulu.

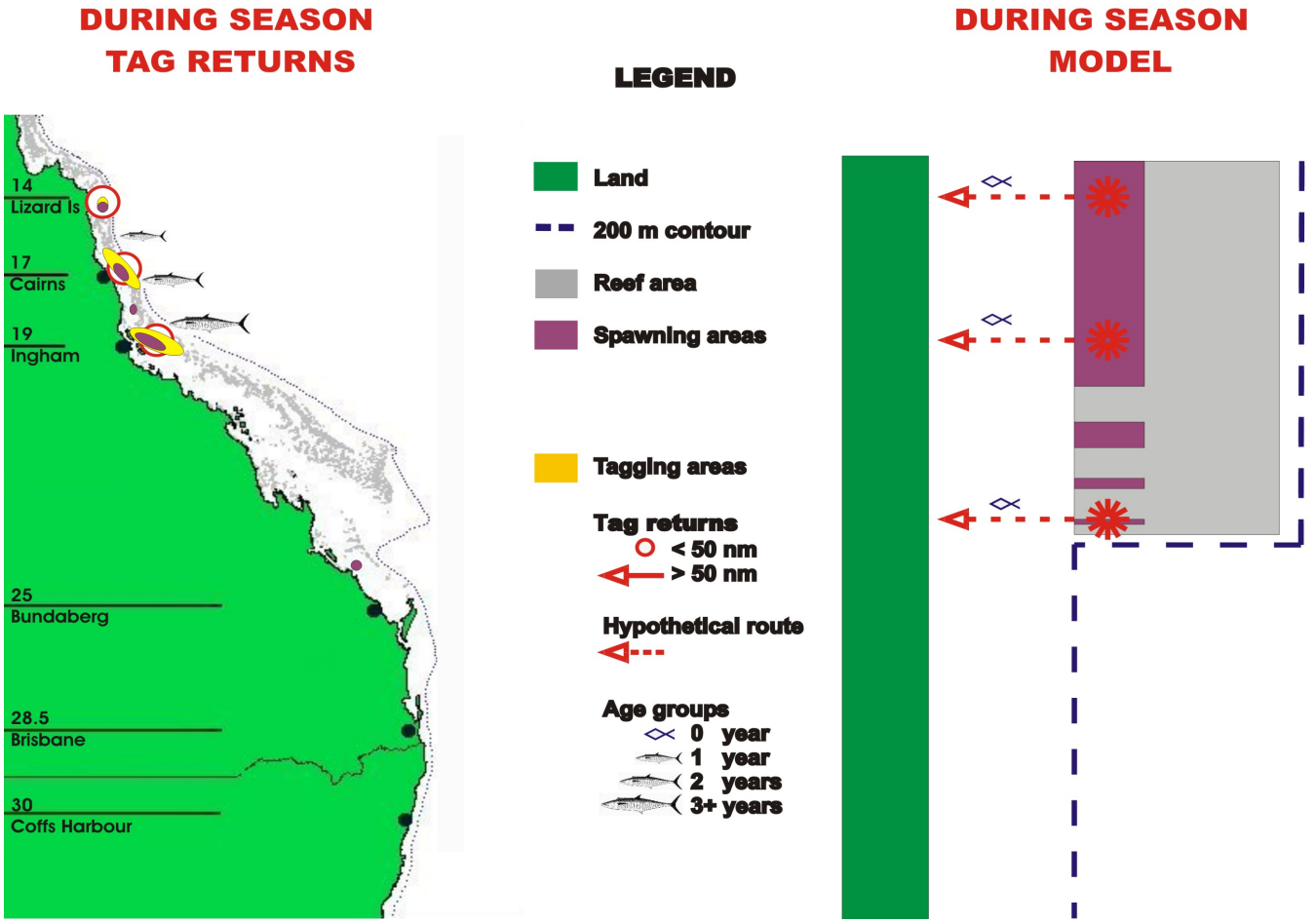
Williams, L.E. (2002). *Queensland's Fisheries Resources. Current Condition and Recent Trends.* Department of Primary Industries, Queensland. Information Series **QI**02012.

Williams, R.L. and Finucane, J.H. (1979). King mackerel migrations (Abstract). **In**: *Proceedings of the Colloquium on the Spanish and King mackerel Resources of the Gulf of Mexico, March 16, 1978, Brownsville Texas,* Nakamura, E.L. and Bullis, H.R. (eds). Pp. 43. Gulf States Fisheries Commission **4**, 111pp.

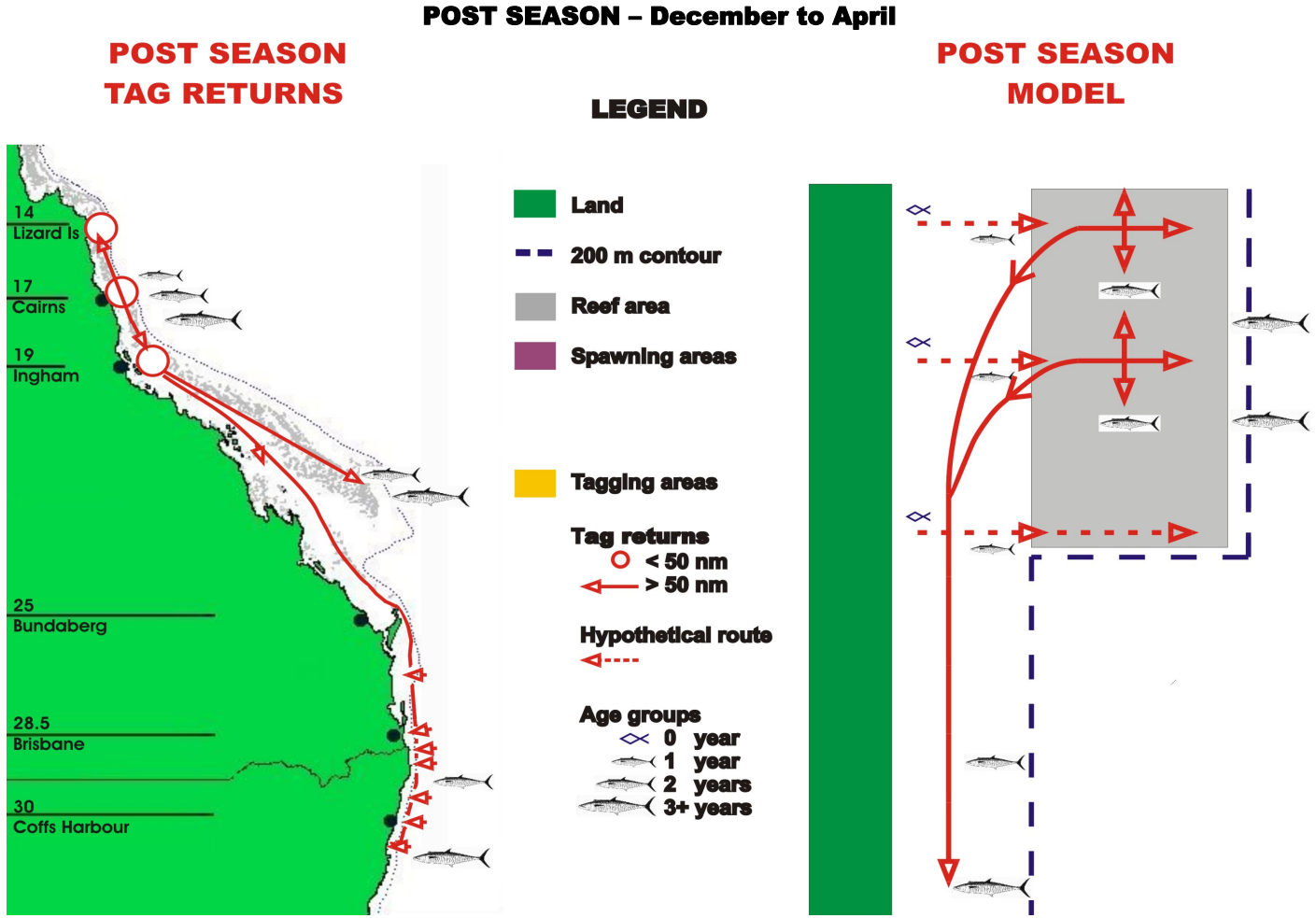
Wilson, M.A. (1981). The biology, ecology and exploitation of longtail tuna *Thunnus tonggol* (Bleeker) in Oceania. MSc Thesis, Macquarie University, Melbourne, Australia, 195pp.

APPENDIX 1: DURING SEASON FISH MOVEMENT MODEL

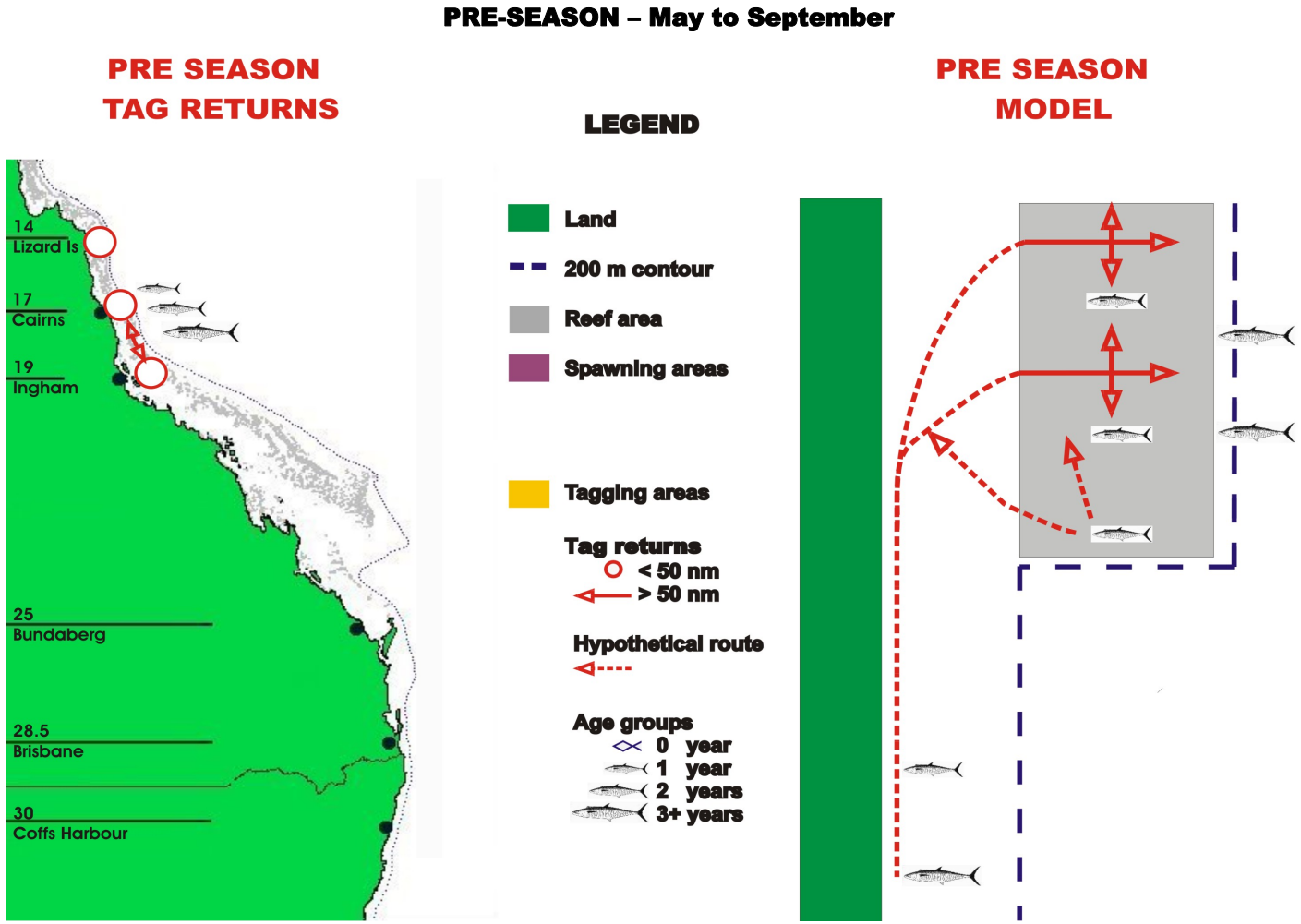
DURING SEASON – October to November



APPENDIX 2: POST-SPAWNING SEASON FISH MOVEMENT MODEL



APPENDIX 3: PRE-SPAWNING SEASON FISH MOVEMENT MODEL



Chapter 3

SPATIAL SUBDIVISION OF ADULT ASSEMBLAGES OF SPANISH MACKEREL, *SCOMBEROMORUS COMMERSON* (PISCES: SCOMBRIDAE) FROM WESTERN, NORTHERN AND EASTERN AUSTRALIAN WATERS THROUGH STABLE ISOTOPE RATIO ANALYSIS OF SAGITTAL OTOLITH CARBONATE

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ABSTRACT

This study investigated the use of stable $\delta^{13}C$ and $\delta^{18}O$ isotopes in the sagittal otolith carbonate of narrow-barred Spanish mackerel, *Scomberomorus commerson* as indicators of population structure across northern and western Australia. Discrete location-specific stable isotope signatures were evident among adult assemblages of *S. commerson* across northern and western Australia. These discrete location-specific signatures indicate that fish from a number of sites sampled across northern and western Australia (Cervantes-Abrolhos Islands, Shark Bay-Exmouth-Thevenard Island, Port Hedland, Eighty Mile Beach, Lord Mayor Shoal-Barcoo Shoal, White Island-Rob Roy Reef, Bassett-Smith Shoal, Fog Bay, Flat Top Bank-Cape Van Diemen-Cape Wessel, Groote Eylandt, Sir Edward Pellew Group, Mornington Island, Lost City, Weipa-Torres Strait, Cairns, Townsville) and from Indonesia (Kupang) are significantly different. The significant differences in the isotopic signatures of *S. commerson* demonstrate that there is unlikely to be any substantial movement of fish among these spatially discrete adult assemblages. A lack of temporal variation between sampling dates in otolith isotope ratios among those locations for which replicate samples are available indicates that *S. commerson* do not undergo longshore spatial shifts in distribution during their life history. Furthermore, the persistence through time of several of these spatially explicit stable isotopic signatures indicates that at these spatial scales the population units sampled comprise functionally distinct independent management units or separate 'stocks' for many of the purposes of fisheries management. However, these location-specific isotope signatures do not preclude the possibility of inshore-offshore movements or cycling during the life history of S. commerson. The persistent spatial structure present among populations of *S. commerson* across northern and western Australia indicates that it may be advantageous to consider *S. commerson* population dynamics and fisheries management from a meta-population perspective.

Keywords: Spanish mackerel, *Scomberomorus commerson*, Stock structure, Stable isotopes, Oxygen, Carbon, Otoliths, Fisheries management.

INTRODUCTION

The narrow-barred Spanish mackerel, *Scomberomorus commerson* (Lacepède), an Indo-West Pacific member of the Family Scombridae, is widely distributed throughout the region from South Africa to the Red Sea in the western Indian Ocean region eastwards throughout the Indo-Australian Archipelago to Fiji in the western central Pacific, around Australia to the south and north to China and Japan (Collette and Nauen 1983). *Scomberomorus commerson*, also known as kingfish, king mackerel, king seer, tiger mackerel, tazar, tanguigue (the official International Game Fishing Association name), and seerfish, is a coastal epipelagic, neritic species which can attain a maximum size of at least 2.4 m and 45 kg (Collette and Nauen 1983) and ages in excess of 22 years (McPherson 1992, Mackie unpublished data).

Across northern Australia, *S. commerson* are a commercially important species from as far south as the Abrolhos Islands area (23°30'S), north and east across northern Australia, down the length of the Great Barrier Reef to the waters of northern New South Wales (Kailola et al. 1993, Mackie unpublished data). Fisheries for *S. commerson* are presently managed across northern Australia by three state-based fishery management agencies. Each state supports highly valuable commercial and recreational troll line based fisheries for *S. commerson* with regional segregation of each fishery through separate management arrangements.

Shaklee et al. (1990) identified two discrete genetic stocks of *S. commerson* in northern and eastern Australian waters. An east coast genetic stock of *S. commerson* included fish from south of Torres Strait southward as far as northern New South Wales. Shaklee et al. (1990) considered that *S. commerson* populations inhabiting Gulf and Torres Strait waters belong to a second genetic stock that extends from southern Papua New Guinea to the west coast of Australia. However, recent genetic analyses (Ovenden et al., this report) suggest that the Torres Strait population is a distinct genetic entity separate from stocks to the west and south-east. Current management arrangements make no allowance for migratory fish or overlapping stocks across either state or intra-state fishery boundaries. Furthermore, foreign (Indonesian) fishing grounds lie adjacent to the territorial waters of north-western Australia. Consequently, there is a need to identify individual stocks or management units within these areas in order to assess the impacts of fishing in each area should competitive fishing practices develop. Therefore, it is important to determine whether post-juvenile populations of *S. commerson* remain discrete and independent or whether there is evidence of mixing of adult fish among different locations. In this paper, we refer to a fish 'stock' specifically as post-juvenile fish populations that remain discrete and non-mixing (i.e. independent) and therefore comprise a functionally distinct management unit capable of independent exploitation.

The information recorded in fish otoliths can be used as an indication of the home range, spatial distribution and stock structure of a fish species (Edmonds et al. 1999). Fish otoliths are composed primarily of calcium carbonate (in the aragonite phase) and contain a small percentage of proteinaceous matter. Otoliths are metabolically inert and their elemental composition is unaffected by post-deposition processes, as they are not subject to resorption,

remodelling or regeneration (e.g. Campana and Neilson 1985). In addition, the isotopic composition of the calcium carbonate can be influenced by a number of factors including the physical and chemical environment of the surrounding water body, the deposition temperature and biological factors such as physiological regulation (Kalish 1989). Analyses of the elemental and stable isotopic composition of teleost otoliths, provide a mechanism for deducing stock structure (e.g. Campana et al. 1994, Kalish et al. 1996, Edmonds and Fletcher 1997, Edmonds et al. 1999, Newman et al. 2000). Measurement of the stable isotopes of oxygen and carbon of the whole sagittal otolith carbonate avoids biases associated with other techniques such as trace element analysis. Moreover, as the whole otolith is used in the analysis, its isotopic signature represents the entire ontogenetic history and has the potential to reflect the home range of each individual fish.

The stable oxygen isotopes of otolith carbonates have been demonstrated to precipitate in isotopic equilibrium with δ^{18}O of ambient water (Kalish 1991a,b, Patterson et al. 1993, Thorrold et al. 1997). Therefore, the stable oxygen isotopes in otolith carbonate will reflect the environmental characteristics of the water body in which the fish resides. Because the physical and chemical composition of seawater varies spatially, otolith isotope chemistry provides a record of the seawater characteristics specific to the area in which the fish resides. Edmonds and Fletcher (1997) showed that differences in sea surface temperature provided the basis for different oxygen isotope signatures in the otolith carbonate of the pilchard *Sardinops sagax* from south-western Australia and hence demonstrated separation of stocks of adult fish.

Carbon isotope data derived from fish otoliths may provide information on diet, growth and metabolic rates (Kalish 1991b, Gauldie 1996, Thorrold et al. 1997). Thorrold et al. (1997) demonstrated that metabolic effects can generate large isotopic disequilibria in δ^{13}C values, with these carbon isotopes not being deposited in equilibrium with the surrounding water (Kalish 1991b). The interpretation of δ^{13}C data can be complicated due to the intrinsic biological and metabolic effects that can influence otolith δ^{13}C values, in addition to ambient environmental conditions and the behaviour of fish in response to environmental change.

The delineation of stock structure from analysis of the stable isotopic composition of teleost otoliths generally assumes that geographically distinct stocks possess a characteristic isotopic signature that reflects the temperature of the water body in which the fish is resident. However, knowledge of the causal mechanisms responsible for the stable isotopic composition of teleost otolith carbonate is not necessary for any measured differences to delineate population subdivision (Edmonds and Fletcher 1997, Newman et al. 2000).

It was therefore considered *a priori* that differences in the isotopic composition of *S. commerson* otoliths sampled from different locations across northern and western Australia would be indicative of location fidelity and a phenotypic measure of population separation. This study was also designed to determine whether any spatial differences in isotopic signatures of the sagittal carbonate were persistent through time. If spatial differences were consistent through time, it would suggest that the populations comprise functionally distinct management units capable of independent exploitation for fishery management purposes.

MATERIALS AND METHODS

Sampling design

Otoliths were collected from twenty-five locations across northern and western Australia extending from the lower west coast of Western Australia across northern Australian waters to the east coast of Australia, including samples from Indonesia (Figure 3.1a–e). For ease of analysis, the coastline of northern and western Australia was divided into four subregions: Mid Western Australia, North Western Australia, Northern Australia and the Gulf of Carpentaria, and Eastern Australia. Seven locations were sampled within Mid Western Australia (Cervantes, Abrolhos Islands, Shark Bay, Exmouth, Thevenard Island, Port Hedland and Eighty Mile Beach); nine in North Western Australia and Indonesia (Lord Mayor Shoal, Barcoo Shoal, White Island, Rob Roy Reef, Bassett-Smith Shoal, Fog Bay, Flat Top Bank, Cape Van Diemen and Kupang (Timor Island, Indonesia)); six in Northern Australia and the Gulf of Carpentaria (Cape Wessel, Groote Eylandt, Sir Edward Pellew Group, Mornington Island, Lost City, Weipa) and three in Eastern Australia (Torres Strait, Cairns and Townsville). Where possible, samples were collected from each location on two separate occasions a minimum of six months apart. Otoliths were collected from approximately 40 fish (20 females and 20 males) at each sampling site on each occasion (Table 3.1 – Table 3.3).

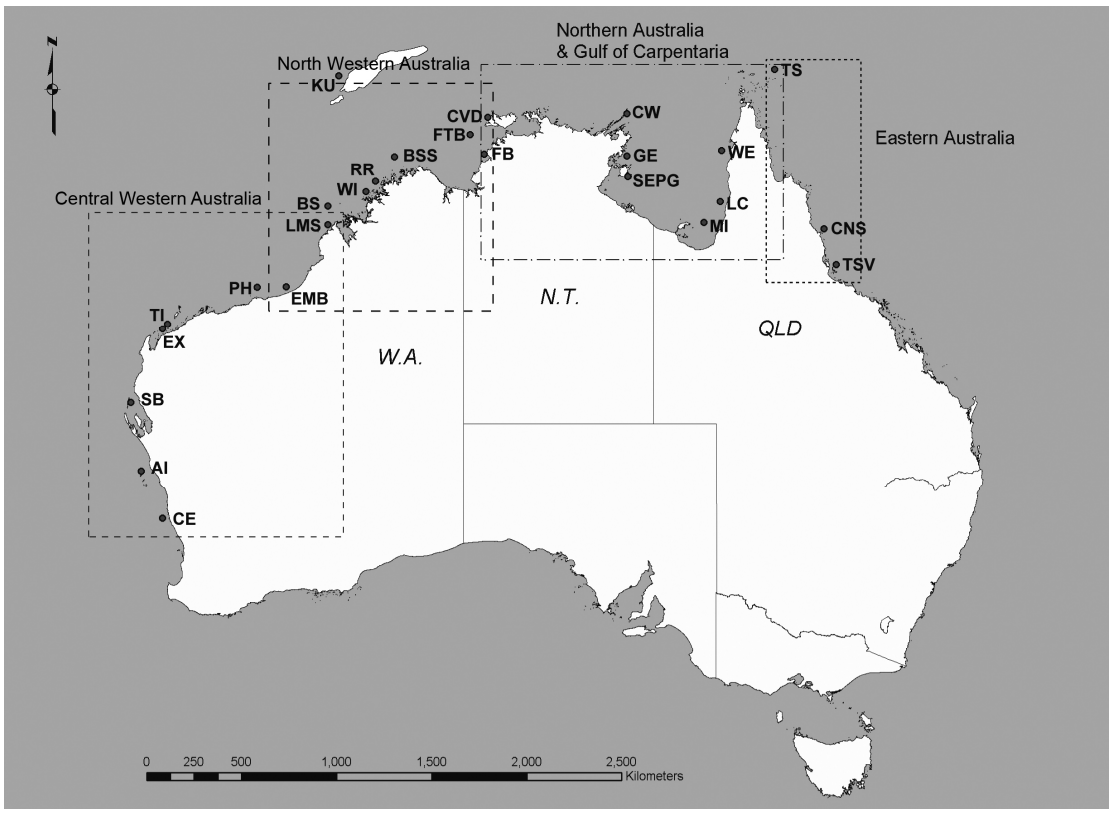

Figure 3.1a. Sampling locations of *Scomberomorus commerson* from western and northern Australia showing each of the four subregions

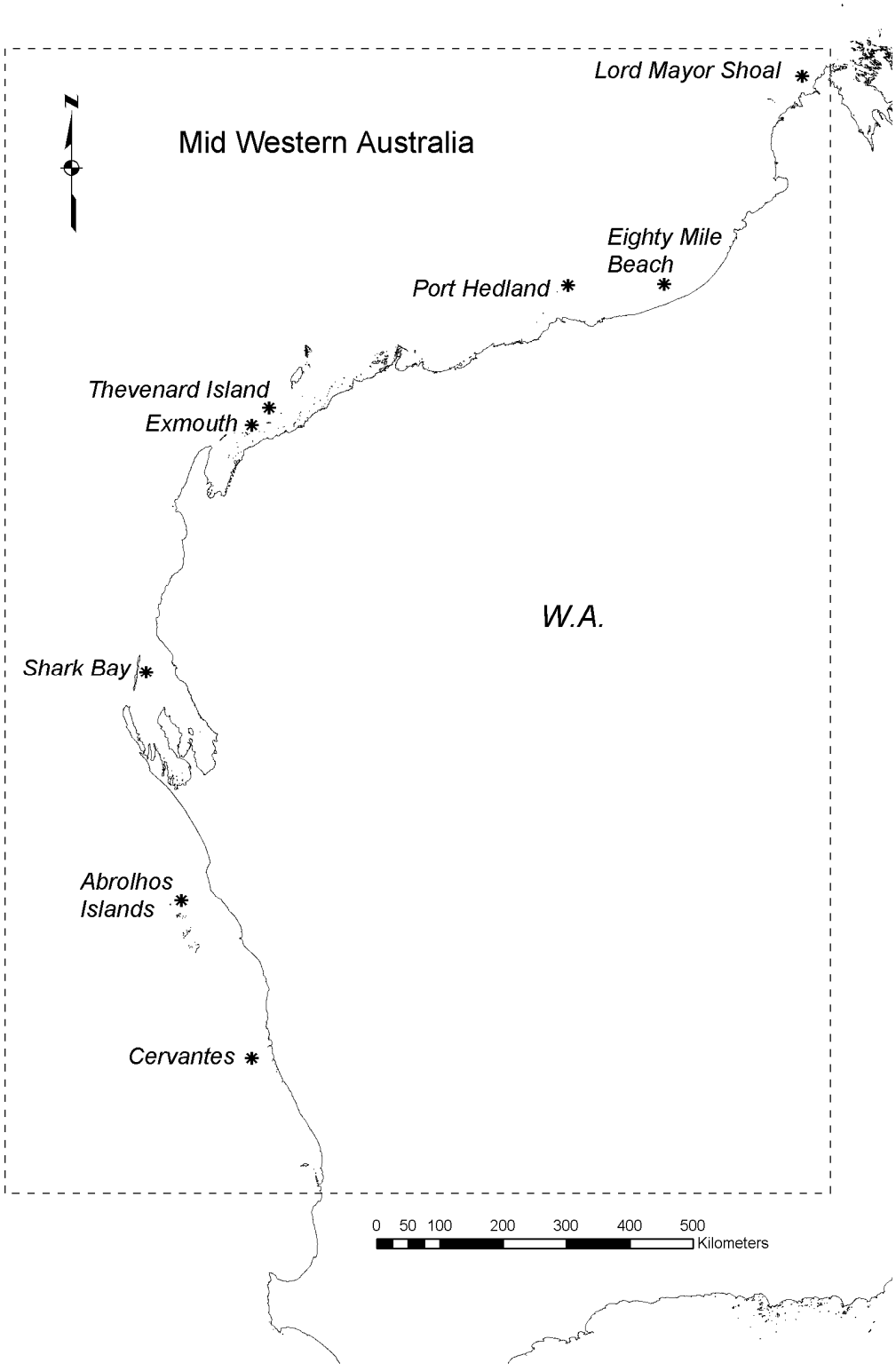

Figure 3.1b. Sample locations of *S. commerson* in Mid Western Australia

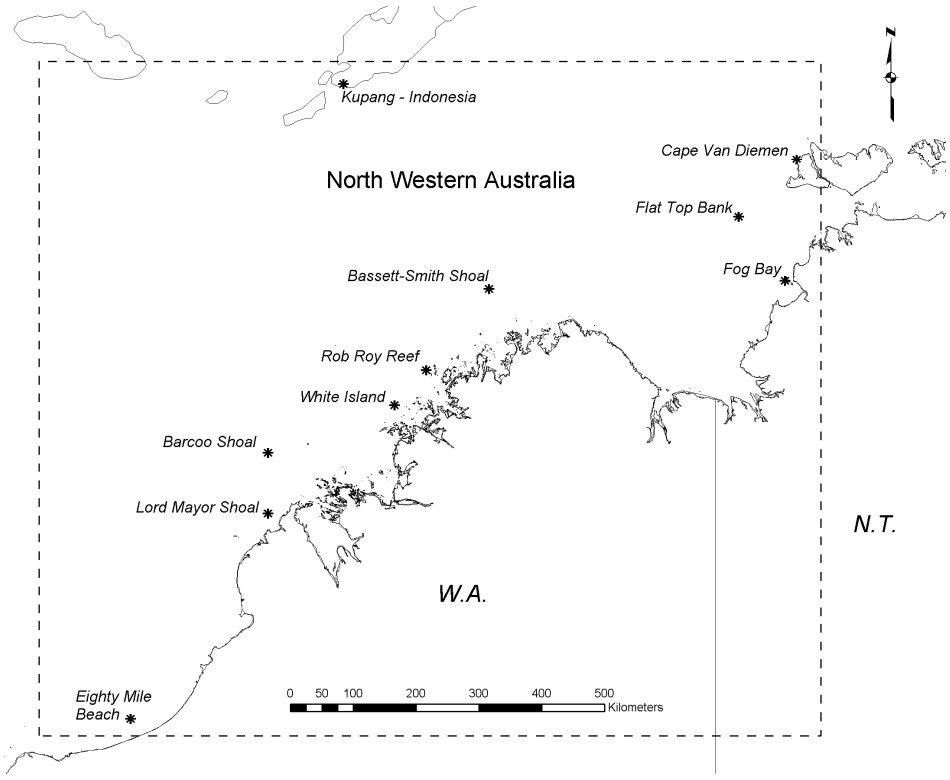

Figure 3.1c. Sample locations of *S. commerson* in North Western Australia

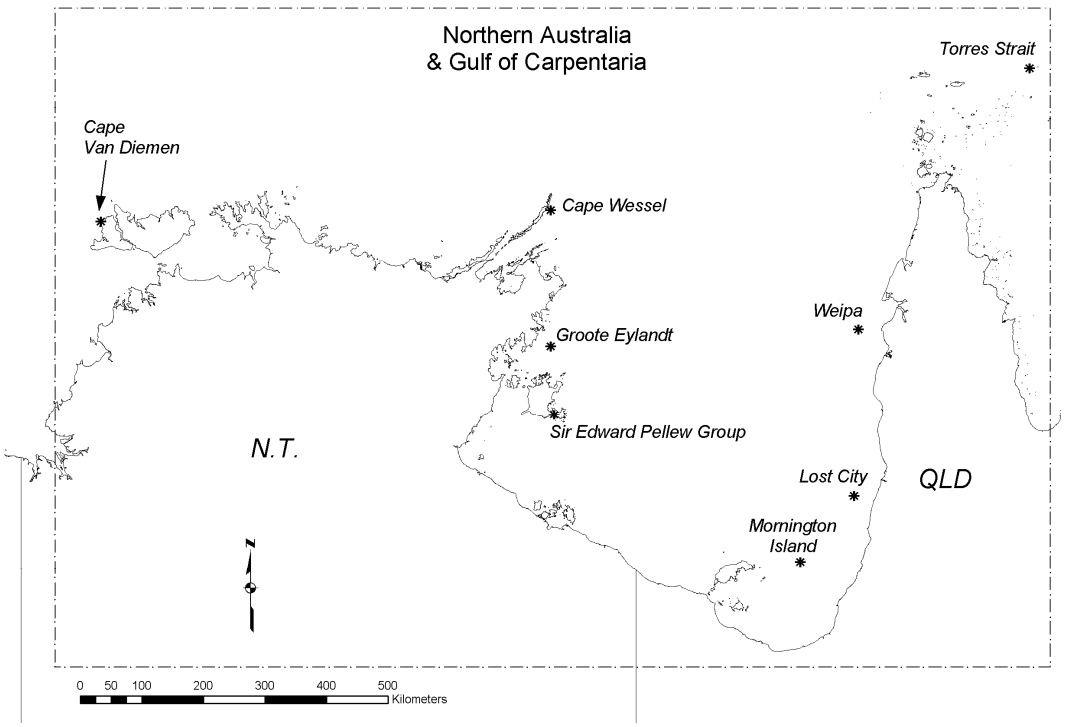

Figure 3.1d. Sample locations of *S. commerson* in Northern Australia and the Gulf of Carpentaria

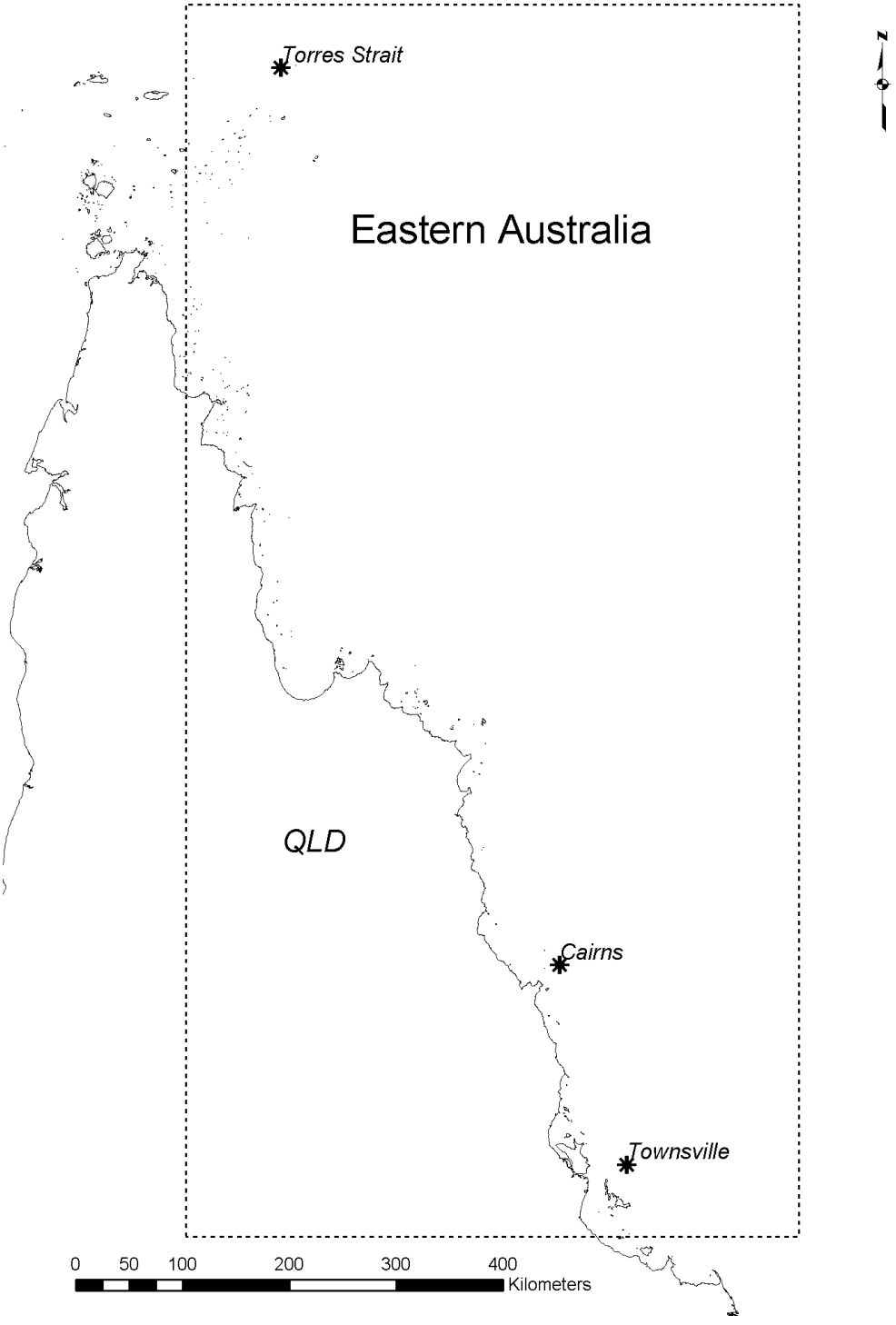

Figure 3.1e. Sample locations of *S. commerson* in Eastern Australia

Table 3.1. Summary of the sampling data, and results from the stable isotope analyses of the sagittal otolith carbonate of *Scomberomorus commerson* from locations in Mid Western Australia (SST = mean annual sea surface temperature [average of the 12-monthly means from 1991 to 2000])

Location (code)	Position (latitude, longitude)	Date of sampling	N	Fork Length (FL, mm) mean (range)	Otolith weight (mg) mean (range)	$\delta^{18}O$ (‰ PDB) mean (range)	$\delta^{13}C$ (‰ PDB) mean (range)	SST (°C)
Cervantes (CE)	30°30'S, 114°45'E	Apr. 2000	18	1158 (860 – 1360)	76.68 (36.5 – 140.9)	-0.59 (-1.21 to -0.10)	-4.66 (-5.29 to -3.88)	21.55
Abrolhos Islands (AI)	28°15'S, 113°45'E	July 1998	12	1058 (950 – 1190)	54.66 (40.3 – 65.5)	-0.43 (-0.93 to -0.13)	-4.59 (-5.19 to -4.20)	22.18
		Aug. 1999	16	1067 (670 – 1400)	63.57 (22.7 – 114.7)	-0.74 (-1.48 to -0.20)	-4.68 (-5.18 to -3.99)	
Shark Bay (SB)	25°00'S, 113°15'E	July 1998	34	1088 (780 – 1460)	54.87 (23.9 – 136.1)	-0.78 (-1.54 to -0.18)	-4.55 (-5.33 to -3.25)	23.65
		June 1999	40	1004 (815 – 1280)	53.16 (33.1 – 110.5)	-0.79 (-1.55 to -0.15)	-4.68 (-5.88 to -3.74)	
Exmouth (EX)	21°30'S, 114°45'E	Aug. 1998	28	988 (113 – 1500)	53.76 (25.7 – 105.5)	-0.81 (-1.33 to -0.23)	-4.54 (-5.02 to -3.98)	25.98
		Sep. 1999	16	1091 (668 – 1500)	64.03 (22.9 – 129.6)	-0.73 (-1.42 to -0.32)	-4.27 (-4.98 to -3.27)	
Thevenard Island (TI)	21°15'S, 115°00'E	Aug. 1998	40	1014 (517 – 1435)	61.83 (17.1 – 135.8)	-0.84 (-1.22 to -0.36)	-4.37 (-5.21 to -3.23)	25.98
		Jan. 1999	40	1043 (581 – 1498)	72.89 (23.0 – 171.8)	-0.67 (-1.69 to 0.07)	-4.46 (-5.20 to -3.48)	
Port Hedland (PH)	19°30'S, 119°15'E	July, 1999	30	1102 (900 – 1500)	75.47 (37.6 – 161.1)	-0.96 (-1.59 to -0.50)	-4.30 (-4.99 to -3.30)	27.16
Eighty Mile Beach (EMB)	19°29'S, 120°37'E	Sep. 2000	40	1062 (793 – 1425)	58.56 (24.7 – 134.5)	-1.20 (-1.99 to -0.83)	-4.47 (-5.42 to -3.50)	27.31

Table 3.2. Summary of the sampling data, and results from the stable isotope analyses of the sagittal otolith carbonate of *Scomberomorus commerson* from locations in North Western Australia and Indonesia (SST = mean annual sea surface temperature [average of the 12-monthly means from 1991 to 2000])

Location (code)	Position (latitude, longitude)	Date of sampling	N	Fork Length (FL, mm) mean (range)	Otolith weight (mg) mean (range)	$\delta^{18}O$ (‰ PDB) mean (range)	$\delta^{13}C$ (‰ PDB) mean (range)	SST (°C)
Lord Mayor Shoal (LMS)	16°32'S, 122°35'E	July 2000	22	988 (704 – 1311)	61.72 (31.2 – 120.1)	-1.41 (-1.92 to -1.07)	-4.38 (-5.10 to -3.61)	28.13
Barcoo Shoal (BS)	15°39'S, 122°35'E	Feb. 1999	27	914 (798 – 1055)	49.76 (36.8 – 103.6)	-1.30 (-1.64 to -0.42)	-4.41 (-5.04 to -2.87)	28.28
White Island (WI)	14°58'S, 124°24'E	Oct. 1998	40	945 (770 – 1180)	50.69 (27.3 – 93.6)	-1.45 (-2.02 to -0.95)	-4.65 (-5.37 to -4.04)	28.28
		July 1999	15	914 (832 – 1008)	47.32 (35.5 – 56.0)	-1.53 (-1.67 to -1.31)	-4.74 (-5.10 to -3.90)	
Rob Roy Reef (RR)	14°28'S, 124°51'E	Aug. 1999	21	921 (669 – 1102)	48.15 (22.7 – 74.0)	-1.51 (-1.97 to -1.20)	-4.76 (-5.51 to -3.92)	28.42
Bassett-Smith Shoal (BSS)	13°17'S, 125°45'E	Sept. 1999	40	1018 (760 – 1175)	64.75 (38.4 – 97.8)	-1.29 (-1.64 to -0.95)	-4.47 (-5.30 to -3.69)	28.63
Fog Bay (FB)	13°10'S, 130°00'E	Aug. 2000	40	1072 (850 – 1370)	52.72 (33.7 – 73.9)	-1.66 (-2.11 to -1.37)	-4.64 (-5.49 to -3.67)	28.83
Flat Top Bank (FTB)	12°15'S, 129°20'E	Aug. 1998	29	1011 (870 – 1220)	60.28 (35.2 – 107.2)	-1.47 (-1.82 to -1.13)	-4.51 (-5.52 to -3.51)	28.85
		July 1999	35	945 (710 – 1230)	53.18 (28.9 – 107.5)	-1.60 (-2.14 to -1.01)	-4.54 (-5.18 to -3.88)	
Cape Van Diemen (CVD)	11°25'S, 130°10'E	Oct. 1999	30	942 (790 – 1300)	53.25 (34.8 – 94.1)	-1.54 (-1.80 to -1.12)	-4.48 (-5.70 to -3.83)	28.75
Kupang (KU)	10°20'S, 123°40'E	Apr. 1998	21	841 (425 – 1376)	28.72 (10.2 – 46.9)	-1.77 (-2.10 to -1.47)	-5.25 (-6.33 to -4.67)	28.63
		Sep. 1999	40	598 (350 – 970)	23.51 (10.0 – 53.0)	-1.84 (-2.07 to -1.10)	-5.55 (-6.38 to -4.43)	
		June 2000	17	895 (710 – 1170)	48.77 (31.0 – 76.9)	-1.64 (-1.81 to -1.35)	-5.11 (-5.78 to -4.04)	

Table 3.3. Summary of the sampling data, and results from the stable isotope analyses of the sagittal otolith carbonate of *Scomberomorus commerson* from locations in North Western Australia and Indonesia (SST = mean annual sea surface temperature [average of the 12-monthly means from 1991 to 2000])

Location (code)	Position (latitude, longitude)	Date of sampling	N	Fork Length (FL, mm) mean (range)	Otolith weight (mg) mean (range)	$\delta^{18}O$ (‰ PDB) mean (range)	$\delta^{13}C$ (‰ PDB) mean (range)	SST (°C)
Cape Wessel (CW)	11°15'S, 136°45'E	Nov. 1999	40	1074 (710 – 1320)	71.64 (28.2 – 119.8)	-1.44 (-1.93 to -1.07)	-4.38 (-5.71 to -3.54)	28.24
		Oct. 2000	36	989 (800 – 1350)	59.93 (36.1 – 121.6)	-1.60 (-2.00 to -1.15)	-4.71 (-5.68 to -3.99)	
Groote Eylandt (GE)	13°15'S, 136°45'E	Aug. 1998	36	1020 (700 – 1340)	62.45 (28.5 – 105.3)	-1.12 (-1.67 to -0.77)	-4.58 (-5.33 to -3.31)	28.04
		Aug. 1999	36	963 (550 – 1220)	54.31 (17.6 – 85.8)	-1.23 (-1.76 to -0.69)	-4.81 (-5.71 to -3.79)	
Sir Edward Pellew Group (SEPG)	14°15'S, 136°48'E	Oct. 1999	40	967 (800 – 1350)	54.02 (32.5 – 115.7)	-1.39 (-1.75 to -0.78)	-4.76 (-6.00 to -2.58)	27.87
Mornington Island (MI)	16°25'S, 140°24'E	Oct. 2000	40	1041 (770 – 1350)	47.95 (22.6 – 81.8)	-1.71 (-2.27 to -1.16)	-4.86 (-5.74 to -3.60)	27.43
Lost City (LC)	15°27'S, 141°11'E	July 1999	40	972 (690 – 1260)	54.45 (22.6 – 103.2)	-1.34 (-1.89 to -0.84)	-4.68 (-5.68 to -3.76)	27.60
Weipa (WE)	13°00'S, 141°15'E	Nov. 2000	40	944 (730 – 1260)	51.38 (30.8 – 89.0)	-1.71 (-2.23 to -1.26)	-4.94 (-5.99 to -3.96)	28.06
Torres Strait (TS)	9°10'S, 143°45'E	Sep. 1998	32	927 (550 – 1170)	58.51 (17.2 – 96.1)	-1.65 (-2.83 to -1.06)	-4.95 (-6.33 to -3.73)	27.40
		Oct. 1999	40	973 (800 – 1270)	60.23 (36.6 – 105.3)	-1.62 (-2.46 to -1.26)	-4.75 (-6.17 to -3.98)	
Cairns (CNS)	16°44'S, 146°06'E	Oct. 1998	29	889 (610 – 1240)	50.98 (24.1 – 100.8)	-1.04 (-1.53 to -0.60)	-4.29 (-5.48 to -2.53)	26.58
Townsville (TSV)	18°25'S, 146°40'E	Nov. 1999	36	967 (640 – 1320)	58.44 (28.8 – 124.4)	-1.00 (-1.34 to -0.54)	-4.45 (-5.27 to -3.82)	26.24

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 high purity (Milli-Q) water, air-dried (50°C) and powdered in an agate mortar and pestle. Powdered sagittae were deproteinated by treatment with hydrogen peroxide and analysed for $^{18}O/^{16}O$ and $^{13}C/^{12}C$ ratios by standard mass spectrometric techniques (CSIRO Division of Water Resources, Perth) after the carbonate was decomposed to CO_2 with 100% phosphoric acid. Stable isotope values are reported using the international standard delta (δ) notation relative to the PDB-1 standard for carbonates (i.e. $\delta^{18}O$ and $\delta^{13}C$).

Statistical analysis

Multivariate analysis of covariance (MANCOVA) of the stable isotope values of $\delta^{18}O$ and $\delta^{13}C$ were undertaken using otolith weight as a covariate in order to examine potential confounding temporal differences (between sampling dates) among samples in their otolith isotopic composition (α = 0.05). The two factors in the analysis were location and sampling date. The two sampling occasions were compared regardless of when they were sampled at each different locality. Thus, location and sampling date were treated as fixed and orthogonal factors in the analysis. Otolith weight is considered to be a proxy for age and therefore was used as a covariate in analyses to adjust treatment means and control error variance across the age range of fish sampled. In order to remove any confounding effects likely to be associated with a bias of young fish sampled at a particular location, to improve homogeneity and normality and to make treatment effects additive, only mature fish were included in the analysis (i.e. fish older than two years or with an otolith weight >= 45 mg).

One-way fixed effects analyses of variance (ANOVA) were undertaken in each subregion to interpret any significant differences in the $\delta^{18}O$ and $\delta^{13}C$ data detected by the MANOVA analyses. To further interpret any significant differences detected by the ANOVA's, the means of $\delta^{18}O$ and $\delta^{13}C$ values between adjacent locations across northern and western Australia were compared using Hotelling's multivariate T^2 test (Hotelling's T^2 test allows comparison of the within group variance matrices of both $\delta^{18}O$ and $\delta^{13}C$ values). The rationale for analysing adjacent locations in a nearest neighbour based approach was that if spatial differences were present among adjacent sites it would more clearly reflect spatial differences than comparison of widely separate sites.

An overall mean $\delta^{18}O$ value was calculated for mature fish from each location and plotted against the mean annual sea surface temperature (for the 10-year period from 1991 to 2000) for that location. The annual sea surface temperature (SST) for the years 1991 to 2000 were the average of the 12-monthly means in each of the 10 years obtained by the methods described by Reynolds and Smith (1994). SST values are assumed to approximate the water temperature where the fish reside. The relationship between $\delta^{18}O$ values and SST for *S. commerson* was compared with that derived for *Pristipomoides multidens* (Newman at al. 2000), a demersal species, in similar waters across northern and western Australia.

RESULTS

Locations sampled, sample numbers, collection dates and results of the $\delta^{18}O$ and $\delta^{13}C$ analyses of the sagittal carbonate are summarised in Table 3.1 – Table 3.3. Codes for each location are also described in Table 3.1 – Table 3.3. All δ18O values were plotted against δ13C values for each subregion and revealed grouping of values among locations (Figure 2.2 – Figure 2.5). Plots of the relationship between $\delta^{18}O$ values and $\delta^{13}C$ values against otolith weight (Figure 3.6 - Figure 3.13) for each subregion also showed location groupings. Moreover, both $\delta^{18}O$ and $\delta^{13}C$ values increase in a linear form with increasing otolith weight (Figure 2.6 – Figure 3.13).

MANCOVA of $\delta^{18}O$ and $\delta^{13}C$ values of adult *S. commerson* from those locations where temporal samples were obtained (Shark Bay, Kupang, Flat Top Bank, Cape Wessell, Groote Eylandt, Torres Strait, Townsville) across northern and western Australia indicated that there were no significant interactions between location sampled and the date of sampling (Table 3.4). In addition, there were no significant temporal differences observed in isotope values, thereby providing no evidence of any temporal variation in $\delta^{18}O$ and $\delta^{13}C$ values, although isotope values were significantly different among locations (Table 3.4). Univariate analyses showed the same results for both $\delta^{18}O$ and $\delta^{13}C$ revealed by ANCOVA (Table 3.5) with otolith weight a significant covariate. Given that there were no confounding temporal differences (between sampling dates) among samples in their otolith isotopic composition all subsequent analyses examined location effects only, using univariate ANOVA on all locations within each subregion.

ANCOVA of the $\delta^{18}O$ and $\delta^{13}C$ values from all *S. commerson* from Mid Western Australia in the selected otolith weight range showed that the location effect was highly significant (Table 3.6, Figure 3.14). CE samples were not significantly different from AI samples (T^2, $p > 0.95$). AI samples were significantly different to SB (T^2, $p < 0.05$). SB and EX (T^2, $p > 0.45$), and EX and TI (T^2, $p > 0.35$) were not significantly different, while TI was significantly different to PH (T^2, $p < 0.0001$). PH was significantly different to EMB (T^2, $p < 0.05$). Bi-variate plots of mean $\delta^{18}O$ and $\delta^{13}C$ values from each location in Mid Western Australia are illustrated in Figure 3.14. Results from all comparisons among locations using Hotelling's T^2 test are summarised in Table 3.10.

ANCOVA of the $\delta^{18}O$ and $\delta^{13}C$ values from all *S. commerson* from North Western Australia using the selected otolith weight data showed that the location effect was highly significant (Table 3.7, Figure 3.15). EMB samples were significantly different from LMS samples (T^2, $p < 0.0001$). LMS samples were not significantly different from BS samples (T^2, $p > 0.70$). BS samples were significantly different from WI samples (T^2, $p < 0.05$). WI samples were not significantly different from RR samples (T^2, $p > 0.25$). RR samples were significantly different to BSS (T^2, $p < 0.001$). BSS was significantly different to FB (T^2, $p < 0.0001$). FB was significantly different to FTB (T^2, $p < 0.05$). FTB was not significantly different to CVD (T^2, $p > 0.45$). Bi-variate plots of mean $\delta^{18}O$ and $\delta^{13}C$ values from each location in North Western Australia are illustrated in Figure 3.15. Both mainland Australian sites that were closest to the Indonesian sampling site had significantly different isotope values (BSS sig. diff. to KU, T^2, $p < 0.001$; CVD sig. diff. to KU, T^2, $p < 0.01$).

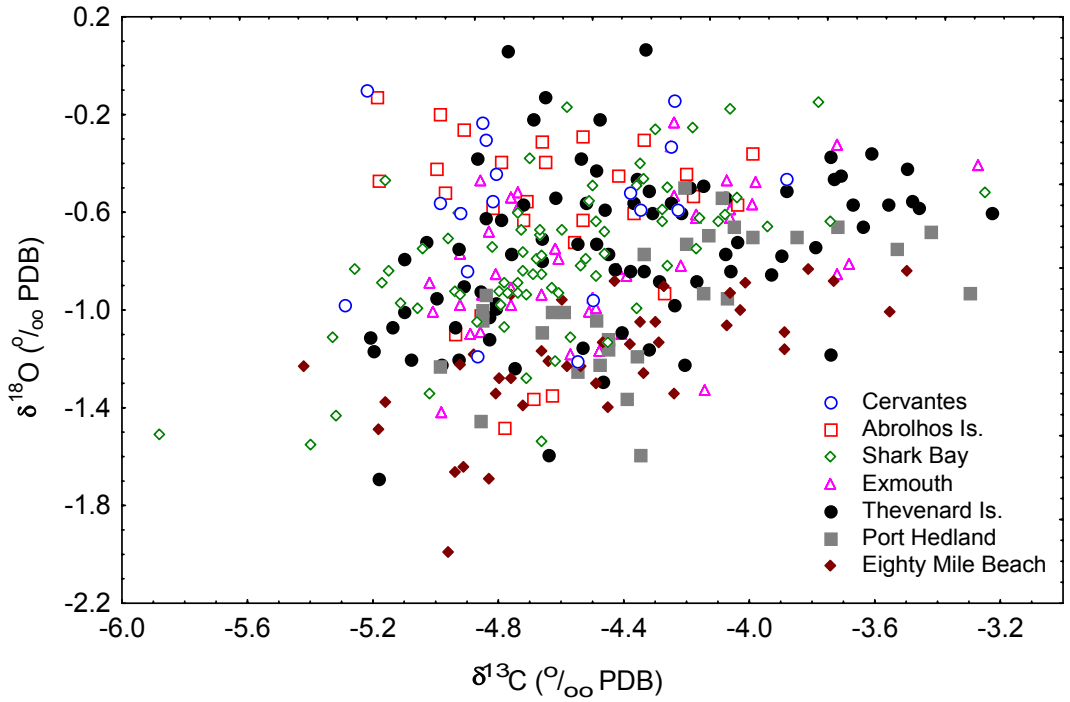

Figure 3.2. $\delta^{18}O$ values versus $\delta^{13}C$ values of *S. commerson* sagittal carbonate from all data for each location in Mid Western Australia

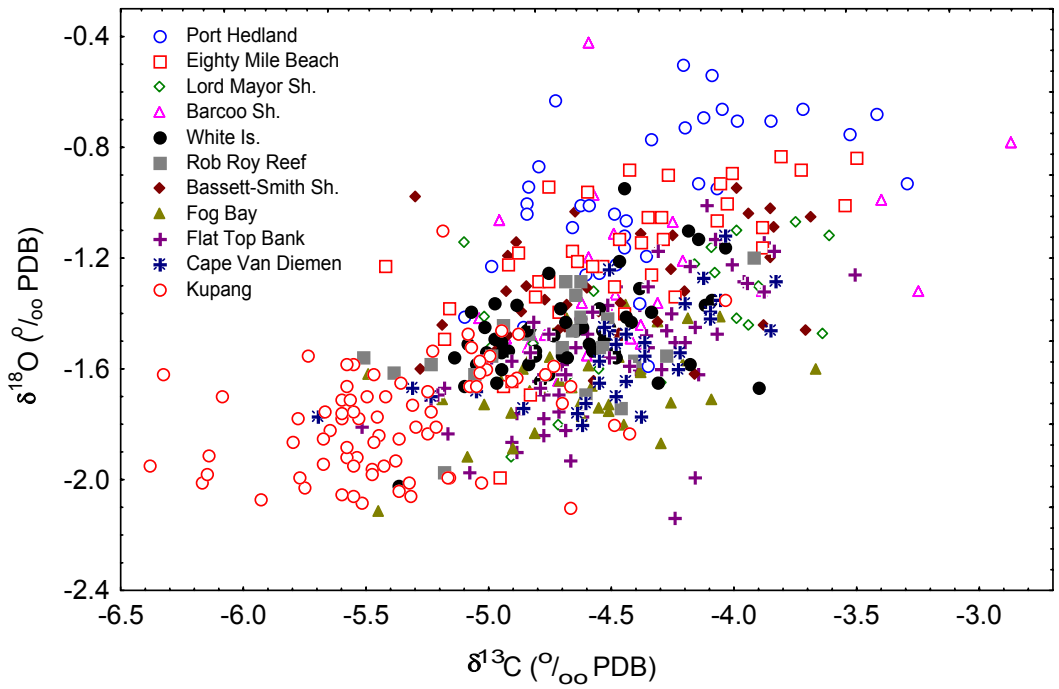

Figure 3.3. $\delta^{18}O$ values versus $\delta^{13}C$ values of *S. commerson* sagittal carbonate from all data for each location in North Western Australia

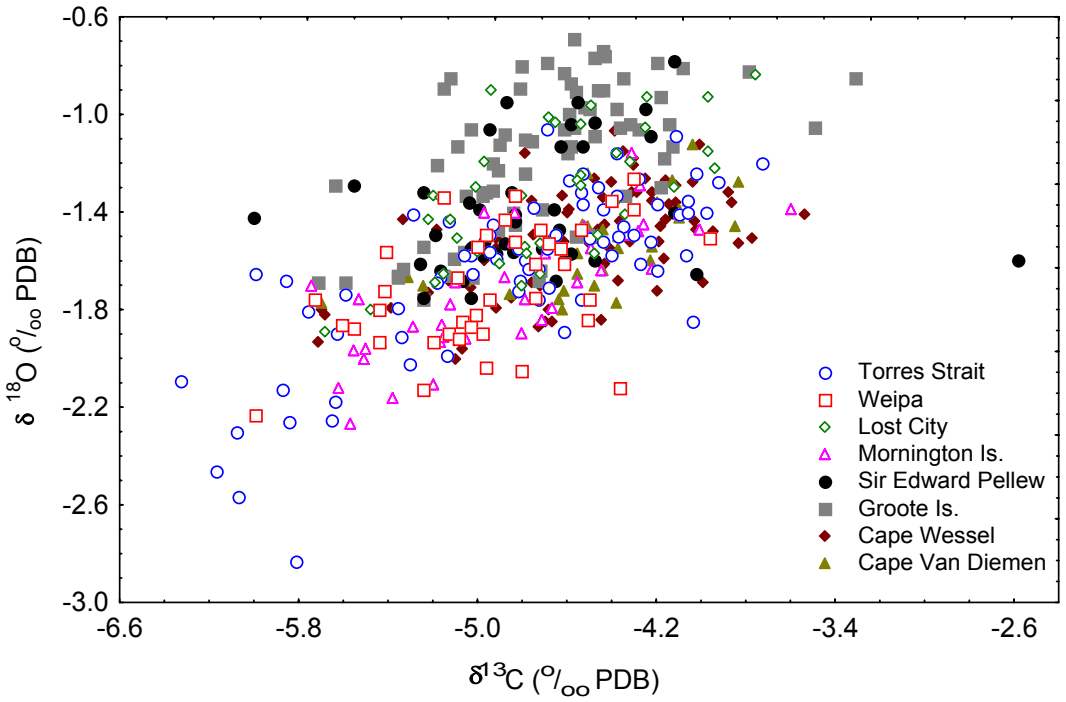

Figure 3.4 $\delta^{18}O$ values versus $\delta^{13}C$ values of *S. commerson* sagittal carbonate from all data for each location in Northern Australia and the Gulf of Carpentaria

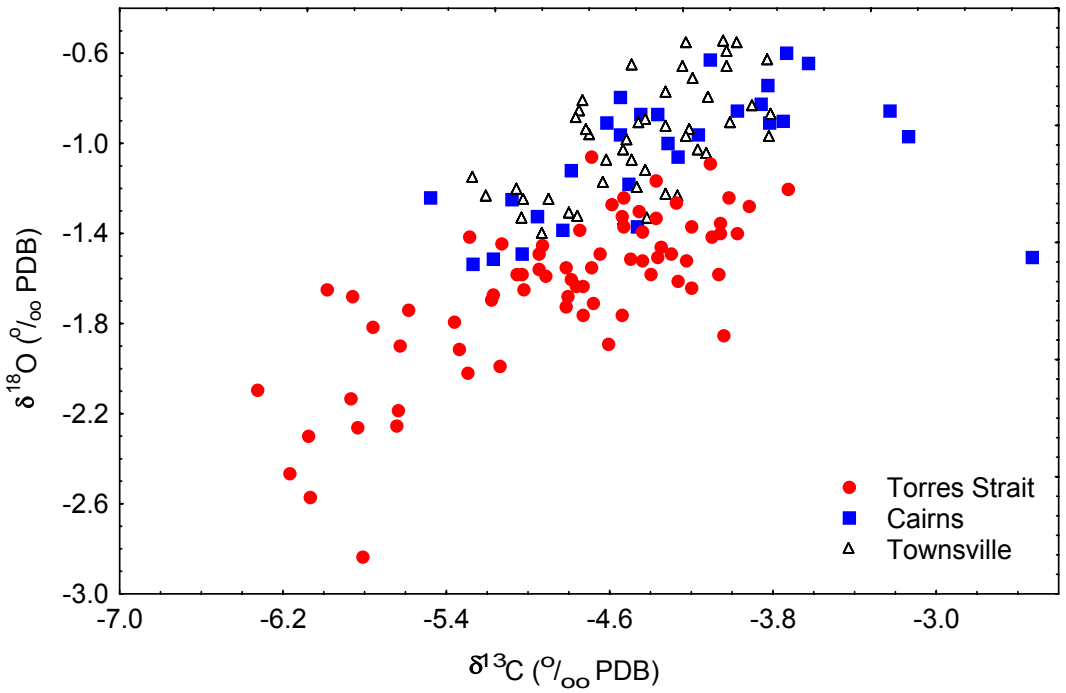

Figure 3.5. $\delta^{18}O$ values versus $\delta^{13}C$ values of *S. commerson* sagittal carbonate from all data for each location in Eastern Australia

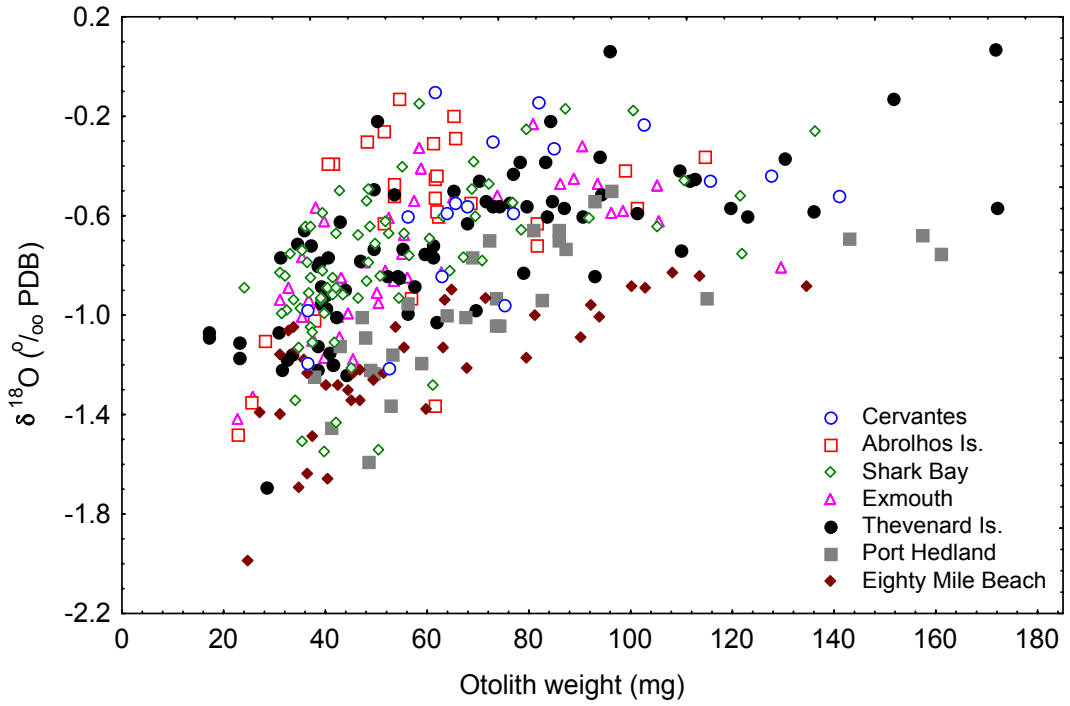

Figure 3.6. $\delta^{18}O$ values of *S. commerson* sagittal carbonate versus otolith weight from all data for Mid Western Australia

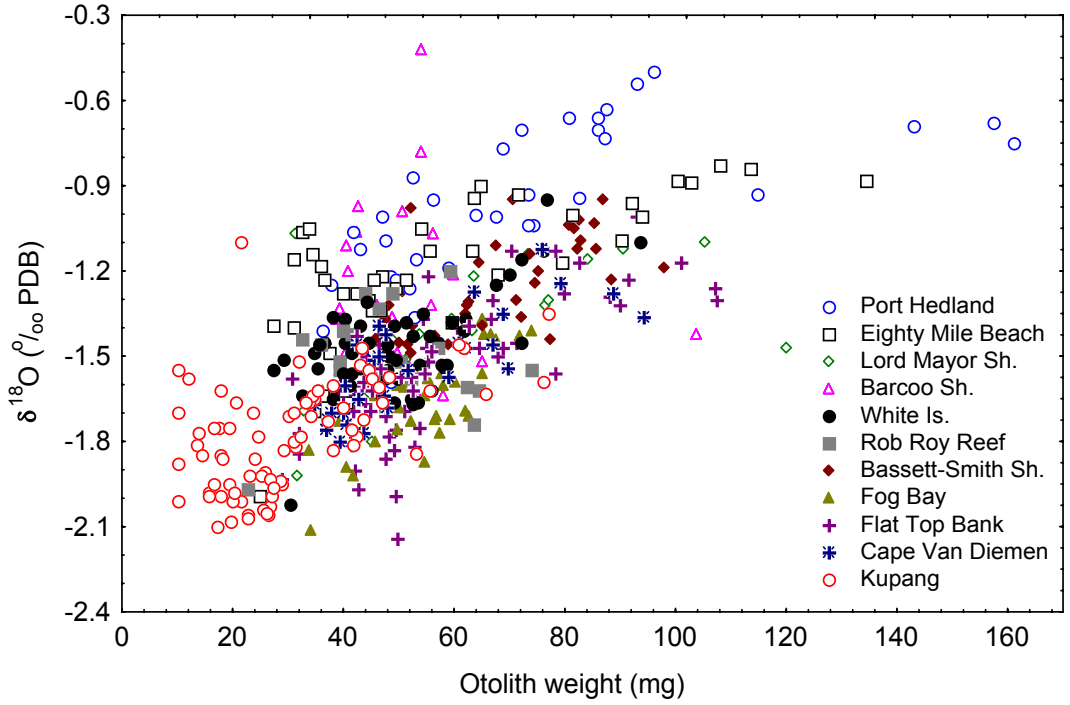

Figure 3.7. $\delta^{18}O$ values of *S. commerson* sagittal carbonate versus otolith weight from all data for North Western Australia

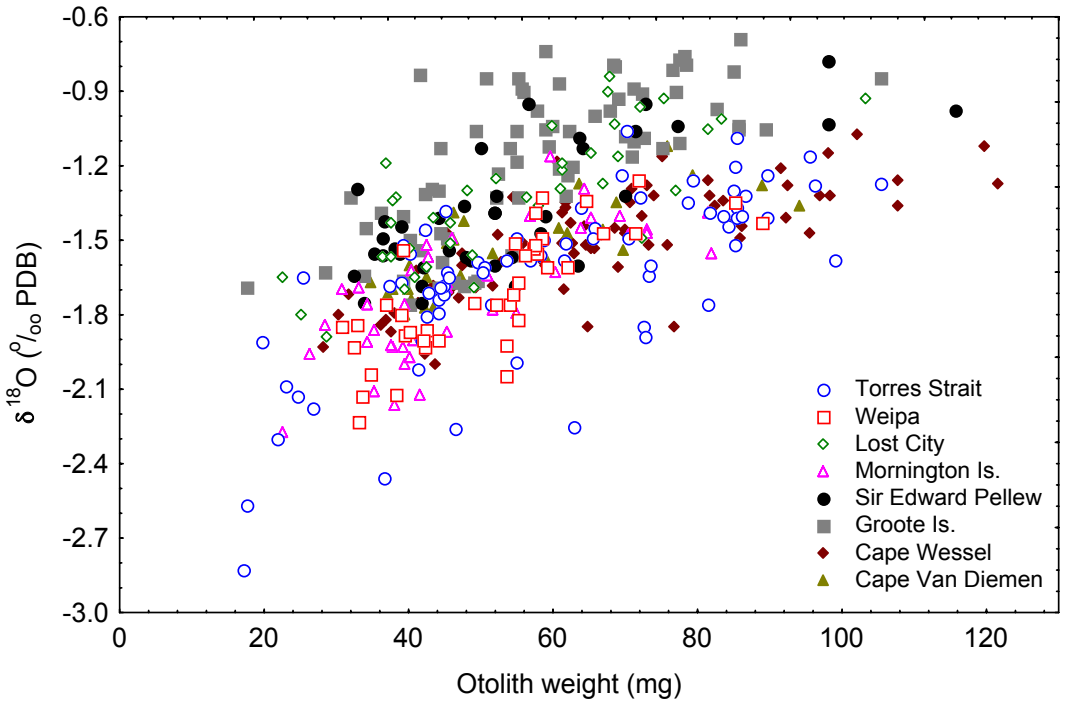

Figure 3.8. $\delta^{18}O$ values of *S. commerson* sagittal carbonate versus otolith weight from all data for Northern Australia and the Gulf of Carpentaria

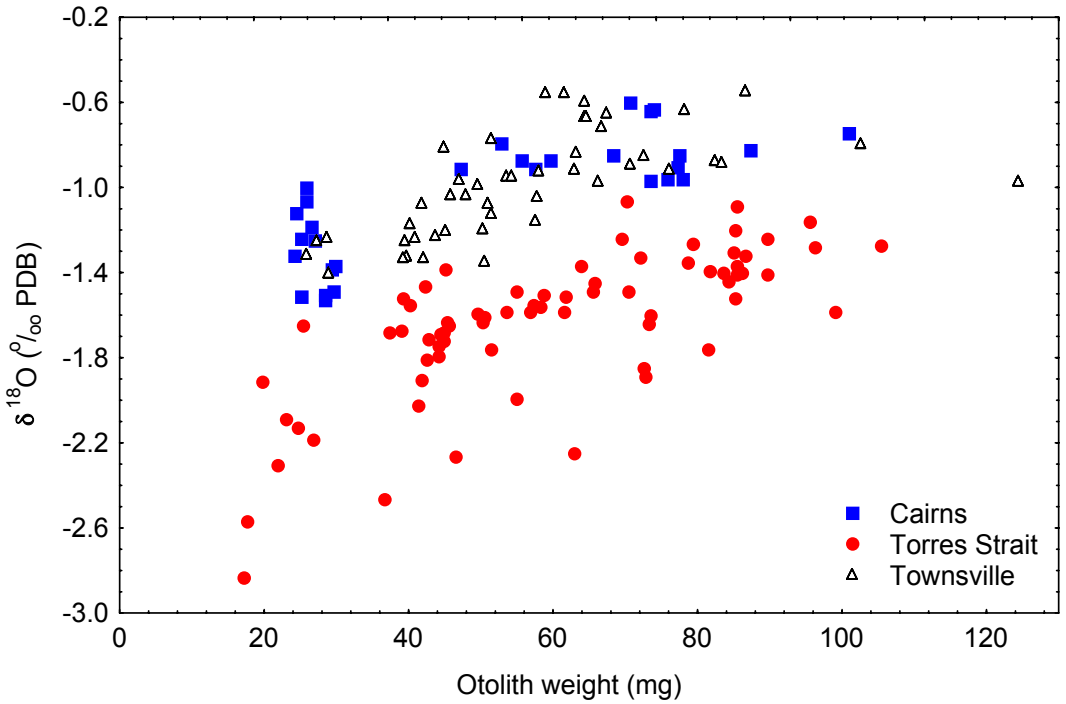

Figure 3.9. $\delta^{18}O$ values of *S. commerson* sagittal carbonate versus otolith weight from all data for Eastern Australia

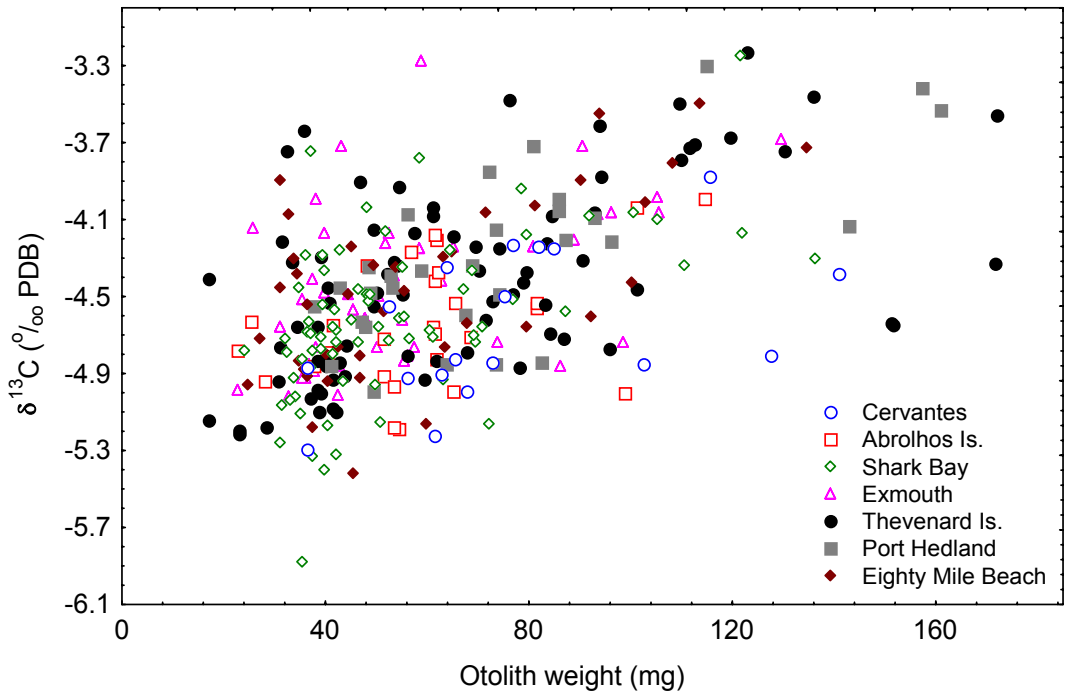

Figure 3.10. $\delta^{13}C$ values of *S. commerson* sagittal carbonate versus otolith weight from all data for Mid Western Australia

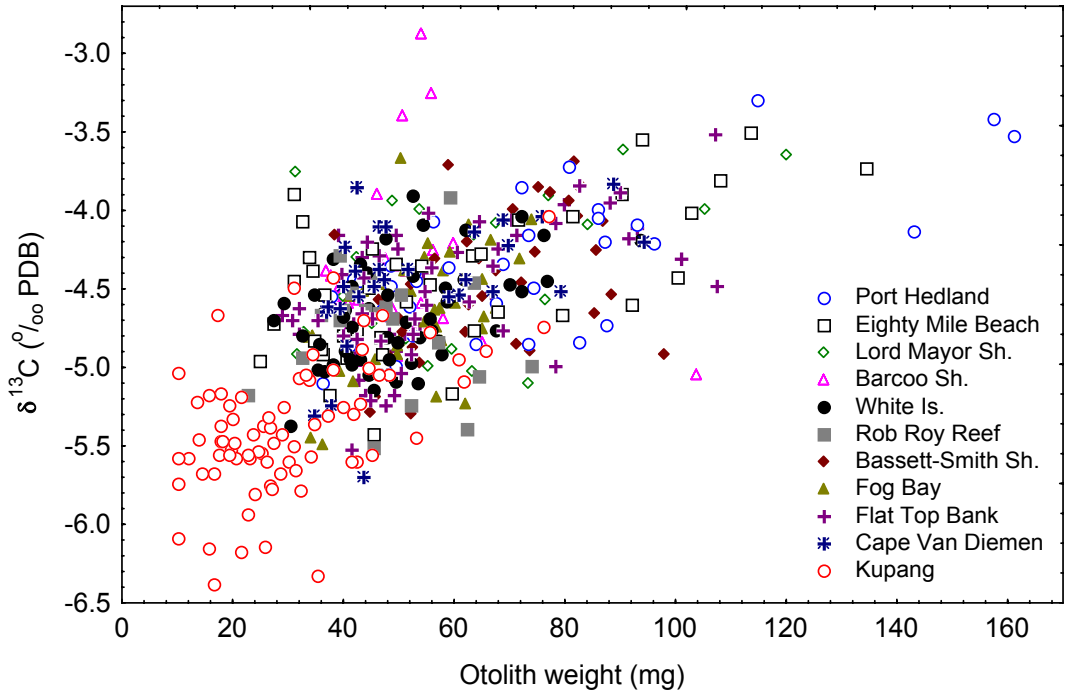

Figure 3.11. $\delta^{13}C$ values of *S. commerson* sagittal carbonate versus otolith weight from all data for North Western Australia

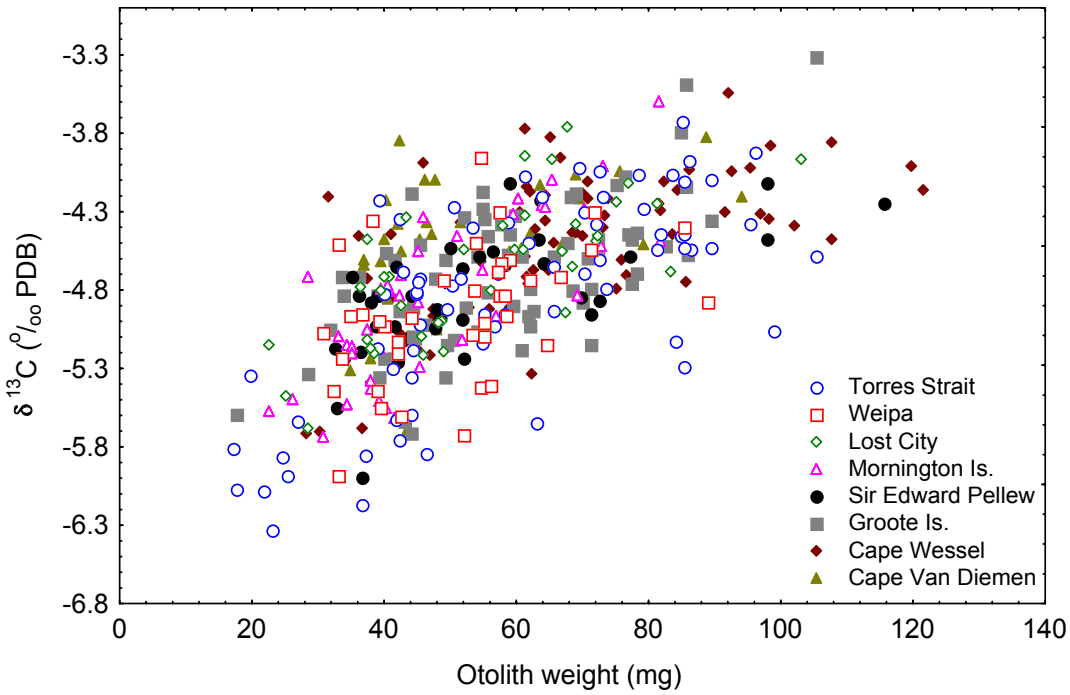

Figure 3.12. $\delta^{13}C$ values of *S. commerson* sagittal carbonate versus otolith weight from all data for Northern Australia and the Gulf of Carpentaria

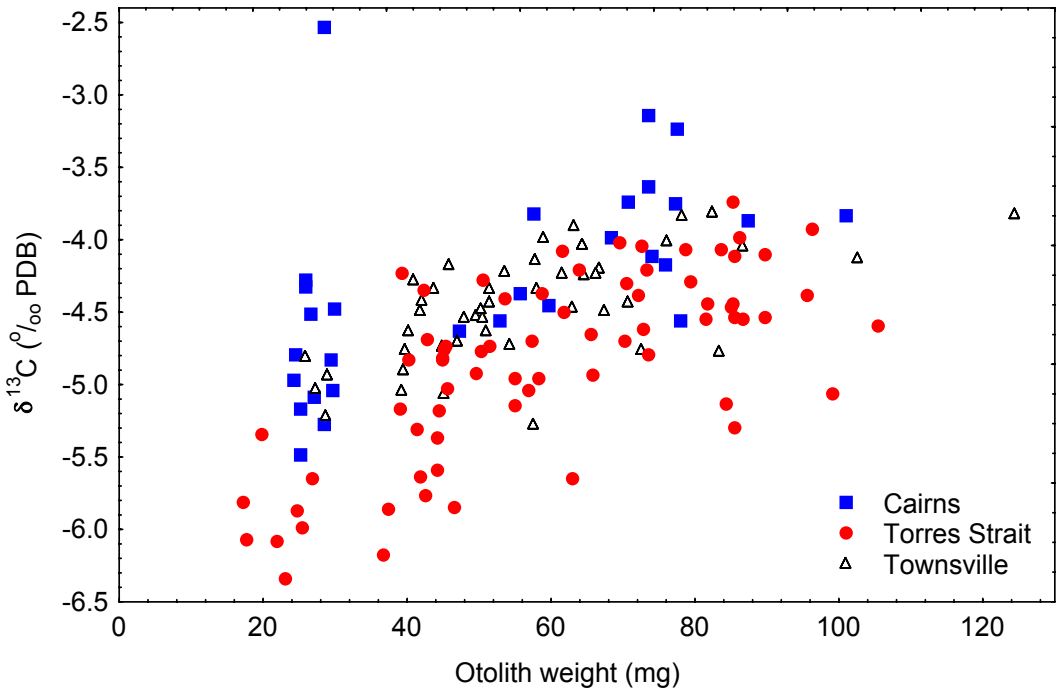

Figure 3.13. $\delta^{13}C$ values of *S. commerson* sagittal carbonate versus otolith weight from all data for Eastern Australia

ANCOVA of the $\delta^{18}O$ and $\delta^{13}C$ values from all *S. commerson* from Northern Australia and the Gulf of Carpentaria using the selected otolith weight data showed that the location effect was highly significant (Table 3.8, Figure 3.16). CVD samples were not significantly different from CW samples (T^2, $p > 0.55$). CW samples were significantly different to TS (T^2, $p < 0.05$). CW samples were significantly different to GE (T^2, $p < 0.0001$). GE samples were significantly different to SEPG (T^2, $p < 0.01$). SEPG samples were significantly different to MI (T^2, $p < 0.01$). MI samples were significantly different to LC (T^2, $p < 0.0001$). LC samples were significantly different to WE (T^2, $p < 0.0001$). WE samples were not significantly different to TS (T^2, $p > 0.20$). Bi-variate plots of mean $\delta^{18}O$ and $\delta^{13}C$ values from each location in Northern Australia and the Gulf of Carpentaria are illustrated in Figure 3.16.

ANCOVA of the $\delta^{18}O$ and $\delta^{13}C$ values from all *S. commerson* from Eastern Australia using the selected otolith weight data showed that the location effect was highly significant (Table 3.9, Figure 3.17). TS samples were significantly different from CNS samples (T^2, $p < 0.0001$). CNS samples were significantly different to TSV samples (T^2, $p < 0.02$). Bi-variate plots of $\delta^{18}O$ and $\delta^{13}C$ values from each location in Eastern Australia are illustrated in Figure 3.17.

Table 3.4. MANCOVA results of temporal and spatial comparisons of $\delta^{18}O$ and $\delta^{13}C$ values from the sagittal otolith carbonate of *Scomberomorus commerson* from locations across Northern and Western Australia (adult fish only, otolith weight greater than or equal to 45 mg)

Source	df	Wilks' λ	F	p
Location	12, 546	0.2253	50.354	< 0.0001
Date	2, 273	0.9855	2.003	0.137
Location × Date	12, 546	0.9510	1.15	0.311
Otolith weight	2, 273	0.6366	77.938	< 0.0001

Note: Wilks' λ can assume values in the range of 0 (perfect discrimination) to 1 (no discrimination).

Table 3.5. ANCOVA results of temporal and spatial comparisons of $\delta^{18}O$ and $\delta^{13}C$ values from the sagittal otolith carbonate of *Scomberomorus commerson* from locations across Northern and Western Australia (adult fish only, otolith weight greater than or equal to 45 mg)

	Source	df	MS	F	p
δ18O	Location	6	5.5571	136.09	< 0.0001
	Date	1	0.0954	2.34	0.128
	Location × Date	6	0.0435	1.07	0.383
	Otolith weight	1	4.4915	109.99	< 0.0001
	Residual (Error)	274	0.0408		
δ13C	Location	6	0.4817	3.88	< 0.001
	Date	1	0.3448	2.77	0.097
	Location × Date	6	0.1549	1.25	0.283
	Otolith weight	1	11.0216	88.67	< 0.0001

	Residual (Error)	274	0.1243

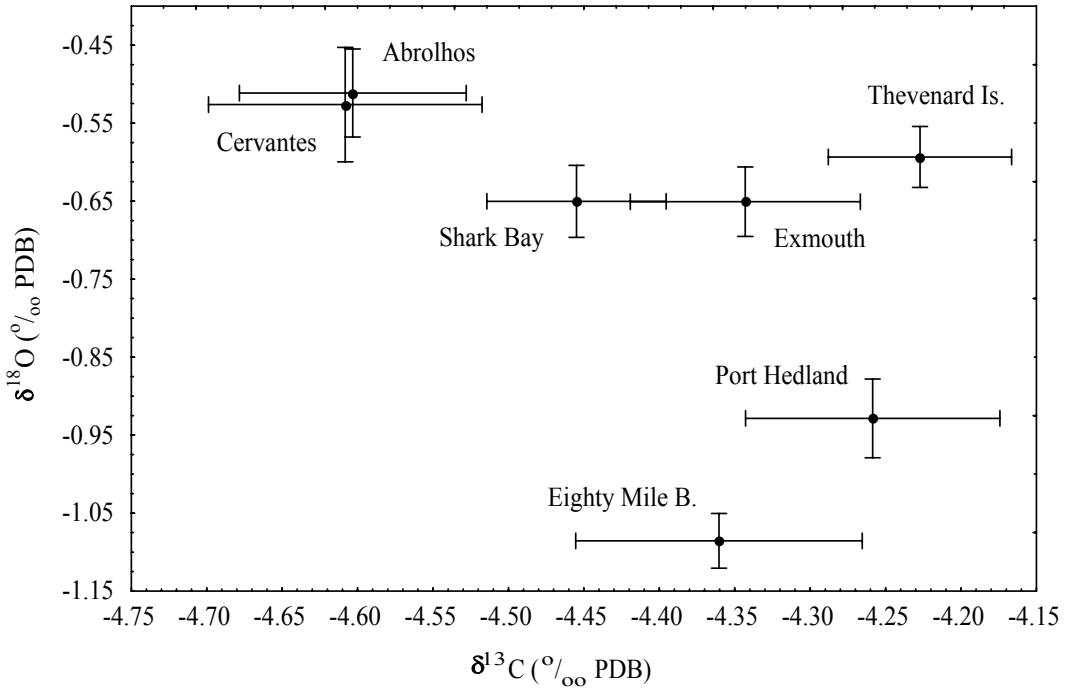

Figure 3.14. Bi-variate plots of mean $\delta^{18}O$ and mean $\delta^{13}C$ values (± standard error) of *S. commerson* sagittal carbonate for each location in Mid Western Australia (selected data; otolith weight >= 45 mg)

Table 3.6. ANCOVA results of $\delta^{18}O$ and $\delta^{13}C$ values from the sagittal otolith carbonate of *Scomberomorus commerson* from locations in Mid Western Australia (adult fish only, otolith weight greater than or equal to 45 mg)

	Source	df	SS	MS	F	p
$\delta^{18}O$	Location	6	7.117	1.186	20.292	< 0.0001
	Otolith weight	1	2.022	2.022	34.591	< 0.0001
	Residual (Error)	198	11.573	0.058		
	Total	205	20.718			
$\delta^{13}C$	Location	6	2.488	0.415	3.222	< 0.01
	Otolith weight	1	7.868	7.868	61.145	< 0.0001
	Residual (Error)	198	25.477	0.129		
	Total	205	37.100			

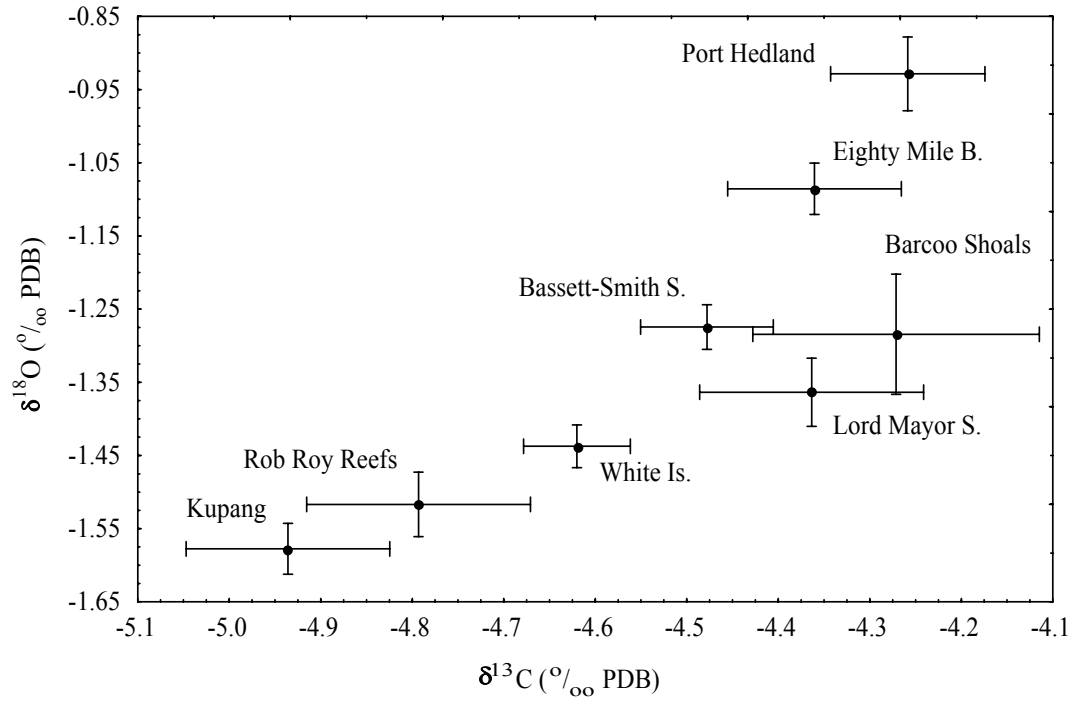

Figure 3.15. Bi-variate plots of mean $\delta^{18}O$ and mean $\delta^{13}C$ values (± standard error) of *S. commerson* sagittal carbonate for each location in North Western Australia (selected data; otolith weight >= 45 mg)

Table 3.7. ANCOVA results of $\delta^{18}O$ and $\delta^{13}C$ values from the sagittal otolith carbonate of *Scomberomorus commerson* from locations in North Western Australia (adult fish only, otolith weight greater than or equal to 45 mg)

	Source	df	SS	MS	F	p
$\delta^{18}O$	Location	10	7.188	0.719	25.291	< 0.0001
	Otolith weight	1	3.531	3.531	124.250	< 0.0001
	Residual (Error)	268	7.617	0.028		
	Total	279	22.953			
$\delta^{13}C$	Location	10	4.413	0.441	3.171	< 0.001
	Otolith weight	1	10.797	10.797	77.584	< 0.0001
	Residual (Error)	268	37.297	0.139		
	Total	279	55.348			

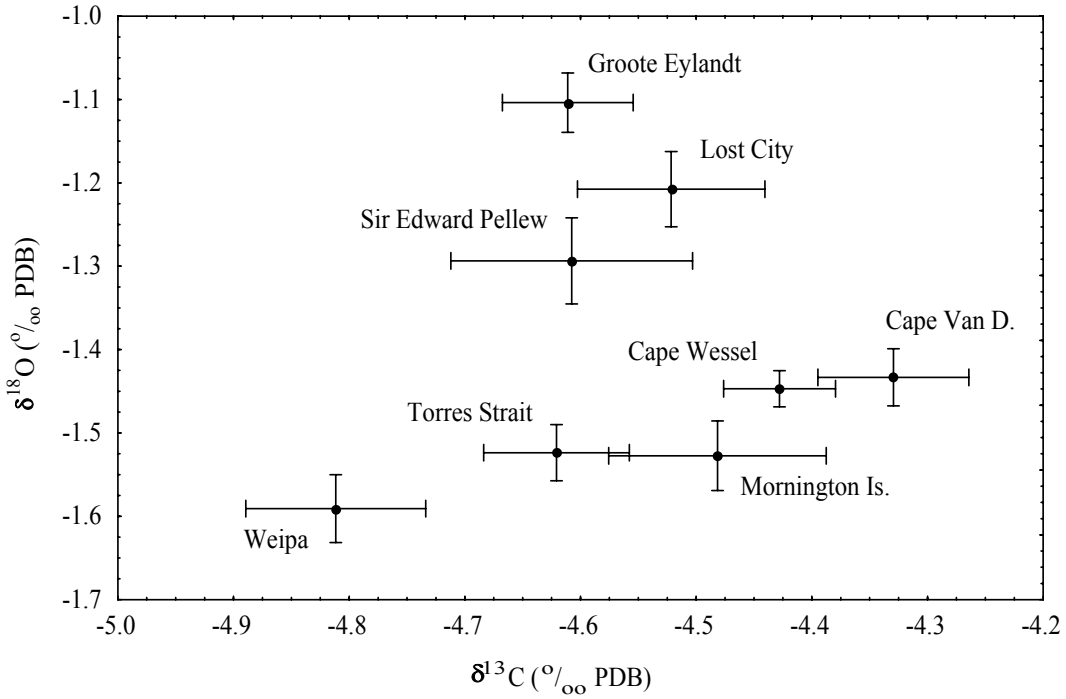

Figure 3.16. Bi-variate plots of mean $\delta^{18}O$ and mean $\delta^{13}C$ values (± standard error) of *S. commerson* sagittal carbonate for each location in Northern Australia and the Gulf of Carpentaria (selected data; otolith weight >= 45 mg)

Table 3.8. ANCOVA results of $\delta^{18}O$ and $\delta^{13}C$ values from the sagittal otolith carbonate of *Scomberomorus commerson* from locations in Northern Australia and Gulf of Carpentaria (adult fish only, otolith weight greater than or equal to 45 mg)

	Source	df	SS	MS	F	p
$\delta^{18}O$	Location	7	9.327	1.333	40.899	< 0.0001
	Otolith weight	1	4.934	4.934	151.451	< 0.0001
	Residual (Error)	277	9.025	0.033		
	Total	285	22.273			
$\delta^{13}C$	Location	7	3.288	0.470	3.417	< 0.01
	Otolith weight	1	11.134	11.134	81.004	< 0.0001
	Residual (Error)	277	38.074	0.138		
	Total	285	53.386			

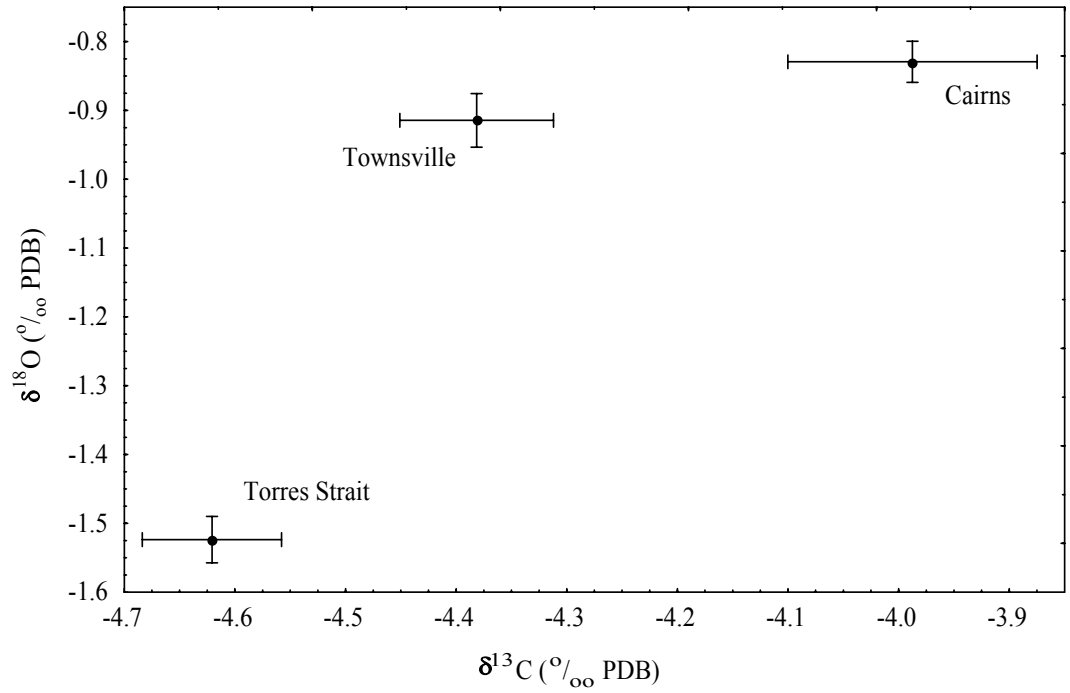

Figure 3.17. Bi-variate plots of mean $\delta^{18}O$ and mean $\delta^{13}C$ values (± standard error) of *S. commerson* sagittal carbonate for each location in Eastern Australia (selected data; otolith weight >= 45 mg)

Table 3.9. ANCOVA results of $\delta^{18}O$ and $\delta^{13}C$ values from the sagittal otolith carbonate of *Scomberomorus commerson* from locations in Eastern Australia (adult fish only, otolith weight greater than or equal to 45 mg)

	Source	df	SS	MS	F	p
$\delta^{18}O$	Location	2	10.157	5.079	136.079	< 0.0001
	Otolith weight	1	1.058	1.058	28.341	< 0.0001
	Residual (Error)	93	3.471	.0373		
	Total	96	14.434			
$\delta^{13}C$	Location	2	4.998	2.499	16.855	< 0.0001
	Otolith weight	1	3.983	3.983	26.863	< 0.0001
	Residual (Error)	93	13.788	0.148		
	Total	96	22.895			

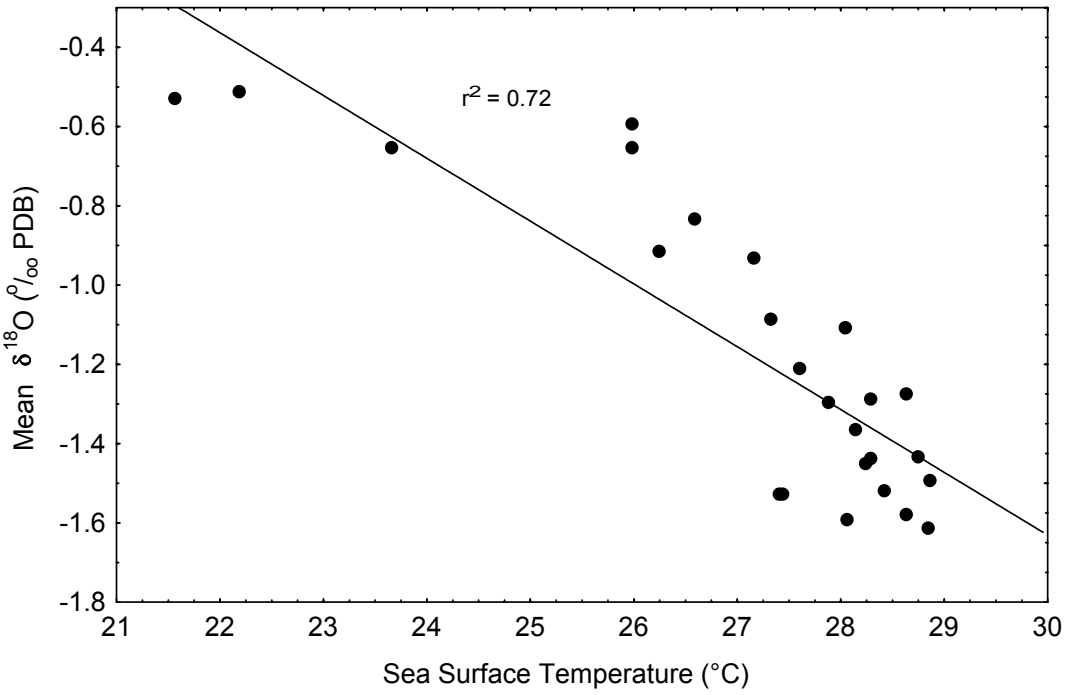

Figure 3.18. Mean $\delta^{18}O$ values of *S. commerson* sagittal otolith carbonate for each location (otolith weight >= 45 mg) versus mean sea surface temperatures from 1991 to 2000 for each location

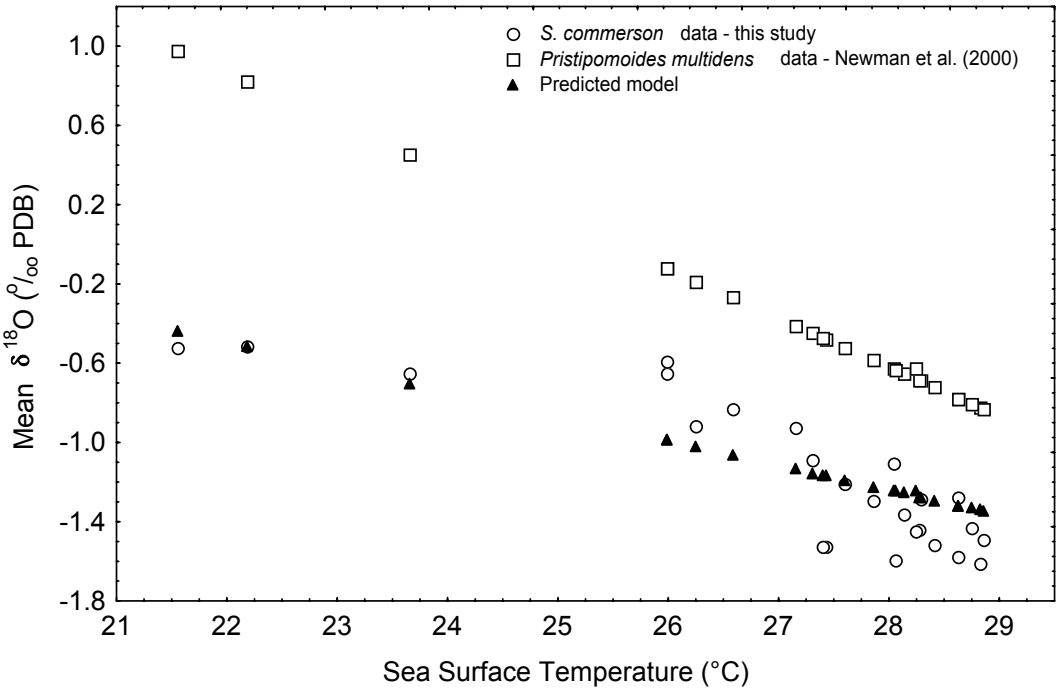

Figure 3.19. Relationships between mean $\delta^{18}O$ values and SST for *S. commerson* (this study) and *Pristipomoides multidens* (Newman et al. 2000) with the incorporation of a predictive model

Mean $\delta^{18}O$ values for each location were plotted against the mean of the annual sea surface temperatures from 1991 to 2000 for each location (Figure 3.18). The relationship between $\delta^{18}O$ values and SST was linear, with an $r^2 = 0.72$. Comparison of these data with the relationship between $\delta^{18}O$ values and SST derived for *P. multidens* ($\delta^{18}O = 6.3088 – [0.24745 \times SST]$; Newman et al. 2000) revealed that the *S. commerson* $\delta^{18}O$ values were on average lower (more depleted) for a given SST (Figure 3.19). These lower values are out of the expected range. In addition, the slopes of each relationship were somewhat different, suggesting that the observed $\delta^{18}O$ values in *S. commerson* may be reflecting a combination of internal body temperature and ambient seawater temperature whereas in *P. multidens* $\delta^{18}O$ values reflect only ambient seawater temperature. To investigate this hypothesis, a predictive model encompassing $\delta^{18}O$ values and a combination of SST values and a constant temperature value was formulated from the *P. multidens* equation. The predictive model considered that the $\delta^{18}O$ values present in *S. commerson* sagittal otoliths were derived from an equal combination of water temperature (as measured by SST) and a constant internal body temperature. The resulting predictive model that provided the best relationship with the observed data incorporated a constant internal body temperature (BT) of 33°C and is described by the equation: $\delta^{18}O = 6.3088 – (0.24745 \times \{[SST \times 0.5] + [BT \times 0.5]\})$; where BT = 33°C (Figure 3.19).

DISCUSSION

There is a large and consistent difference in $\delta^{18}O$ and $\delta^{13}C$ values with increasing otolith weight that ranges across the distribution of *S. commerson* in the study (Figure 3.6 – Figure **3.13**). Both the $\delta^{18}O$ and $\delta^{13}C$ values have a similar linear relationship with otolith weight that indicates the effect may be metabolic. Therefore, smaller *S. commerson* may have a higher metabolic rate and consequently either have a higher internal body temperature or are more active (resulting in lower or depleted $\delta^{18}O$ and $\delta^{13}C$), than larger *S. commerson* (higher $\delta^{18}O$ and $\delta^{13}C$), thus the relationship between $\delta^{18}O$ and $\delta^{13}C$ values with increasing otolith weight is linear as metabolic activity changes (Figure 3.6 – Figure **3.13**). Similar trends in $\delta^{18}O$ and $\delta^{13}C$ values with increasing otolith weight are evident in demersal reef fish such as *Pristipomoides multidens* (Newman et al. 2000), *Lutjanus sebae* and *Epinephelus multinotatus* (Stephenson et al. 2001), though they are not as pronounced. In order to remove any potential bias in comparison among locations with a preponderance of smaller fish, the otoliths weight range analysed was restricted so that only adult fish were compared. This procedure adjusted treatment means across the age range of fish sampled and prevented any confounding effects.

Metabolic rate is an important consideration in understanding the observed $\delta^{18}O$ values in *S. commerson* relative to other marine species. The predictive model indicates that the internal body temperature of *S. commerson* is maintained at or close to 33°C, warmer than ambient seawater. In order to maintain an internal body temperature above that of ambient seawater, both heat reduction and heat retention systems are required. These systems are not known

within the Scomberomorini (Collette and Nauen 1983). However, in the related tuna tribe Thunini, all four genera possess counter-current heat exchange systems that allow the retention of metabolic heat above that of ambient seawater (Collette and Nauen 1983). The large body size and locomotory style of *S. commerson* suggest that convective and conductive heat exchange may be present to some degree. However, the physiological mechanisms that may facilitate the maintenance of the internal body temperature in *S. commerson* remain to be elucidated.

Otolith $\delta^{18}O$ values reported in this study represent a mean value integrated over the entire ontogenetic life history of each individual fish, which may range in age from two to 22 years. Variation in $\delta^{18}O$ values reflected differences among locations that were related to the relative differences in ambient seawater temperature across northern and western Australia. The lowest $\delta^{18}O$ values corresponded to the highest mean water temperatures that were present in fish from Fog Bay, Flat Top Bank and Cape Van Diemen (Figure 3.19). Conversely, the highest $\delta^{18}O$ values corresponded to the lowest mean water temperatures that were present in fish from Cervantes and the Abrolhos Islands (Figure 3.19). Thus, the relationship between sea surface temperature and $\delta^{18}O$ values was linear (Figure 3.19). Once metabolic effects are taken into account, the stable oxygen isotopes of otolith carbonate in *S. commerson* are deposited in equilibrium with ambient conditions within the fish.

Variations in $\delta^{13}C$ values of *S. commerson* across all regions were more attributable to otolith weight than the location of sampling. This suggests that $\delta^{13}C$ values are dependent to a large degree on the metabolic rate (see also Kalish 1991b). However, $\delta^{13}C$ represents a combination of carbon sources and is derived principally from dissolved inorganic carbon (~80%) in addition to metabolic carbon and may vary as a consequence of diet, temperature and physiology (Weidman and Millner 2000).

The isotope signatures of *S. commerson* among regions were significantly different for a number of locations, indicating that adult *S. commerson* remain resident within specific home ranges that reflect different environmental conditions (e.g. ambient temperature, prey sources). If adult fish were mixing among locations then isotopic signatures amongst locations would be similar (Edmonds et al. 1999). Adult *S. commerson* populations comprise spatially distinct and non-mixing assemblages and thus can be considered as independent management units or stocks for the purposes of fisheries management.

The $\delta^{18}O$ and $\delta^{13}C$ data from fish collected near Kupang (Indonesia), demonstrated that those fish were significantly different from fish collected from the nearest mainland Australia locations. Newman et al. (2000) found a similar difference between Australia and Indonesia in the deepwater demersal fish, *Pristipomoides multidens*, and suggested that the Timor Trench (~ 2000 m deep) is an effective barrier to the movement of adult fish between the continental shelf waters of Australia and the islands of Indonesia. The results of this study also indicate that the Timor Trench provides an effective barrier to the movement of adult *S. commerson* between the continental shelf waters of Australia and the islands of Indonesia. However, it is not known whether adult populations of *S. commerson* in southern Indonesia and Papua New Guinea that have no deepwater barrier separating them from Australian

continental shelf waters are significantly different from the northern Australian populations of *S. commerson* (samples could not be obtained from these areas to test this hypothesis). Given the separation of adult assemblages within the Gulf of Carpentaria, there is a low probability of connectedness; however this assumption remains to be tested.

The stable isotope composition of fish otoliths provides a record of the environmental variation that a fish experiences throughout its life history, such as changes in temperature, depth, habitat and diet (Kalish 1991a,b, Schwarcz at al. 1998). Significant differences in the stable isotopic composition of whole otoliths between geographically separate groups of fish indicates that distinct environmental conditions are occupied throughout their life history, thus providing an indirect measure of population subdivision or 'stock' separation (Campana 1999, Edmonds et al. 1999, Newman et al. 2000). Moreover, since the results from the stable isotope analyses of otoliths from different regions provide no evidence of a latitudinal cline (that is, *S. commerson* populations do not form part of a continuous distribution in which the otoliths undergo a progressive linear change in isotope values, modified by latitudinal environmental variation, with the regions sufficiently far apart for these characteristics to be significantly different), it is evident that *S. commerson* form discrete management units and occupy a distinct location for most of their life history. This does not preclude the possibility of some genetic exchange among adult management units.

The significant differences in isotopic values between samples from different geographic locations in this study indicate that variable metabolic and environmental conditions were experienced by *S. commerson* throughout their spatial distribution. In addition, this study has provided further evidence that measurement of stable isotope ratios in teleost sagittal carbonate is a valuable tool in discerning fishery management units of adult fish where the range of distribution of the species includes waters with variable temperature regimes.

The population units defined by otolith stable isotope analysis infer that the level of mixing within populations of *S. commerson* is variable among regions. For example, in the cooler southern waters off Western Australia at the limit of their distribution population units of *S. commerson* range over large distances (ranging over approximately 300 km), while in the more northern locations population units of *S. commerson* range over much smaller distances (approximately 100 km; Table 3.10). The variable distance over which adult population units of *S. commerson* range is likely to be a reflection of habitats optimal for growth and survival and hence availability of prey items.

FISHERY MANAGEMENT IMPLICATIONS

Oxygen and carbon isotope values from the whole otolith analysis of *S. commerson* in this study have shown distinct location-specific spatial signatures, which indicates the presence of multiple functionally distinct independent management units (FDIMU) of *S. commerson* across western and northern Australia. These location-specific spatial signatures were persistent through time for a number of widespread locations and thus a multi-FDIMU

Table 3.10. Comparison of the means of $\delta^{18}O$ and $\delta^{13}C$ values from the sagittal otolith carbonate of *Scomberomorus commerson* between adjacent locations across Northern and Western Australia using Hotelling's T^2 test (Hotelling's T^2 test is based on the within group variance matrices of both $\delta^{18}O$ and $\delta^{13}C$ values; locations codes are described in Table 3.1 – Table 3.3)

Groups	T^2	df	F	p	Distance (km)
Mid Western Australia					
CE vs AI	0.031	2, 35	0.015	0.9849	290
AI vs SB	6.96	2, 58	3.42	< 0.0395	375
SB vs EX	1.58	2, 63	0.78	0.4636	425
EX vs TI	1.90	2, 74	0.94	0.3957	85
TI vs PH	27.29	2, 74	13.46	< 0.0001	510
PH vs EMB	6.80	2, 49	3.33	< 0.0439	150
North Western Australia					
EMB vs LMS	31.93	2, 39	15.57	< 0.0001	395
LMS vs BS	0.74	2, 30	0.36	0.7022	100
BS vs WI	7.85	2, 48	3.85	< 0.0282	215
WI vs RR	2.84	2, 45	1.39	0.2592	75
RR vs BSS	17.66	2, 48	8.65	< 0.001	165
BSS vs KU	28.59	2, 47	14.00	< 0.0001	400
BSS vs FB	77.79	2, 66	38.31	< 0.0001	475
FB vs FTB	6.48	2, 73	3.20	< 0.0466	125
FTB vs CVD	1.63	2, 60	0.80	0.4529	130
CVD vs KU	25.32	2, 27	12.21	< 0.001	700
Northern Australia and Gulf of Carpentaria					
CVD vs CW	1.05	2, 77	0.52	0.5977	730
CW vs TS	6.76	2, 113	3.35	< 0.0386	780
CW vs GE	117.52	2, 117	58.26	< 0.0001	220
GE vs SEPG	10.26	2, 80	5.07	< 0.0085	110
SEPG vs MI	13.94	2, 41	6.80	< 0.0028	470
MI vs LC	40.06	2, 41	19.56	< 0.0001	140
LC vs WE	40.03	2, 47	19.60	< 0.0001	270
WE vs TS	3.25	2, 76	1.61	0.2076	510
Eastern Australia					
TS vs CNS	118.43	2, 67	58.35	< 0.0001	880
CNS vs TSV	10.16	2, 40	4.95	< 0.0120	200

complex of *S. commerson* can be discerned across western and northern Australia. These locations can be separated into a number of distinct stocks or FDIMU's; (1) Cervantes-Abrolhos Islands; (2) Shark Bay-Exmouth-Thevenard Island; (3) Port Hedland; (4) Eighty Mile Beach; (5) Lord Mayor Shoal-Barcoo Shoal; (6) White Island-Rob Roy Reef; (7) Bassett-Smith Shoal; (8) Fog Bay; (9) Flat Top Bank-Cape Van Diemen-Cape Wessel; (10) Groote Eylandt; (11) Sir Edward Pellew Group; (12) Mornington Island; (13) Lost City; (14) Weipa-Torres Strait; (15) Cairns; (16) Townsville and (17) Kupang, Indonesia. The results of this study do not support the likelihood of extensive longshore migratory patterns in this species. This is in contrast to earlier reported studies (e.g. Lewis 1981). However, Collette and Nauen (1983) reported the likely presence of permanent resident populations of *S. commerson*.

Further subdivision may be revealed between Cape Van Diemen and Cape Wessel. These locations are over 700 km apart and are similar in latitude and may also encounter similar environmental conditions from the nearby Arafura Sea region. Therefore, the possibility exists that the lack of contrast in environmental variables between these widespread locations may have resulted in a null difference when the locations are in fact different, given that comparisons among other locations reveal separation on a much finer spatial scale. In this context the precautionary management approach should be to consider these locations as functionally distinct. Analysis of material from locations between these widespread areas would be necessary to determine the extent of partitioning.

Allozyme and mitochondrial DNA (mtDNA) analyses of *S. commerson* population structure have shown that *S. commerson* from Kupang and from the east coast of Australia are genetically distinct from the northern and western Australian populations (Ovenden et al., this report). There was no allozyme or mtDNA evidence to support genetically distinct stocks among populations of *S. commerson* sampled from Shark Bay in Western Australia to the Gulf of Carpentaria in northern Australia. MtDNA haplotype frequency differences among fish sampled from the southern and western Gulf of Carpentaria, Torres Strait and Townsville were pronounced, suggesting genetic distinctiveness over a spatial scale that is similar to that reported here for differentiation of populations *S. commerson* through otolith isotopic composition. In the study by Ovenden et al. (this report), no genetic marker was found to differentiate the FDIMU's reported in this study across northern and western Australia. However, the concordance between genetic and otolith isotopic composition results for the north-eastern Australian populations of *S. commerson* suggests that a more extensive range of genetic markers, for example microsatellites (Ovenden and Broderick *pers. comm.*), may reveal genetic subdivision among northern and western Australian populations.

The presence of spatial genetic subdivision among northern and western Australian populations of *S. commerson* would indicate that these populations are likely to be experiencing low levels of gene flow despite the action of long-shore currents such as the Leeuwin current or the Indonesian through-flow, although this remains to be confirmed. Consequently, the presence of genetic homogeneity among *S. commerson* populations across northern and western Australia suggests there is a high probability of intermixing by early life-history stages and thus each FDIMU would contribute to a common pool of recruits

among locations or within regions. This assumption implies that the size of the total adult spawning population (i.e. the combined sum of each of the separate FDIMU) could impact recruitment in each region. Thus, fishing on any one FDIMU could impact fishing on any other FDIMU through subsequent recruitment (resulting from a reduced spawner biomass in the meta-population). However, direct impacts of fishing on one FDIMU should not affect an adjacent FDIMU (or any fishing impact should be negligible). The presence of spatial subdivision among *S. commerson* populations indicates that management can be applied separately to each of the FDIMU at the regional or location level along the northern and western Australian coast. Fishery managers need to consider maintaining adequate levels of the total adult spawning biomass within each FDIMU, thus limiting indirect effects of fishing.

Populations of *S. commerson* are currently managed separately by state-based management agencies in the Northern Territory and Queensland (Northern and Eastern Australia). The process of management within Western Australia is in the development phase, with the fishery for *S. commerson* across the state currently under review as part of Western Australia's Integrated Fisheries Management framework. However, little spatial structure is currently assumed in those states with formal management arrangements in place. The use of bag limits and/or size limits that are uniform for all fishers across their range in each state are based on the implicit assumption that each population represents a single homogenous stock and that demographic parameters do not vary substantially. The presence of spatial subdivision among adult assemblages of *S. commerson* and thus the formation of distinct management units suggest that the spatial scale of management needs to be reviewed. For example, the state of Western Australia is divided in separate bioregions for fishery management purposes. Each of these bioregions contains several functionally distinct adult management units. Fishery managers need to therefore consider the relevant spatial scales of management approaches and review whether a state-wide approach will meet management objectives or whether different approaches are required for each bioregion. Given the spatial subdivision present among populations of *S. commerson* across northern and western Australia, it may be advantageous to consider *S. commerson* population dynamics and fisheries management from a meta-population perspective.

Coral reef associated fish are generally considered to form meta-populations of sedentary adult populations linked by pelagic larval dispersal (Allison et al. 1998). The spatial segregation of adult populations of *S. commerson* indicates that each management unit is likely to be exposed to different environmental, biological and ecological processes that underpin the spatial differences evident in this study. Under a precautionary approach to management, fisheries for *S. commerson* across Australia and throughout their range need to be managed with consideration given to both the existence of functionally distinct independent management units that comprise a large spawning meta-population. Fisheries management in each area should therefore aim to maintain population subdivision and hence demographic structure as well as adequate levels of the spawning stock.

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REFERENCES

Allison, G.W., Lubchenco, J. and Carr, M.H. (1998). Marine reserves are necessary but not sufficient for marine conservation. *Ecological Applications* **8**:79-92.

Campana, S. E. (1999). Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Marine Ecology Progress Series* **188**:263-297.

Campana, S.E. and Neilson, J.D. (1985). Microstructure of fish otoliths. *Canadian Journal of Fisheries & Aquatic Sciences* **42**:1014-1032.

Campana, S. E., Fowler, A.J. and Jones, C.M. (1994). Otolith elemental fingerprinting for stock identification of Atlantic cod (*Gadus morhua*) using laser ablation ICPMS. *Canadian Journal of Fisheries & Aquatic Sciences* **51**:1942-1950.

Collette, B. B. and Nauen, C.E. (1983). *FAO species catalogue. Vol. 2. Scombrids of the world. An annotated and illustrated catalogue of tunas, mackerels, bonitos and related species known to date*. FAO Fisheries Synopsis No. 125 Volume 2. Rome, FAO. 1983. 137p.

Edmonds, J.S. and Fletcher, W.J. (1997). Stock discrimination of pilchards *Sardinops sagax* by stable isotope ratio analysis of otolith carbonate. *Marine Ecology Progress Series* **152**:241-247.

Edmonds, J.S., Steckis, R.A., Moran, M.J., Caputi, N. and Morita, M. (1999). Stock delineation of pink snapper *Pagrus auratus* and tailor *Pomatomus saltatrix* from Western Australia by analysis of stable isotope and strontium/calcium ratios in otolith carbonate. *Journal of Fish Biology* **55**(2):243-259.

Gauldie, R.W. (1996). Biological factors controlling the carbon isotope record in fish otoliths: principles and evidence. *Comparative Biochemistry & Physiology* **115B**:201-208.

Kailola, P.J., Williams, M. J., Stewart, P.C., Reichelt, R.E., McNee, A. and Grieve, C. (1993). *Australian fisheries resources*. Bureau of Resource Sciences, Department of Primary Industries and Energy, and the Fisheries Research and Development Corporation. Canberra, Australia. 422p.

Kalish, J.M. (1989). Otolith microchemistry: validation of the effects of physiology, age and environment on otolith composition. *Journal of Experimental Marine Biology & Ecology* **132**:151-178.

Kalish, J.M. (1991a). Oxygen and carbon stable isotopes in the otoliths of wild and laboratory-reared Australian salmon (*Arripis trutta*). *Marine Biology*. **110**:37-47.

Kalish, J.M. (1991b). ^{13}C and ^{18}O disequilibria in fish otoliths: metabolic and kinetic effects. *Marine Ecology Progress Series* **75**:191-203.

Kalish, J.M., Liningston, M.E. and Schofield, K.A. (1996). Trace elements in the otoliths of New Zealand blue grenadier (*Macruronus novaezelandiae*) as an aid to stock discrimination. *Marine & Freshwater Research* **47**:537-542.

Lewis, A.D. (1981). Population studies in northern Australian pelagic species utilising the electrophoretic approach. **In**: *Northern Pelagic Programme*, C.J. Grant and D.G. Walter (eds.). Australian Government Publishing Service, Canberra.

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

Newman, S.J., Steckis, R.A., Edmonds, J.S. and Lloyd, J. (2000). Stock structure of the Goldband snapper, *Pristipomoides multidens* (Pisces: Lutjanidae) from the waters of northern and western Australia by stable isotope ratio analysis of sagittal otolith carbonate. *Marine Ecology Progress Series* **198**:239-247.

Patterson, W.P., Smith, G.R. and Lohmann, K.C. (1993). Continental paleothermometry and seasonality using the isotopic composition of aragonitic otoliths of freshwater fishes. *Geophysical Monographs* **78**:191-202.

Reynolds, R.W. and Smith, T.M. (1994). Improved global sea surface temperature analyses using optimum interpolation. *Journal of Climate* **7**:929-948.

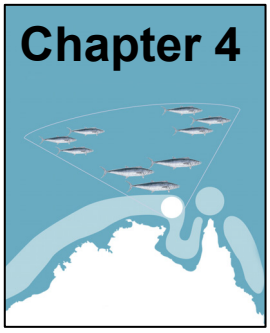
Schwarcz, H.P., Gao, Y., Campana, S., Browne, D., Knyf, M. and Brand, U. (1998). Stable carbon isotope variations in otoliths of Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries & Aquatic Sciences* **55**:1798-1806.

Shaklee, J.B., Phelps, S.R. and Salini, J. (1990). Analysis of fish stock structure and mixed-stock fisheries by the electrophoretic characterization of allelic isozymes. pp. 173-196. **In**: *Electrophoretic and Isoelectric Focusing Techniques in Fisheries Management*, Whitmore DH (ed.). CRC Press, Boca Raton, FL.

Stephenson, P.C., Edmonds, J.S., Moran, M.J. and Caputi, N. (2001). Analysis of stable isotope ratios to investigate stock structure of Red emperor and Rankin cod in northern Western Australia. *Journal of Fish Biology* **58**:126-144.

Thorrold, S.R., Campana, S.E., Jones, C.M., Swart and P.K. (1997). Factors determining $\delta^{13}C$ and $\delta^{18}O$ fractionation in aragonitic otoliths of marine fish. *Geochim Cosmochim Acta* **61**:2909-2919

Weidman, C. R. and Millner, R. (2000). High-resolution stable isotope records from North Atlantic cod. *Fisheries Research* **46**:327-342.



GENETIC POPULATION STRUCTURE OF SPANISH MACKEREL

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ABSTRACT

Spanish mackerel (*Scomberomorus commerson*) is found in continental shelf waters throughout the Indo-West Pacific where it supports commercial, recreational and artisanal fisheries. In Queensland 600-1000 tonnes are harvested per year by the commercial fleet, primarily from spawning aggregations. Recreational fishers take large, but unquantified numbers. Similar fisheries operate in the Northern Territory and Western Australia. Allozyme and mitochondrial DNA (mtDNA) population genetics were used to test the expectation of several separate populations along the extensive northern and western Australian coast as well as a separate population on the Queensland east coast. Short-term (one year) and long-term (15-20 years) tests of the temporal stability of allozyme genic and genotypic frequencies were incorporated. Approximately 100 commercially harvested fish were sampled in 1998 from five roughly equidistant locations along the northern Australian coastline between Townsville (Queensland) in the east, and Shark Bay (Western Australia) in the west. Collections were repeated in 1999, with the addition of fish from the Kimberley coast in north-western Australia, and from Kupang, West Timor. Heterogeneity in allozyme frequencies for ten loci and mtDNA control region sequence data strongly supported the genetic distinctiveness of Kupang mackerel, and to a lesser degree the distinctiveness of fish from the east coast of Queensland fish. There was no evidence of distinct stocks among populations sampled from Shark Bay in the west to the Gulf of Carpentaria. Fish collected from the Torres Strait were similarly related to adjacent stocks and mtDNA RFLP frequency data suggested that the population could be an admixture. Comprehensive comparison to a previous allozyme analysis of Spanish mackerel suggested a shift away from genetic equilibrium (Hardy-Weinberg) conditions in the intervening 15-20 years. Current disequilibrium may be due to a Wahlund effect indicating the presence of undetected finer scale genetic subdivision. It is unclear why equilibrium conditions have changed through time unless falling spawner numbers due to harvesting has increased the geographic scale of mixing during aggregation for spawning. Enhanced genetic drift as a consequence of fewer spawners is consistent with the observed short and long-term instability in allele frequencies for at least two of ten loci for four of the five collections that were repeated one-year later.

In this chapter, the ‘Background’ section places the study in context, and describes hypotheses to test. The ‘Need’ section says why this study is important, and the ‘Objectives’ define what we set out to do.

In the allozyme sections, the ‘Methods’ and ‘Results’ are in two parts; part one describes the analysis of the population structure of Spanish mackerel from samples taken in 1998 and 1999 from six locations around eastern, northern and western Australia. Part two compares this study with a similar one performed in the 1980s by Jim Shaklee, then working at CSIRO Marine Laboratories. The process of comparison consisted of firstly standardising our allozyme mobilities against his to ensure data homology, and secondly analysing the two data sets to test for changes in genetic composition that may have occurred during the intervening 20 years.

The ‘Discussion’ evaluates the results in context. ‘Conclusions and Recommendations’ contain outputs for management of the fishery.

INTRODUCTION

Population genetics for fisheries stock structure analyses

There are compelling reasons for using genetic analyses for the identification of fisheries stocks. Stock assessments, which aim to predict the outcomes on fisheries of management controls, make the assumption that the fish stock is a group of individuals of a species which can be regarded as a separate breeding population of a species (Froese and Pauly, 2001). This working definition of a fish stock corresponds well to the genetic definition of a randomly breeding, or panmictic, population (Hedrick, 2000). Consequently, genetic marker methodology used by population geneticists is applicable to fisheries science and can play a role in fisheries stock assessment and management.

Allozyme genetics as a tool for population genetics pre-dates the use of mitochondrial DNA, microsatellites and other nuclear loci (Ward and Grewe, 1994). Unlike more recent methods that directly or indirectly examine fish genetic material (DNA), allozyme genetics infers DNA or genetic similarity between fish from electrophoretic banding patterns of specific proteins. The electrophoretic mobility of a specific protein (encoded by a genetic locus) changes when a DNA mutation leads to the replacement of one or more protein building-blocks (amino acids); however, not all mutations necessarily lead to this type of change. A typical allozyme study sums genetic similarities (or differences) across numerous proteins (loci) for fish sampled from the same or different geographical localities, often in subsequent seasons or years. It requires high quality, non-degraded tissue (eg. retina, muscle, liver etc.) from each fish, which is more expensive and time-consuming to collect in the field, and maintain in the lab, than the tissue required for DNA methods. Non-lethal tissue sampling is not normally an option for allozyme studies, although muscle biopsies are a good source of some protein loci (Frentiu et al., 2001).

Since its inception in the 1970s (Hubby and Lewontin, 1966; Lewontin and Hubby, 1966; Shaw and Prasad, 1970) allozyme genetics has been extensively applied to fisheries populations, but examples where research outcomes have been incorporated into management practises are relatively rare. This is due, in part, to the high levels of gene flow generally experienced by marine populations (Waples, 1998), which practically means that the discovery of genetic stocks in the marine context are not all that common (Shaklee and Bentzen, 1998). In these cases, genetic studies are used to reinforce the assumption of stock cohesion for stock assessment studies. It is also partly due to the slow cycle of management that requires managers to evaluate and integrate incoming data and obtain political clearance before implementing new controls. North American and European salmonid fisheries managers have been the biggest users of genetic data for fisheries management, where genetic monitoring has become an essential management tool (Shaklee et al., 1999).

In Australia, marine vertebrate and invertebrate populations have been subjected to allozyme electrophoresis to achieve fisheries related objectives since the 1980s. Most relevant to this study of Spanish mackerel are genetic results from finfish inhabiting in-shore or reef habitats on the northern Australian coastline, whereas ocean-going pelagics such as tuna (Grewe et

al., 1997; Richardson, 1983; Ward et al., 1994) and deep water species such as orange roughy (Smith and Benson, 1997) have different or unknown environmental and life history constraints that are not generally relevant to the formulation of hypotheses for this study. Results from DNA studies are also relevant to this survey of allozyme literature, as the correspondence between the outcomes of the two methods has been demonstrated (Buonaccorsi et al., 1999; Scribner et al., 1998).

The Torres Strait region off northern Cape York features prominently in genetic stock boundaries of some northern Australian coastal species. The tropical saddle-tail sea perch (*Lutjanus malabaricus*) is a demersal reef species that occurs widely on the northern Australian coastline and through the Indo-Pacfic. Elliot (1996) found allozyme gene frequency differences between the Kimberley coast (North West Shelf, Western Australia) and Gulf of Carpentaria populations; mtDNA haplotypes further differentiated the east coast of Queensland samples. Similarly, Chenoweth (1998b) reported a genetic 'break' in mtDNA patterns to the east and west of Cape York (northern Queensland) in the barramundi (*Lates calcarifer*) following the description by Shaklee (1993) and Keenan (1994) of extensive genetic subdivision among Australia-wide estuarine populations from allozyme data. Gene flow in these species may be presently attenuated by the shallow seas of the Torres Strait, and would have ceased entirely between eastern and northern Australia populations at the height of the last glaciation about 15,000 years ago when there was a land bridge connecting Cape York to southern Papua New Guinea (Jones and Torgersen, 1988). Spanish mackerel occur to the east and west, and throughout, Torres Strait. If the region is a transition zone between putative eastern and western stocks of Spanish mackerel, hybridisation and subsequent introgression of allozyme or mtDNA alleles may be found in the Torres population.

The wide continental shelf along the north-western coast of Australia is largely uninterrupted by geological features that could influence gene flow between stocks. Yet, unpublished mtDNA data from Salini, Haryanti and Ovenden on the red snapper (*L. erythropterus*) suggests distinct genetic stocks on the northern Australian coast around Weipa in the eastern Gulf of Carpentaria compared to Darwin, Northern Territory. Ovenden (in press) used mtDNA to test for genetic stocks among goldband snapper (*Pristipomoides multidens*) and found that the Kimberley population may be unique, although they had difficulty presenting historical, biogeographical or environmental correlates to explain the result. Spanish mackerel are thought to be capable of movements over large distances (Tobin, 2000), although it is unclear whether they return to breed in the same location (philopatry) after a feeding migration, or whether the dispersers return to participate in breeding. In the absence of philopatry, highly mobile mackerel could be responsible for large amounts of gene flow along the north-western Australian coastline and the apparent absence of separate genetic stocks in this region.

There have been two published allozyme studies of stock structure in Australian mackerel species; jack mackerel (Richardson, 1982, *Trachurus declivis*) and school and spotted mackerel (Begg et al., 1998, *Scomberomorus queenslandicus* and *S. munroi*). Begg and colleagues sampled mackerel from the east and north coast of Australia. In both species they reported significant allozyme differences between eastern and northern populations.

Additionally, for school mackerel only, they reported a complex stock structure on the east coast with each genetic stock being associated with particular coastal embayments. Water circulation patterns in inshore and estuarine spawning grounds may operate to retain larvae, and maintain stock separation. Their interpretation was backed up by additional data that they collected on the movement patterns of adults, concurrent spawning at different locations and regional differences in growth and elemental otolith composition. The combination of genetics, otolith and parasite analyses in this study of Spanish mackerel has the potential to provide data as detailed as those collected by Begg and colleagues on school mackerel, and to resolve fine-scale stock structure if it exists in this species.

Temporal comparisons of allozyme data

Population genetics studies are rarely repeated, as there are relatively few workers, a multitude of taxa and in many cases limited research funds. However, there are important reasons for re-collecting allozyme data. Estimates of effective population size can be made from the variance in allozyme gene frequency change through time (Waples, 1989). Repeatability is a big issue for fisheries scientists who often view population genetics with suspicion. The image of allozyme genetics as 'black magic' has not helped the acceptance of population genetics as a tool for fisheries science. Allozyme genetics has a further image problem as field biologists and cooperative fishermen, not geneticists, obtain samples for allozyme studies often at great expense and effort. Allozyme workers who plan to repeat earlier projects need to standardise allele scoring between studies as differences are known to occur among running buffers and from laboratory to laboratory (Shaklee and Phelps, 1990; White and Shaklee, 1991). This study reports a series of standardisation experiments for Spanish mackerel allozymes with data from a previous study (Shaklee et al., 1990; Shaklee, unpublished, Appendix 4).

A compelling reason for repeating allozyme studies on the same taxa in subsequent years is that genetics can detect cryptic changes in population structure and size, which may provide advance warning to fisheries managers about imminent stock collapse. Smith (1991) monitored the change in allozyme genetics of New Zealand orange roughy (*Hoplostethus atlanticus*) over six years when intense harvesting pressure reduced virgin biomass by 70%. They reported a significant reduction in genetic diversity over that period. Populations of the gag grouper (*Mycteroperca microlepis*) on the South Atlantic coast of the United States and Mexico were out of genetic equilibrium (Hardy-Weinberg) and showed a mosaic pattern of genetic differentiation that could not be explained by life history and environmental characteristics (Chapman et al., 1999). The authors concluded that grouper were experiencing inbreeding in size-limited populations. Australian populations of Spanish mackerel have experienced intense harvesting pressure since the 1950's, often during spawning aggregrations (Tobin, 2000). If the species is sedentary or philopatric, overharvesting could have affected the allozyme genetics of the species in the time since samples were collected for a previous study in the early 1980s (Shaklee et al., 1990).

History of allozyme genetics of Spanish mackerel

Dr James Shaklee analysed the population genetic structure of Spanish mackerel during his tenure at CSIRO Marine Laboratories, Cleveland and later at Washington Department of Fish and Wildlife, Olympia, WA, USA. His objectives were to test for the presence of multiple genetic stocks and define the geographic boundaries of the stocks throughout the range of the species in Australian and southern Papuan New Guinea waters. Eighteen populations were sampled during 1981 to 1985 from the mid-western Australian coast at Shark Bay (Cape Cuvier), northern Australia and the eastern Queensland coast to Brisbane. Over 2,300 fish from these collections were genotyped at 28 polymorphic allozyme loci. His results indicated the presence of two Australian genetic stocks; one on the east coast of Queensland, and the other across northern and western Australia from the Gulf of Carpentaria in the east to Shark Bay in the west and including southern Papua New Guinea. Shaklee believed that fisheries management should be based around these stocks, and he recommended that the fisheries authorities in the states of Western Australia, the Northern Territory and Queensland co-operatively manage the single northern and western stock, in conjunction with Papua New Guinea (Shaklee, unpublished, Appendix 4).

Our genetic analyses of Spanish mackerel were similar to Shaklee's study. This study used allozyme loci; six of which were directly comparable between the two studies. This study sampled across the same range as Shaklee's had done, with our samples being taken 13 to 19 years later (1981-85 compared to 1998-2000). Furthermore, our samples were obtained within 500km or less of his sampling locations at Shark Bay, Kimberleys, Northern Australia adjacent to Darwin, Torres Strait and on the east Queensland coast adjacent to Cairns and Townsville. Our sampling locales were within less than 50km (approx) of his at Shark Bay and Torres Straits. This study also analysed an mtDNA marker that was not analysed by Shaklee.

The outcomes of this study are strengthened by the availability of Shaklee's raw data and analyses for direct comparison with the results of this study, and by the combination of genetically independent nuclear (allozyme) and mitochondrial loci. Shaklee's Spanish mackerel genetic research also accomplished the tedious process of evaluating running buffers for optimal resolution for each locus for different tissue types that normally precedes allozyme analyses, and searching for loci that were polymorphic.

NEED

Allozyme and mitochondrial loci are widely used molecular markers for fisheries stock discrimination (Ovenden, 1990; Richardson et al., 1986), and their analysis has, in several cases, been shown to lead to concordant results. However, both systems can be subject to selective pressures (Koehn et al., 1983; Nigro and Prout, 1990; Tishkoff and others, 2001) that can potentially bias the results of population subdivision analyses. The most robust fisheries stock analyses are from studies where concordant results are obtained from independent marker sets; allozymes and mtDNA markers, for example. The usefulness of two genetic

markers in fisheries science is explained by Buonaccorsi (1999). Allozyme loci are diploid and biparentally inherited, whereas mtDNA is effectively a single locus, with haploid maternal inheritance resulting in a fourfold lower effective population size than allozyme markers. Natural selection may influence polymorphism at all these loci if it is subject to functional constraints or tightly linked to regions that are influenced by selection. Because selection tends to act independently on individual loci, the influence of selection on genetic tests of population structure and estimates of gene flow are minimised when data is used from several loci.

The genetic analyses of the stock structure of Spanish mackerel reported in this Chapter were conducted on the same specimens that were analysed for chemical composition of otoliths (Chapter 3) and parasite load (Chapter 6) as part of the overall project. Each technique has the potential for reciprocal verification, but also for viewing stock discrimination at a different level. Parasite analyses track similarities among adult fish. Otolith chemistry reveals similarities among individuals large enough to possess otoliths, normally from juveniles and adults. Both techniques reflect similarities in the environment inhabited by the individual. Otolith studies reflect the chemical environment, while the parasites reflect biological characteristics of the ecosystem. Genetic methods are independent of environmental and ecological correlates and can detect similarities among all life stages of the fish as well as reflecting the similarity among recent ancestors of those fish. Geographically, and thus environmentally distinct populations can be genetically similar because they have shared recent ancestors, or because they are exchanging individuals as migrants. All three methods of stock discrimination used simultaneously can lead to exceptionally powerful results.

OBJECTIVES

1. To test the expectation of genetic homogeneity within northern and western Australian populations of Spanish mackerel.
2. To confirm the presence of two Australian Spanish mackerel stocks (East Coast, northern and western), and define the spatial boundary between them.
3. To test for temporal stability of the allozyme genetic composition of Spanish mackerel populations over a period of 15-20 years. To achieve this objective, allozyme methodology was standardised between this study and Shaklee (unpublished, Appendix 4) to ensure allele correspondence between studies.

METHODS

Genetic population ptructure

Sampling

Allozymes. Spanish mackerel were genotyped from six collection locales in 1998 and 1999 (Figure 4.1a – e). The collection locales were

- East Coast (EC, off Townsville, approximate lats and longs 148 ° 30' 18 °30'),
- Torres Strait (TS, around Bramble Cay and Ashmore Reef, 144°00' 9°30'),
- Northern Territory (NT, off north-east Arhnem Land and Gove, 137°00' 12°30'),
- Northern Australia (NA, around Bathurst and Melville Islands, north-west of Darwin, 130°30' 12°00'),
- North Kimberley (NKimb, north-western Australia, 124°30' 13°00'), and
- Shark Bay (SB, western Australia off Shark Bay, Exmouth and Dampier, 114°00' 24°00').

In addition, fish were sampled from Kupang, West Timor, Indonesia in 1999.

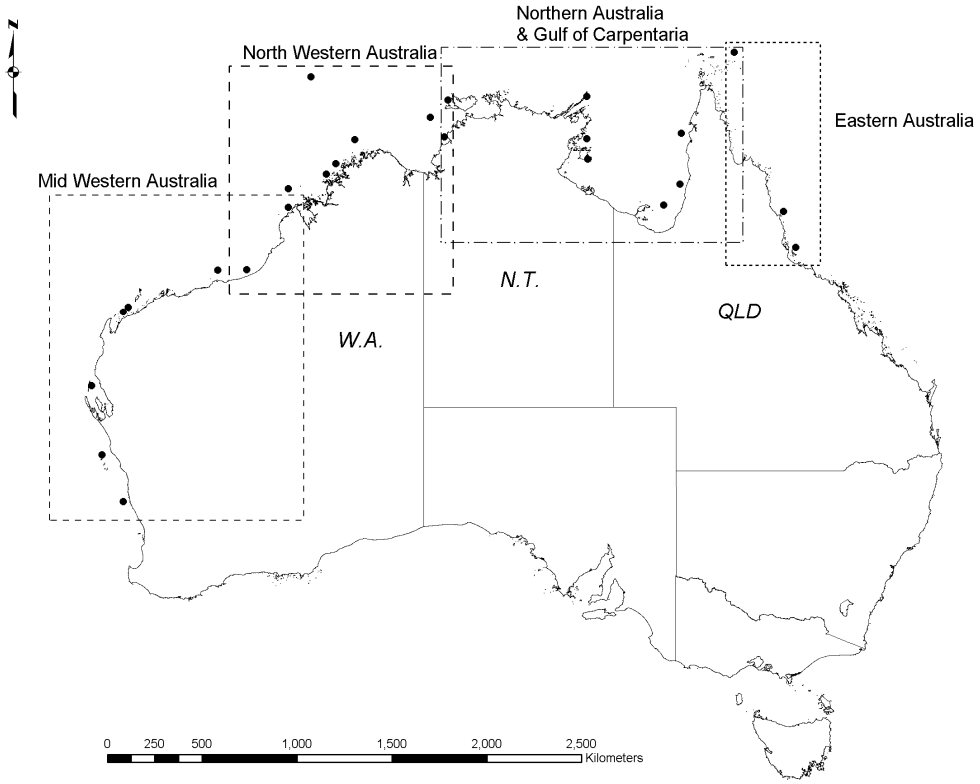


Figure 4.1a. Sampling locations of *Scomberomorus commerson* from western and northern Australia showing each of the four subregions (Figure taken from S Newman et al., Chapter 3)

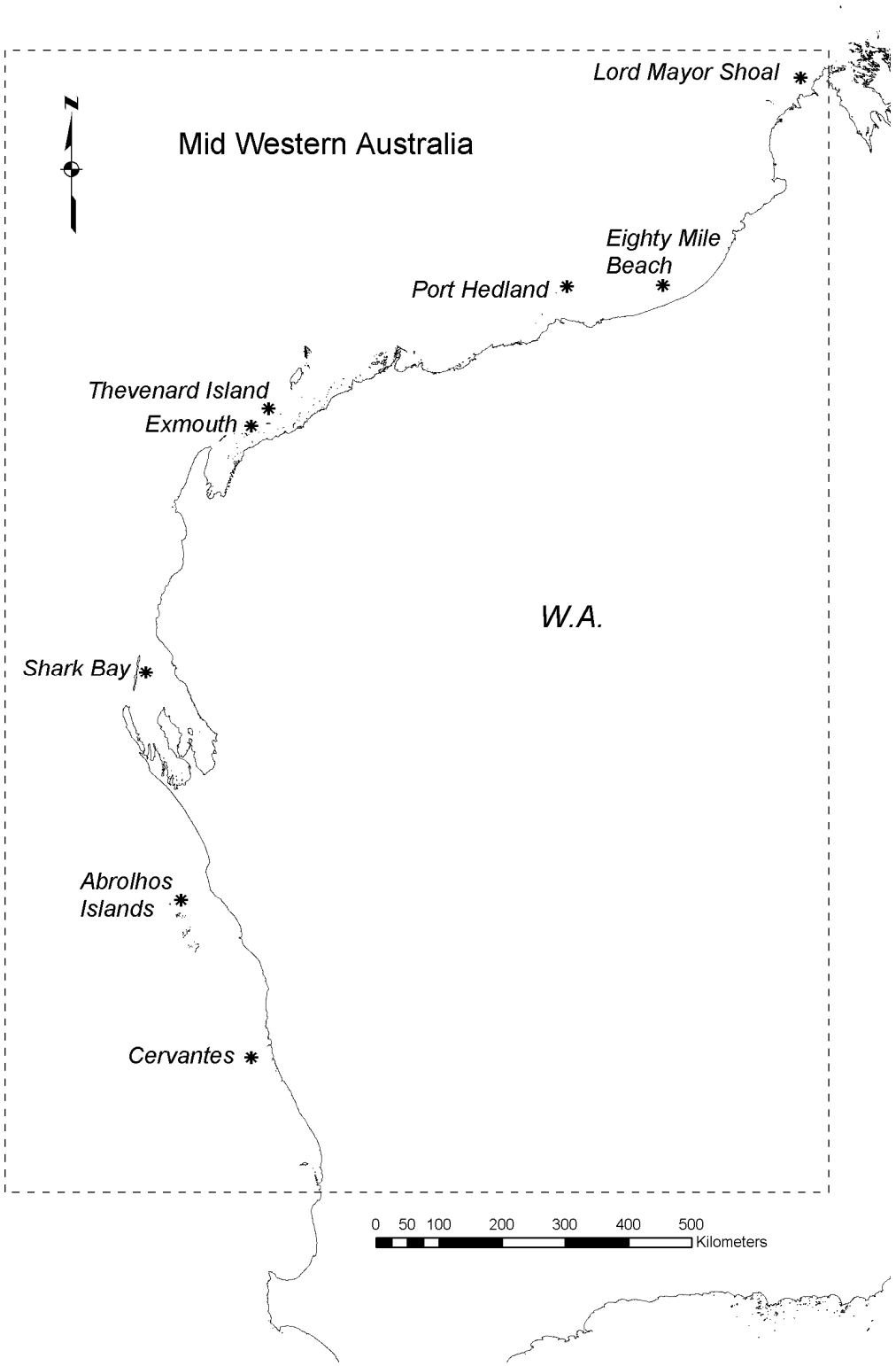


Figure 4.1b. Sample locations of *S. commerson* in Mid Western Australia (Figure taken from S Newman et al., Chapter 3).

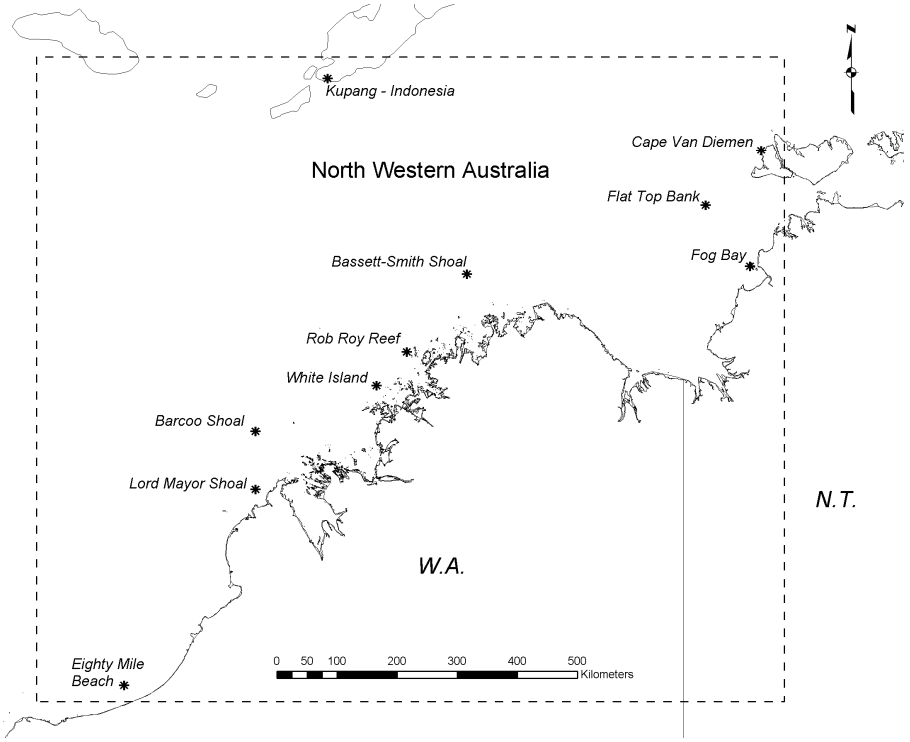


Figure 4.1c. Sample locations of *S. commerson* in North Western Australia (Figure taken from S Newman et al., Chapter 3).

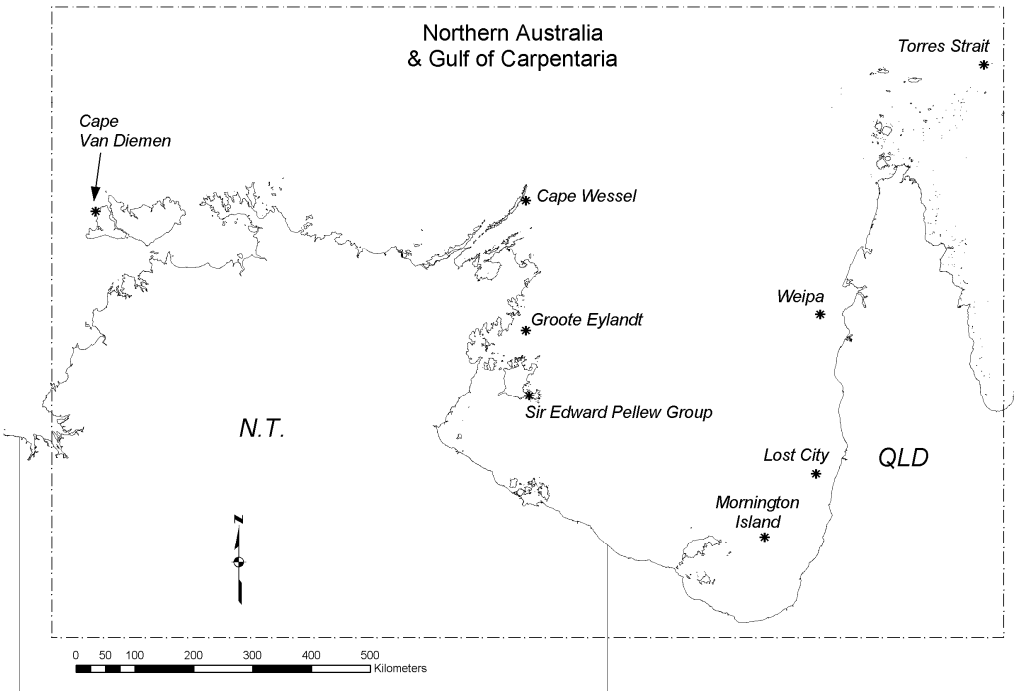


Figure 4.1d. Sample locations of *S. commerson* in Northern Australia and the Gulf of Carpentaria (Figure taken from S Newman et al., Chapter 3).

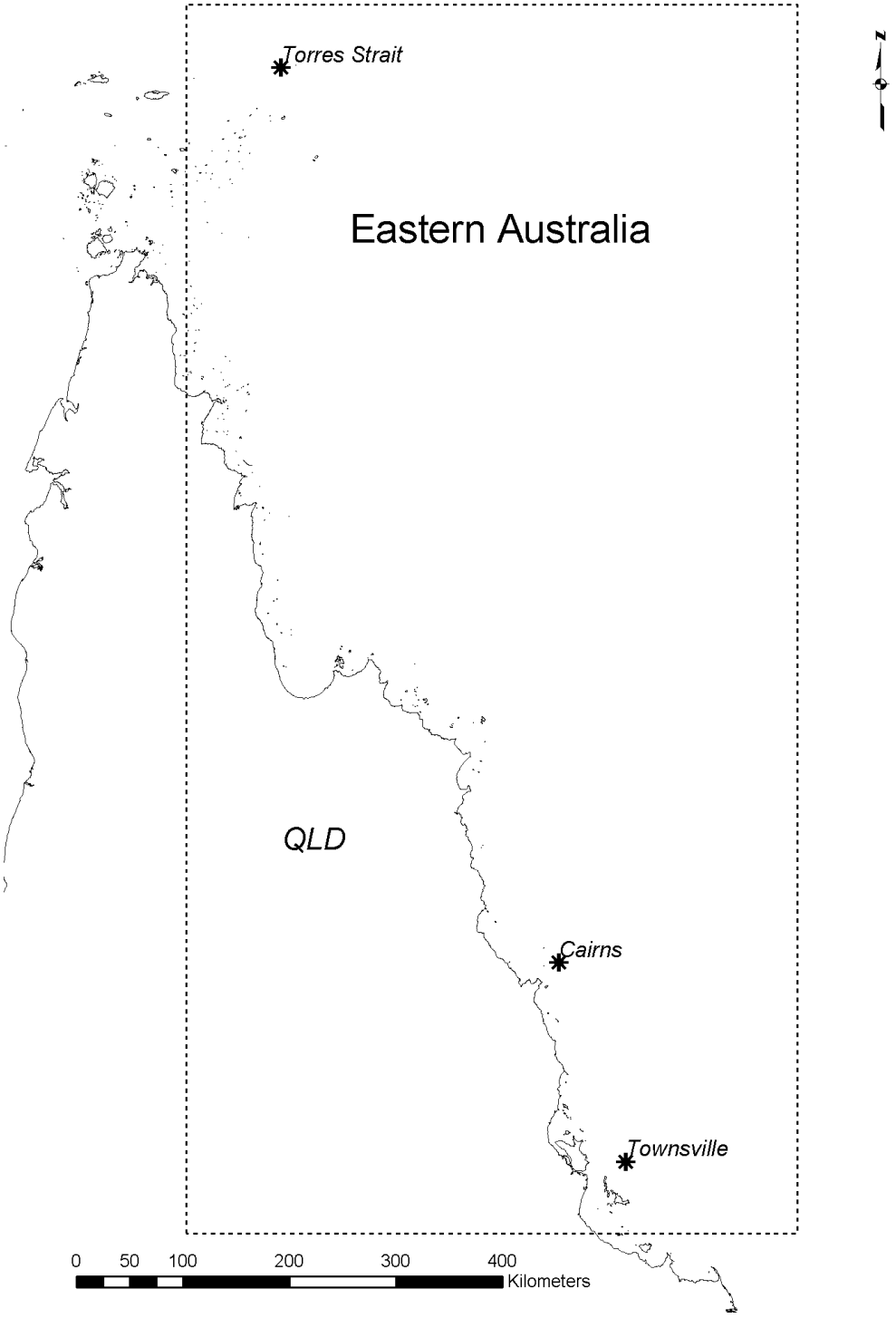


Figure 4.1e. Sample locations of *S. commerson* in Eastern Australia (Figure taken from S Newman et al., Chapter 3).

Fish were sampled from commercial catches by observers. The majority of fish at a site were sampled in less than two weeks, sometimes over only two days. The East Coast was sampled over 13 days in 1998 (20th Oct – 1st Nov) and 14 days in 1999 (27th Oct – 9th Nov). The Torres Strait was sampled over 11 days in 1998 (15th – 25th Sept) and over nine days in 1999 (19th – 27th Oct). The Northern Territory was sampled over two days in 1998 (4 – 5th Aug) and over eight days in 1999 (10th – 17th Nov). The Northern Australia locale was sampled over three days in 1998 (31st Aug – 2nd Sept) and 14 days in 1999 (23rd Jul – 5th Aug). The Kimberley locale was sampled over seven days in 1998 (17th – 23rd Oct) and 16 days in 1999 (7th – 22nd Sept).

The Shark Bay samples were sampled over a greater temporal scale. In 1998 fish were taken from three general areas; 17 fish over 23 days (1st – 22nd June), 25 fish over three days in August (25th – 27th) and 12 fish from another locations over about 10 weeks (11th June – 28th August). In 1999, 51 fish were sampled over three weeks from one location (15th Jul – 2nd Aug) and another 52 fish over 12 weeks (7th May – 25th Jul) from another location.

About 100 fish were genotyped from each geographic locale. Binomial theory describes the probability of collecting an allele of frequency '*p*' as:

$$N = \left\lfloor \frac{\ln(1-\alpha)}{\ln(1-p)} \right\rfloor / 2$$

where *N* is the number of individuals required and 'α' is the confidence level desired (Bartley et al., 1995). For example, a minimum of 74 fish is needed to capture a rare allele of frequency 0.02 at a 95% confidence level.

Three tissue samples (muscle, liver and retina) were taken in the field from each fish and stored at or below –70°C. Histochemical staining, electrophoresis buffers and tissue types used closely followed Shaklee (unpublished, Appendix 4; 1986).

MtDNA. Spanish mackerel analysed for mtDNA were a subset of those genotyped for allozymes. Briefly, six primary collection locales were sampled in 1998 and 1999; East Coast (EC, off Townsville), Torres Strait (TS, around Bramble Cay and Ashmore Reef), the Northern Territory (NT, off north-east Arhnem Land and Gove), Northern Australia (NA, around Bathurst and Melville Islands, north-west of Darwin), North Kimberley (NKimb, north-western Australia) and Shark Bay (SB, western Australia off Shark Bay, Exmouth and Dampier, Figure 4.1a – e). Fish genotyped for mtDNA were also genotyped for allozymes, but not all fish were genotyped for both loci. However, fish from one secondary locale (two fish, Southern Gulf of Carpentaria, approximately 16°S 139°E) and from Kupang, West Timor, Indonesia in 1998 (21 fish) were not genotyped for allozymes. Tissue samples were stored at –80°C.

Laboratory Protocol

Allozymes. Starch gels were prepared by mixing 30g of hydrolysed potato starch (Starch Art Corporation P.O. Box 268, Smithville, TX 78957 US) with 300 ml of buffer. The gel mixture was heated over a flame, whilst being continually swirled, until it reached a clear, liquid consistency. The mixture was degassed under vacuum for 30 seconds and was poured into a

glass mould 238 mm long, 138 mm wide and 6 mm deep. Directly before use, the gel was cut in a straight-line 4 cm in from the cathodal end to create an origin for sample application.

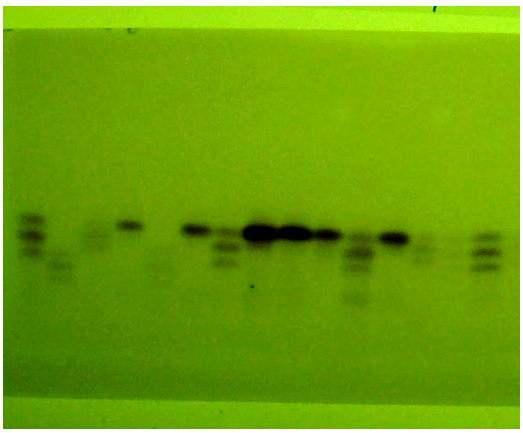
Unmacerated tissue sample plus homogenising buffer (50-100 microlitres) was centrifuged at 10,000 rpm for five minutes at 4°C. The processed samples were stored at -70°C and kept on ice during use to reduce enzyme degradation. The tissue supernatant was absorbed onto a filter paper wick, 10 mm long and four mm wide. A wick dipped in bromophenol dye interspaced sets of ten sample wicks. The last ten individuals of each gel also were loaded onto the next gel to ensure correspondence among alleles. The gel was kept at 4°C during electrophoresis to prevent enzyme degradation. A direct current was applied to the gel using an electrophoresis power pack.

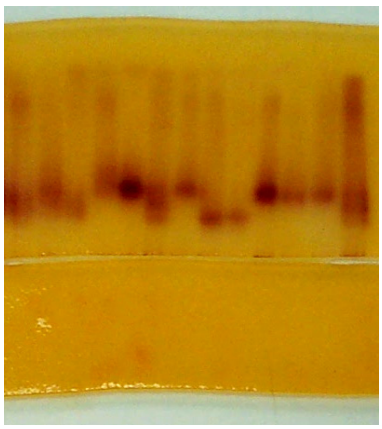
Following electrophoresis, gels were cut into thin horizontal slices using nylon thread. Each slice was placed on a plate of glass. A stain was applied to each slice (Appendix 1), after which the slice was incubated at 37°C until bands appeared. Stained gel slices were then scored and photographed. Scoring consisted of making a line drawing of the banding phenotype and assigning a presumed genotype to each phenotype. All gels were scored and interpreted by the same operator (RS). The distance travelled from the origin in millimetres was recorded for each band (allele). This was converted to relative mobility by assigning '100' to the most common allele. Presumed enzyme structure was used to infer genotypes (Shaklee and Keenan, 1986). Genotype data for each fish was entered onto a MS-Excel spreadsheet that was subsequently printed and proof read against gel photographs and corresponding line drawings (Figure 4.2).

MtDNA. *DNA isolation*. Genomic DNA was isolated from 50-100mg of muscle using 500µl of 10% Chelex 100 solution, and 5µl Protease K (20mg/ml). This solution was incubated for three hours or overnight at 55°C, with gentle mixing from time to time. It was boiled for eight mins, cooled to room temperature, and 55µl TE solution was added. The solution was again gently mixed and centrifuged for five mins at 13,000 rpm. Supernatant was removed into clean-labelled tubes and stored at -20°C.

DNA amplification. Approximately 400bp of the 5' end of the control region (D-loop) and 340 bp of the cytochrome b region was amplified as described (Ovenden et al., in press), except that the annealing temperature was 55°C for the control region, and 45°C for the cytochrome b region. The primers used were Pro889U20 (CCW CTA ACT CCC AAA GCT AG) and TDKD1291L21 (CCT GAA ATA GGA ACC AAA TGC) for the control region (Ovenden et al., in press). Primers CB1L (CCA TCC AAC ATC TCA GCA TGA TGA AA) and CB2H (CCC TCA GAA TGA TAT TGG TCC TCA) were used to amplify the cytochrome b region.

DNA sequencing. Forward and reverse PCR primers for the control region and the cytochrome b region were used to sequence the purified PCR product. Sequences were obtained with an ABI automated sequencer using the chain-termination method with big-dye terminators. Sequence data was aligned with Sequencher v 3.12 (Anon, 2000).





PGDH			
llane	**fish#**	**score**	**genotype**
1	TS2710	+/s	100/085
2	TS2714	s/vs	085/079
3	TS2715*	+/m	100/095
4	TS2716	+/+	100/100
5	TS2717	m/vs	095/079
6	TS2718*	+/+	100/100
7	TS2719	+/s	100/085
8	TS2720	+/+	100/100
9	TS2721*	+/+	100/100
10	TS2722*	+/+	100/100
11	TS2723*	+/s	100/085
12	TS2724	+/+	100/100
13	TS2725	+/m	100/095
14	TS2726	+/s	100/085
15	TS2727*	+/s	100/085

ACP			
lane	**fish#**	**score**	**genotype**
1	TS2545	+/s	100/100
2	TS2546*	+/s	100/100
3	TS2547	+/s	100/020
4	TS2548	+/f	100/200
5	TS2579*	+/+	100/100
6	TS2580*	+/s	100/020
7	TS2581	+/+	100/100
8	TS2582*	s/s	020/020
9	TS2583	s/s	020/020
10	TS2584*	+/+	100/100
11	TS2585*	+/+	100/100
12	TS2586*	+/+	100/100
13	TS2587	+/s	100/020

Figure 4.2. Spanish mackerel banding phenotypes on starch gels stained for PGDH (buffer CAAPM pH 6, liver tissue) and ACP (buffer CAEA pH 7, liver tissue), and genotypic interpretation

DNA RFLP analysis. The 5' end of the control region was amplified as above in a 50μl reaction. One unit of *Rsa*I (GT$^{\downarrow}$AC) digested 10μl of the unpurified control region amplicon at 37^{o}C for three hours. The digestion products were electrophoresed in a 3% agarose gel, stained with ethidium bromide and photographed with a polaroid camera. EC (East Coast) clade fish possessed a band at 139bp, while non-clade members possessed a band at 149bp. A representative sample of east coast clade fish that were identified using the RFLP technique was confirmed as clade members by nucleotide sequencing.

Analysis Methods

Allozymes. *Allele Frequencies.* Allele frequencies were calculated from raw data using Genepop-on-the-web (Morgan, 2000) based on Genepop v. 3.3 (Raymond and Rousset, 1995).

Linkage Disequilibrium. Linkage was tested using goodness of fit procedures using Genepop-on-the-web (Morgan, 2000). The null hypothesis was that genotypes at one locus are independent from genotypes at the other locus.

Heterozygosity per locus. Two measures of heterozygosity were calculated: observed heterozygosity and expected heterozygosity. Observed heterozygosity was the proportion of individuals in the sample that were observed to be heterozygous by direct counting. The expected heterozygosity was the proportion of heterozygous individuals expected under Hardy-Weinberg expectations. Observed and expected heterozygosity at each locus and for each population was calculated using Genepop-on-the-web.

Hardy-Weinberg Equilibrium. Spatially and temporally defined populations were tested for conformance to Hardy-Weinberg proportions for each allozyme locus. The complete enumeration method was used when the total number of alleles was less that five; otherwise a Markov chain method was used to calculate p-values implemented using Genepop-on-the-web.

Spatial and temporal subdivision. Analyses were performed to detect differentiation of allelic and genotypic frequencies between spatially and temporally distinct populations. An analog of Fisher's exact test, the log-likelihood G-test, and Fst's were performed using Genepop-on-the-web.

MtDNA. The filter taxa utility of Macclade (Maddison and Maddison, 1992) was used to determine the number of haplotypes from sequence data. Estimation of nucleotide diversity was done in Arlequin v 2.0 (Schneider et al., 2000).

Analysis of molecular variance {Excoffier, 1992; AMOVA} was used to estimated the stock composition with differing geographical groupings by quantifying the inter- and intragroup component of total variance using Φ - statistics in Arlequin v 2.0 (Schneider et al., 2000).

Maximum parsimony trees were constructed from sequence data using the heuristic (control region) or exhaustive (cytochrome b) search option of PAUP (Swofford, 1999). Single base pair indels were regarded as 'fifth' bases. Characters were not weighted. The strict consensus method was used to condense control region MP trees.

Temporal comparison of allozyme data

Standardisation

Sample, Locus, Buffer and Tissue Selection. Standardisation was performed in order to directly compare the data from this study to the unpublished data of Shaklee to test for temporal stability over the intervening 15 to 20 years between sampling. From this study two population samples were chosen for standardisation trials; the Torres Strait samples from 1998 and 1999.

Four loci were selected for standardisation (mAAT, ACP, sIDHP and PGDH) because they were resolved on different buffers, and sometimes on different tissues in this study and Shaklee's. The remaining six of ten loci in common between the two studies were resolved on Poulik (CK-A, GPI-A, LDH-B and PGM), LiOH (ADH) and EBT (EST-1) buffers.

For standardization, the Torres Strait samples were run on CAAPM and CAEA gels. These buffers were chosen because Shaklee used CAEA for the four loci, with the addition of CAAPM for ACP and TC-1 for sIDHP. This study used CAAPM for ACP and PGDH, but TRIC for mAAT and TRIC and TC-1 for sIDHP.

Sets of 15 muscle and 15 liver samples from the same 15 fish were run on each gel using wide wicks (5mm wide by 6.5mm high) for increased band resolution. This study used liver for mAAT, while Shaklee used muscle, and this study used muscle for sIDHP while Shaklee used liver. The same tissue was used by both studies (muscle for CK-A, GPI-A, LDH-B and PGM: liver for ACP, ADH and PGDH and retina for EST) for the remaining eight common loci.

Gels were photographed with a digital camera. Otherwise, gel running and staining conditions were as described above.

Gel Scoring and Recording. Standardisation gels were scored independently by two workers (Raewyn Street and Jenny Ovenden). When scoring differences occurred scores were reconciled by reciprocal cross-checking and then by comparison to the actual gels in the laboratory. Standardised scores were compared to the original data and a list of inconsistencies developed. When an inconsistency was detected it was classified (see below), in part by examination of original photographs and gel scoring sheets.

Analysis Methods. *Classes of Inconsistencies.* Inconsistencies between the original and the standardised data were placed into one of the following types.

1. For the same fish, allele mobility or scoring differed between standardised and original data due to running buffer substitution for two loci, mAAT and sIDHP.
2. For the same fish, allele mobility or scoring differed for the standardised data between CAAPM and CAEA gels.
3. For the same fish, alleles were not scored for both original and standardised data. This occurred due to lack of staining, or because the sample was too poorly resolved to be scored. This category does not necessarily address the issue of scoring discrepancies as it relates to the absence of a score in one case, and presence in another. It is included here, however, as it allowed us to test the hypothesis that poorly resolved heterozygotes may be inadvertently scored as homozygotes.
4. For the same fish, allele mobility differed between liver and muscle tissue.
5. Allelic differences between original and standardised data as a result of
   a. human error, where original gels were scored incorrectly or where pairs of samples were reversed during loading, and
   b. poor resolution of either original or standardised gels that lead to the incorrect scoring.

Inconsistencies 1, 2 and 4 relate to standardisation between this study and Shaklee's. Inconsistencies 3, 5a and 5b relate to data quality and repeatability within this study.

Determination of the correspondence between alleles. To determine possible correspondences between the data in this study and those of Shaklee, Jenny Ovenden and Jim Shaklee worked side-by-side in Olympia, WA U.S. from 19-22nd September 2001. For each locus, allele frequency distributions were compared as an initial guide to allele homology. For example, the most common allele was always '100' and its frequencies in this data and Shaklee's were checked to determine if it had the same range, for example, 95 – 99% across populations. Photographs of both Shaklee's gels and those from the present study and the relative mobilities of bands were used to check the consistency of allele identification. The results of standardisation gels, subsequently run as part of this study in Australia were used to further validate homologies.

Temporal Analysis

Genepop-on-the-web was used to compare allelic and genotypic proportions between 1980s and 1990s data. Estimates of the observed amount of allele frequency variance over the elapsed time between sampling events was made using the moments-based or F-statistic method (equation 9) of Waples (1989). Equation 16 was used to calculate confidence intervals.

RESULTS

Genetic population structure

Allozymes

Raw Data. Approximately 100 mackerel were genotyped from each spatio-temporal sample (population NT 99, mean number of fish genotyped at the ten loci = 98.0; EC 98, 106.0; EC 99, 83.8; KMB, 90.4; TS 99, 112.5; TS 98, 125.4; Kupang, 93.6; NA 99, 137.1; NA 98, 105.6; SB 99, 98.5). Sampling problems reduced the number of fish from the Northern Territory sample in 1998 (40 fish) and the Shark Bay sample in 1998 (52 fish).

Fish were genotyped at ten allozyme loci (Table 4.1). One locus (mAAT) was characterised by cathodal alleles only and locus ADH had two cathodal (-100, -280) and one anodal allele (040). All other loci had anodal alleles. The number of alleles per locus varied from two (LDH-B) to seven (sIDHP, Table 4.2).

Table 4.1. Enzyme loci for *S. commerson*, including name (Shaklee, unpublished, Appendix 4) European Enzyme Commission numbers (E.C. No), presumed tertiary structure, tissue type and electrophoresis buffers (Appendix 1)

Enzyme Name	E.C. No.	Abbrev.	Structure	Tissue	Buffer and pH
Aspartate Aminotransferase	2.6.1.1	mAAT	Dimer	Liver	TRIC 7.2
Acid Phosphatase	3.1.3.2	ACP	Dimer	Liver	CAAPM 6
Alcohol Dehydrogenase	1.1.1.1	ADH	Dimer	Liver	LiOH 8.3
Creatine Kinase	2.7.3.2	CK-A	Dimer	Muscle	Poulik 8.8
Esterase	3.1.1.-	EST-1	Dimer	Retina	EBT 8.6
Glucose-6-phosphate Isomerase	3.5.1.9	GPI-A	Dimer	Muscle	Poulik 8.8
Isocitrate dehydrogenase	1.1.1.42	sIDHP	Dimer	Muscle	TC-1 7/
TRIC 7.2					
Lactate Dehydrogenase	1.1.1.27	LDH-B	Tetramer	Muscle	Poulik 8.8
Phosphogluconate Dehydrogenase	1.1.1.44	PGDH	Dimer	Muscle	CAAPM 6
Phosphoglucomutase	5.4.2.2	PGM	Monomer	Muscle	Poulik 8.8

Note: Although CK-A is a dimer, the presumed heterozygote in muscle exhibits a two-banded phenotype that is typical of monomers.

Approximately equal numbers of female and male Spanish mackerel were genotyped (Table 4.3). The exception to this was the Northern Australia and Shark Bay samples where there were about twice the number of females compared to males. This may reflect a bias in the composition of fish in spawning aggregations, or a bias in the fishing method, as samples for genotyping were selected at random from the total number collected. For each spatial sample, the allele frequencies of female and male fish were not significantly different, except for Shark Bay females and males for loci CK-A (p=0.00178) and GPI-A (p=0.00387) and Northern Australian females and males for locus CK-A (p=0.00523). Lack of equal numbers of males and females at these locales may have affected the power of the tests; for example there were 99 females but only 49 males in the Shark Bay sample.

Linkage Disequilibrium. For each pair of loci it was not possible to reject the null hypothesis of linkage equilibrium.

Hardy-Weinberg Equilibrium and Levels of Heterozygosity. The observed genotypic proportions were in general agreement with expected proportions predicted by Hardy-Weinberg equilibrium for the majority of the 12 spatially and temporally distinct population samples for the ten loci (Table 4.4). Three samples; North Kimberley (PGDH, p=0.0002), Torres Strait 1999 (Est, p=0; GPI-A, p=0.001) and Northern Australia 1999 (PGDH, p=0.002) were out of equilibrium for at least one locus at a level of significance adjusted for multiple tests p=0.005 (0.05/10 loci). Gel scoring errors did not contribute to disequilibrium for PGDH data for North Kimberley and Northern Australia, as the data was verified by re-collection during the process of standardisation reported elsewhere in this chapter.

Table 4.2. Tables of allelic frequencies for each locus. Alleles are designated by their relative mobility

Locus	Population	Allele Designation					2N
mAAT		-33	40	-80	-100	-132	
	Nth Territory 98	0	0	0.026	0.962	0.013	78
	Nth Territory 99	0	0	0	1.000	0	194
	East Coast 98	0	0	0	1.000	0	222
	East Coast 99	0	0	0	1.000	0	162
	Nth Kimb	0	0	0	1.000	0	172
	Torres 99	0.008	0	0	0.992	0	242
	Torres 98	0	0.004	0	0.984	0.012	246
	Kupang	0	0	0	1.000	0	174
	Nth Aust 99	0	0	0	0.996	0.004	262
	Nth Aust 98	0.010	0	0	0.990	0	208
	Shark Bay 99	0	0	0	1.000	0	198
	Shark Bay 98	0	0	0	0.980	0.020	102
Locus	**Population**	**Allele Designation**					**2N**
ACP		**20**	**100**	**200**			
	Nth Territory 98	0.275	0.637	0.087			80
	Nth Territory 99	0.295	0.632	0.074			190
	East Coast 98	0.332	0.605	0.064			220
	East Coast 99	0.399	0.530	0.071			168
	Nth Kimb	0.295	0.631	0.074			176
	Torres 99	0.335	0.619	0.047			236
	Torres 98	0.289	0.645	0.066			242
	Kupang	0.245	0.651	0.104			192
	Nth Aust 99	0.319	0.616	0.065			276
	Nth Aust 98	0.315	0.645	0.040			200
	Shark Bay 99	0.362	0.574	0.064			188
	Shark Bay 98	0.360	0.600	0.040			100
Locus	**Population**	**Allele Designation**					**2N**
ADH		**40**	**-100**	**-280**			
	Nth Territory 98	0.013	0.938	0.050			80
	Nth Territory 99	0	0.914	0.086			198
	East Coast 98	0.009	0.927	0.064			220
	East Coast 99	0.006	0.942	0.052			172
	Nth Kimb	0.017	0.892	0.091			176
	Torres 99	0	0.955	0.045			244
	Torres 98	0.020	0.904	0.076			250
	Kupang	0	0.984	0.016			192
	Nth Aust 99	0.007	0.918	0.075			280
	Nth Aust 98	0.029	0.859	0.112			206
	Shark Bay 99	0.020	0.894	0.086			198
	Shark Bay 98	0.020	0.890	0.090			100

Table 4.2 (continued). Tables of allelic frequencies for each locus. Alleles are designated by their relative mobility

Locus	Population	Allele Designation						2N
CK-A		**87**	**91**	**100**	**113**			
	N^{th} Territory 98	0.050	0	0.950	0			80
	N^{th} Territory 99	0.105	0	0.895	0			200
	East Coast 98	0.140	0.009	0.851	0			222
	East Coast 99	0.135	0	0.865	0			170
	N^{th} Kimb	0.097	0	0.903	0			186
	Torres 99	0.144	0	0.856	0			202
	Torres 98	0.083	0	0.917	0			252
	Kupang	0.076	0	0.918	0.005			184
	N^{th} Aust 99	0.087	0	0.913	0			276
	N^{th} Aust 98	0.121	0	0.879	0			214
	Shark Bay 99	0.105	0	0.895	0			200
	Shark Bay 98	0.093	0	0.907	0			108
Locus	**Population**	**Allele Designation**						**2N**
EST-1		**88**	**91**	**95**	**100**	**103**	**105**	
	N^{th} Territory 98	0	0	0.013	0.900	0	0.087	80
	N^{th} Territory 99	0	0	0.020	0.828	0.030	0.121	198
	East Coast 98	0.005	0	0.005	0.846	0	0.145	214
	East Coast 99	0	0	0.018	0.910	0	0.072	166
	N^{th} Kimb	0	0	0.034	0.904	0.006	0.056	178
	Torres 99	0.004	0	0.008	0.891	0	0.097	238
	Torres 98	0	0	0.008	0.898	0	0.094	254
	Kupang	0	0	0.048	0.892	0.038	0.022	186
	N^{th} Aust 99	0.018	0	0.018	0.871	0.036	0.058	278
	N^{th} Aust 98	0	0	0.005	0.953	0	0.042	212
	Shark Bay 99	0.005	0.005	0.005	0.897	0	0.088	194
	Shark Bay 98	0	0	0.010	0.902	0.010	0.078	102
Locus	**Population**	**Allele Designation**						**2N**
GPI-A		**78**	**91**	**100**	**110**	**130**		
	N^{th} Territory 98	0	0.162	0.837	0	0		80
	N^{th} Territory 99	0.010	0.100	0.875	0.015	0		200
	East Coast 98	0	0.131	0.851	0.018	0		222
	East Coast 99	0.006	0.151	0.801	0.042	0		166
	N^{th} Kimb	0.022	0.145	0.801	0.032	0		186
	Torres 99	0.010	0.134	0.847	0.010	0		202
	Torres 98	0.012	0.155	0.821	0.012	0		252
	Kupang	0.005	0.277	0.644	0.069	0.005		188
	N^{th} Aust 99	0	0.148	0.841	0.011	0		270
	N^{th} Aust 98	0.005	0.154	0.832	0.009	0		214
	Shark Bay 99	0.020	0.155	0.810	0.015	0		200

Table 4.2 (continued). Tables of allelic frequencies for each locus. Alleles are designated by their relative mobility

Locus	Population	Allele Designation							2N
sIDHP		**57**	**85**	**89**	**100**	**116**	**120**	**131**	
	N^{th} Territory 98	0	0	0.013	0.962	0.025	0	0	80
	N^{th} Territory 99	0.005	0	0.010	0.918	0.010	0.056	0	196
	East Coast 98	0	0	0.023	0.927	0	0.050	0	218
	East Coast 99	0	0	0.006	0.929	0	0.060	0.006	168
	N^{th} Kimb	0	0	0.006	0.983	0	0.011	0	178
	Torres 99	0	0.025	0	0.912	0	0.063	0	238
	Torres 98	0	0.004	0.004	0.956	0.012	0.024	0	252
	Kupang	0	0	0	0.990	0	0.010	0	194
	N^{th} Aust 99	0	0	0.004	0.954	0	0.043	0	280
	N^{th} Aust 98	0	0.010	0.005	0.947	0.005	0.034	0	208
	Shark Bay 99	0	0	0.010	0.970	0	0.020	0	198
	Shark Bay 98	0	0.010	0.020	0.931	0.010	0.029	0	102
Locus	**Population**	**Allele Designation**							**2N**
LDH-B		**100**	**149**						
	N^{th} Territory 98	0.988	0.013						80
	N^{th} Territory 99	1.000	0						196
	East Coast 98	0.995	0.005						222
	East Coast 99	1.000	0						172
	N^{th} Kimb	0.989	0.011						186
	Torres 99	1.000	0						200
	Torres 98	0.992	0.008						254
	Kupang	1.000	0						190
	N^{th} Aust 99	0.996	0.004						276
	N^{th} Aust 98	1.000	0						216
	Shark Bay 99	0.990	0.010						200
	Shark Bay 98	1.000	0						108
Locus	**Population**	**Allele Designation**							**2N**
PGDH		**79**	**85**	**95**	**100**	**108**			
	N^{th} Territory 98	0.013	0.192	0.218	0.577	0			78
	N^{th} Territory 99	0.043	0.085	0.186	0.660	0.027			188
	East Coast 98	0.014	0.145	0.138	0.696	0.007			138
	East Coast 99	0.006	0.019	0.253	0.708	0.013			154
	N^{th} Kimb	0.011	0.120	0.245	0.592	0.033			184
	Torres 99	0.020	0.150	0.171	0.626	0.033			246
	Torres 98	0.031	0.079	0.213	0.650	0.028			254
	Kupang	0.005	0.319	0.071	0.582	0.022			182
	N^{th} Aust 99	0.007	0.141	0.267	0.570	0.015			270
	N^{th} Aust 98	0.023	0.139	0.134	0.676	0.028			216
	Shark Bay 99	0.015	0.113	0.165	0.660	0.046			194
	Shark Bay 98	0.020	0.078	0.216	0.647	0.039			102

Table 4.2 (continued). Tables of allelic frequencies for each locus. Alleles are designated by their relative mobility

Locus	Population	Allele Designation						2N
PGM		47	76	85	87	100	119	
	N^{th} Territory 98	0.013	0.025	0	0	0.962	0	80
	N^{th} Territory 99	0	0.030	0	0	0.960	0.010	200
	East Coast 98	0	0.014	0.005	0.005	0.973	0.005	222
	East Coast 99	0	0.024	0	0	0.976	0	170
	N^{th} Kimb	0	0.011	0	0	0.984	0.005	186
	Torres 99	0	0.020	0.005	0	0.970	0.005	202
	Torres 98	0	0.004	0	0	0.988	0.008	252
	Kupang	0	0	0	0	0.995	0.005	190
	N^{th} Aust 99	0	0.011	0	0	0.989	0	274
	N^{th} Aust 98	0.005	0.005	0	0	0.991	0	218
	Shark Bay 99	0	0.015	0	0	0.980	0.005	200
	Shark Bay 98	0	0.019	0	0	0.981	0	108

Table 4.3. Numbers and ratios of females (F), males (M) and juveniles (J) genotyped from 10 spatio-temporal Spanish mackerel samples

A small number of fish from the East Coast, North Australia and Shark Bay were not sexed.

Population		Female	Male	Juvenile	F/M (all samples)	Spatially distinct samples
N^{th} Territory 98	NT 98	24	16	-	1.5	1.5
N^{th} Territory 99	NT 99	60	40	-	1.5	
East Coast 98	EC 98	52	58	-	0.9	1.1
East Coast 99	EC 99	51	35	-	1.5	
N^{th} Kimberly	NKmb	39	51	-	0.8	0.8
Torres Strait 98	TS 98	57	70	-	0.8	
Torres Strait 99	TS 99	59	65	-	0.9	0.9
Kupang	KUP	23	19	55	1.2	1.2
N^{th} Australia 98	NA 98	76	32	-	2.4	
N^{th} Australia 99	NA 99	87	49	2	1.8	2.0
Shark Bay 98	SB 98	28	23	3	1.2	
Shark Bay 99	SB 99	72	28	-	2.6	2.0

Table 4.4. P-values for Hardy-Weinberg Equilibrium for 10 spatially and temporally distinct samples for ten allozyme loci

Monomorphic loci are indicated (-).

Population	Locus									
	mAAT	ACP	ADH	CK-A	Est	GPI	sIDHP	LDH-B	PGDH	PGM
Nth Territory 98	0.013	0.744	1.000	0.076	1.000	0.045	1.000	-	0.045	1.000
Nth Territory 99	-	0.078	0.528	0.284	0.126	0.595	0.030	-	0.047	1.000
East Coast 98	-	0.015	0.444	0.215	0.022	0.355	0.447	-	0.052	1.000
East Coast 99	-	0.025	0.021	0.641	0.501	0.651	0.033	-	0.089	0.035
Nth Kimberly	-	0.112	1.000	1.000	0.393	0.318	1.000	1.000	0.0002	1.000
Torres Strait 98	1.000	0.157	0.705	1.000	0.183	0.253	1.000	1.000	0.016	1.000
Torres Strait 99	1.000	0.230	1.000	0.421	0	0.001	1.000	-	0.026	0.074
Kupang	-	0.170	1.000	0.148	0.132	0.578	1.000	-	0.041	-
Nth Australia 98	1.000	0.197	0.690	0.649	0.031	0.575	0.038	-	0.020	1.000
Nth Australia 99	-	0.135	0.626	0.598	0.391	0.018	1.000	-	0.002	0.011
Shark Bay 98	0.010	0.628	0.460	0.367	1.000	0.589	0.149	-	0.590	1.000
Shark Bay 99	-	0.146	1.000	0.284	0.658	0.347	1.000	1.000	0.080	1.000

Loci ACP and PGDH had the highest levels of heterozygosity (proportion of total number of expected heterozygotes; ACP, 0.513; PGDH, 0.544). Loci mAAT and LDH-B were monomorphic for six of the 12 populations and loci sIDHP and PGM had similarly low levels of heterozygosity (IDH, 0.100; PGM, 0.039). The heterozygosities of the remaining loci were 0.151 for ADH, 0.191 for CK-A, 0.201 for EST and 0.306 for GPI-A.

The mean expected heterozygosity across the 12 populations was 0.252 (standard deviation = 0.014) for eight polymorphic loci, excluding loci mAAT and LDH-B that were monomorphic for numerous populations.

Genetic Subdivision. A global test for genetic subdivision between all pairs of population samples was performed with pooled 1998 and 1999 data. Data was pooled in line with the probable likelihood of temporal stability at collection locations. The allele frequencies of the fish in the Kupang collection differed from the five Australian collections at numerous loci. Three loci were significantly different between Kupang and North Kimberley (ADH, GPI-A and PGDH) while seven loci differed between Kupang and East Coast collections (ACP, ADH, CK-A, EST-1, GPI-A, sIDHP and PGDH, Table 4.5). Allele frequencies at loci EST-1 and sIDHP were significantly different between the East Coast sample and other Australian collection locations, except those from Shark Bay. Similarly, Est-1 allele frequencies differentiated the Torres Strait sample from the remainder, except Shark Bay and the East Coast. Two other pairs of locations had significantly different allele frequencies; the Northern

Territory and Northern Australia (loci Est-1 and PGM) and Northern Australia and North Kimberley (GPI-A)

Table 4.5. Allozyme loci that show significant (p<0.05) allele frequency variation between pairs of Spanish mackerel populations

The populations are East Coast (EC; 1998&1999), Torres Strait (TS, 1998&1999), the Northern Territory (NT; 1998&1999), northern Australia (NA; 1998&1999), North Kimberley (Nkimb, 1999), Shark Bay (SB; 1998&1999) and Kupang, West Timor (Kup, 1999).

	Torres Strait	Northern Territory	northern Australia	Nth Kimberly	Shark Bay	Kupang, West Timor
East Coast	IDH	Est	Est	Est, IDH		ACP, ADH, CK-A, Est, GPI-A, IDH, PGDH
Torres Strait	—	Est	Est	Est		ACP, ADH, Est, GPI-A, PGDH
Northern Territory		—	Est, PGM			ADH, Est, GPI-A, IDH, PGM
Northern Australia			—	GPI-A		ACP, ADH, Est, GPI-A, PGDH
Nth Kimberly				—		ADH, GPI-A, PGDH
Shark Bay					—	ACP, ADH, Est, GPI-A, PGDH

Table 4.6. Probability of genic differentiation for eastern and north-west Australian genetic stocks of Spanish mackerel for ten allozyme loci

P-values relate to the inclusion of the Torres Strait population in either the eastern or north-west genetic stock. The eastern genetic stock was represented by East Coast 1998 & 1999 samples, and the north-western stock represented by Northern Territory 1998 & 1999, northern Australia 1998 & 1999, North Kimberley 1999, and Shark Bay 1998 & 1999 samples. The Torres Strait was represented by Torres Strait samples collected in 1998 & 1999.

	p over ten loci	Loci where p<0.05	
East Coast and Torres Strait v Northern and Western	0.00277	CK-A ADH Est	p=0.03350 p=0.03824 p=0.00012
East Coast v Torres Strait and Northern and Western	0.00173	CK-A PGDH IDH	p=0.00207 p=0.03439 p=0.02041

Table 4.7. Fst's between Kupang, East Coast (pooled East Coast and Torres Strait) and North and West Coast (pooled Northern Territory, North Kimberley, Northern Australian and Shark Bay) samples, for ten allozyme loci

Locus	Kupang vs East Coast	Kupang vs North and West Coast	East Coast vs North and West Coast
AAT	-0.0006	-0.0023	-0.0011
ACP	0.0079	0.0045	-0.0007
ADH	0.0180	0.0340	0.0045
CKA	0.0066	-0.0011	0.0025
Est	0.0194	0.0081	0.0013

GPI	0.0755	0.0791	-0.0010
IDH	0.0183	0.0085	0.0022
LDH	-0.0010	-0.0003	-0.0008
PGDH	0.0588	0.0472	0.0006
PGM	0.0028	0.0030	-0.0009
All loci	0.0344	0.0305	0.0006

Shaklee's (unpublished, Appendix 4) hypothesis of genetic stock structure consisting of an east coast stock (represented by collections from Cairns and Moreton Bay) and a north-western stock of Spanish mackerel could not be rejected by the data collected in our study. In our study, grouped east coast samples (EC 98&99, Townsville area only) were significantly different from grouped northern and western samples (NT98&99, NA98&99, NKimb, SB98&99). However, the relationship of the Torres population to either stock was ambiguous in our data set. When the Torres population (TS98&99) was included with our east coast collections, and compared to the north-west populations the probability of genic differentiation between the two groups over ten loci was 0.00277, with three loci (ADH, p=0.03824; CK-A, p=0.03350 and EST, p=0.00012) having significant p-values (Table 4.6). Conversely, when the Torres population was included with the north-west samples the overall p-value was 0.00173, again with three loci having significant p-values (CK-A, p=0.00207; PGDH, p=0.03439; sIDHP, p=0.02041).

Fst values reflected the major genetic break between Kupang and Australian stocks where the Fst over all loci was 0.0344 (Kupang v East Coast) and 0.0305 (Kupang v North and west coast, Table 4.7). The Fst between the East Coast and North and West coast stock was a small but positive 0.0006 over all loci, ranging from a high of 0.0045 for ADH, 0.0025 for CK-A and 0.0013 for EST (Table 4.7) to negative values for five loci (mAAT, ACP, GPI-A, LDH-B and PGM).

MtDNA

Intra-population genetic diversity. In 434 bp at the 5' end of the control region (GenBank accession no. AY205244), 107 variable sites were observed, including five single base pair indels in 179 Spanish mackerel (Table 4.8). Of 148 haplotypes, there were 16 haplotypes that were represented by more than one fish (Table 4.9). The distribution of the observed number of differences between pairs of haplotypes was multimodal, most likely reflecting the random nature of gene trees and suggesting that, except for the Kupang population, recent population expansion has not occurred (Figure 4.3).

As expected, sequence variation in the cytochrome b sequence was less extensive than the control region sequences. In 343 bp (GenBank accession no. AY205243) only ten variable characters were observed among 38 fish. There were six haplotypes that were represented by more than one fish (Table 4.10), and two fish had unique sequences.

Intrapopulation control region sequence divergence was high for the Australian sampling locations (Table 4.11). The East Coast and Torres Strait populations had mean sequence diversities around 5 to 6%. The northern and western intrapopulational sequence diversity

ranged from 3 to 4%. The Kupang population was an order of magnitude below this, at 0.5% sequence diversity.

Table 4.8. Populations and sampling year from which mtDNA nucleotide sequence (Control region, cytochrome b) or RFLP data was obtained

Population	N		
	Control Region		Cyt b
	Sequence	RFLP	Sequence
Northern Territory 98	21	25	2
Northern Territory 99	12	45	0
East Coast 98	23	44	6
East Coast 99	3	64	0
Nth Kmberly 98	20	0	2
Torres Strait 98	23	46	9
Torres Strait 99	2	67	0
Kupang 97	0	0	4
Kupang 98	21	0	0
north Australia 98	20	26	5
north Australia 99	10	45	0
Shark Bay 98	22	0	10
Shark Bay 99	0	0	0
Southern Gulf of Carpentaria 99	2	43	0
Total	179	405	38

Table 4.9. Numbers of identical fish control region sequences in each of 16 haplotypes, and the population from which the fish was collected. The populations were East Coast (EC; 1998&1999), Torres Strait (TS, 1998&1999), Northern Territory (NT; 1998&1999), northern Australia (NA; 1998&1999), North Kimberley (Nkimb, 1999), Shark Bay (SB; 1998&1999) and Kupang, West Timor (Kup, 1998)

Haplotype Group No. / Region	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
East Coast	1				5	1	1	2								
Torres Strait						1		1	1					1		1
North Australia			1				1			1		1		2	1	
Northern Territory		1					1				1			2	2	1
BR	1	1	1	2												
K		3											2			

Shark Bay	1	1	1	1

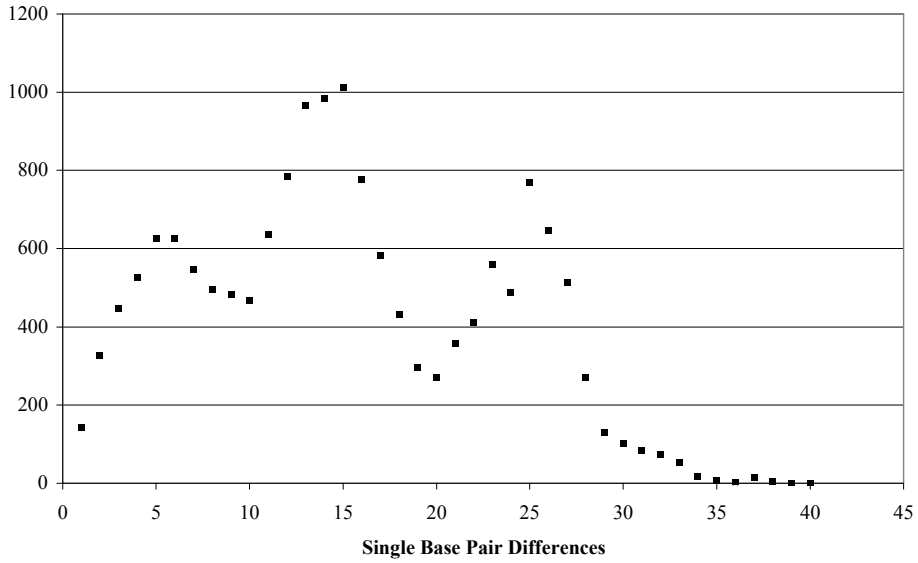


Figure 4.3. Mismatch distribution for 179 control region sequences from Spanish mackerel representing seven Australian and one Indonesian populations

Table 4.10. Numbers of identical fish cytochrome b sequences in each of six haplotypes, and the population from which the fish was collected. The populations were East Coast (EC; 1998), Torres Strait (TS, 1998), Northern Territory (NT; 1998), northern Australia (NA; 1998), North Kimberley (Nkimb, 1999), Shark Bay (SB; 1998) and Kupang, West Timor (Kup, 1997)

Haplotype Group No. / Region	1	2	3	4	5	6
East Coast	1		4			
Torres Strait	1		3	2	1	
North Australia		1		3		1
Northern Territory	1	1				1
BR				1	1	
K				4		
Shark Bay	2	1		6		

Table 4.11. Intra-population nucleotide diversity (Kimura's equivalent to gene diversity at the nucleotide level) for 5' end of the control region. Standard deviations are for both sampling and stochastic processes

	N	Mean	Std Dev
Northern Territory 98, 99	33	0.035830	0.018381
East Coast 98, 99	26	0.050451	0.025687
North Kmberly, 98	20	0.037867	0.019779

Torres Strait 99, 99	25	0.058980	0.030039
Kupang 98	21	0.005904	0.003747
north Australia 98, 99	30	0.031343	0.016235
Shark Bay 98	22	0.038403	0.020028
SG 99	2	0.004831	0.005900
Total	179		

Spatial Molecular Variance. *Stock subdivision between West Timor and Australia.* An AMOVA was performed to test various hypotheses of population structure. Combining the Australian mackerel sequences and comparing them to the Kupang sequences gave the highest Φ estimate (0.19), confirming the distinctiveness of stocks across the national boundary between West Timor (Indonesia) and Australia (Table 4.12).

Similarity among northern and western Australian populations. There was no evidence of genetic population structure among the Shark Bay, Kimberley, north Australian and Northern Territory stocks. The Φ estimate was small (0.007) and not significant (Table 4.12).

Stock subdivision across Cape York. As for the allozyme genetic results, the mtDNA data shows that the Torres Strait population has affinities to the northern and western stock, as well as to the east coast stock. In the AMOVA analysis the Torres Strait population was included in the northern and western stock and compared to the east coast stock, and then with the east coast population, and compared to the northern and western stock. Both combinations yielded similar Φ estimates (Table 4.12). However, the AMOVA also revealed that the Torres Strait population was subdivided from the northern and western stock (Φ_{ST} = 0.045, p=0.0004), and was genetically similar to the east coast stock (Φ_{ST} = 0.003, p=0.24).

Phylogeny. Of the 107 polymorphic nucleotide positions in from the control region, 80 were parsimony informative. The shortest MP tree found was 340 character state changes in length (CI 0.0371, HI 0.629). Three well-characterised clades of haplotypes were present on a strict consensus tree of 10,414 trees each of which was 340 in length (Figure 4.4). Clade A (east coast clade) consisted of 19 sequences from 12 east coast, six Torres Strait and one Northern Territory fish. In 500 bootstrap replicates, this clade was present in 100% of NJ

Table 4.12. AMOVA[1] to test for genetic population structure among populations from the East Coast (EC; 1998&1999), Torres Strait (TS, 1998&1999), Northern Territory (NT; 1998&1999), northern Australia (NA; 1998&1999), North Kimberley (NKimb, 1999), Shark Bay (SB; 1998&1999) and Kupang, West Timor (Kup, 1998), using control region sequence. Structure refers to the geographical hierarchy given below. Some statistics were not applicable (NA).

Structure	Φ_{CT}[2]	*p*	Φ_{SC}	*p*	Φ_{ST}	*p*
(Kup, EC, TS, NT, NA, NKimb, SB)	NA	NA	NA	NA	0.15841	0.00000
(Kup) (EC, TS, NT, NA, NKimb, SB)	0.19807	0.14211	0.10743	0.00000	0.28422	0.00000
(EC, TS)(NT, NA, NKimb, SB)	0.14892	0.06655	0.01031	0.00006	0.15769	0.00000
(EC)(TS, NT, NA, NKimb, SB)	0.14579	0.00000	0.04265	0.00000	0.18222	0.00000
(TS, NT, NA, NKimb, SB)	NA	NA	NA	NA	0.04589	0.00044
(NT, NA, NKimb, SB)	NA	NA	NA	NA	0.00723	0.22435
(EC, TS)	NA	NA	NA	NA	0.00308	0.24003

Note: [1] Analysis of molecular variance.
[2] Φ_{ST} is the correlation of random haplotypes within populations relative to that of random pairs of haplotypes drawn from the complete data set analysed, Φ_{CT} is the correlation of random haplotypes within a group of populations relative to that of random pairs of haplotypes drawn from the complete data set analysed, and Φ_{SC} is the correlation of the molecular diversity of random haplotypes within a population relative to that of random pairs of haplotypes drawn from the region. Significance of Φ estimates is obtained by permutations of the original data.

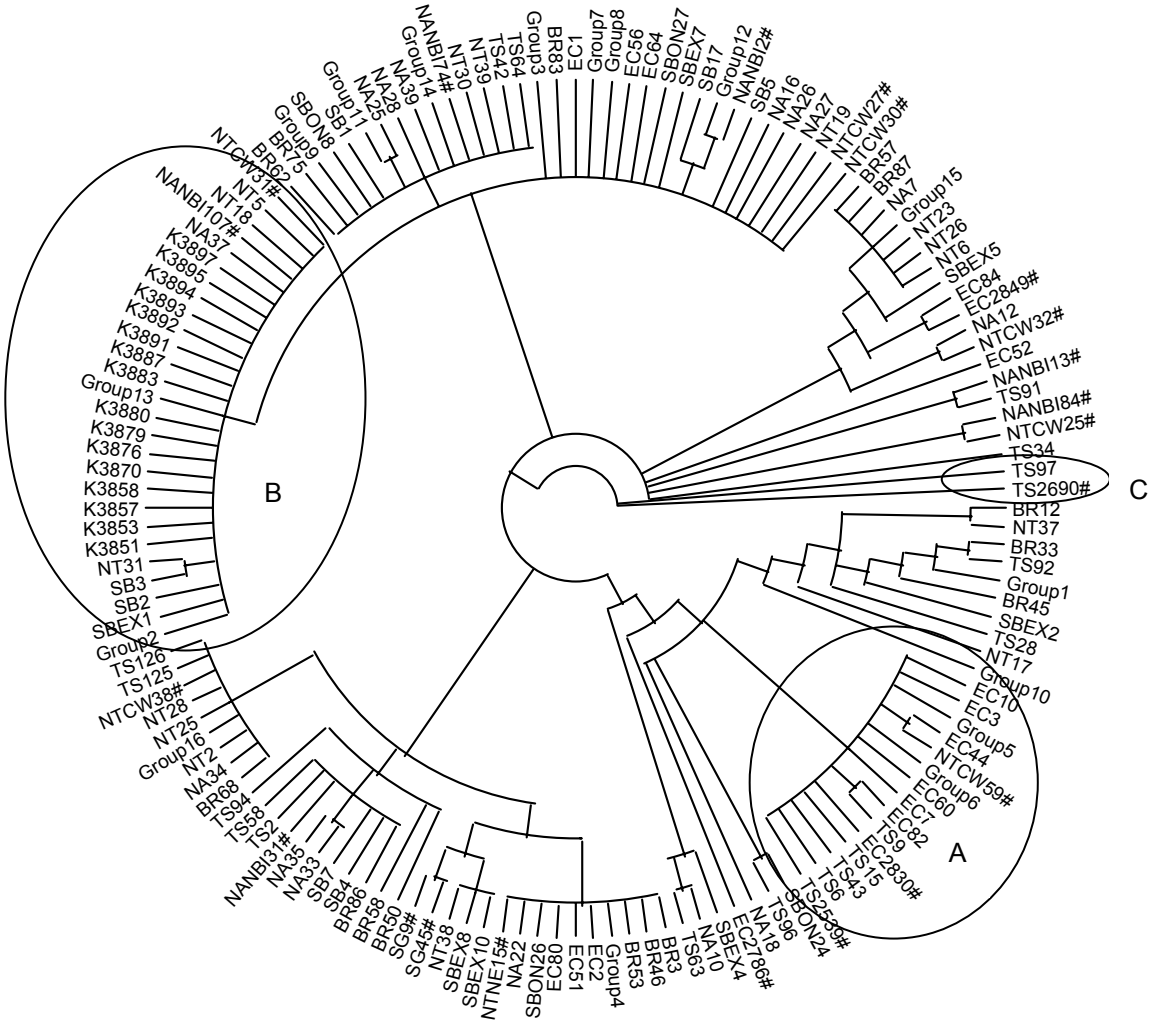


Figure 4.4. Clades A, B and C on the strict consensus of 10,414 maximum parsimony trees, rooted with TS97 and TS2690 and constructed from control region sequence data
Each branch represents a fish or group of identical fish (Table 4.9) from East Coast (EC), Torres Strait (TS), Northern Territory (NT) northern Australia (NA), North Kimberley (BR), Shark Bay (SB), Southern Gulf of Carpentaria (SG) and Kupang, West Timor (K). Fish collected in 1998 and 1999 are marked.

trees (not shown) and was fully or partially supported by eight characters (6, 25, 26, 40, 53, 55, 74 and 102). Further support for the relatedness of fish in the east coast clade (clade A) comes for the cytochrome b sequence data. Seven of the clade fish that were assayed for cytochrome b sequence were identical (haplotype group 3, Table 4.10), but were distinct from the remainder (Figure 4.5).

Clade B (Kupang clade, Figure 4.4) consisted of all of the fish sequenced from Kupang (21), seven sequences from Northern Territory fish, four sequences from North Australian fish and three sequences from Shark Bay fish. Three variant sites (36, 84 and 91) supported clade B.

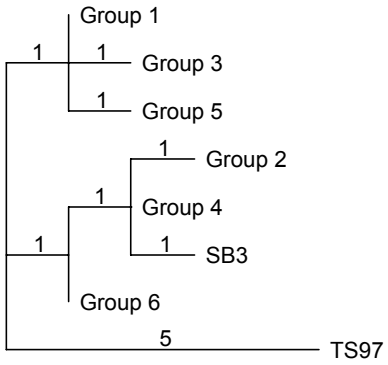


Figure 4.5. Maximum parsimony tree constructed from cytochrome b sequence data and rooted with TS97
Each branch represents a fish (Torres Strait, TS; Shark Bay, SB) or group of identical fish (Table 4.2B). There were ten polymorphic nucleotides or characters, of which six were parsimony-uninformative. The length of the tree was 12 and the number of character states changes along each branch is shown.

Table 4.13. Frequencies of the RFLP east coast haplotype among East Coast (EC), Torres Strait (TS), southern Gulf of Carpentaria (SG), Northern Territory (NT) and northern Australia (NA) populations in 1998 and 1999
See Table 4.8 for the number of fish assayed using the RFLP technique for each population.

	1998	1999
East Coast	0.25	0.20
Torres Strait	0.09	0.10
Southern Gulf of carpentaria	NA	0
Northern TerritoryT	0	0.02
northern Australia	0.04	0

Table 4.14. Estimates of the standardised allele frequency variance (Fˆ) for the East Coast (EC), North Australia (NA), Torres Strait (TS), Shark Bay (SB) and North Kimberley (NKimb). Estimates apply to temporal change between samples taken in 1980s (Shaklee, unpublished, Appendix 4), between samples taken in the 1990s (this study) and between the pooled 1980s and 1990s samples for data from the six standardised allozyme loci (see text for details). Confidence intervals for F are presented, along with F's that are adjusted for sampling error by subtracting 1/S, where S is the sample size

Method	Between 80s samples			Between 90s samples			Pooled 80s to pooled 90s		
	F	CI	F-1/S	F	CI	F-1/S	F	CI	F-1/S
East Coast	0.0034	0.001, 0.019	-ve	0.0258	0.009, 0.196	0.0155	0.0064	0.002, 0.038	0.0021
North Australia	-	-	-	0.0188	0.007, 0.138	0.0107	0.0132	0.005, 0.098	0.0070
Torres Strait	0.0079	0.003, 0.053	0.0001 1	0.0146	0.005, 0.108	0.0066	0.0051	0.002, 0.035	0.0012
Shark Bay	-	-	-	0.0087	0.003, 0.064	-ve	0.0088	0.003, 0.065	0.0027

North Kimberly	-	-	-	-	-	-	0.0117	0.004, 0.085	0.0015

Clade C consisted of two fish collected from the Torres Strait. It was well supported by nine control region characters (partially by 3, 10, 73, 85 and 97; fully by 8, 33, 50 and 89) and was present in 100% bootstrap replicates of the NJ tree (not shown). One of the clade C fish (TS97) had a distinct cytochrome b sequence, that varied by five characters (Figure 4.4).

The control region sequence divergence among the 19 Clade A (east coast clade) fish was 0.007830 (±0.004932) compared to an overall diversity for 139 non-east coast clade fish from Australia of 0.038371 (±0.019179).

RFLP Haplotype Frequencies. The RFLP test was used to further identify Clade A (east coast clade) fish. Eleven clade members where found among a further 44 East Coast fish collected in 1998 and 13 were found among 64 fish collected in 1999 (Table 4.6). This was in addition to the 12 east coast clade fish identified by sequencing from 25 fish collected in 1998 and 1999. For the Torres Strait collection in 1998, the RFLP test revealed that four fish from 46 were east coast clade members, and seven of 67 were members in 1999. The number of east coast clade fish identified from sequencing data in the Torres Strait in 1998 and 1999 was six out of 25. The proportion of east coast clade fish identified using sequencing (East Coast, 0.48; Torres, 0.24) was higher than with the RFLP's (East Coast, 0.22; Torres, 0.10), but in both cases, east coast clade fish were about twice as frequent on the East Coast compared to the Torres Strait.

East coast clade fish were found at low frequencies at the Northern Territory (0.01) and the North Australia (0.01, Table 4.13). They were not found among 43 fish from the southern Gulf of Carpentaria collected in 1999.

Temporal comparison of allozyme data

Standardisation

Raw Data. Between 124 and 127 fish from the two sampling localities (Torres Strait 98 and 99) were re-scored for four loci (MAAT, ACP, sIDHP and PGDH), except only 26 fish were scored from Torres Strait 99 for ACP. We were unable to replace an essential ingredient for the ACP stain (Black K salt). Gel photos, scoring and comparisons between original and standardised data are presented in Appendix 3.

Allele scoring was affected by running buffer type for loci mAAT and sIDHP (Inconsistency #1). Six of 249 fish were scored differently for mAAT on the different buffers (CAEA and CAAPM). Eight fish were scored differently for sIDHP depending on whether CAEA and CAAPM, or TC-1 and TRIC buffers were used. Similarly, two fish of 151 for ACP and one fish of 250 for sIDHP were scored differently depending on whether CAEA or CAAPM buffers were used (Inconsistency #2).

Nineteen fish out of 250 were scored either during standardisation, or otherwise, but not on both occasions (Inconsistency #3). These fish were not consistently heterozygotes, as may have been the case if heterozygotes were more likely to be weakly stained or poorly resolved and thus generally less scorable. Three homozygote fish and two heterozygote fish were scored in 1999 and 2000, but not during standardisation in 2001. Nine heterozygote and five homozygote fish were scored in 2001, but not previously.

Liver tissue from one fish of 250 was resolved on CAEA and CAAPM buffers during standardisation as +/f genotype for PGDH. Muscle tissue from the same fish was resolved as f/f genotype (Inconsistency #4).

As a consequence of the standardisation, we had a valuable opportunity to measure, and subsequently improve data accuracy by comparing the scores between original and standardised gels. Human error (Inconsistency #5a) was responsible for incorrect scorings in 0.8% (2/250, locus sIDHP), 1.3% (2/151, locus ACP) and 1.6% (4/250, locus PGDH) of fish. No incorrect scoring was detected for locus mAAT. Scoring errors associated with poor gel resolution (Inconsistency #5b) did not occur for loci mAAT or sIDHP, but occurred in 2.6% (4/151) of the fish for locus ACP and 3.6% (9/250) for locus PGDH. These error rates are similar to those reported by White (1991). To control this error rate

- the original data was corrected from the standardised data (TS 98 and 99 samples for loci (MAAT, ACP, SIDHPand PGDH) were necessary,
- selected original data was re-scored from original photographs (EC 98 and 99 samples for PGDH, NA 98 and 99 for EST-1)
- selected original data was re-scored from repeated starch gels (NA 98 and 99 samples for PGDH).

Inconsistencies.

1. **For the same fish, allele mobility or scoring differed between standardised and original data due to buffer substitution for two loci; mAAT and sIDHP.**
    a. For sIDHP on 24th October 2001, TS026 was scored as 100/116. In 1999 on TC-1 and TRIC buffers it was scored as 100/120.
    b. For mAAT, TS039 was scored as 100/132 in 1999 on TRIC, but was scored on 24th October 2001 as a mf (104), not an f (132) heterozygote on CAEA and CAAPM buffers. ‘104’ was a rare allele that Shaklee observed (Shaklee, unpublished, Appendix 4), but which was not previously reported by this study.
    c. For mAAT, TS015 was scored as 100/040 in 1999 on TRIC, but was clearly 100/100 in 17th October 2001 on CAEA and CAAPM buffers.
    d. For mAAT, TS035 was scored at 100/132 on 24th October 2001 on CAEA and CAAPM buffers, but as 100/100 in 1999 on TRIC.

e. For mAAT, a s/s fish (080/080, TS054, 24th October 2001 on CAEA and CAAPM buffers) was not detected in 1999 where it was scored as 100/100 on TRIC in 1999.
f. For sIDHP on 25th October 2001, the fast allele of TS070 and TS086 may not be 116, as it was in 1999 on TRIC and TC-1 buffer, but be 120 (relative mobility on 2001 CAEA gel = 32/26). Similarly, the slow allele of TS81 may not be 089, but 085.
g. For sIDHP, TS2584 was scored as 100/100 on clearly resolved TRIC and TC-1 gels in 2000, but was clearly resolved as 100/085 on 31st October 2001 on CAEA and CAAPM buffers.
h. For mAAT, TS2540 was a 033/100 het in 1999 on TRIC buffer, but a 100/100 on 31st October 2001 on CAEA and CAAPM buffers.
i. For mAAT, TS2701 was scored as a clearly resolved 100/100 in 2000 on TRIC buffer, but as a clearly resolved 100/132 on 7th November 2001 on CAEA and CAAPM buffers.
j. For sIDHP, fish TS2630 was scored as a clearly resolved homozygote in 100/100 in 1999 on TC-1 and TRIC buffers, but was scored as a clearly resolved 100/120 on 1st November 2001 on CAEA and CAAPM buffers.
k. For sIDHP, fish TS2648 was scored as 100/100 in 1999 from a well resolved band using TC-1 and TRIC buffer, but as 100/120 in a well-resolved gel on 1st November 2001 using CAAPM and CAEA buffers.
l. For sIDHP, TS2745 that was 120/100 in 1999 on TC-1 and TRIC, but clearly 100/100 on 8th November 2001 on CAAPM and CAEA.

In the following five cases, buffer substitution and poor gel resolution led to inconsistencies.

m. For mAAT on 17th October 2001, TS021 was scored as 132/100 in 1999 and 132/132 in 2001. Either interpretation could be correct, as both gels had marginal resolution.
n. For sIDHP, TS2544 was 120/100, but was 100/100 on 31st October 2001. TS2581 was 100/100, but was scored in 2001 as 120/100. The photo shows that previous gel resolution for TS2544 and 2581 was poor.
o. For mAAT, TS123 appeared to be a 132/132 on 31st October 2001, but a 132/100 het in 1999. The 1999 score is probably correct as it was a clearer gel.
p. For sIDHP, fish TS2638 was scored as 100/085 in 1999, but as 100/100 on 1st November 2001. However the 1999 gel photo shows that it was scored from a faint band.
q. For sIDHP, fish TS2646 was scored as 100/085 in 1999 from poorly resolved bands, but as 100/100 1st November 2001.

2. **For the same fish, allele mobility or scoring differed for the standardised data between CAAPM and CAEA gels.**

a. For ACP, a medium fast allele (TS109) was not observed in 1999 on CAAPM buffer, and on 31st October 2001 it was only observed on the CAEA buffer (TS109). This fish was scored as a 100/100 based on the CAAPM result.
b. For ACP on 31st October 2001, TS2544 appears as a heterozygote (100/200) on CAEA buffer, but as a 100/100 on CAAPM.
c. For sIDHP (1st November) using CAAPM buffer, fish TS2689 had a 131 (very fast) allele, however on CAEA the same fish had a 120 (fast) allele. The fast, not the very fast allele, was scored for this fish in 2000 on buffers TC-1 and TRIC.

3. For the same fish, alleles could not be scored for both original and standardised data. This occurred due to lack of staining, or because the sample was too poorly resolved to be scored.

a. TS054 did not stain on 24 October 2001, but did stain in 1999 for sIDHP where it was 100/100.
b. For PGDH on 24th October 2001 on gel #1 five samples could not be scored, that could be scored in 1999. Three were heterozygotes, and two were homozygotes.
c. For mAAT on 24th October 2001, TS6 could not be scored, whereas in 1999 it was scored as a 100/100.
d. For PGDH, TS93 was scored as 100/095 on 25th October 2001, but not scored in 1999.
e. For mAAT, fish TS86 was scored on 25th October 2001 (100/100), but failed in 1999.
f. For sIDHP, TS2545 was resolved on 31st October 2001 (100/100), but not scored in 1999.
g. For PGDH, TS2462 & 3 were scored on 31st October 2001 as heterozygotes (100/085 and 100/095), but they were not scored in 1999 as they did not stain on the gel.
h. For ACP, TS121 was scored as 200/100 in 1999, but could not be scored on 31st October 2001.
i. For sIDHP, fish TS2645 not scored in 2000, but was scored as a 100/085 heterozygote on 1st November 2001.
j. For PGDH (1st November), TS2680 was not scored in 2000, but was scored as 085/095 in 2001. This is a class of heterozygote that may not be detected on poorly resolved gels as the mobility difference between 085 and 095 is small, and consequently a three-banded pattern may not be detected.
k. For PGDH on 7th November, TS2702 was scored as 100/100 in 2001 although it was faint. It was not scored at all in 2000.
l. For PGDH, TS2733&4 could not be scored in either 2000 or 8th November 2001. This would not have resulted in a net change in the number of heterozygotes as in each case the pattern was TS2733 100/095 (2001) & no score (2000) and TS2734 no score (2001) and 100/095 (2000).

m. For PGDH on 8th November 2001, TS021 could not be genotyped; it was 085/085 in 1999.

4. **For the same fish, allele mobility differed between liver and muscle tissue.**
    a. For PGDH, TS035 was scored as 100/079 on liver in 1999 and again on 24th October 2001, but as 100/085 on muscle in 2001. Gel photos suggest that liver samples produce cathodal sub-bands that may be responsible for the '079' score.
    b. For PGDH on 25th October 2001, TS104 was scored as a 108/108 on muscle on CAEA, and 100/108 on liver on both CAEA and CAAPM. The 100/108 score was consistent with the 1999 score.
5. **Allelic differences between original and standardised data as a result of**
    a. human error, where original gels were scored incorrectly or where pairs of samples were reversed during loading
        i. For PGDH (24th October 2001), TS60 was scored as 085/100 in 1999, but was scored as 095/100 in 2001. TS60 was repeated in 1999 at both ends of the gel, and it was scored a second time at 095/100. It is likely that the 2001 score is correct.
        ii. For ACP, TS29 and 30 (24th October 2001) were scored incorrectly in 1999.
        iii. For sIDHP, sample TS104 was detected as a 100/120 heterozygote on 25th October 2001, but was scored as a 100/100 in 1999. Examination of the 1999 photo shows that the 1999 score was incorrect; it is a 100/120 on the photograph.
        iv. For PGDH on 25th October 2001, two heterozygote fish (TS98/9) were scored as 100/085 in 1999, but 100/095 in 2001. However, the 1999 photo shows they should have been 095/100, so the 1999 score was incorrect.
        v. For PGDH on 25th October 2001, TS66 and TS67 were scored from muscle and they differed from the 1999 scores. However, the 1999 and 2001 scores from liver were identical (TS66; 100/100; TS67 095/100), so it is likely that the muscle samples were reversed when loaded onto the gel.
        vi. For sIDHP, fish TS 112 was scored as 100/100 in 1999, but as 100/120 on 31st October 2001. The 1999 photo shows that it should have been scored as a heterozygote.
        vii. For PGDH, scores for TS2544/5 may have been reversed in either 1999 or 2001 (TS2544 no score > 100/095, TS2545 100/095 > no score).
    b. poor resolution of either original or standardised gels.
        i. For ACP, TS25, 27 and 28 were poorly resolved in 1999, but could be scored clearly from the 24th October 2001 gel.
        ii. For ACP in 1999 five fish were scored as 020/020 (TS32-34, 42, 44), but on 24th October 2001 they were scored as 100/200. After checking these on 1999 photos they were all poorly resolved in 1999. A further four fish

(TS48, 49, 51 and 52) were poorly resolved in 1999, but well resolved in 2001 as 100/020.

iii. For ACP on 25th October 2001, TS93 was scored as 020/020, but in 1999 it was scored as 020/100. TS94 was scored in 2001 as 100/100, but was scored in 1999 as 100/020. The 1999 gel was smeary, and the 2001 gel was clear so the 2001 scores are most likely more reliable.

iv. For PGDH, TS110 was scored as 085/085 in 1999, but 095/095 on 31st October 2001. Neither 1999 nor 2001 gels were well resolved, and the sample was entered as 'no data'.

v. For PGDH, TS2464 was 100/095 in 2000, but was 100/085 on 31st October 2001; the 2000 gel was too weak to score reliably. TS2500 was 100/095 in 2000, but is 095/095 as the 2000 gel was too weak to score reliably.

vi. For ACP, TS2541 was 020/020 in 1999, but was 100/100 in 2001. TS2579 was 200/100 in 1999, but was 100/100 in 2001. TS 2514 was 020/020 in 1999 but was 100/100 in 2001. These three fish were all feint on the 1999 gel photo, so the 2001 scores were used.

vii. For PGDH, fish TS2629 was scored as 108/100 in 2000, but as 108/108 on 1st November 2001. The 1999 gel was clearer than 2001, so the 1999 score is probably the more reliable.

viii. For PGDH, fish TS2666 was 100/100 on 1st November 2000, now 108/108 on this gel. The 2000 gel was faint, so the 2001 gel is likely to give the most correct score.

ix. For PGDH, TS2677 was 085/085 in 2000, and was 085/095 on 1st November 2001. Similarly, TS2678 was 100/085, now 100/095. These two fish were previously feint on the 2000 gel.

x. For PGDH on 7th November 2001, TS2695 was well resolved as a 100/100, but in 2000 it was scored as 100/079 in 1999. On the 2000 gel the sample was faint.

xi. For PGDH, TS2719 was scored as 100/095 in 2000 from a faint gel, and 100/085 on 8th November 2001.

xii. For PGDH, TS 2748 was scored as 085/085 in 1999, but as 095/085 on 8th November 2001. As both the 1999 and 2001 gels were poorly resolved, the score for this fish was 'no data'.

xiii. For PGDH, TS012 was scored as 015/079 in 1999, and 095/079 on 8th November 2001. As both the 1999 and 2001 gels were poorly resolved, the score for this fish was 'no data'.

Comparability. A data set was created following the process of standardisation that allowed direct tests for temporal genotypic and genic stability across the 15-20 intervening years. The standardised data set consisted of six loci ACP, ADH, CK-A, GPI-A, LDH-B and PGDH (loci mAAT, Est-1, sIDHP and PGM were excluded). The first of the five sampling locations was

the East Coast, where the 1981 and 1982 sampling sites (Cairns #1 and Cairns #2 of Shaklee) were about 300 kilometres north of the 1998 and 1999 sampling sites (off Townsville) from this study. The Torres Strait samples were taken from Bramble Cay in 1983, 1984, 1998 and 1999. North Australian samples were taken from Cape Croker in 1984 and about 300 km to the west of there in 1998 and 1999 around Bathurst and Melville Islands. The North Kimberley sampling sites were separated by about 200 km (1983; Holothurian Banks, 1999; Kimberley coast). The Shark Bay samples were taken from Capes Cuvier and Quobba in 1985 and within a 500 km radius in 1998 and 1999 (Shark Bay, Exmouth and Dampier coast).

The inclusion of loci in the standardised data was based upon the following.

mAAT. For this locus, this study used TRIC as a running buffer, while Shaklee used CAD (another acronym for CAEA). This study used liver tissue and Shaklee used muscle tissue. Standardisation experiments showed that allele identification was dependent on the type of running buffer. For example, fish TS039 was genotyped as 100/132 on TRIC buffer during 1999 gels, but as 100/104 on CAEA and CAAPM buffer during standarisation gels on 24th October 2001 (Appendix 3). Several other inconsistencies were noted, such as fish TS015 that was alternatively genotyped as 100/040 or 100/100 and fish TS054 (100/100 compared to 080/080), suggesting that results from this study for this locus that were obtained on TRIC buffer were not comparable to the results of Shaklee. Therefore, this locus was not used during comparisons between the studies.

ACP. Both studies used identical buffers; CAAPM (CAAPM is identical to CAM-6) for this locus, except that Shaklee used CAEA as well. Both studies used liver tissue. Standardisation experiments revealed possible cases of inconsistencies between buffers, human errors and mis-scoring associated with poor-gel resolution (above, and Appendix 3). Despite these, there was a high degree of comparability between the data from this study and Shaklee's data.

ADH. Both studies used LiOH buffer and liver tissue. Shaklee reported the alleles described in this study with the addition of a few rare cathodal alleles (for example, -180 and –280) that were pooled in his analysis. Alleles from this study that resolved near the origin had different mobilities from those reported by Shaklee, for example our allele 020 was Shaklee's –090. Differences in the amount of electroendo-osmosis between batches of starch may explain this effect. This study may not have scored some rare alleles, either because we did not include many fish from non-Australian locales where Shaklee observed many of his rare alleles, or because this study did not routinely stain the cathodal part of the gel. Data from this locus was judged to be comparable between studies.

CKA. Both this study and Shaklee's study used Poulik running buffer with muscle tissue for this locus. Data from this locus was judged to be comparable between studies.

Est-1. For this locus, EBT was used as a running buffer in this study and Shaklee's study and both studies used retina tissue. Mobility differences among alleles were small for this

locus and consequently it was difficult to score. This could have led to scoring differences between this study and that of Shaklee. This locus was dropped from comparisons.

GPI-A. Both this study and that of Shaklee used Poulik running buffer with muscle tissue samples. This study scored all alleles found in Shaklee's study with the addition of a rare allele (130) that this study found once among the Kupang samples. This locus was comparable between the two studies.

sIDHP. This locus was included in standardisation experiments because this study used TC-1 and TRIC running buffers, while Shaklee reported mobilities from TC-1 and CAEA buffers. Also this study used muscle samples and Shaklee used livers. The results of the standardisation experiments show that nine out of 11 inconsistencies could be accounted for by differences in running buffers, with the balance being caused by human error. Consequently, data from this locus was excluded from comparisons between this study and that of Shaklee.

LDH-B. For this locus, both studies used Poulik running buffer and muscle tissue. The 149 allele of this study is almost certainly the 146 allele of Shaklee. In addition, Shaklee found three additional rare alleles (157, 116 and 014; one fish for each) that were pooled into the 146 mobility class. The data for this locus was considered comparable between this study and Shaklee's.

PGDH. This study resolved PGDH on CAAPM running buffer because the buffer that was used by Shaklee (CAEA) was available in Australia. Both studies used liver samples. Allele scorings for this locus were reconciled during standardisation experiments by comparing CAAPM and CAEA scores for the same fish on the same gels. In addition to the most common allele (100), there was one fast allele and three slow alleles in common between the two studies. The medium slow allele (m) was designated 095 in this study and 085 in Shaklee's. The slow allele (s) was 085 in this study, and 079 in Shaklee's and the very slow allele (vs) was 079 or 065. The fast allele (f) was 108 in both studies. Shaklee found two further, rarer alleles (050 and 095) that were not found in this study. Most of the inconsistencies (13/14) reported during standardisation experiments were associated with human errors and mis-scoring due to poor gel resolution. Scoring of this locus was considered to be consistent between the two studies.

PGM. Poulik buffer was used during this study to resolve PGM, while Shaklee used both CAD (CAAPM) and Poulik buffers, in combination, to resolve all the alleles recognised. Both studies used muscle and found many rare alleles. To get to a comparable data set it would have been necessary to compare Poulik only scores from both studies. The low level of genetic variation at this locus did not warrant this effort, so this locus was excluded from comparisons between the two studies.

Hardy-Weinberg Equilibrium. The standardised data sets, consisting of data from this study (1998/99) and data from Shaklee's study and consisting of six common loci, were tested for deviation from Hardy-Weinberg equilibrium using exact tests with Markov chain estimation of exact p-values. Seven of Shaklee's spatio-temporal samplings were analysed,

as they were close-by the sampling sites used in this study. For these seven, three deviated for ADH, two for PGDH and one for GPI-A($p<0.05$). For the nine Australian spatio-temporal samplings, one deviated for ADH, two deviated for ACP and GPI-A and seven deviated for PGDH. Of the seven PGDH deviations, two (North Kimberley 1999, $p=0.0001$ and North Australia 1999, $p=0.0067$) remained significant following the adjustment of the p-value for multiple tests. For the PGDH locus there was an increase in the proportion of spatio-temporal samples out of equilibrium at $p=0.05$ from the time of Shaklee's study (early 1980s; 2/7 or 28%) to this study (late 1990s; 7/9 or 78%). Data from five of the seven locations were re-collected during the standardisation process in this study, ruling out the likelihood of disequilibrium due to gel scoring errors

Temporal Stability. *Short-term.* Four of the five Australian collections of mackerel were repeated after one year to test the null hypothesis that genic and genotypic proportions were stable ($p<0.05$). The allelic and genotypic distribution of the East Coast samples in 1998 and 1999 differed at the PGDH locus (Figure 4.6). They also differed in allelic distribution for the Est-1 locus. The Torres Strait samples taken in 1998 and 1999 differed in allelic and genotypic proportions for loci sIDHP and ADH. The Northern Territory samples differed at the mAAT locus in allelic proportions, and at the PGDH loci in genotypic proportions. Sampling error could have been responsible in the Northern Territory, as only 39 mackerel were genotyped for these loci in 1998, compared to close to 100 for the 1999 sample (97, mAAT and 94, PGDH). The northern Australian samples taken in 1998 and 1999 differed at the Est and

PGDH (Figure 4.6) loci for both allelic and genotypic proportions. However, the samples from the Shark Bay area taken in 1998 and 1999 were not different in allelic or genotypic proportions at any of the ten loci assayed.

The lack of short-term genetic stability at three of the four collection locations sampled in this study is mirrored by the magnitude of their allele frequency variance. The *F* for the East Coast, North Australia and Torres Strait collections made one year apart was 0.0258, 0.0188 and 0.0146, compared to the Shark Bay collection whose *F* was 0.0087 (Table 4.14). The unstable three populations (EC, TS and NA) retained non-zero *F's* after correction for sampling error. The allele frequency variance of samples collected from the East Coast and Torres Strait in the 1980s (Shaklee, unpublish.) was less than in this study of 1990s samples (Table 4.14).

Long-term. Using the standardised data set, it was possible to test the null hypothesis that allelic proportions have been stable in the long term at four sampling locations for six loci (ACP, ADH, CK-A, GPI-A, LDH-B and PGDH). The temporal interval between sampling varied from 14 to 18 years.

Allelic proportions were not significantly different between the pooled 1980s and pooled 1990s, except at the Northern Australian location for CK-A ($p=0.03460$) and PGDH ($p=0.04242$) and at the North Kimberley location for CK-A ($p=0.04359$, Figure 4.6). At the North Australia sampling site in 1984 the frequencies of the two CK-A alleles were 0.94 and

0.06, compared to the pooled 1998/1999 data the frequencies were 0.90 and 0.10. For the North Kimberley mackerel sampled in 1983 the CK-A frequencies were 0.96 and 0.04, but 16 years later in 1999 the frequencies were 0.90 and 0.10. In adjacent sampling localities, the frequency of the most common CK-A allele was 0.90 in the Northern Territory in 1999 and 0.89 (1985) and 0.90 (1998/9) in Shark Bay.

The variance of allele frequencies (*F*) from the pooled 1980s to the pooled 1990s data was highest for North Australia (0.0132) and North Kimberley (0.0117, Table 4.14), as expected from the number of significantly different allele frequency comparisons. For the other collection locations (EC, TS and SB), *F* was less (0.0064, 0.00551 and 0.0088), but remained non-zero when corrected for sampling error (Table 4.14). For the East Coast at least, short instability in allele frequencies in the 1990s samples masked the measurement of long-term instability, as the 1998 and 1999 allele frequencies were pooled in the analysis. For example, at the PGDH locus the frequency of allele *095* was 0.1377 in 1998 and 0.2533 in 1999, giving a pooled frequency of 0.1986 that was similar to the frequency of that allele in the pooled 1980s (0.1759, Table 4.15).

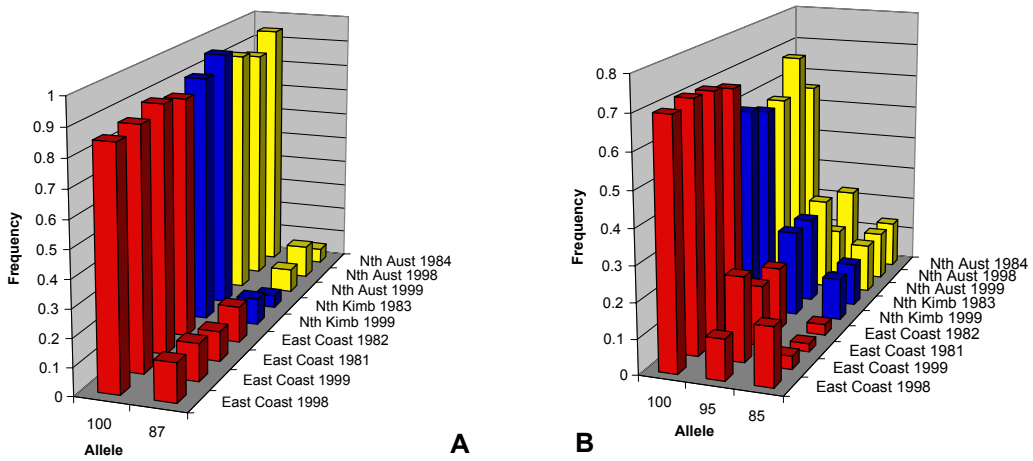


Figure 4.6. Allele frequencies (A; locus CK-A, B; locus PGDH) for three sampling locations (East Coast, North Kimberly and Northern Australia) for mackerel collected in the 1980s (Shaklee, unpub.) and in the 1990s (this study)

Table 4.15. Allele frequencies for six standardised loci for samples taken off the East Coast of Queensland in 1980s (1981 Cairns #1 and 1982 Cairns #2; Shaklee, unpublished, Appendix 4) and the 1990s (1998 and 1999, Townsville; this study)
The 100 allele from both studies corresponded directly, as did the ADH, CK-A, GPI-Aalleles. For ACP, the 300 allele of Shaklee (unpublished, Appendix 4) corresponds to 200, -90 to 020 and -20 of Shaklee was not detected in 1990s samples. For LDH-B, the 146 allele of Shaklee (unpubl.) corresponds to 149. For PGDH, the 85 allele of Shaklee (unpublished, Appendix 4) corresponds to 095, 85 to 079 and 65 to 079. The 95 allele of Shaklee was not detected in 1990s samples (*).

	ACP		ADH		CK-A		GPI-A		LDH-B		PGDH	
	Allele	**Freq**	**Allele**	**Freq**	**Allele**	**Freq**	**Allele**	**Freq**	**Allele**	**Freq**	**Allele**	**Freq**
1981												
	100	0.5901	-100	0.9000	100	0.8919	100	0.8378	100	0.9910	100	0.7027
	020	0.3423	-280	0.0909	087	0.1081	110	0.0090	116	0.0090	085	0.0901
	200	0.0631	040	0.0091			091	0.1441			108	0.0225
	-20	0.0045					078	0.0090			095	0.1712
											079	0.0135
1982												
	100	0.6062	-100	0.8899	100	0.8687	100	0.8616	100	0.9972	100	0.6844
	020	0.3240	-280	0.0863	087	0.1313	110	0.0113	116	0.0028	085	0.0894
	200	0.0615	040	0.0238			091	0.1215			108	0.0307
	-20	0.0028					078	0.0056			095	0.1788
	-250	0.0056									079	0.0140
											95*	0.0028
Pooled 1980s												
	100	0.6000	-100	0.8939	100	0.8776	100	0.8524	100	0.9948	100	0.6914
	020	0.3310	-280	0.0881	087	0.1224	110	0.0104	116	0.0052	085	0.0897
	200	0.0621	040	0.0180			091	0.1302			108	0.0276
	-20	0.0034					078	0.0069			095	0.1759
	-250	0.0034									079	0.0138
											95*	0.0017
1998												
	100	0.6046	-100	0.9273	100	0.8514	100	0.8514	100	0.9955	100	0.6957
	020	0.3318	-280	0.0636	087	0.1396	110	0.0180	149	0.0045	085	0.1449
	200	0.0636	040	0.0091	091	0.0090	091	0.1306			095	0.1377
							078	0.0000			108	0.0072
											079	0.0145
1999												
	100	0.5298	-100	0.9419	100	0.8647	100	0.8012	100	1.0000	100	0.7078
	020	0.3988	-280	0.0523	087	0.1353	110	0.0422	149	0.0000	085	0.0195
	200	0.0714	040	0.0058	091	0.0000	091	0.1506			095	0.2533
							078	0.0060			108	0.0130
											079	0.0065
Pooled 1990s												
	100	0.5722	-100	0.9337	100	0.8571	100	0.8299	100	0.9975	100	0.7021
	020	0.3608	-280	0.0587	087	0.1378	110	0.0284	149	0.0025	085	0.0788
	200	0.0670	040	0.0077	091	0.0051	091	0.1392			108	0.0103
							078	0.0026			095	0.1986
											079	0.0103

DISCUSSION

Population structure

Two major genetic stocks were recognised among Australian Spanish mackerel populations by Shaklee (Shaklee et al., 1990; Shaklee, unpublished, Appendix 4). One genetic stock extended along the east coast of Australia, and the other included populations from Torres Strait to western Australia. The allozyme and mtDNA data from this study support the presence of a distinct stock on the east coast stock and the lack of further genetic stocks along the northern and western Australian coastline. However, the geographic definition of the genetic break in Torres Strait between eastern and northern and western stocks appears to differ between this study and Shaklee's previous study.

Our data does not support the placement of the Torres Strait population in the northern and western genetic mackerel stock as proposed by Shaklee (unpublished). The allozyme and mtDNA genetics of this population suggest affinities to both the east coast stock, and to the northern and western stock. However, the similarity of mtDNA RFLP allele frequencies between the East Coast and the Torres Strait samples, and the mtDNA AMOVA suggest that the Torres Strait population is not part of the northern and western stock. For example, the RFLP haplotype that is present in about 10% of Torres Strait fish and 20-25% of East Coast fish is absent or at low frequencies in samples taken to the west of the Torres Strait; the southern Gulf of Carpentaria and the coast of the Northern Territory. Explanations for the difference between the outcomes of the two studies include Shaklee's sampling strategy that may have amplified the relative distinctiveness of the East Coast stock by the inclusion of fish from Moreton Bay and the increased resolution to the allozyme data that is provided here by mtDNA.

The mtDNA evidence, especially the RFLP data, suggests that the Torres Strait region does not belong to the northern and western stock of Spanish mackerel, but what is its relationship to the East Coast stock? The Torres Strait is a zone of genetic transition for several marine species (Chenoweth et al., 1998a; Chenoweth et al., 1998b; Elliott, 1996), and it is possible that the Torres Strait population is a mixture of fish from the two main stocks. This would account for its intermediate RFLP frequency and its overall affinity with both stocks. If it is a mixed population, the proportion of fish from each stock that contribute to the spawning population each year must stay relatively constant, as the RFLP frequencies for Torres Strait were similar in 1998 and 1999. Furthermore, based on its RFLP allele frequencies and that of adjoining stocks, approximately equal numbers of fish from the two adjacent stocks may contribute each year. Alternatively, the Torres Strait population may be a discrete population experiencing little or no gene flow with the neighbouring populations, which accounts for the distinctiveness of its RFLP allele frequencies. Otolith and parasite data from elsewhere in this study generally support the presence of discrete populations within this species, however the position of the population between two major stocks, its intermediate RFLP allele frequencies and its overall genetic affinity with the two major stocks supports the hypothesis that it is a zone of admixture.

The use of the non-recombining mitochondrial genome to confirm the genetic distinctiveness of the East Coast stock allows a glimpse into the possible evolutionary history of the species through phylogenetic analyses of sequence data. Among the samples taken from the Queensland east coast and Torres Strait, there was a group of fish whose mitochondrial genomes shared the same evolutionary history (Clade C, Figure 4.4). These fish were taken side-by-side from spawning aggregations with fish with a different and more heterogeneous past history. One explanation for the existence of a distinct clade on the east coast is that they are the descendants of a population of Spanish mackerel that were geographically isolated on the eastern Australian coast during past intervals of low-sea level when a land-bridge connected Cape York to southern Papua New Guinea (Jones and Torgersen, 1988). Under this scenario, non-clade fish may be the descendants of immigrants who moved eastwards through the Torres Strait when sea levels rose. However, there is no evidence of a corresponding westward movement of clade fish as would be expected. Alternatively, the observed phylogenetic structure could be the result of sampling error at the individual or genome level, or of the random process of mutation and drift in mtDNA.

Like the study of Shaklee (unpublished), this study found significant genetic distinctiveness between Australian Spanish mackerel and those from nearby countries; Papua New Guinea in Shaklee's case, and Indonesia in this study. The mtDNA of several Australian fish from the northern and western stock were phylogenetically related to fish from Kupang, Indonesia suggesting that one-way movement from Indonesia to Australia may occur. The reverse movement appears not to occur as the mtDNA of Kupang fish was not phylogenetically similar to Australian clades, and the Kupang population has a significantly lower mtDNA diversity that could not be maintained if it was receiving immigrants from Australia. An identical hypothesis of movement between Australia and Indonesia was proposed independently by Lester (2001) from parasite data as part of this overall study. The observed genetic distinctiveness between Australian and Indonesian stocks would be eroded by high rates of interchange, so dispersal may be a rare phenomenon or related to recent perturbations among populations. The species is rarely found in depths greater than 100 metres (Tobin, 2000). Mitochondrial genomes are inherited without recombination so past dispersal events are preserved in the genetic characteristics of modern specimens.

Two fish from Torres Strait (TS097 and TS2690) had aberrant mtDNA sequence. These fish were not representatives of another mackerel species, as their ten-locus allozyme genotype was identical to the remainder. Shaklee (1990) used allozyme loci, including CK-A, GPI-A and PGDH, to distinguish among three other mackerel species and the wahoo (*Acanthocybium solandri*) that were inadvertently included among his samples. The most likely explanation is that these fish were back-crossed descendents of hybrids between Australian and non-Australian Spanish mackerel, or between Australian Spanish mackerel and another mackerel species.

Allozyme temporal stability

Comparability and Data Quality

In this study a direct comparison was made between the allele frequencies in mackerel samples collected from the fishery in the early 1980s to those collected in the late 1990s. Genetic samples were serendipitously taken from fish at the same or similar locations about 15 years apart and following an extensive process of standardisation, we believe that the allozyme data from the 1980s and 1990s (for six loci) are directly comparable. We are indebted to Jim Shaklee for sharing his methodology for allozyme loci that we implemented on our 1990s samples, for providing his 1980s raw data in its many forms for comparison, and particularly for his practical assistance with the process of standardisation.

Genetic disequilibrium

This study presents evidence that suggests that the allozyme genetics of Australian Spanish mackerel populations sampled in the late 1990s may have deviated from genetic equilibrium that was a demonstrated feature of the resource in the early 1980s. In a population that is at equilibrium (Hardy-Weinberg, HW) genotype frequencies within one generation will follow the multinomial distribution, with allele frequencies as the distribution parameters (Lessios, 1992). The constancy of allele frequencies from generation to generation, and therefore of the genotypic composition of the population, is the single most important implication of the HW law. It implies that in the absence of specific evolutionary forces to change allele frequency, the mechanism of Mendelian inheritance, by itself, keeps the allele frequencies constant and thus preserves genetic variation (Hartl and Clark, 1989).

The previous study analysed genotypic proportions in spatially and temporally distinct samples of Australian Spanish mackerel collected in the early 1980s (Shaklee et al., 1990; Shaklee, unpublished, Appendix 4). The collections were shown to conform to HW equilibrium, except for a small number of deviations that would be expected due to the repeated application of statistical testing. Following the process of standardisation reported here, Shaklee's 1980s data set and the data from this study were re-analysed using the same methodology to test for conformance to HW expectations. Both standardised data sets contained comparable allele scorings for six allozyme loci representing five geographic localities throughout much of the range of the species in Australian waters; from the east, north and west. The most heterozygous locus in both studies, PGDH, was at equilibrium in the 1980s standardised data (p=0.05, two departures from seven spatio-temporally distinct Australian populations), but was not in equilibrium in the 1990s standardised data (p=0.05, seven departures from nine populations). Furthermore, in the non-standardised ten-locus 1990s data set, three populations were out of equilibrium for one to two loci at a p-value that was adjusted for multiple statistical testing.

Evidence of disequilibrium in the 1990s sampling of the Australian Spanish mackerel resource comes predominantly from the PGDH locus that was the most polymorphic locus assayed. The locus had five alleles; two (85 and 95) of which had relatively small mobility

difference. A heterozygote fish might not have been scored correctly if the three-banded heterozygote pattern was not clearly resolved; it could be assumed to be an overstained homozygote, and have been scored as 85/85 or 95/95. This could lead to a deficiency of heterozygotes for this genotypic class and contribute to HW disequilibrium. The expected frequency of 85/95 heterozygotes was low (mean per population = 4.5±1.9%) precluding a robust statistical test of observed compared to expected numbers with our sample sizes. Furthermore, there was no evidence from standardisation trials that heterozygotes were more likely than homozygotes to be absent from gels due to weak enzymatic activity.

It is unlikely that gel scoring, or other errors led to the non-conformance with Hardy-Weinberg proportions. Allozyme data from five of the seven populations, which were subsequently shown not to conform to HW proportions for locus PGDH, was collected twice in our study; once in October 1999 (TS98 and NA98) or April 2000 (Nkimb, TS99, NA99) and again in October and November 2001 (TS98, TS99, NA98, NA99, Nkimb) during standardisation trials. Errors in PGDH data due to incorrect scoring (Inconsistency 5a, four fish out of 250) and poor gel resolution (Inconsistency 5b, nine fish out of 250) were detected during standardisation trials were relatively rare and were corrected in the final data set. Non-conformance may also have been associated with selection operating on the PGDH locus, but it was not exclusively confined to this locus, and the Shaklee had previously not recorded a PGDH-type effect for Australian mackerel.

The combination of a shift away from equilibrium in the standardised 1990s data compared to the standardised 1980s data, and the significant deviations from equilibrium in the ten-locus 1990s data set requires careful evaluation against a background of intense harvesting pressure on the species. Populations could be out of HW equilibrium because genotypes are not selectively equivalent, for example if the proportion of heterozygote individuals that survived to breeding age may be less than for homozygotes. Smith (1991) observed a decline in the number of heterozygote individuals in the orange roughy fishery in New Zealand over a period of six years that coincided with intense harvesting pressure. They suggested that fishing activities might differentially remove the older and more heterozygous individuals from the virgin stock. Ward (1993) were unable to convincingly demonstrate that larger Australian orange roughy were more heterozygous than smaller fish although the power of their study to detect a small effect may have been limited by small sample sizes. Subsequently, Pogson (1998) found a significant correlation between growth rate and heterozygosity in the Atlantic cod, *Gadus morhua*. The proportion of large Spanish mackerel in the fishery has decreased (O'Neill and McPherson, 2000), but a link between size and heterozygosity in this species has not been investigated. If the decrease in the proportion of heterozygotes is accompanied by a decrease in one or more classes of homozygotes then, under some circumstances, the population will remain in HW proportions (Hartl and Clark, 1989, p 36) however this is unlikely to be the case for harvested fish populations.

The Wahlund effect is a special case of an observed deficiency of heterozygotes compared to expectation under the HW rule. It is a consequence of sampling a mixture of individuals from two or more populations that have differences in allelic frequencies, even though each

population may be in HW equilibrium. Only the heterozygote frequency at those loci with allelic frequency divergence among subpopulations should show the Wahlund effect (Hedrick, 2000). One locus in the data, PGDH, was more likely to deviate from HW expectations, and its allelic frequency was shown to vary between north-west and east coast genetic stocks. Undetected fine-scale genetic structure could exist among Spanish mackerel populations especially as otolith results presented elsewhere in this report suggest that populations may be sedentary, in some places on a fine spatial scale. The Wahlund explanation is compromised, however, as Shaklee's 1980s samples encompassed the same or similar geographic range as here and his study found no evidence of the presumed Wahlund effect. If harvesting has led to significantly fewer spawners since the 1980s, and fish have to travel larger distances to form spawning aggregations, samples taken from current spawning aggregations may represent a greater range of populations and be more likely to display a Wahlund effect.

Temporal Genetic Instability

Shaklee (unpublished) sampled three localities in consecutive years in the early 1980s; Moreton Bay, Cairns and Torres Strait, and tested for temporal stability of allele frequencies. His study failed to find significant heterogeneity between years. This study analysed temporal replicates for five of the six localities sampled, and found significant allele frequency heterogeneity between 1998 and 1999 samples at three of the five localities. Furthermore, direct comparisons of the standardised 1990s data to the standardised 1980s data show that locus CK-A allele frequencies from two locations (North Kimberley and North Australia) have significantly changed over a period of approximately 15 years. Although standardisation trials have ensured allele comparability between 1980s and 1990s data, tests of temporal stability may have been compromised by lack of spatial correspondence. Only the Torres Strait population was sampled from exactly the same location in the 1980s as the 1990s; Bramble Cay. Other location pairs were separated by one to two hundred kilometres that may invalidate the temporal comparison. It is unlikely that sampling error is responsible for the observed instability between the 1990s and 1980s samples as samples sizes were similar (CK-A locus: North Australia, 1980s n=118 and 1990s n=123; North Kimberley, 1980s n=101 and 1990s n=93).

Genetic drift is the random change in allele frequencies that results from the sampling of gametes from generation to generation. The magnitude of the standardised variance in allele frequency variance between the 1980s and 1990s samples, and between the 1998 and 1999 may suggest that random genetic drift has been enhanced at East Coast, Torres Strait, North Australia and North Kimberley locations, but not at Shark Bay. It most likely occurred between the birth dates of the fish included in the 1980 and 1990 sampling events; say 1975 to 1996 assuming spawning mackerel are between two and five years of age. It is also more likely to have occurred after the 1980s, as allele frequencies were stable among the 1980s samples. In highly fecund species with a large variance in family survival, such as Spanish mackerel, restrictions in spawner numbers or larval survival could lead to enhanced genetic drift. The proposed reduction does not have to be cumulative through time, however, for a

species such as Spanish mackerel that has overlapping generations, spawner numbers or larval survival would have to be low for several successive years to cause enhanced genetic drift.

CONCLUSIONS AND RECOMMENDATIONS

Standardisation

To ensure allozyme data of the highest quality in future studies, and to ensure comparability, we recommend

- double gel scoring by independent workers,
- buffer and tissue evaluation on at least a portion of the samples and loci to assess rates of inconsistencies,
- preservation of catalogued raw data to allow future temporal comparisons, and
- retention of at least representative tissue samples for use as controls in future studies.

Population structure

- This study supports the management of the east coast population of Spanish mackerel as a distinct unit.
- Management of the stocks adjacent to the Torres Strait may impact on the viability of that population as it likely that this population is an admixture of surrounding populations. The stocks, including the Torres Strait stock, need to be managed with caution until its status can be clarified.
- The Spanish mackerel resource of the Northern Territory and Western Australia appear to be the same stock, implying that reciprocal gene flow is occurring.

Genetic disequilibrium and instability

The Australian Spanish mackerel resource may have generally moved away from genetic equilibrium due to the effect of harvesting of the resource upon its hypothesised metapopulation structure. Consequently, demographic parameters may have altered to such as extent that a previously undetectable effect is now measurable.

Observed short and longer term genetic instability may be evidence of enhanced genetic drift that could be consistent with the affects of overexploitation. Ultimately enhanced genetic drift could compromise genetic diversity of the species. Preservation of the genetic diversity of the species is integral to disease resistance, resilience to harvesting and future unknown environmental perturbations.

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REFERENCES

Anon (2000). Sequencher v 3.12 Sequence analysis software Gene Codes Corporation Ann Arbor, MI USA.

Bartley D.M., Kent, D.B. and Drawbridge, M.A. (1995). Conservation of genetic diversity in a white seabass hatchery enhancement program in southern California. **In**: *Uses and effects of cultured fishes in aquatic ecosystems.* (eds. Schramm HL, Piper RG), pp. 249–258. American Fisheries Society, Betheseda, MD, USA.

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

Buonaccorsi, V.P., Reece, K.S., Morgan, L.W. and Graves, J.E. (1999). Geographic distribution of molecular variance within the blue marlin (*Makaira nigricans*): A hierarchical analyisis of allozyme , single-copy nuclear DNA and mitochondrial DNA markers. *Evolution* **53**, 568–579.

Chapman, R.W., Sedberry, G.R., Koenig, C.C. and Eleby, B.M. (1999). Stock identification of gag, *Mycteroperca microlepis*, along the southeast coast of the United States. *Marine Biotechnology* **1**:137–146.

Chenoweth, S.F., Hughes, J.M., Keenan, C.P. and Lavery S. (1998a). Concordance between dispersal and mitochondrial gene flow: isolation by distance in a tropical teleost, *Lates calcarifer*. *Heredity* **80**:187–197.

Chenoweth, S.F., Hughes, J.M., Keenan, C.P. and Lavery, S. (1998b). When oceans meet: a teleost shows secondary intergradation at an Indian–Pacific interface. *Proceedings of the Royal Society of London B* **265**:415–420.

Elliott, N. (1996). Allozyme and Mitochondrial DNA Analysis of the Tropical Saddle-tail Sea Perch, Lutjanus malabaricus (Schneider), from Australian Waters. *Marine & Freshwater Research* **47**:869–876.

Excoffier, L., Smouse, P.E. and Quattro, J.M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* **131**:479-491.

Frentiu, F.D., Ovenden, J.R. and Street, R. (2001). Australian lungfish (*Neoceratodus forsteri* : Dipnoi) have low genetic variation at allozyme and mitochondrial DNA loci: a conservation alert? *Conservation Genetics* **2**:63–67.

Froese, R. and Pauly, D. (2001). FishBase. World Wide Web electronic publication. 02 February 2002 www.fishbase.org

Grewe, P.M., Elliot, N.G., Innes, B.H. and Ward, R.D. (1997). Genetic population structure of southern bluefin tuna (Thunnus maccoyii). *Marine Biology* **127**:555–561.

Hartl, D. and Clark, A. (1989). *Principles of Population Genetics. 2nd Ed* Sinauer, Sunderland MA.

Hedrick, P.W. (2000). *Genetics of populations.* Second Edition edition. Jones and Bartlett, Sudbury Massachusetts.

Hubby, J.L. and Lewontin, R.C. (1966). A molecular approach to the study of genic heterozygosity in natural populations. I. The number of alleles at different loci in *Drosophila pseudoobscura*. *Genetics* **54**:577–594.

Jones, M.R. and Torgersen, T. (1988). Late Quaternary evolution of Lake Carpentaria on the Australia-New Guinea continental shelf. *Australian Journal of Earth Sciences* **35**:313–324.

Keenan, C.P. (1994). Recent evolution of population structure in Australian Barramundi, *Lates calcarifer* (Bloch): An example of isolation by distance in one dimension. *Australian Journal of Marine & Freshwater Research* **45**:1123–1148.

Koehn, R.K., Zera, A.J. and Hall, J.G. (1983). Enzyme polymorphism and natural selection. **In**: *Evolution of Genes and Proteins*, Nei M and Koehn R (eds). Sinauer, Sunderland MA.

Lessios, H.A. (1992). Testing electrophoretic data for agreement with Hardy-Weinberg expectations. *Marine Biology.* **112**:517–523.

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

Lewontin, R.C. and Hubby, J.L. (1966). A molecular approach to the study of genic heterozygosity in natural populations II Amount of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscura*. *Genetics* **54**:595–609.

Maddison, W. and Maddison, D. (1992). MacClade v 3.1.1 Sinauer & Assoc. Inc. Sunderland, Massachusetts.

Morgan, E. (2000). Genepop on the web. v http://wbiomed.curtin.edu.au/genepop Curtin Univeristy of Technology Perth, WA, Australia.

Nigro, L. and Prout, T. (1990. Is there selection on RFLP differences in mitochondrial DNA? *Genetics* **125**:551–555.

O'Neill, M. and McPherson, G. (2000). A review of stock assessment requirements for Spanish Mackerel; A model for Queensland based on available data. **In**: *Towards the Sustainable Use of Fishery Resources: Review Workshop* (ed. Walters CJ). Northern Territory Fisheries, Darwin.

Ovenden, J.R. (1990). Mitochondrial DNA and marine stock assessment: a review. *Australian Journal of Marine & Freshwater Research* **41**:835–853.

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

Pogson, G.H. and Fevolden, S.E. (1998). DNA heterozygosity and growth rate in the Atlantic cod Gadus morhua. *Evolution* **52**:915–920.

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

Richardson, B.J. (1982). Geographical distribution of electrophoretically detected protein variation in Australian commercial fishes. I. Jack mackerel [*Trachurus declivis* (Jenyns)]. *Australian Journal of Marine & Freshwater Research* **33**:917–926.

Richardson, B.J. (1983). Distribution of protein variation in skipjack tuna (*Katsuwonus pelamis*) from the central and south-western Pacific. *Australian Journal of Marine & Freshwater Research* **34**:231–251.

Richardson, B.J., Baverstock, P.R. and Adams, M. (1986). *Allozyme electrophoresis: a handbook for animal systematics and population studies* Academic Press, Sydney.

Schneider, S., Kueffer, J.M., Roessli, D. and Excoffier, L. (2000). Arlequin: A software for population genetic data analysis. v 2.0 Genetics and Biometry Lab, Department of Anthropology, University of Geneva Geneva.

Scribner, K.T., Crane, P.A., Spearman, W.J. and Seeb, L.W. (1998). DNA and allozyme markers provide concordant estimates of population differentiation: analyses of U.S. and Canadian populations of Yukon River fall-run Chum salmon. *Canadian Journal of Fisheries & Aquatic Sciences* **55**:1748–1758.

Shaklee, J., Beacham, T., Seeb, L. and White, B. (1999). Managing fisheries using genetic data: case studies from four species of Pacific salmon. *Fisheries Research* **43**:45–78.

Shaklee, J., Phelps, S., Salini, J. (1990). Analysis of fish stock structure and mixed-stock fisheries by the electrophoretic characterisation of allelic isozymes. **In**: *Electrophoretic and Isoelectric Focussing Techniques in Fisheries Management* (ed. Whitmore D), pp. 173–196. CRC Press, Boston.

Shaklee, J.B. and Bentzen, P. (1998). Genetic identification of stocks of marine fish and shellfish. *Bulletin of Marine Science* **62**:589–621.

Shaklee, J.B. and Keenan, C.P. (1986). A practical laboratory guide to the techniques and methodology of electrophoresis and its application to fish fillet identification, pp. 1–59. CSIRO Marine Research Laboratories., Hobart, Tas. Australia.

Shaklee, J.B. and Phelps, S.R. (1990). Chinook salmon NADP super(+)-dependent cytosolic isocitrate dehydrogenase: Electrophoretic and genetic dissection of a complex isozyme system and geographic patterns of variation. *Biochemical Genetics* **30**:455–490.

Shaklee, J.B., Salini, J. and Garrett, R.N. (1993). Electrophoretic characterisation of multiple genetic stocks of Barramundi perch in Queensland, Australia. *Transactions of the American Fisheries Society* **122**:685–701.

Shaw, C.R. and Prasad, R. (1970). Starch gel electrophoresis for enzymes - a compilation of recipes. *Biochemical Genetics* **4**:297–320.

Smith, P. and Benson, P. (1997). Genetic diversity in Orange roughy from the east of New Zealand. *Journal of Fisheries Research* **31**:197–213.

Smith, P.J., Francis, R.I. and McVeagh, M. (1991). Loss of genetic diversity due to fishing pressure. *Fisheries Research* **10**:309–316.

Swofford, D.L. (1999). PAUP*: Phylogenetic Analysis Using Parsimony. v 4.0 Sinauer Associates Champaign, Illinios.

Tishkoff, S.A. (2001). Haplotype diversity and linkage disequilibrium at human G6PD: recent origin of alleles that confer malarial resistance. *Science* **293**:455–462.

Tobin, A. (2000). Review of Spanish mackerel (*Scomberomorus commerson*) in Queensland waters: life history and biology, historical catch and effort, and recent trends., p. 33. Queensland Fisheries Service, Brisbane, Queensland.

Waples, R.S. (1989). A generalized approach for estimating effective population size from temporal changes in allele frequency. *Genetics* **121**:379–391.

Waples, R.S. (1998). Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *Journal of Heredity* **89**(5):438-450.

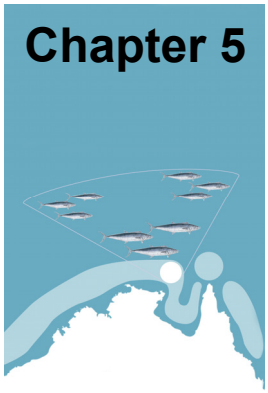
Ward, R.D. and Elliot, N.G. (1993). Heterozygosity and morphological variability in the Orange Roughy, *Hoplostethus atlanticus* (Teleostei: Trachichthyidae). *Canadian Journal of Fisheries & Aquatic Sciences* **50**:1641–1649.

Ward, R.D., Elliott, N.G., Grewe, P.M. and Smolenski, A.J. (1994). Allozyme and mitochondrial DNA variation in yellowfin tuna (*Thunnus albacares*) from the Pacific Ocean. *Marine Biology* **118**:531–539.

Ward, R.D. and Grewe, P.M. (1994). Appraisal of molecular genetic techniques in fisheries. *Reviews in Fish Biology and Fisheries* **4**:300–325.

White, B.A. and Shaklee, J.B. (1991). Need for replicated electrophoretic analyses in multiagency genetic stock identification (GSI) programs: Examples from a pink salmon (*Oncorhynchus*

gorbuscha) GSI fisheries study. *Canadian Journal of Fisheries & Aquatic Sciences* **48**:1396–1407.



Chapter 5

MALE-BIASED GENE FLOW IN NARROW-BARRED SPANISH MACKEREL (*SCOMBEROMORUS COMMERSON*, PERCIFORMES; TELEOSTEI) BASED ON MITOCHONDRIAL DNA AND MICROSATELLITE MARKERS

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ABSTRACT

Narrow-barred Spanish mackerel (*Scomberomorus commerson*) are found in coastal waters throughout the Indo-West Pacific supporting commercial, recreational and artisanal fisheries. Understanding of spatial population dynamics is important to resolve responsibility issues with straddling stocks, and for ensuring management at appropriate scales. In Australia, *S. commerson* are distributed across the tropical north, from the mid-west to the mid-east coast. We used restriction site fragment length polymorphism of the control region of the mitochondrial genome and six microsatellite loci to examine the extent and nature of gene flow at a major northern Australian marine biogeographic boundary and point of contact between genetic stocks of this species, the Torres Strait. F-statistics based on haplotype and microsatellite allele frequencies confirmed the genetic boundary originally proposed from an allozyme analysis. The F_{ST} from the two nuclear markers (allozyme and microsatellites) were of the same order of magnitude (0.0014 and 0.0039). Additionally, mtDNA haplotype frequencies identified a new genetic stock in the Torres Strait. This was not, however, supported by the microsatellite data. We propose that this lack of concordance between mitochondrial and nuclear markers is due to a bias in dispersal capacity towards males. This is evidenced by higher female-only global F_{ST}'s, based on microsatellite data from this study, and supported in the literature where parasite abundance on male and female *S. commerson* caught in the same location were different. Dispersal rates that differ between sexes and genetic stock structure increase the vulnerability of this species to overfishing.

Keywords: male-biased dispersal, narrow-barred Spanish mackerel, *Scomberomorus commerson*, Australia, mtDNA, microsatellites

INTRODUCTION

Australia has a significant responsibility to manage fisheries species that occur within the Australian Fishing Zone, an area of about nine million square kilometres extending 200 nautical miles from the shore including Australia's external territories except the Antarctic Territory. The Zone is third largest in the world after those of France (because of its external territories) and the USA. Its high marine faunal diversity arises from its geographic extent, wide range of habitat types and Australia's history of isolation from other continents. Comparatively, Australia's fisheries resources are not abundant or productive; marine habitats are low in nutrients due to little run off from the dry Australian continent, the continental shelf is narrow except in the north and the region lacks permanent upwellings. Since 1992 when the *Fisheries Management Act 1991* came into effect, the Bureau of Rural Science have reported the status of a consistent set of 74 species whose management is the responsibility of the Federal, as opposed to the State or Territory, government. In 2004, 17 species were classified as overfished compared to five in 1992. Furthermore, the status of 40 of the 74 species is listed 'uncertain' due to the lack of sufficient biological information to determine status (Caton and McLoughlin, 2004). The list includes narrow-barred Spanish mackerel, *Scomberomorus commerson*.

Narrow-barred Spanish mackerel (*Scomberomorus commerson*) are large, epi-pelagic predators distributed across tropical Australia. They grow rapidly and reach maturity in about two years at a length of around 80 cm (FL). East coast fish have been estimated to attain ages of at least 10 (males) to 14 (females) years (McPherson, 1992) and a male of 22 years was observed in the Pilbara region of Western Australia (Mackie et al. 2003). Fecundity is presumed to be high, but not known in detail. Larval duration is two to four weeks. The species is harvested by commercial and recreational fishers; total Australian catches are between 200 - 3000 tonnes (Buckworth 2004). Concerns about the sustainability of this harvest centre around (1) the 'hyperstability' (Hilborn, Walters, 1992) of catch rates in fisheries for the species, where large catch rates can be maintained despite falling total biomass because fishers almost exclusively target inshore spawning aggregations that occur on specific reefs in spring and summer, and (2) recent evidence that juveniles and adults show limited movement (Lester et al., 2001; Moore et al., 2003, Newman et al. 2007). These attributes are likely to increase the susceptibility of populations to serial depletion. The spatial extent of interbreeding populations, or stocks, that comprise fisheries species is one of the most important biological parameters that must be known prior to an assessment of the conservation status of the species. Shaklee (Appendix 4) tested for the presence of multiple genetic stocks of narrow-barred Spanish mackerel throughout the range of the species in Australian and southern Papua New Guinea waters. Eighteen populations were sampled during 1981 to 1985 from the mid-western Australian coast at Shark Bay (Cape Cuvier), northern Australia and the eastern Queensland coast to Brisbane. These collections (over 2,300 fish) were genotyped at 28 polymorphic allozyme loci. Two Australian genetic stocks were reported; one on the east coast of Queensland, and the other from Western Australia

across northern Australia and the Gulf of Carpentaria to Torres Strait in the east including southern Papua New Guinea.

The Torres Strait is culturally and strategically important to Australia, and represents one of Australia's most vulnerable borders, especially in terms of quarantine. It is a biologically productive area and yields large amounts of seafood for local consumption and for sale in Australia and overseas (Caton et al., 2004). *Scomberomorus commerson* populations in the Torres Strait were identified as the eastern-most extent of the geographically widespread northern stock from an allozyme study (Shaklee, submitted); consequently the Torres Strait is a population boundary for this species. The Torres Strait features prominently in genetic stock boundaries of other marine species including the tropical saddle-tail sea perch (*Lutjanus malabaricus*, Elliott, 1996), barramundi (*Lates calcarifer*, Chenoweth et al., 1998) and green turtle (*Chelonia mydas*, Fitzsimmons et al., 1997). The last has unique gender differences in behaviour that could lead to sex-biased dispersal (Avise, 1994). Gene flow in these and other co-distributed species would have ceased at the height of the last glaciation about 15,000 years ago, when there was a land bridge connecting Cape York to southern Papua New Guinea (Jones, Torgersen, 1988). The purpose of this research was to examine the gene flow dynamics of *S. commerson* in the Torres Strait area using genetic markers with higher resolving power than allozymes, namely mtDNA control region sequence polymorphism and microsatellite markers.

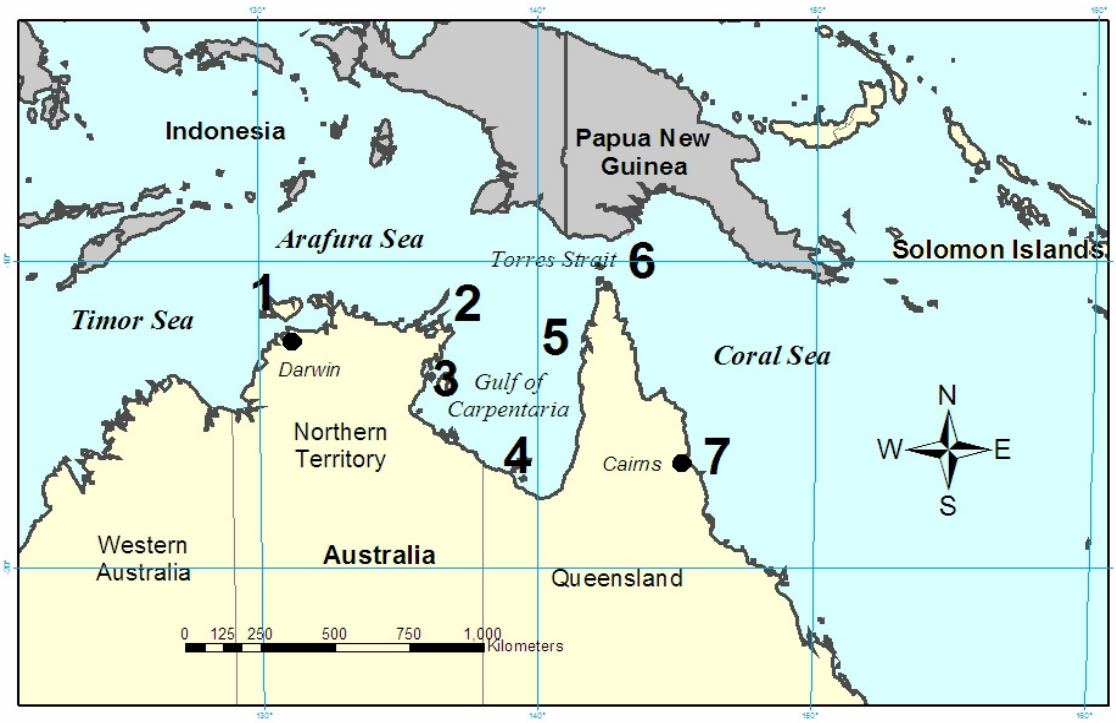

Figure 5.1. *Scomberomorus commerson* sampling locations: **1,** Bathurst Island; **2**, Cape Wessel; **3**, Groote Eylandt; **4**, Mornington Island; **5**, Lost City; **6**, Bramble Cay; and **7**, Cairns

MATERIALS AND METHODS

Population sampling

Spawning adult *S. commerson* were sampled at random from commercial catches during 1999. Gender of dissected fish was recorded, tissue samples stored in liquid nitrogen and transferred to a laboratory freezer at -70^{o}C. The sampling locations were selected to focus on the junction between the northern stock and the eastern stock, but constrained by areas accessed by commercial fishermen. From west to east the sampling locations were Bathurst Island (near Darwin, 130^{o}E 11^{o}S), Cape Wessel (north-east Northern Territory, 137^{o}E 11^{o}S) Groote Eylandt (western Gulf of Carpentaria, 136.5^{o}E 14^{o}S), Mornington Island (southern Gulf of Carpentaria, 137^{o}E 14.5^{o}S), Lost City (eastern Gulf of Carpentaria, 140^{o}E 14^{o}S), Bramble Cay (north-eastern Torres Strait, 144^{o}E 9^{o}S) and Cairns (eastern coast of Queensand, 146.5^{o}E 18^{o}S) (Figure 5.1).

DNA isolation

Muscle tissues stored at –70^{o}C were used for total genomic DNA extraction: 10-50mg from each sample was digested in 50μl of a suspension of 5% Chelex-100 (w/v) in 5mM Tris.Cl ph8.0, 0.5mM EDTA. Proteinase K (100mg) was added and the tissue digested to completion at 55^{o}C for one hour on a shaking platform. The mixture was boiled for five minutes then centrifuged at 1200g for five minutes to precipitate Chelex resin and cellular debris. The supernatant was removed to a fresh tube for subsequent manipulation and storage.

DNA amplification

The 5' end of the mtDNA control region (D-loop) was amplified as described by Ovenden et al. (2002b) except that the annealing temperature was 55^{o}C. The primers used were Pro889U20 (CCW CTA ACT CCC AAA GCT AG) and TDKD1291L21 (CCT GAA ATA GGA ACC AAA TGC). Polymerase chain reaction amplification was carried out in 25μl volumes, using the following reagent concentrations: dNTP's, 100μM each; primers, 100nM each; and $MgCl_2$, 3.0mM. Each reaction contained 0.5 Units of Taq DNA polymerase and the reaction buffer supplied by the manufacturer (Fisher Biotech, Australia). Thermal cycling conditions consisted of an initial denaturation, 94°C for two minutes followed by 30 cycles of 92°C for 10 seconds, 55°C for 30 seconds and 70°C for one minute, with a final extension step of 70°C for five minutes. Cycling was performed in a PTC200 DNA Engine (MJ Research, USA) and the gradient function of this machine was used to initially optimise annealing temperature for these primers within the range of 51°C to 60°C.

RFLP analysis

Amplification products were digested to completion with five units of *Rsa*I restriction enzyme (New England Biolabs) added directly to each PCR reaction mixture immediately after amplification. Digestions were carried out for one hour at 37°C with a final step of 70°C for 10

minutes to inactivate the enzyme. Restriction fragment length polymorphisms (RFLP's) were detected by electrophoresis of 10μl of the restriction digest on 8% polyacrylamide gels (acrylamide:bis = 19:1) in 1xTAE buffer on a vertical polyacrylamide gel electrophoresis (PAGE) apparatus (BioRad, USA). Gels were run for 1.5hours at 200V. DNA was visualised by staining gels with ethidium bromide after electrophoresis and exposing them to UV light. Samples from each gel that demonstrated unique restriction patterns were run on a series of final line-up gels to validate comparisons between gels.

DNA sequencing

The sequence of mtDNA control region amplicons corresponding to RFLP-defined haplotypes was determined using Megabace Dye Terminator chemistry (Amersham Biosciences) with fragment separation carried out by capillary electrophoresis (MegaBace, Amersham Biosciences) under conditions recommended by the manufacturer except that the quantity of template used was 100-150fmol.

Microsatellite data collection

Narrow-barred Spanish mackerel samples were genotyped with six di-nucleotide microsatellite loci (*Bst6.39TG, Sca30, Sca37, Sca8, SM3* and *SM37*) described in Broderick et al. (in press). Microsatellite amplifications were performed in 96-well plates using a Perkin Elmer 9700 thermocycler. Reactions (20μl) contained 2μl of PCR buffer ® (Qiagen P/L) containing Tris-HCl (pH 8.7), KCl and $(NH_4)_2SO_4$; 4mM $MgCl_2$; 0.2μM forward primer (labelled); 0.2μM reverse primer; 0.6 units *Taq* DNA polymerase (Qiagen P/L); 125μM dNTP (Pharmacia Biotech); 1% bovine serum albumin and approximately 50ng genomic DNA template. The DNA template and enzyme were denatured at 92^{o}C for one min., followed by 35 cycles consisting of 92^{o}C for 10 sec at the locus-specific annealing temperature for 20 sec and 72^{o}C for 30 sec. A final extension at 72^{o}C for five min was used to ensure complete addition of adenine to the PCR product, essential for consistent allele calling during genotyping.

Microsatellite gel separation and scoring were performed by the AGRF (Australian Genome Research Facility, Melbourne Division, Walter and Eliza Hall Institute, Post Office Royal Melbourne Hospital, Victoria 3050). The PCR amplicons were resolved on gels that were 36 cm long and 0.2 mm thick with 96 lanes. The gels were 4.5% polyacrylamide (19:1 acrylamide-bis) with 6M urea. The running buffer was 1X TBE diluted from 10x TBE solution containing tris-base (108.0g), boric acid (55.0g), and Na_2EDTA (9.3g) per litre. Gels were run at 3,000 volts for about two and a half hours at 51^{o}C on a 377 ABI Prism DNA sequencer. For sample loading procedure, 10μl of H_2O was added to the amplification reaction. From diluted samples, 2.5μl was taken and 0.3μl Genescan-500® TAMRA size standard, 0.5μl blue dextran and 1.7μl formamide was added. This solution was then denatured for three min at 95^{o}C and then placed on ice for five min. 1μl was loaded onto the gels. The software used for analysis was Genescan® v3.1.2 and Genotyper® v2.1.

Statistical analysis

Data analysis focussed on testing the genetic relationship of the Torres Strait population (Bramble Cay) to populations from the northern stock (Bathurst Island, Cape Wessel, Groote Eylandt, Mornington Island and Lost City) and the eastern stock (Cairns). The significance of mtDNA haplotype frequency differences between population samples, pooled or otherwise, was tested with pair-wise exact tests (Raymond, Rousset, 1995) using the software Arlequin v2.0 (Schneider et al., 2000) with 5000 dememorisation and 100000 Markov steps. Concordance between mtDNA control region RFLP haplotype and geographical location was tested by calculating Wright's F_{ST} (Wright, 1978) from frequency data. In situations where sufficient time has not elapsed to allow for independent sequence evolution of mtDNA haplotypes in genetically isolated populations, estimates of F_{ST} made from haplotype frequencies may be more accurate than equivalent estimates made using sequence information as well (Rosel et al., 1999). Scoring of microsatellite alleles was verified by graphical representation of allele size measured to two decimal places against bin size. Alleles were consistently two base pairs apart, as expected from the di-nucleotide loci used, and there were clear cut-off points between successive allele sizes. The software Microchecker (Van Oosterhout et al., 2004) was used to test for the presence of null alleles by comparing observed and expected frequencies of each heterozygote genotype. Tests were also made for heterozygotes missed by excessive stuttering by comparing the frequencies of heterozygotes consisting of alleles one to many repeat units apart. Genetic diversity was characterised by the number of alleles per locus, allele richness per locus per sample, allele size range in base pairs, expected heterozygosity (H_E) and observed heterozygosity (H_O). Deviations from Hardy-Weinberg equilibrium were examined for each population at each locus using Fisher's exact test. Microsatellite data was used to investigate population structure by calculating F_{ST} (Weir, Cockerham, 1984) using the software Microsatellite Analyser (Dieringer, Schlotterer, 2003). Non-parametric bootstrapping, with strict Bonferroni correction, was implemented for p-values. For the microsatellite data, analyses were conducted using both sexes and then repeated separately for each sex. Pairwise F_{ST}'s for mtDNA and microsatellite data were treated as surrogate distances between populations and were clustered using the average distance method (UPGMA, Nei, 1987 p. 293).

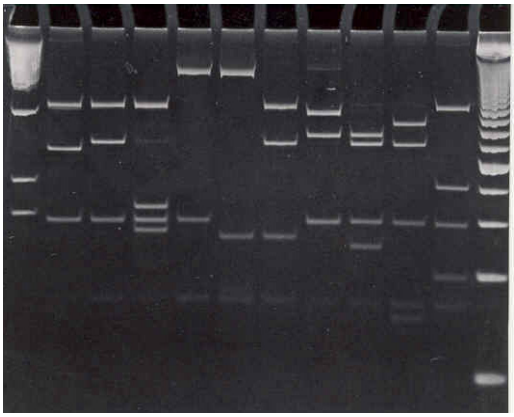

Figure 5.2. *Rsa*I restriction fragment patterns for mtDNA control region amplification products from 10 Spanish mackerel samples separated by PAGE. Lane 1, size marker, 100bp ladder (Fermentas Inc.); lanes 2-11, sample Ec2751 (haplotype a), Sg1 (b), Sg9 (c), Ec2821 (d), Ts2690 (e), Nei17 (f), Sg30 (g), Scsg9008 (h), Scsg9027 (j) and Nei05 (k); lane 12, size marker, 25bp ladder (Invitrogen Corp.).

RESULTS

Mitochondrial control region RFLP haplotypes

Ten mtDNA control region haplotypes were identified by *Rsa*I restriction fragment patterns (Figure 5.2). Sequence data for the three most common haplotypes (*a*, *b* and *c*) plus a rare haplotype (*g*) confirmed the fragment sizes observed by PAGE. The full length of the fragment (including primers) was 448 or 449 base pairs (bp), depending on a one base pair insertion/deletion polymorphism. The absolute number of pairwise base differences (unadjusted for intra-haplotype sequence variation) between the two most common haplotypes, *a* and *b,* was 24. Haplotypes *a* and *b* were present at all sampling locations, excepting the absence of haplotype *a* from the Groote Eylandt location. The frequency of haplotype *a* varied between locations: 24% at Cairns, 12% at Bramble Cay in Torres Strait and 0 – 3% in the remaining locations in the Gulf of Carpentaria and northern Australia (Table **5.1**). Heterogeneity of haplotype frequencies across collection locations was confirmed by pairwise exact tests (Table 5.2). The sampling locations were sorted into three groups based on their haplotype *a* frequencies: groups 1 and 2 contained one location each (Cairns and Bramble Cay, respectively) and group 3 contained the remaining locations. Pairwise F_{ST}'s between the groups revealed significant genetic structure based on polymorphism in the mtDNA control region (Table 5.3, Figure 5.3).

Table 5.1. Mitochondrial haplotype counts by geographic location

Location	a	b (+f)[1]	c (+k)	d (+e)	g	h	j	Total
Cairns	20	58	1	4				83
Bramble Cay	12	82		2				96
Lost City	3	88	2	1	2			96
Cape Wessel	3	83	4					90
Groote Eylandt		84	5			1		90
Mornington Is.	1	91			1	2	1	96
Bathurst Is	3	86	2		1			92

Note: [1] Rare haplotypes *f, k,* and *e* were pooled with *b, c* and *d* respectively based on common possession of restriction fragments larger than 130 base pairs.

Table 5.2. *p*-values for a pairwise exact test for homogeneity of haplotype frequencies. p-values that were significant after Bonferroni correction for multiple tests are in bold

Sampling location	Cairns	Bramble Cay	Lost City	Cape Wessel	Groote Eylandt	Mornington Island
Cairns	–					
Bramble Cay	0.06380	–				
Lost City	**0.00015**	0.01785	–			
Cape Wessel	**0.00000**	**0.00695**	0.73845	–		
Groote Eylandt	**0.00000**	**0.00000**	0.10425	0.21725	–	
Mornington Island	**0.00000**	**0.00020**	0.31225	0.10280	0.04110	–
7. Bathurst Island	**0.00000**	0.01375	1.00000	0.94750	0.12020	0.31295

Microsatellites

Summary statistics for allelic variation at the six microsatellite loci are shown in Table 5.4. Microsatellite variation within populations was high compared to other nuclear markers, for example allozyme loci assayed by Shaklee (Appendix 4). The number of alleles per locus ranged from four (*Bst6.39TG*) to 25 (*Sca30*). All but one population sampling for one locus (Cairns, locus *Sca8*) conformed to Hardy-Weinberg equilibrium proportions. No departures from expected genotype frequencies or scoring errors were found with Microchecker software.

There was evidence for population structure among the seven populations based on the microsatellite data. The global F_{ST} over all loci was 0.003952 ($p < 0.001$), indicating a small, but significant, amount of population subdivision. Three loci showed significant F_{ST}: *Bst6.39TG* ($F_{ST} = 0.040438$, $p < 0.001$), *Sca30* ($F_{ST} = 0.001767$, p-value < 0.05) and *SM3* ($F_{ST} = 0.003649$, $p < 0.02$). When the data was separated into males and female fish, the

Table 5.3. Pairwise F_{ST}'s with significance levels (above diagonal) based on mtDNA RFLP haplotype frequencies for three groups of sampling locations

Sampling location	Cairns	Bramble Cay	Remainder
Cairns	–	< 0.02	< 0.001
Bramble Cay	0.04198	–	< 0.001
Remainder	0.21991	0.04939	–

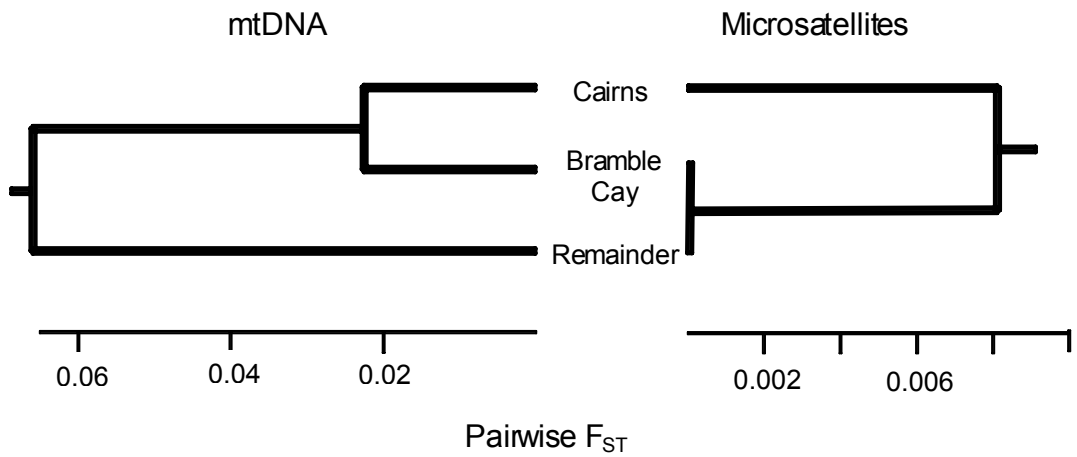

Figure 5.3. Average distance (UPGMA) tree showing relationships between two populations of *Scomberomorus commerson* (Bramble Cay and Cairns) and a group of populations representing locations in the Gulf of Carpentaria (Lost City, Mornington Island and Groote Eylandt) and northern Australia (Cape Wessel and Bathurst Island

Pairwise FST's were calculated from mtDNA RFLP haplotype frequencies or microsatellite data (Table 5.3 and Table 5.5). Non-significant FST values were set to zero. **Note** that *X*-axis scales differ.

Female global F_{ST} (0.005057, $p < 0.001$) was greater than for both sexes combined and greater than for males alone (F_{ST} = 0.003655, $p < 0.001$). Three loci had significant F_{ST}'s in the global female comparison (N=324; *Bst6.39TG*, F_{ST} = 0.034937, $p < 0.001$; *Sca30*, F_{ST} = 0.006358, $p < 0.005$ and *SM3*, F_{ST} = 0.005468, $p < 0.02$). One locus was significant in the male global F_{ST} estimation (N=333; *Bst6.39TG*, F_{ST} = 0.041441, $p < 0.001$).

Microsatellite data for both sexes was used to test the population structure suggested by the frequency of mtDNA RFLP haplotype *a*. The first two groups contained single populations; Bramble Cay and Cairns, respectively. The five remaining collection locations comprised a single group representing populations in the Gulf of Carpentaria and northern Australia. The F_{ST} between Cairns and the Gulf/northern Australia group was comparatively large and significant (0.011806, *p*-value < 0.001). The F_{ST} between Cairns and Bramble Cay was smaller, but significant nevertheless (0.004519, *p*-value < 0.005), while the F_{ST} of the final comparison, between Bramble Cay in Torres Strait and the Gulf/northern Australia group was not significant (Table 5.5, Figure 5.3).

Table 5.4. Summary statistics for six *Scomberomorus commerson* microsatellite loci[1]

	Bst6.39TG	Sca30	Sca37	Sca8	SM3	SM37
Cape Wessel						
n	89	78	83	83	59	56
A	4	25	19	24	14	15
Rs	.045	.320	.229	.289	.237	.268
As	149-157	134-190	182-270	174-242	193-219	270-298
H_E	22.6158	72.0711	73.1213	71.8546	50.6838	51.7027
H_O	23	68	71	67	48	51
HW	0.5215	0.1277	0.5441	0.2409	0.8352	0.6408
Cairns						
n	81	83	82	78	78	65
A	5	20	22	15	15	18
Rs	.06	.241	.268	.192	.192	.277
As	142-157	130-180	188-310	186-230	193-221	270-312
H_E	43.3168	74.5031	72.1102	66.7936	68.2129	59.0697
H_O	46	72	73	63	72	63
HW	0.5992	0.3513	0.4390	0.0000	0.7759	0.7823
Bathurst Island						
n	91	66	90	88	51	79
A	4	21	21	20	13	21
Rs	.044	.318	.233	.227	.254	.265
As	149-157	134-180	182-278	174-232	193-219	260-326
H_E	17.7735	61.0611	79.2960	76.8458	43.3663	73.4013
H_O	16	61	76	75	39	73
HW	0.0811	0.2571	0.7334	0.6862	0.2870	0.8018

Table 5.4 (continued). Summary statistics for six *Scomberomorus commerson* microsatellite loci[1]

	Bst6.39TG	Sca30	Sca37	Sca8	SM3	SM37
Groote Eylandt						
n	89	84	92	88	87	43
A	5	22	23	24	13	17
Rs	.056	.261	.25	.273	.149	.395
As	149-159	134-190	194-312	186-236	193-219	270-328
H_E	17.7062	76.2575	81.5791	78.0459	77.3931	39.2470
H_O	19	74	81	77	77	39
HW	1	0.0101	0.6201	0.9107	0.8561	0.7895
Mornington Island						
n	95	77	89	81	41	44
A	4	24	21	19	12	14
Rs	.042	.312	.236	.235	.293	.318
As	149-157	134-188	194-276	174-238	193-217	260-298
H_E	13.3333	71.1699	80.2769	69.4659	34.9012	39.7931
H_O	14	70	80	65	32	43
HW	1	0.2030	0.3320	0.0735	0.0375	0.3776
Lost City						
n	96	94	95	93	95	90
A	4	21	24	22	13	19
Rs	.042	.223	.252	.237	.137	.211
As	149-157	134-180	182-300	174-234	193-219	270-306
H_E	13.3770	85.2568	86.2013	80.5890	80.6878	83.7821
H_O	14	85	84	77	84	85
HW	1	0.5544	0.3861	0.0577	0.6591	0.5989
Bramble Cay						
n	93	95	95	94	79	90
A	5	24	23	24	14	21
Rs	.054	.253	.242	.255	.177	.233
As	145-157	130-188	188-278	174-236	193-219	264-324
H_E	26.1459	87.2011	85.8731	82.0536	68.7198	83.7821
H_O	29	89	83	84	68	84
HW	1	0.9325	0.1992	0.7902	0.1019	0.7289

Note: [1] The number of individuals genotyped at each sampling location (*n*), the number of alleles *(a*), allele richness per locus per sample *(Rs),* allele size range in base pairs *(As)*, expected number of heterozygotes *(H_E)*, observed number of heterozygotes *(H_O)* and probability value of concordance with Hardy-Weinberg expectation (*HW*, significant departures from Hardy-Weinberg expectations are shown in bold type) are given for each locus.

Table 5.5. Pairwise F_{ST}'s (below diagonal) with significance levels (above diagonal) based on microsatellite allele frequencies for six loci for three groups of sampling locations

Sampling location	Cairns	Bramble Cay	Remainder
Cairns	–	< 0.005	< 0.001
Bramble Cay	0.004519	–	NS
Remainder	0.011806	0.001032	–

DISCUSSION

The combination of female philopatry and male dispersal is common in many species, particularly mammals. Male biased dispersal can cause the genetic structure of populations to differ between the maternally inherited mitochondrial DNA (mtDNA) and the bi-parental nuclear genome (Lyrholm et al., 1999). A lack of correspondence between the population genetic structure revealed by nuclear and mitochondrial genetic markers is strong evidence for a difference in dispersal rates between males and females. Male-biased dispersal deduced from genetic evidence has been reported in marine vertebrates such as great white sharks (*Carcharodon carcharias*, Pardini et al., 2001), harbour porpoises (*Phocoena phocoena*, Rosel et al., 1999), sperm whales (Lyrholm et al., 1999) and green turtles (*Chelonia mydas*, Bowen et al., 1992; Karl et al., 1992). In turtles, gene flow is thought to be biased towards males because matings occur away from rookeries presumably on feeding grounds or other locations spatially separate from breeding sites. Dispersal patterns in great white sharks and harbour porpoises were shown to be sex-biased, with females being more philopatric or site-associated than wide-roving males. Sex-biased dispersal has not been extensively reported for fishes. Several studies report differences in male compared to female dispersal rates in anadromous salmonid species (Bekkevold et. al, 2004 for example) and there is one report of differing dispersal rates in Lake Malawi cichlids (Knight et al., 1999). The latter study compared relatedness in males and females of two species and found than over the same spatial scale females were more related that males. There are no reports of male-biased dispersal in mackerels.

We provide mtDNA evidence that the *S. commerson* population centred on Bramble Cay in north-east Torres Strait is a separate stock from those in northern and eastern Australia. However, neither microsatellite allele frequencies reported here, nor allozyme data from a previous study on samples also collected from Bramble Cay (Shaklee, submitted), support this conclusion. We believe that this conundrum is explained by a difference in rates of gene flow between males and females where *S. commerson* males are more wide-roving than females and where females tend to be philopatric. Our case is strengthened by the difference between male and female global F_{ST}'s from microsatellite alleles in this study, the higher number of individual loci that support population subdivision among females only and by previous analyses of parasite infestation rates in this species. Lester et al. (2001) found that in four of nine collections of mackerel around Australia, the abundance of parasites with a short association with the host were not similar between males and females from the same location. Males showed more similarity to fish in adjacent areas than to females in the same area that they were caught. They concluded that on the west coast of Australia, at least, male and female *S. commerson* had different dispersal dynamics. The difference in male compared to female mediated gene flow between *S. commerson* stocks is likely to be subtle. However it is presumably sufficient to lead to nuclear gene homogeneity within populations in the waters of the Northern Territory, the Gulf of Carpentaria and Torres Strait, yet low enough to allow the separate Torres Strait stock to retain a unique assemblage of maternally inherited mtDNA

haplotypes. This study once again shows the importance of combining information from more than one class of genetic marker when investigating population genetic structure. However, as highlighted by Avise (1994 p 229), a full appreciation of the geographic population structure of a species requires integration of data from genetic, behavioural and tagging studies. The former contains information about the evolutionary connectedness of populations, while the latter uncovers contemporary demographic parameters that may be invisible to conventional genetic studies. While a genetic tagging study of *S. commerson* is underway (Ovenden et al., 2002a), very little known about the behavioural ecology of this species.

Two genetic stocks were recognised among Australian Spanish mackerel populations by Shaklee (Appendix 4). One genetic stock extended along the east coast of Australia, and the other included populations along the northern Australian coastline from Torres Strait to western Australia. MtDNA polymorphism and allelic variation at microsatellite loci presented in this study strongly support this stock structure. The degree of population structuring is modest, but it is consistent between two classes of nuclear markers; allozymes and microsatellites. Shaklee (Appendix 4) reported an F_{ST} for 22 polymorphic allozyme loci across 12 Australian populations of 0.0014 while this study reported an F_{ST} of 0.0039 for six microsatellite loci for seven populations. The microsatellite F_{ST} may be comparatively higher as this study excluded sampling locations from the north-western Australian coastline that were included in the study by Shaklee (Appendix 4). Similar population structure has been reported for other species of fish in the same region. The tropical saddle-tail sea perch (*Lutjanus malabaricus*), for example, is a demersal reef species that occurs widely on the northern Australian coastline and through the Indo-Pacific. Elliott (1996) found allozyme gene frequency differences between the Kimberley coast (north-western Australia) and Gulf of Carpentaria populations; mtDNA haplotypes further differentiated the east coast of Queensland samples. Similarly, Chenoweth (1998) reported a genetic 'break' in mtDNA patterns to the east and west of Cape York (northern Queensland) in the barramundi (*Lates calcarifer*) following the description by Shaklee et al. (1993) and Keenan (1994) of extensive genetic subdivision among Australia-wide estuarine populations from allozyme data. The distribution of Spanish mackerel in northern Australia includes Torres Strait and the evolutionary history of this species would presumably also have been influenced by this marine biogeographic boundary (Ovenden, Street, Submitted).

The genetic characteristics of the Torres Strait population compared to neighbouring stocks on the east and north coasts suggest a discrete stock of *S. commerson* in that location. The population has significantly different mtDNA haplotype *a* frequencies compared to surrounding populations. Chemical analyses of otolith composition (Newman et al. 2007) and parasite load estimates (Lester et al., 2001; Moore et al., 2003) generally support the presence of discrete populations within this species, including the Torres Strait area. Amoung the genetic information, only mtDNA data supports this proposal. Neither allozyme nor microsatellite data support a separate stock in Torres Strait. While concordant support across genetic marker types for population structure is common in the literature (Buonaccorsi et al., 2001), there are numerous explanations for the reverse situation, including differing rates of mutation and fixation. The important message, however, is that evidence from only one class of marker is

needed to propose a distinct population. The proposed *S. commerson* Torres Strait stock would be centred to the north of the Cape York region in Torres Strait around the sampling location of Bramble Cay. It would lie north of Cairns (Queensland east coast) and does not extend westward into the Gulf of Carpentaria or into the waters of the Northern Territory. The allozyme study by Shaklee (Appendix 4) showed that genetically distinct populations of *S. commerson* do occur in the Indo-Pacific region – to the north of Papua New Guinea, for example. But, could there be an alternative explanation that would explain the frequencies of mtDNA haplotypes? About 10% of Torres Strait fish are characterised by haplotype *a*, which is intermediate in frequency between the east-coast stock (24%) and the northern Australian stock represented by populations from the Gulf of Carpentaria and the coast of the Northern Territory (0 - 3%). These frequencies would be consistent with the movement at spawning season of equal numbers of females from the east coast stock and the northern stock into the Torres Strait. If this were a consistent feature of spawning behaviour, along with the dispersal of juveniles spawned in the Torres Strait, the genetic distinctiveness of the Torres Strait population would soon be lost as gene flow would homogenise mtDNA haplotype frequencies. In the absence of further data to support a hybrid or admixed origin for the Torres Strait population, our preferred interpretation of the data is a newly identified genetic stock in Torres Strait.

Narrow-barred Spanish mackerel are large, active, schooling predators that are open water, broadcast spawners with high fecundity. Commercial and recreational harvest of the species is common throughout its range in western, northern and eastern Australia. Several lines of evidence from this study backed up by the work of others suggest that dispersal rates in *S. commerson* differ between the sexes, with the males likely to be the most active dispersers. This paper also presents mtDNA and microsatellite evidence that gene flow between eastern and northern Australian populations through the narrow Torres Strait between Cape York and southern Papua New Guinea is attenuated. Furthermore, mtDNA haplotype frequencies of a population in the Torres Strait are distinct from eastern and northern populations, and a separate stock status is proposed for *S. commerson* in this region. The finding of this paper in combination with others (Lester et al. 2001; Moore et al. 2003; Newman et al. 2007; Shaklee submitted) suggest this species exists as sedentary assemblages possibly occupying non-overlapping territories of shallow coastal continental shelf waters and experiences attenuated levels of reciprocal gene flow that is biased towards males. We consider this to be compelling data to be incorporated into management plans to protect populations of the species from serial depletion by over-fishing.

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REFERENCES

Avise, J.C. (1994). *Molecular Markers, Natural History and Evolution* Chapman & Hall, New York.

Bekkevold, D., Hansen, M.M. and Mensberg, K.L.D. (2004). Genetic detection of sex-specific dispersal in historical and contemporary populations of anadromous brown trout *Salmo trutta*. *Molecular Ecology* **13**:1707-1712.

Bowen, B.W., Meylan, A.B., Ross, J.P., Limpus, C.J., Balazs, G.H. and Avise, J.C. (1992). Global population structure and natural history of the green turtle (*Chelonia mydas*) in terms of matriarchal phylogeny. *Evolution* **46**:865-881.

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

Buckworth, R.C. (1998). Age structure of the commercial catch of Northern Territory narrow-barred Spanish mackerel. Project T94/015. Final Report to the Fisheries Research & Development Corporation. Northern Territory Department of Primary Industry and Fisheries, Darwin, Australia. Fishery Report 42, 28 p. ISBN 0 7245 3037 1.

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

Buonaccorsi, V.P., McDowell, J.R. and Graves, J.E. (2001). Reconciling patterns of inter-ocean molecular variance from four classes of molecular markers in blue marlin (*Makaira nigricans*). *Molecular Ecology* **10**:1179-1196.

Caton, A., Findlay, J., Williams, G., McLoughlin K., Tilzey R. and Larcombe J. (2004). Other fisheries. **In**: *Fishery Status Reports 2004: Status of Fish Stocks Managed by the Australian Government,* Caton A, McLoughlin K (eds). Bureau of Rural Sciences., Canberra.

Caton, A. and McLoughlin, K. (2004). *Fishery Status Reports 2004: Status of Fish Stocks Managed by the Australian Government*. Bureau of Rural Sciences, Canberra.

Chenoweth, S.F, Hughes J.M., Keenan C.P. and Lavery, S. (1998). Concordance between dispersal and mitochondrial gene flow: isolation by distance in a tropical teleost, *Lates calcarifer*. *Heredity* **80**:187-197.

Dieringer, D. and Schlotterer, C. (2003). Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes* **3**:167-169.

Elliott, N. (1996). Allozyme and mitochondrial DNA analysis of the tropical saddle-tail sea perch, *Lutjanus malabaricus* (Schneider), from Australian waters. *Marine & Freshwater Research* **47**:869-876.

Fitzsimmons, N.N., Moritz, C., Limpus, C.J., Pope, L and Prince, R. (1997). Geographic structure of mitochondrial and nuclear gene polymorphisms in Australian green turtle populations and male-biased gene flow. *Genetics* **147**:1843-1854.

Hilborn, R. and Walters, C.J. (1992). *Quantitative Fisheries Stock Assessment. Choice, Dynamics and Uncertainty.* Chapman and Hall, New York.

Jones, M.R. and Torgersen, T. (1988). Late Quaternary evolution of Lake Carpentaria on the Australia-New Guinea continental shelf. *Australian Journal of Earth Sciences* **35**:313-324.

Karl, S.A., Bowen, B.W. and Avise, J.C. (1992). Global population genetic structure and male mediated gene flow in the green turtle (*Chelonia mydas*): RFLP analysis of anonymous nuclear loci. *Genetics* **131**:163-173.

Keenan, C.P. (1994). Recent evolution of population structure in Australian barramundi, *Lates calcarifer* (Bloch): An example of isolation by distance in one dimension. *Australian Journal of Marine & Freshwater Research* **45**:1123-1148.

Knight, M.E., Van Oppen, M.J.H., Smith, H.L., Rico, C., Hewitt, G.M. and Turner, G.F. (1999). Evidence for male-biased dispersal in Lake Malawi cichlids from microsatellites. *Molecular Ecology* **8**:1521-1527.

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

Lyrholm, T., Leimar, O., Johanneson, B. and Gyllensten, U. (1999). Sex-biased dispersal in sperm whales: contrasting mitochondrial and nuclear genetic structure of global populations. *Proceedings of the Royal Society of London Series B-Biological Sciences* **266**:347-354.

Mackie, M., Lewis, P. D., Gaughan, D. J., and Buckworth, R. (2003). *Stock assessment of Spanish mackerel (*Scomberomorus commerson*) in Western Australia.* Final Report, Fisheries Research and Development Corporation Project 1999/151. Fisheries Department of Western Australia.240 pp. ISBN 1 877098 20 5

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

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

Nei, M. (1987). *Molecular Evolutionary Genetics* Columbia University Press, New York.

Newman, S.J., Buckworth, R.C., Mackie, M., Lewis, P.D. Wright, I. W., Williamson, P.C., Bastow, P.D., Gaughan, D.J. and Ovenden, J.R. (2007). Spatial subdivision among assemblages of Spanish mackerel, *Scomberomorus commerson* (Pisces: Scombridae) from western, across northern Australia: implications for fisheries management. *Global Ecology and Biogeography* (**in review**)

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

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

Ovenden, J.R. and Street, R. (2007). Genetic population structure of Spanish mackerel. **In**: *The stock structure of northern and western Australian Spanish mackerel*,

Buckworth, R.C., Newman, S.J., Ovenden, J.R., Lester, R.J.G. and McPherson, G.R. (eds). Final report for FRDC 98/159 Pp. 91-140. Fisheries Group, Department of Business, Industry and Resource Development, Northern Territory Government, Darwin, Australia.

Pardini, A.T., Jones, C.S., Noble, L.R., Kreiser, B., Malcolm, H., Bruce, B.D., Stevens, J.D., Cliff, G., Scholl, M.C., Francis, M., Duffy, C.A.J. and Martin, A.P. (2001). Sex-biased dispersal of great white sharks - **In** some respects, these sharks behave more like whales and dolphins than other fish. *Nature* **412**:139-140.

Raymond, M. and Rousset, F. (1995). An exact test for population differentiation. *Evolution* **49**:1280-1283.

Rosel, P.E., France, S.C., Wang, J.Y. and Kocher, T.D. (1999). Genetic structure of harbour porpoise *Phocoena phocoena* populations in the northwest Atlantic based on mitochondrial and nuclear markers. *Molecular Ecology* **8**:S41-S54.

Schneider, S., Kueffer, J.M., Roessli, D. and Excoffier, L. (2000). Arlequin: A software for population genetic data analysis. Genetics and Biometry Lab, Department of Anthropology, University of Geneva, Geneva.

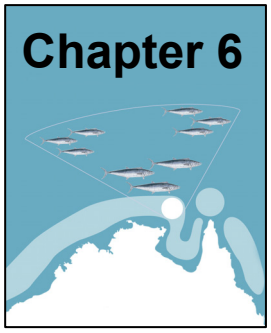
Shaklee, J.B., Salini, J. and Garrett, R.N. (1993). Electrophoretic characterisation of multiple genetic stocks of Barramundi perch in Queensland, Australia. *Transactions of the American Fisheries Society* **122**:685-701.

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M. and Shipley, P. (2004). MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**:535-538.

Weir, B.S. and Cockerham, C.C. (1984). Estimating F-stastistics for the analysis of population structure. *Evolution* **38**:1358-1370.

Welch, D.J., Hoyle, S.D., McPherson, G.R. and Gribble, N.A. (2002). *Preliminary assessment of the Queensland east coast Spanish mackerel fishery*, Northern Fisheries Centre, Cairns. Department of Primary Industries, Qld, Australia, 44pp.

Wright, S. (1978). *Evolution and the Genetics of Populations. Vol. 4. Variability Within and Among Natural Populations* Univ. of Chicago Press, Chicago.



STOCK STRUCTURE AND SPATIAL RELATIONSHIPS OF NORTHERN AUSTRALIA NARROW-BARRED SPANISH MACKEREL, AS INDICATED BY PARASITOLOGY

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ABSTRACT

The parasite fauna of 996 mackerel was evaluated for evidence of fish movement and migration. Adult mackerel carry hundreds of parasites (harmless to man) mostly in the stomach wall and gills. Some of these survive for the life of the fish.

Parasites were particularly common in fish from the NT and northern WA and almost absent from fish from Kupang, Indonesia, enabling us to conclude that there had been no movement of adult fish from Australia into the population from which our Kupang samples had come. Whether Kupang fish moved to Australia we could not determine from the parasite data.

Comparison of the parasite faunas of fish from 16 sites around northern and western Australia, from the Torres Strait to the Abrolhos Islands, revealed that in northern areas the fish had distinct parasite faunas. This indicates that once fish have recruited to an area, they do not move much. In contrast, down the west coast the parasite fauna was similar from several adjacent areas which would be consistent with the fish moving between these sites.

A detailed study of long-lived parasites from six sites in the Gulf of Carpentaria suggested some exchange between locations near the Sir Edward Pellew Group and Groote Eylandt, but no mixing between these areas and Mornington Island, Weipa, Torres Strait and the Wessel Islands, all of which had distinct parasite faunas.

Analysis of parasite data from fish caught in the same area on different half-days indicated that variability was the same for fish within half days as it was between half days. Hence there was no evidence that fish formed into semi-permanent schools.

In conclusion, the parasite data indicated that in the tropics the fish were sedentary and once they had recruited to an area they probably moved little.

METHODS

The Parasitology segment was completed in three phases. The first examined the parasite faunas of a total of 459 fish from nine primary sites sampled in 1998 from the Abrolhos Islands to Torres Strait to evaluate the likely movement of fish between sites. The second investigated movement between Indonesia and Australia using 124 fish (5 sites, collected 1999-2001), and the third concentrated on the Gulf of Carpentaria and adjacent coasts (413 fish, eight sites, 1999-2000).

Spanish mackerel carry hundreds of parasites (harmless to man), mostly in the stomach wall and gills. Counts of up to 14 species provided clues to the history of the fish and more importantly allowed comparisons to be made of the history of fish from different areas. Some of the species were considered short-lived parasites on the fish and referred to as 'temporary'. These were adult monogeneans and copepods on the gills. Others were considered long-lived and referred to as 'permanent'. These were larval or juvenile stages of helminths encapsulated in the stomach wall or gills.

The principle of the approach is that where the parasite fauna of fish from two areas is the same, the fish either have a common history or have grown in a similar habitat. Where the fauna is different, the history of the fish is different according to the time scale of the parasite counted, recent history for temporary parasites and longer term history for permanent parasites. For more details see Lester et al. (2001).

RESULTS: PARASITOLOGY

Phase 1: Primary sites

Analysis of both temporary (7 species) and permanent parasites (7 species) in fish from four adjacent sites along the west coast, Abrolhos, Shark Bay, Exmouth and Onslow, showed that there were strong similarities between the areas suggesting that either the fish were mixing or that the habitat was relatively uniform. In contrast, widely spaced samples across northern Australia, specifically Broome, Groote Eylandt, Torres Strait and East Coast were significantly different from the west coast samples and from each other, indicating that fish from these areas were not mixing and were each acquiring their own parasite faunas independently.

A sample of 37 relatively small fish from Kupang, Indonesia (mean length 705 mm compared to 963 mm in the Australian samples), suggested that Indonesian fish had fewer parasites than Australian fish. This was investigated further in Phase 2.

Male/female movement

On the west coast, Bray-Curtis similarity indices indicated that males in each area showed greater similarities to females in an adjacent area than to females in the same area. This suggested that males and females may move independently. There was also evidence of a small number of 'rogue' or 'disperser' fish which had parasite faunas more like those in an adjacent area than in their own area. No such phenomena were detected in the northern samples, possibly because they were more widely spaced. This was investigated in the third part of the parasite work, the Gulf of Carpentaria study (Phase 3).

For other details on Phase 1 see Lester et al. (2001).

Phase 2: Indonesia/Australia exchange

To evaluate the amount of exchange between Kupang mackerel and fish from adjacent areas in northern Australia, a new sample of 24 Kupang fish, mean length 911mm, carefully matched in length to Australian samples was compared to new samples from four Australian areas (Table 6.1).

The mean numbers of the first four parasites are very similar between Kupang and the four Australian sites. All four means for Kupang (0.4, 2, 0.4 and 2) fall well within the ranges of the means for the Australian sites. The first three parasites, *Pseudocycnoides armatus*, *Pricea multae*, and *Gotocotyle bivaginalis*, are external adult parasites on the gills of the fish. The fourth parasite, *Anisakis simplex* occurs as an encapsulated juvenile on the viscera; adults are found in dolphins. Thus, these four parasites provide no power to discriminate between Indonesian and Australian fish.

The next four parasites, however, paint a very different picture. The parasites are all larval or juvenile stages which are embedded within the flesh of the fish, are long-lived and probably survive as long as the host. All four are abundant in Australian fish and absent or nearly so from the Kupang fish suggesting total separation of Australian and Kupang stocks. It could be possible for Kupang fish to move to Australia, pick up local parasites and become indistinguishable from fish that originated in Australia. The reverse could occur if fish migrated when they were very small before they had begun to accumulate the four indicator parasites. However, it is clear that no adult fish move from Australia to Kupang.

Table 6.1. Mean numbers of eight parasites in samples of Spanish mackerel from five areas. Key to parasites: *P.armatus* = *Pseudocycnoides armatus* (copepod); *P. multae* = *Pricea multae* (monogenean); *G. bivaginalis* = *Gotocotyle bivaginalis* (monogenean); *A. simplex* = *Anisakis simplex* (nematode); *O. cysticum* = *Otobothrium cysticum* (trypanorhynch); *G. branchi* = *Grillotia branchi* (trypanorhynch); *Terranova* sp. (nematode); *Pterobothrium* sp. (trypanorhynch)

	No. of fish	Mean length	*P. armatus*	*P. multae*	*G. bivaginales*	*A. simplex*	*O. cysticum*	*G. branchi*	*Terranova* sp.	*Pterobothrium* sp.
Kupang	24	911	0.4	2	0.4	2	1	2	1	0
Bathurst I.	40	915	0.2	2	0.3	2	127	47	61	5
Flattop B.	28	913	1.4	2	0.6	5	77	34	41	3
Holothurian	9	939	0.4	1	1.4	5	65	35	78	6
C. Leveque	23	923	1.2	3	1.5	1	122	31	60	3

Phase 3: Gulf of Carpentaria

This study used nine parasites; four 'temporary' (gill copepods and monogeneans) and five 'permanent' (juvenile trypanorhynchs). Nematodes were ignored to speed up the dissection process. Analyses of the numbers of permanent parasites indicated that distinct parasite

faunas (95% level of confidence) occurred in six groups from the eight areas. They were Fog Bay/Bathurst Island, Cape Wessel, Groote Eylandt/Sir Edward Pellew Islands, Mornington Island, Weipa and Torres Strait. Analyses of the temporary parasites also demonstrated little similarity between the areas indicating minimal short term exchange between neighbouring groups of fish. The data thus suggest that fish in northern Australia are relatively sedentary.

'Schools' of mackerel were defined as all the fish in a sample (usually 10) taken in the same morning or afternoon at one site. Using this definition we had data from six 'schools' from each of three areas, Sir Edward Pellew Group, Torres Strait and Mornington Island. One way ANOVA analysis found no differences in any of the nine parasite species between schools from the first two sites at the 95% level of confidence. Two schools in the Mornington Island samples differed with respect to one of the permanent parasites *Grillotia branchi*. At the 95% level of confidence one would expect 5% of tests to give a false positive and this is probably the explanation here. Thus we conclude that in the Gulf, fish caught within minutes of each other do not have a history that is more similar to each other than to other fish in the same area. In other words, mackerel do not show prolonged school integrity as is found in other scombrids.

The results from Phase 1 suggested that on the West coast males and females moved separately, and there was evidence of rogue fish that were more similar to fish of other areas than other fish in the same area. We sought evidence for these two phenomena in the Gulf of Carpentaria. Neither phenomenon was supported by the data, suggesting that fish in the tropics may be more sedentary than those in the sub-tropics.

This information is examined in greater detail in Moore et al. (2003).

DISCUSSION

The absence of four permanent parasites from the Kupang fish and their abundance in neighbouring Australian fish means that few if any Australian fish are recruited to the Kupang mackerel fishery. A possible explanation for the absence of the permanent parasites lies in the definitive hosts of these parasites. Three of the parasites are juvenile trypanorhynch tapeworms. These mature only in the spiral valves of specific elasmobranchs. The fourth, *Terranova* sp. belongs to a genus of nematodes most of which mature in elasmobranchs. Very few sharks are caught off Kupang (C. Bryce, *pers. comm.*). Low numbers of these evidently make it impossible for the parasite populations to survive and hence their general absence from Kupang mackerel.

The parasite faunas indicated limited movement of fish along the West coast of Australia, and virtually no movement of fish in the tropics. One possible exception was that fish from the Gulf of Carpentaria could move into the Torres Strait, accumulate the parasite fauna specific to the area, and thus become indistinguishable from fish that have spent their whole life within the Strait. This is because three permanent parasites, *Grillotia branchi, Otobothrium cysticum* and *Paranybelinia balli* were more abundant in the Torres Strait than at Weipa so

that fish could move into Torres Strait, accumulate parasites and become undetectable parasitologically, though the reverse could not occur. The hypothesis is also consistent with the temporary parasite data which showed similarities between fish from Weipa and the Torres Strait. On a note of caution, however, though the parasitological data does not reject a Weipa to Torres Strait hypothesis, neither does it support it.

BENEFITS

Three students achieved BSc (Hons)

FURTHER DEVELOPMENT

Parasitological examination of mackerel on the east coast was undertaken under FRDC 2001/19 ('Stock discrimination of east coast Spanish mackerel') by Rissa Williams, BSc (Hons.) student with Bob Lester. A second student, Stephanie Rawlinson, used parasites to investigate movement of mullet *Mugil cephalus* along the east coast. Mark Robertson, honours student with Steve Barker (UQ) and Jenny Ovenden (QDPI) examined genetic markers for mackerel species in Australasia.

ACKNOWLEDGMENTS

The honours students whose theses were associated with the project, Craig Thompson, Helen Moss and Bradley Moore, were supported by Australian Postgraduate Awards from the Department of Education, Science and Training, Government of Australia. The Australia Research Council Grant, (1999, R Lester and S Barker, $13000) *Are the parasites of Spanish mackerel suitable markers of stocks of this species* provided additional information to this project.

STAFF

Dr Bob Lester (University of Queensland (UQ)); Dr Steve Barker (UQ); Mr Craig Thompson (UQ); Ms Helen Moss (UQ), Mr Brad Moore (UQ); and Mr C. Bryce, Department of Primary Industries, Fisheries and Mines, Northerm Territory Government.

REFERENCES

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

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

Appendix1. Intellectual Property

There are no intellectual property considerations arising from this project.

Appendix 2. Staff

DEPARTMENT OF PRIMARY INDUSTRY, FISHERIES AND MINES (NT)

(Previously Department of Primary Industry and Fisheries and Department of Business, Industry and Resource Development)

Dr Rik Buckworth	Principal Investigator, Fisheries Scientist
Mr Charles Bryce	Fisheries Technician
Mr Damian White	Fisheries Technician

DEPARTMENT OF PRIMARY INDUSTRIES & FISHERIES (QLD)

(Previously Department of Primary Industries)

Dr Jennifer Ovenden	Co-Investigator, Fisheries Geneticist
Mr Geoff McPherson	Fisheries Scientist
Ms Raewyn Street	Genetics Technician
Ms Coralie Foschia	Fisheries Geneticist
Mr Damien Broderick	Fisheries Geneticist
Mr Darren Rose	Fisheries Technician

WA FISHERIES

Dr Stephen Newman	Co-Investigator, Fisheries Scientist
Dr Michael Mackie	Fisheries Scientist
Dr Daniel Gaughan	Fisheries Scientist
Dr Trevor Bastow	Fisheries Chemist
Mr Paul Lewis	Fisheries Technician

UNIVERSITY OF QUEENSLAND

Emeritus Prof. Bob Lester	Co-Investigator, Parasitologist
Dr Steve Barker	Molecular Parasitologist
Mr Craig Thompson	Hons student, and Research Assistant
Ms Helen Moss	Hons student, and Research Assistant
Mr Brad Moore	Hons student, and Research Assistant

CONTRIBUTING FISHERS AND VESSELS

Mr Milton Miller, Mr Derryl Andrew	FV Ariel
Mr Bruce Davey, Mrs Juanita Davey	FV Wildcard
Mr Norm Hedditch	FV Taroona II
Mr Stuart McEvoy	FV Rebecca J

Appendix 3. Thesis by Rik Buckworth

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

ABSTRACT

With the world's fisheries in crisis, most fisheries fully- or over-fished, and world catches perhaps exceeding sustainable limits, our capability to monitor and manage fisheries is uncertain. I reviewed these problems, and described ways that spatial complexity compromises monitoring and assessment. Monitoring/ management combinations that are robust to fine-scale dynamics are needed. I developed a closed-loop simulation framework, using the disc equation to distribute fishing effort. A suite of small, spatially-complex fisheries were simulated, and fishery performance was measured, under different monitoring/ management arrangements. Spatial dynamics interacted with monitoring/ control systems, engendering fine scale effects such as biomass erosion, and serial depletion. Performance depended upon control and monitoring information quality. It deteriorated as capacity and hyperstability increased. Poor information / control combinations (CPUE/ TAC) produced poor performance, especially where the stock and effort were concentrated. Effort control with monitoring fishing rates (*F*) by tagging was risk-averse, performing consistently well across all scenarios.

I used a single-stock, age-structured model to assess the Northern Territory Spanish mackerel fishery. This fishery's status is grossly uncertain: available abundance and composition data were uninformative; catch and effort history, biological parameters and stock structure were all uncertain. There was no evidence supporting any increase of the current limit reference point.

Genetic mark-recapture might overcome the limitations of conventional tagging. It is suggested for direct *F* measurement, for routine monitoring. This would entail *in situ* collection of tissue ("tagging") and subsequent screening of catch samples for matches ("recaptures"), using DNA-fingerprinting. I present device designs for *in situ* tissue collection. Success rates (proportions of strikes yielding tissue) relative to design features were examined. Design and the line on the test vessel on which the tool was deployed interacted strongly. Predicted success rates of the best design are 44–85%, depending upon the line used. Industry participation, entailing daily genetic tagging of a set number of fish, would ensure that all members of the fished population have a similar probability of being tagged. In further simulations, genetic tagging outperformed other monitoring methods. Performance improved with small concurrent conventional tagging programs. This methodology could be developed to monitor *F* in many fisheries.

Appendix 4. Papers by Jim Shaklee et al.

Shaklee, J. B. (unpublished). Patterns of allozyme variation in narrow-barred Spanish mackerel (*Scomberomorus commerson*) reveal population subdivision in the Australia – Torres Strait – New Guinea region.

Shaklee, J. B., Ovenden, J. R. and Street, R. (unpublished). Details of allozyme variation in narrow-barred Spanish mackerel (*Scomberomorus commerson*) in the Australia - Torres Strait region.

Patterns of allozyme variation in narrow-barred Spanish mackerel (*Scomberomorus commerson*) reveal population subdivision in the Australia – Torres Strait – New Guinea region

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running title: Australian Spanish mackerel stock structure

Abstract. Patterns of allozyme variation were used to evaluate genetic aspects of stock structure in *Scomberomorus commerson* in the Australia - Papua New Guinea region. Over 2,300 fish from eighteen collections were characterized using 28 variable loci (22 of which were polymorphic at the $\mathbf{P}_{0.99}$ level). Allele frequencies at the three locations sampled more than once were stable over a two- to three-year period. Despite relatively high levels of genetic variation in this species, only modest population subdivision was revealed (e.g., average pairwise F_{ST} = 0.002). Explicit pairwise tests of population differentiation and MDS analyses indicated the existence of three genetic groups in the region: one in northeastern Papua New Guinea (Tufi), a second along the east coast of Queensland (Moreton Bay – Cairns), and the third ranging from the Port Moresby region of southern Papua New Guinea, through the Torres Strait, and across northern Australia (from the Gulf of Carpentaria in Queensland to Cape Cuvier in Western Australia). The results suggest that *S. commerson* harvested in the Torres Strait are part of a stock shared by Australia, Irian Jaya, and Papua New Guinea. Management and conservation of the *S. commerson* fisheries on this stock, and allocation of catch, should reflect its apparent shared nature.

Extra keywords: stock structure, biochemical genetics, fisheries management.

Introduction

The narrow-barred Spanish mackerel (*Scomberomorus commerson*) is a large epipelagic Indo-west Pacific species found from South Africa and the Red Sea in the west, throughout the Indian Ocean to Japan, Australia, and Fiji in the western Pacific (Collette and Russo 1984). Although permanently resident populations are thought to occur, the species is known to undertake significant coastal migrations in many parts of its range. In Australia, substantial migrations occur along the west (Tobin 2000) and east coasts (McPherson 1981).

S. commerson is exploited in commercial, subsistence, and recreational fisheries throughout its range. Major fisheries for this species occur in Indonesia, the Philippines, Sri Lanka, Yemen, and Pakistan (Collette and Russo 1984). Commercial harvests in Queensland in the 1960s and 1970s ranged from 700 to 1,000 tonnes per year (McPherson 1981). A recent analysis of revised commercial catch data for eastern Queensland (Hoyle 2003) showed a progressive increase in harvest from approximately 300 tonnes per year in 1936 to about 1,100 tonnes in 2001. The combined commercial and recreational catch in eastern Australia has been estimated at 2,500 tonnes per annum (McPherson 1988). This species is also subjected to significant fisheries in the Torres Strait (McPherson 1986). Unresolved questions regarding stock structure and stock contributions to fisheries occurring between northern Australia and southern Irian Jaya and Papua New Guinea and the need to develop rational and equitable fishery management programs in this international area led to the present investigation.

Fish stocks are often defined as panmictic breeding components of species (Berst and Simon 1981; Shaklee et al. 1990b). Given this definition, genetic tests can be expected to

provide insights regarding the number and boundaries of stocks for any given species (see reviews by Shaklee and Bentzen [1998] and Shulman [1998]). Electrophoretic investigations of patterns of allelic variation in enzymes and other proteins have proven to be a powerful and cost-effective approach for defining genetic aspects of stock structure for a diversity of marine species (Johnson et al. 1986; Salini and Shaklee 1988; Shaklee et al. 1993; Benzie et al. 1992; Johnson and Joll 1993; Benzie and Ballment 1994; Doherty et al. 1994; Lavery et al. 1995; Shulman and Bermingham 1995; Planes and Fauvelot 2002) and for estimating stock-group contributions to fisheries for commercially exploited anadromous fishes (Shaklee et al. 1999).

Fishery management, both domestically within Australia, Irian Jaya, or Papua New Guinea and internationally in the Torres Strait region between these countries, should be based on information regarding genetic stock status of the species in these areas in order to avoid overexploitation of weaker stocks. This study was undertaken to provide information and answers to four specific questions regarding *S. commerson* in Australia and the New Guinea region:

1) Do multiple genetic stocks of narrow-barred Spanish mackerel occur in this region?
2) Are genetic characteristics of collections from specific localities stable from year to year?
3) If multiple stocks exist, how many stocks occur and what are their geographic distributions and genetic interrelationships?
4) What stock or stocks contribute to fishery harvests in the Torres Strait?

Materials and Methods

Sample Collection and Tissue Processing

Eighteen separate collections of narrow-barred Spanish mackerel, consisting of 2,363 individuals, were obtained from cooperating fishermen and biologists from1981-1985 (Table 1; Figure 1). Collections from two or more consecutive years were obtained from three locations to allow tests of temporal variation/stability. All fish sampled were adults, and were obtained using commercial fishing gear. Nearly all fish were presumed to be in immediate pre- or post-spawning condition, based on dates of capture and gonad morphology. After capture, the fish were measured and sexed before the head and guts of each fish were removed, placed into individual plastic bags, and frozen. Subsequently, these samples were shipped frozen to the laboratory. After thawing, samples of skeletal muscle, eye (primarily retinal tissue), and liver were isolated, homogenized in an approximately equal volume of buffer (Shaklee and Keenan 1986), and centrifuged. Supernatants were stored in glass vials at -70^{o}C until the electrophoretic analyses were completed.

Electrophoresis, Enzyme Staining, Nomenclature, and Gel Scoring

Horizontal starch-gel electrophoresis (11.5 % Connaught starch) and enzyme-specific histochemical staining using standard procedures (Harris and Hopkinson 1976; Shaklee and Keenan 1986) were used to separate and visualize isozymes. Nearly all gels were loaded with samples from two different localities to facilitate consistent allele identification across collections. Details regarding the loci analyzed, alleles scored, and the tissues and buffer

systems used are presented in Appendix 1. Enzyme, locus, and allele nomenclature follow Shaklee et al. (1990a). Esterases (EST) were stained using alpha-naphthyl acetate as substrate, esterase-D (ESTD) was visualized using 4-methylumbelliferyl acetate as substrate, tripeptide aminopeptidase (zinc enzyme) (PEPB) was stained using leucyl-leucyl-leucine as substrate, and x-pro dipeptidase (proline dipeptidase; PEPD) was stained using phenylalanyl-proline as substrate.

Loci encoding multilocus isozyme systems were designated using letters when orthologies were known or assigned numbers beginning with "1" for the locus encoding the most anodal isozyme when orthologies were uncertain. Alleles at each locus were designated according to the electrophoretic mobilities of the homomeric isozymes they encode relative to the mobility of the homomeric isozyme encoded by the most common allele at the locus in the Moreton Bay population.

Several loci expressed multiple alleles encoding isozymes with very similar electrophoretic mobilities (e.g., *ACP**, *sAH**, *EST-1**, *sIDHP**, *LDH-B**, *mMDH**, *MEP**, *PGM**) (Appendix 1). Where necessary, samples were re-electrophoresed on gels with controls to verify allele identification (e.g., ACP and ADAl in Figure 1 & SOD in Figure 2 in Accessory Publication). In cases where one or more alleles were very rare, the rare alleles were pooled with alleles of the closest mobility classes to facilitate data summarization (see Appendix 2 in Accessory Publication) but all statistical testing was done with unpooled alleles.

The enzyme PGM was screened on both a high pH (Poulik; see Figure 1 in Accessory Publication) and a low pH (CAD*7) buffer in all fish allowing a total of 17 different alleles to be resolved during this study. While many of the *PGM** alleles were resolved on both

buffers, some alleles were only resolved on Poulik gels and others only on CAD*7 gels. Appendix 1 relates the relative mobilities observed for each *PGM** allele on the two buffers with the final (composite) allele designations used for the 17 recognized alleles.

Because there are several species of scombrids in the Australian region that are morphologically similar to *S. commerson*, steps were taken to identify and exclude data from any such fish that might have been inadvertently included in any collections. This was accomplished by obtaining reference tissue samples from verified specimens of these other species (broad-barred Spanish mackerel, *Scomberomorus semifasciatus*; Australian spotted mackerel, *S. munroi*; Queensland school mackerel, *S. queenslandicus*; Australian spotted mackerel, *S. guttatus*; and wahoo, *Acanthocybium solandri*), identifying the allozyme characteristics of these species, and using the diagnostic, multi-locus allozyme data to identify and remove such specimens from the narrow-barred Spanish mackerel genetic data set before statistical analysis.

Statistical Analyses

The program WHICHRUN v.3.2 (Banks and Eichert 2000) was used to calculate allele frequencies. MICROSATELLITE ANALYSER (MSA) version 2.65 (Dieringer and Schlotterer 2003) was used to calculate observed and expected heterozygosities and genetic distances. FSTAT v2.93 (Goudet 1995) was used to conduct Hardy-Weinberg equilibrium tests, pairwise genotypic tests of population differentiation and calculations of observed heterozygosity across all loci, allelic richness, and pairwise F_{ST} values. GENEPOP v3.3 (Raymond and Rousset 1995a) was used to identify the individual loci that were significantly different in pairwise genotypic tests of population differentiation (Raymond and Rousset 1995b). NTSYS-pc v.2.02j (Rohlf

1998) was used to conduct a non-metric multidimensional scaling analysis of population interrelationships using the Cavalli-Sforza and Edwards (1967) chord genetic distance data.

Results

Isozyme Patterns and Allele Frequencies

Sixteen of the 44 loci surveyed were not successfully screened in all collections or had minimal variation, therefore, these loci were excluded from the statistical analyses (see Appendix 1 for details). Examples of isozyme banding patterns and genotype scores for CK-A (dimer), ACP (dimer), ADAl (adenosine deaminase-like enzyme, dimer), PGM (monomer), EST-4 (monomer), ADH (dimer), mSOD (tetramer), and sSOD (dimer) are presented in Figures 1 & 2 in Accessory Publication.

All but three of the enzyme systems (CK-A, ADA-like, and PEPB) that were variable exhibited banding patterns in heterozygotes that were consistent with the reported subunit structures of the enzymes. Although the muscle predominant CK-A_2 isozyme of vertebrates is known to be a dimeric protein (Darnall and Klotz 1975; Harris and Hopkinson 1976); two-banded, monomeric patterns of variation, such as those observed in the present study, have been previously documented for the muscle creatine kinases of many teleost fishes (Ferris and Whitt 1978; Utter et al. 1979) and Ferris and Whitt (1978) established the relationship between the observed monomeric patterns of variation characteristic of teleosts and the dimeric structure of this enzyme by molecular hybridization experiments.

The adenosine deaminase-like enzyme screened in this study was a cathodal allozyme

system that appeared in liver extracts only when adenosine, purine-nucleoside phosphorylase, and xanthine oxidase were present in the enzyme stain. Omission of any one of these components resulted in absolutely no staining in this zone. Furthermore, this observed "ADA" staining was distinct from alcohol, lactate, and malate dehydrogenases, guanine deaminase, purine-nucleoside phosphorylase and all other enzymes surveyed in this study. Although the biochemical identity of this enzyme is unknown at present (hence the name "adenosine deaminase-like"), there seems to be no reason not to accept that the observed variation is the result of a simple genetic polymorphism. Indeed, virtually all expected phenotypes were observed during this survey and genotypic proportions were in Hardy-Weinberg equilibrium in all collections (except 1984 Torres Strait). The ADAl enzyme was distinct from the dimeric, muscle predominant adenosine deaminase (ADA).

PEPB exhibited two-banded patterns in presumed heterozygotes (consistent with a monomeric protein). This enzyme has been shown to be dimeric in skipjack tuna (Frick 1984) and presumed heterozygotes commonly exhibit the three-banded patterns expected for a dimeric protein in several fish species (e.g., chinook and chum salmon, cutthroat trout, sardines; unpublished data). However, as noted by Morizot and Schmidt (1990), this enzyme has also been reported to exhibit apparent monomeric variation in some fishes and it has been reported to consist of a mixture of dimers and monomers in pigs (cited in Frick 1984).

A total of 28 variable loci were screened in all collections and allele frequencies at the 18 most variable of these loci are presented in Appendix 3 in Accessory Publication.

Because some fish in several collections exhibited atypical genotypes at multiple loci, the species identity of these fish was verified by comparing their genotypes with those of the known,

reference specimens for several other species of Spanish mackerel and wahoo to identify any fish that were not *S. commerson*. Table 2 documents the genotypes of both the reference specimens and the atypical fish at 19 loci that were informative for distinguishing among the relevant species and identifies the actual species of the 22 fish that were excluded from the data set prior to statistical analysis.

The numbers of alleles per locus in *S. commerson* ranged from four at *CK-A**, *CK-B**, *mIDHP**, and *mSOD** to 17 at *PGM**. Average allelic richness across all populations ranged from 1.04 and 1.06 (*LDH-A** and *sMDH-B**, respectively) to 4.0 and 5.0 (*ACP** and *PGDH**, respectively). Average allelic richness across all loci ranged from 2.00 and 2.01 (Cairns and Moreton Bay populations, respectively) to 2.25 and 2.27 (Bedout and Broome collections, respectively). The overall levels of genetic variation characteristic of these collections were surprisingly similar. Using the 28 variable loci screened in all samples, the percentage of loci polymorphic at the $\mathbf{P}_{0.95}$ level in each collection ranged from 25% in the Tufi collection to 32% (in 12 of the collections). Similarly, the mean observed heterozygosities (direct count) ranged from 9.6% in the 1981 Cairns collection to 11.3% in the Gloma Shoal collection.

Tests of Hardy-Weinberg equilibrium at the 28 variable loci in the 18 collections revealed only three outcomes that were significant after Bonferroni correction for multiple tests (Rice 1989); *EST-4** and *CK-A** in the Lord Mayor Shoal collection (uncorrected p = 0.0009 and 0.0001, respectively) and *ADH** in the Port Moresby collection (uncorrected p = 0.0016) (Appendix 4 in Accessory Publication). Observed and expected heterozygosities at the 28 variable loci in the 14 populations were generally in close agreement throughout the entire data set (Table 3). With the exception of *ADH**, which exhibited a statistically significant deficiency

of heterozygotes when tested across all collections ($X^2_{df=36} = 92.5$), these results provide no basis for concern regarding the genetic models and gel scoring used in data collection at any of the 28 variable loci used in data analysis. A comparison between observed and expected heterozygosities at the twelve most variable loci in each of the 18 collections revealed close agreement in nearly all cases (Appendix 4 in Accessory Publication).

Temporal Comparisons

Multiple collections obtained from three different localities: Moreton Bay, Cairns, and the Torres Strait were used to test for year-to-year stability/variability of allele frequencies within locations. Pairwise genotypic population differentiation tests revealed no convincing differences at any of these three sites (Moreton Bay 1983 vs. 1984, p = 0.451; Moreton Bay 1983 vs. 1985, p = 0.231; and Moreton Bay 1984 vs. 1985, p = 0.027; Cairns 1981 vs. 1982, p = 0.699; and Torres Strait 1983 vs. 1984, p = 0.611). Furthermore, when the annual collections at each location were pooled, tests of Hardy-Weinberg equilibrium at each locus individually revealed no significant departures from expectations (after correction for multiple tests). Furthermore, none of the simultaneous tests across all loci at these three locations were significant (unadjusted p values for Moreton Bay = 0.256; Cairns = 0.999; and Torres Strait = 0.181). Collectively, these results indicated year-to-year stability of genotypic proportions at these three locations. Given the apparent stability of allele frequencies at these three locations, it seemed reasonable to assume temporal stability at other locations and to use allele frequencies of collections to represent the genetic characteristics of Spanish mackerel at geographic localities

(presumed populations), not simply for individual collections.

Population Structure

Genotypic tests of population differentiation (Raymond and Rousset 1995b; Goudet et al. 1996) indicated that the total data set was significantly heterogeneous, both when tested as 18 separate collections using all 28 variable loci (overall unadjusted $p < 0.0000$ with significant differences at five loci [*ACP**; *sAH**; *CK-A**; *PEPD-1**; and *PGDH**]) and tested as 14 locations using the 28 variable loci (overall unadjusted $p < 0.0000$ with significant differences at five loci [*ACP**; *sAH**; CK-A*; *LDH-B**; and *PGDH**] and nearly significant differences at *mAAT** and *sSOD**). A reduced data set consisting of only the Australian and Torres Strait collections (Tufi and Port Moresby omitted) was also significantly heterogeneous when tested as 16 separate collections for all 28 variable loci (unadjusted $p< 0.0000$) with significant differences at four individual loci (*ACP**; *sAH**; *CK-A**; and *PGDH**) and when tested as 12 populations for all 28 variable loci (overall unadjusted $p< 0.0000$; same four loci significant). Genic tests of population differentiation yielded nearly identical results. Review of the pattern and magnitude of significant and non-significant pairwise genotypic tests of population differentiation (Table 4) revealed three major findings: 1) the Tufi, PNG population was significantly different from all but the Port Moresby population, 2) the Port Moresby population (represented by a collection of only 76 fish) was not significantly different from any of the other populations, and 3) the Moreton Bay and Cairns populations (Queensland east coast) were significantly different from all other populations (except each other, Port Moresby, Mornington

Island, Torres Strait, and Bedout Island – but the unadjusted p-values for the latter three sets of comparisons are all quite low). Only four of the 28 pairwise comparisons between the nine northern and western Australian localities (Mornington Island, Cape Croker, Holothuria Banks, Lord Mayor Shoal, Broome, Bedout Island, Gloma Shoal, Monte Bello Islands, and Cape Cuvier) were significant (and one wasn't significant overall but had a significant difference at one locus – Cape Croker vs. Cape Cuvier at *ACP**). This pattern suggests that narrow-barred Spanish mackerel in the Australian - southern Papua New Guinea region can be subdivided into three more-or-less divergent genetic groups: 1) northeastern Papua New Guinea (Tufi), 2) east coast of Queensland (Moreton Bay and Cairns), and 3) northern and western Australia (Mornington Island to Cape Cuvier) plus southern Papua New Guinea (Port Moresby) and the Torres Strait. The fact that Spanish mackerel in the Torres Strait region are not genetically differentiated from those at Port Moresby or from any of those in Australia (except for Lord Mayor Shoal) suggests that the Torres Strait population is part of a widespread northern Australia – southern Papua New Guinea stock (Figure 1).

Pairwise F_{ST} values were all quite small. Twenty-seven of the 91 F_{ST} values between pairs of the14 populations were zero or negative while the largest F_{ST} values were between Tufi and Cairns (0.0133) and Tufi and Moreton Bay (0.0120). When the negative values were converted to zeros, the average pairwise F_{ST} value for the entire 14-population data set was 0.0021 and that for the 12-population Australian data set was only 0.0014.

Pairwise genetic distances (Table 4) and multidimensional scaling (MDS) analysis of these pairwise genetic distance values (Figure 2) were consistent with the generalized pattern of population genetic divergence revealed by the pairwise population differentiation tests. The

MDS plot reinforced this basic three-stock pattern of relationships, although the stress value indicated only a "poor" fit of the plotted population relationships to the actual genetic distance values. The Tufi population was the most distinctive, defining one corner of the plot. The Australian east coast populations (Cairns and Moreton Bay) were the most distant from the Tufi population in this plot while the northern Australian populations (Mornington Island to Cape Cuvier) were more-or-less intermediate. While this depiction of population interrelationships revealed relatively large variance among repeat collections (Moreton Bay 1 vs. 2 vs. 3; Cairns 1 vs. 2; and Torres Strait 1 vs. 2) and some geographic anomalies in the genetic relationships (e.g., the relatively large distance between the Monte Bello Islands and Cape Cuvier collections), it also showed some expected patterns (the Torres Strait collections being more-or-less intermediate between those from eastern Queensland and those from northern and western Australia).

In spite of the large sample sizes for the eastern Queensland (N = 644) and the north and west Australia / south PNG groups (N = 1,439), no new significant deviations from Hardy-Weinberg equilibrium expectations were observed when this was tested in these two large, pooled groups. This result is consistent with the conclusion that each of these groups represents a single panmictic population.

Discussion

Genetic variation

Narrow-barred Spanish mackerel in the Australia-southern PNG region exhibit average to slightly above average levels of genetic variation compared to other species of fish. For

example, average allozyme heterozygosity is approximately 5% in fish as a group (Nevo et al. 1984) yet is about 10.6% across the 28 loci completely screened (listed in Appendix 1) in these Spanish mackerel.

The apparent temporal stability of allele frequencies at three different localities observed in the present study is not surprising given that commercial catches of this species typically contain fish from multiple year classes and only collections from consecutive years were tested. Temporal stability of allele frequencies has also been noted previously in several commercial fish species (Koehn and Williams 1978; Shaklee and Samollow 1984; Salini and Shaklee 1988) but not in all (Kornfield et al. 1982; Chapman et al. 2002). The failure to detect significant differences between narrow-barred Spanish mackerel collections from multiple years at three localities is consistent with an assumption of temporal stability of allele frequencies in this species. Comparison of allele frequencies for 12 loci at five different locations from this study with comparable data from an independent investigation by Ovenden and Street conducted 15-18 years later indicates temporal stability over at least 15 years (Table 1 in Accessory Publication). These expanded data provide strong justification for using data from individual collections to represent geographic locations, allowing comparisons among collections made in different years.

The relatively high level of heterozygosity observed in this species and the fact that 7-9 loci were polymorphic at the $\mathbf{P}_{0.95}$ level in every collection make the present tests of stock structure relatively robust. Additionally, the close agreement between observed and expected genotype distributions for all loci in nearly all collections strengthened the analysis because it is consistent with the assumption that individual collections represented panmictic populations.

This situation contrasts sharply with that reported for Australian jack mackerel, Trachurus declivis, where excesses of heterozygotes at one or more loci characterized nearly all individual collections (Richardson 1982).

Although there have been some reports linking high fishery harvest rates with decreased genetic diversity (heterozygosity, heterozygote deficits) (Smith et al. 1991; Hauser et al. 2002), no such effects were observed in the present study. In fact, there was very close agreement between observed and expected heterozygosity across loci and collections in the present study (Table 3 and Appendix 4 in Accessory Publication) and, as noted above, levels of polymorphism and heterozygosity were relatively high in *S. commerson*. However, because genetic data for these populations prior to fishery exploitation are not available, definitive conclusions regarding possible differences in allelic diversity or heterozygosity between pre- and post-exploitation populations are not possible.

Population structure

Despite the statistically significant stock divergence described above, relatively little genetic differentiation in *S. commerson* was observed over the considerable geographic scope of the present investigation. Indeed, the common variant alleles (frequencies greater than 0.05) were present at each locality sampled and often exhibited similar frequencies. The broad geographic distributions of many of the rare (e.g., *ACP*-20*; *ADH*40*; *EST-1*103*; *sIDHP*73*; *LDH-B*146*; *PEPD-1*83*; *PEPD-2*118* & *112*) and very rare alleles (e.g., *ACP*-250*; *sAH*96*; *EST-1*95* & *88*; *GPI-A*78*; *sIDHP*120* & *82*; *mMDH*260* & *60*; *MEP*92*; *PEPD-1*111* & *91*; *PGM*-118* & *-84*; and *mSOD*180* & *38*) also suggested little genetic

differentiation throughout the region (cf. Appendixes 2 and 3 in Accessory Publication). Furthermore, individual locus F_{ST} values (Wright 1978) in this species were small – 0.003 (at seven different loci) to 0.021 (at *PGDH**) and the mean F_{ST} across all loci was only 0.009 in these samples, hardly indicative of substantial genetic subdivision. For comparison, the mean F_{ST} for the seven recognized genetic stocks of barramundi (*Lates calcarifer*; a catadromous species) in the Northern Territory and Western Australia was reported to be 0.087 (Salini and Shaklee 1988) while that for one southern Papua New Guinea and seven Queensland stocks was 0.031 (Shaklee et al. 1993).

Nevertheless, in addition to the statistically significant overall differences indicated by the pairwise population differentiation tests (Table 4), the geographic distributions of several of the rare and very rare alleles were consistent with partial reproductive isolation (cf. Appendixes 2 and 3 in Accessory Publication). The *mAAT-3*-132* allele was absent in Moreton Bay, very rare in Cairns, but present in all other collections (except Lord Mayor Shoal). The *sAH*90* allele occurred at a frequency of 0.138 in Tufi but was considerably rarer in other eastern locations such as Cairns (0.010), Port Moresby (0.026), Torres Strait (0.033), and Moreton Bay (0.003). The *GPI-A*110* allele was present in all collections except Tufi. The *sAH*80* and *PEPD-1*86* alleles were absent from Moreton Bay, Cairns, and Tufi but present in most other collections (i.e., collections of the northern Australia - southern PNG stock). The *sSOD*112* allele was absent at Moreton Bay and Cairns but present at many other localities. Although the genetic differences distinguishing the three stocks are not large, they are statistically significant and their geographic patterns suggest genetic population differentiation due to partially restricted gene flow among regions. Various life history characteristics of narrow-barred Spanish

mackerel likely contribute to limited gene flow and population differentiation. Among these are the discontinuous distribution of known spawning aggregations and the apparent preference of juveniles for inshore, shallow water habitats; 2-10 cm juveniles being abundant in creeks, estuaries, and mud flats in Queensland and larger juveniles (15-40 cm) being frequently taken by prawn trawls in embayments and coastal waters 6-12 m deep (McPherson 1981; Tobin 2000).

Other relevant studies

Genetic studies have also indicated that several other marine species exhibit population subdivision in this region. Begg et al. (1998a) reported that allozyme data revealed evidence of a complex stock structure in school mackerel (*S. queenslandicus*), with stocks being associated with large embayments (Darwin & Joseph Bonaparte Gulf vs. Weipa vs. Queensland east coast; but evidence for only two stocks of spotted mackerel (*S. munroi*), one along the east coast of Queensland and one at Darwin (Arafura Sea). Subsequent studies of otolith microchemistry of fish from the east coast of Queensland (Begg et al. 1998b) were consistent with these general conclusions regarding stock structure in these two species. Elliott (1996) reported weak allozyme differentiation between Gulf of Carpentaria and NW shelf populations but strong east coast of Queensland vs. Arafura Sea population differences at mtDNA for the saddle-tail sea perch *Lutjanus malabaricus*. MacDonald (1980; cited in Richardson et al. 1986) reported that the snapper (*Chrysophrys unicolor*) in Shark Bay, Western Australia were genetically divergent from all other populations throughout southwestern Australia while Johnson et al. (1986) reported the existence of two distinct stocks of this species within Shark Bay. Benzie et al. (1992 and 1993) reported significant genetic stock divergence in the prawn, *Penaeus monodon*,

between Western Australia (the De Grey River, near Port Hedland) and northern and eastern Australia (Joseph Bonaparte Gulf, Northern Territory to the Clarence River, northeastern New South Wales).

Other, recent studies of stock structure in *S. commerson* have led to the conclusion that multiple stocks exist in Australian and nearby waters, but the number and distributions of hypothesized stocks have varied from study to study. Lester et al. (2001) used the presence or absence of permanent parasites as evidence of five distinct stocks in the region: 1) a west coast stock (found at Abrolhos Island, Shark Bay, Exmouth, and Onslow [just slightly SW of Monte Bello Islands]), 2) Broome, 3) Kupang, Indonesia, 4) a Groote Eylandt-Torres Strait stock, and 5) an east coast of Australia stock.

Ovenden and colleagues have conducted mtDNA studies of population structure in *S. commerson* from Australia, the Torres Strait, and West Timor. They concluded that four distinct stocks exist in the region, one in West Timor, one in northern and western Australia, one in eastern Australia, and a fourth in the Torres Strait (personal communication; J. Ovenden, Southern Fisheries Research Centre, Queenland DPI, Deception Bay, Queensland 4508 Australia).

The differing views of the relationship of the Torres Strait population between the present study and the investigations of Ovenden et al. (personal communication) cannot be accounted for by the sample site used to characterize the Torres Strait population because fish from Bramble Cay were used in both studies. However, the numbers of collections and collection localities for both the east coast of Queensland and for northern (and western) Australia in the two studies differed substantially and this could have been at least partially

responsible for the different results (as could differences between the genetic markers in the two studies. Both the similarity of *S. commerson* parasite burdens between the northwest Australia areas and the Torres Strait (Lester et al. 2001) and the observations of McPherson (1989), who noted easterly movement of fish from the Gulf of Carpenteria toward the Torres Strait are consistent with the genetic similarity of fish in these areas reported here. However, the recognition of three distinct stocks across western and northern Australia by the parasite investigations of Lester et al. (2001) compared to the lack of genetic divergence across this same region noted by the present study and the mtDNA investigations of Ovenden et al. (personal communication) are inconsistent and troublesome because they suggest different fishery management and conservation issues and approaches.

Given the more-or-less continuous range of narrow-barred Spanish mackerel throughout the study region, it is perhaps somewhat surprising that there is any signal of population subdivision. Nevertheless, the present results provide evidence that three somewhat genetically divergent stocks occur in the area; one in the Tufi region of northeastern Papua New Guinea, a second in eastern Queensland (Cairns to Moreton Bay), a third ranging from Port Moresby, PNG through the Torres Strait and across northern Australia from the Gulf of Carpentaria in northern Queensland to the Cape Cuvier region of Western Australia. The somewhat limited and discrete sampling of this study constrains our understanding of the boundaries of these three stocks (see the "??" symbols in Figure 1). How much the current genetic characteristics and stock structure of Spanish mackerel in the Torres Strait region reflect historical geographic isolation due to the presence of a land bridge connecting northern Australia and Papua New Guinea roughly 15,000 years ago (Jones and Torgersen 1988) that would have eliminated all gene flow through this area

and how much they are shaped by recent and ongoing gene flow among populations in the region is unknown. Recolonization of the Torres Strait region by *S. commerson* following the rise in sea level could have involved fish from eastern Queensland, fish from north (and western) Australia, fish from eastern PNG, or some combination of these. The genetic data from this study suggest that fish from northern (and western) Australia played a dominant role in this recolonization.

Despite this uncertainty, and the fact that inter-population gene flow in many marine species ensures that genetic signals of population differentiation will be weak (Waples 1998), even modest genetic evidence of population subdivision raises the need for conservative management because overfishing or other mismanagement of components of a multi-stock fishery can lead to declines or even collapses of important components or even the entire fishery. Biological (and economic) recovery from such negative impacts can require long-term and expensive conservation and management actions.

It should be noted that, in addition to these three closely similar stocks of *S. commerson* in Australia and southern Papua New Guinea, several additional, genetically distinct stocks are recognizable elsewhere throughout the Indo-west Pacific range of this species (Shaklee et al. 1990b; Shaklee 2004; Shaklee, unpublished data).

Marked, large-scale discontinuities in suitable adult and juvenile habitats throughout the Indo-Pacific and isolation by distance are the likely causes of population divergence in shallow water marine fish such as *S. commerson* that occur throughout such large areas (Planes and Fauvelot 2002), the physical factors underlying the modest divergence in the Australia - New Guinea region reported in this study are less clear. It seems likely that several factors are

involved, including: 1) geologically historical barriers, such as the closure of the Torres Strait, caused by periods of glaciation and the accompanying fall in sea levels (Jones and Torgersen 1988; McMillan and Palumbi 1995, and references therein), 2) the presence of several discontinuities/restrictions of the near-shore shallow water (<200m) zone within the Australia - New Guinea region (see Figure 1), 3) isolation by distance (Wright 1943) promoted by the discontinuous distribution of Spanish mackerel spawning aggregations across the region (McPherson 1993), 4) oceanographic currents (both coastal and open ocean) that may limit or even prevent gene flow mediated by larval dispersal (cf. Doherty et al. 1995; Jones et al. 1999; Hoskin 2000; Muss et al. 2001), and 5) life history characteristics, such as the estuarine juvenile stage of *S. commerson* (reviewed by Tobin 2000), that limit dispersal.

Management and conservation implications

Because the data and analyses presented in this report suggest that the narrow-barred Spanish mackerel populations in the Australian region are components of three different reproductive stocks, I believe that long-term fisheries management for this species should be based on recognition of these stocks. This multiple stock model should guide the development and implementation of fishing regulations and harvest quotas, the collection and interpretation of fishery statistics, and the allocation of catch between Australia and Papua New Guinea. Furthermore, the presence of a single, shared stock ranging across the northern Australian coast, within the Torres Strait, and along the southern coast of Papua New Guinea (and Irian Jaya?) indicates that successful long-term management of fisheries throughout this region is a matter of concern to at least Papua New Guinea, Queensland, the Northern Territory, and Western

Australia.

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References

Banks, M.A. and W. Eichert. 2000. WHICHRUN (version 3.2): a computer program for population assignment of individuals based on multilocus genotype data. *Journal of Heredity* **91**:87-89.

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

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

Benzie, J.A.H., Ballment, E. (1994). Genetic differences among black-lipped pearl oyster (*Pinctada margaritifera*) populations in the western Pacific. *Aquaculture* **127**, 145-156.

Benzie, J.A.H., Frusher, S., Ballment, E. (1992). Geographic variation in allozyme frequencies of populations of *Peneus monodon* (Crustacea: Decapoda) in Australia. *Australian Journal of Marine and Freshwater Research* **43**, 715-725.

Benzie, J.A.H., Frusher, S., Ballment, E. (1993). Genetic structure of *Peneus monodon* in Australia: concordant results from mtDNA and allozymes. *Aquaculture* **111**, 89-93.

Berst, A.H., Simon, R.C. (Eds) (1981). Proceedings of the Stock Concept International Symposium (STOCKS). *Canadian Journal of Fisheries and Aquatic Sciences* **38**, 1457-1921.

Cavalli-Sforza, L.L., Edwards, A.W.F. (1967). Phylogenetic analysis: models and estimation procedures. *Evolution* **21**, 550-570.

Chapman, R.W., Ball, A.O., Marsh, L.R. (2002). Spatial homogeneity and temporal heterogeneity of red drum (*Sciaenops ocellatus*) microsatellites: effective population sizes and management implications. *Marine Biotechnology* **4**, 589-603.

Collette, B.B., Russo, J.L. (1984). Morphology, systematics, and biology of the Spanish mackerels (*Scomberomorus*, Scombridae). *NOAA Fishery Bulletin* **82**, 545-692.

Darnall, D.W., Klotz, I.M. (1975). Subunit constitution of proteins: A table. *Archives of Biochemistry and Biophysics* **166**, 651-682.

Dieringer, D., Schlotterer, C. (2003). MICROSATELLITE ANALYSER (MSA): a platform independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes* **3**,

167-169.

Doherty, P.J., Mather, P., Planes, S. (1994). *Acanthochromis polyacanthus*, a fish lacking larval dispersal, has genetically differentiated populations at local and regional scales on the Great Barrier Reef. *Marine Biology* **121**, 11-21.

Doherty, P.J., Planes, S., Mather, P. (1995). Gene flow and larval duration in seven species of fish from the Great Barrier Reef. *Ecology* **76**, 2373-2391.

Elliott, N.G. (1996). Allozyme and mitochondrial DNA analysis of the tropical saddle-tail sea perch, *Lutjanus malabaricus* (Schneider), from Australian waters. *Marine and Freshwater Research* **47**, 869-876.

Ferris, S.D., Whitt, G.S. (1978). Genetic and molecular analysis of nonrandom dimer assembly of the creatine kinase isozymes of fishes. *Biochemical Genetics* **16**, 811-829.

Frick, L. (1984). Isolation and characterization of five cytosolic di- and tripeptidases from the skipjack tuna (*Katsuwonis pelamis*). *Comparative Biochemistry and Physiology* **77B**, 533-540.

Goudet, J. 1995. FSTAT (version 1.2): A computer program to calculate F-statistics. *Journal of Heredity* **86**:485-486.

Goudet, J., M. Raymond, T. de Meeus, and F. Rousset. 1996. Testing differentiation in diploid populations. *Genetics* **144**,1933-1940.

Harris, H., Hopkinson, D.A. (1976). Handbook of Enzyme Electrophoresis in Human Genetics. Amer. Elsevier Publ. Co. New York.

Hauser, L., Adcock, G.J., Smith, P.J., Ramirez, J.H.B., Carvalho, G.R. (2002). Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). *Proceedings of the National Academy of Sciences* **99**, 11742-11747.

Hoskin, M.G. (2000). Effects of the East Australian Current on the genetic structure of a direct developing muricid snail (*Bedeva hanleyi*, Angas): variability within and among local populations. *Biological Journal of the Linnean Society* **69**, 245-262.

Hoyle, S.D. (2003). 2003 update: Management strategy evaluation for the Queensland east coast Spanish mackerel fishery. Queensland Department of Primary Industries Information Series QI03021, Brisbane, 9pp.

Johnson, M.S., Creagh, S., Moran, M. (1986). Genetic subdivision of stocks of snapper, *Chrysophrys unicolor*, in Shark Bay, Western Australia. *Australian Journal of Marine*

and Freshwater Research **37**, 337-345.

Johnson, M.S., Joll, L.M. (1993). Genetic subdivision of the pearl oyster *Pinctada maxima* (Jameson, 1901) (Mollusca: Pteriidae) in northern Australia. *Australian Journal of Marine and Freshwater Research* **44**, 519-526.

Jones, G.P., Milicich, M.J., Emslie, J.J., Lunow, C. (1999). Self-recruitment in a coral reef fish population. *Nature* **402**, 802-804.

Jones, M.R., Torgersen, T. (1988). Late Quaternary evolution of Lake Carpenteria on the Australia-New Guinea continental shelf. *Australian Journal of Earth Sciences* **35**, 313-324.

Koehn, R.K., Williams, G.C. (1978). Genetic differentiation without isolation in the American eel, *Anguilla rostrata*. II. Temporal stability of geographic patterns. *Evolution* **32**, 624-637.

Kornfield, I., Sidell, B.D., Gagnon, P.S. (1982). Stock definition in Atlantic herring (*Clupea harengus harengus*): Genetic evidence for discrete fall and spring spawning populations. *Canadian Journal of Fisheries and Aquatic Sciences* **39**, 1610-1621.

Lavery, S., Moritz, C., Fielder, D.R. (1995). Changing patterns of population structure and gene flow at different spatial scales in *Birgus latro* (the coconut crab). *Heredity* 74, 531-541.

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

MacDonald, C.M. (1980). Population structure, biochemical adaptation and systematics in temperate marine fishes of the genera *Arripes* and *Chrysophrys* (Pisces: Perciformes). Ph.D. thesis. Australian National University, Canberra 336pp.

McMillan, W.O., Palumbi, S.R. (1995). Concordant evolutionary patterns among Indo-West Pacific butterflyfishes. *Proceedings of the Royal Society of London B* **260**, 229-236.

McPherson, G.R. (1981). Preliminary report: Investigations of Spanish mackerel *Scomberomorus commerson* in Queensland waters. In 'Northern Pelagic Fish Seminar'. (Eds C.J. Grant and D.G. Walter) pp.51-58. (Australian Government Publishing Service: Canberra).

McPherson, G.R. (1986). The Torres Strait Spanish mackerel fishery: A review of Australian development, production and research. In 'Torres Strait Fisheries Seminar'. (Eds A.K. Bain, G.C. Williams, and D. Coates) pp.151-164. (Australian Government Publishing Service: Canberra).

McPherson, G.R. (1988). A review of large coastal pelagic fishes in the south Pacific region, with special reference to *Scomberomorus commerson* in north-east Australian waters. Workshop on Pacific Inshore Fishery Resources. Workshop Proceedings 15, 41pp. (South Pacific Commission: Noumea, New Caledonia, 14-25 March).

McPherson, G.R. (1989). North-eastern Australian mackerel (*Scomberomorus*) fishery. Proceedings of the workshop Australia - Mexico on Marine Sciences (Ed E.A. Chavez), Mexico. pp. 341-348.

McPherson, G.R. (1993). Reproductive biology of the narrow barred Spanish mackerel (*Scomberomorus commerson* Lacepede, 1800) in Queensland waters. *Asian Fisheries Science* **6**, 169-182.

Morizot, D.C., Schmidt, M.E. (1990). Starch gel electrophoresis and histochemical visualization of proteins. In 'Electrophoretic and Isoelectric Focusing Techniques in Fisheries Management'. (Ed D.H. Whitmore) pp.24-80. (CRC Press: Boca Raton. 350pp.)

Muss, A., Robertson, D.R., Stepien, C.A., Wirtz, P. Bowen, B.W. (2001). Phylogeography of *Ophioblennius*: the role of ocean currents and geography in reef fish evolution. *Evolution* **55**, 561-572.

Nevo, E., Beiles, A., Ben-Shlomo, R. (1984). The evolutionary significance of genetic diversity: ecological, demographic and life history correlates. *Lecture Notes in Biomathematics* **53**, 13-213.

Planes, S., Fauvelot, C. (2002). Isolation by distance and vicariance drive genetic structure of a coral reef fish in the Pacific Ocean. *Evolution* **56**, 378-399.

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

Raymond, M., Rousset, F. (1995b). An exact test for population differentiation. *Evolution* **49**, 1280-1283.

Richardson, B.J. (1982). Geographical distribution of electrophoretically detected protein variation in Australian commercial fishes: I. Jack mackerel, *Trachurus declivis* Jenyns. *Australian Journal of Marine and Freshwater Research* **33**, 917-926.

Richardson, B.J., Baverstock, P.R., Adams, M. (1986). Allozyme Electrophoresis: A Handbook for Animal Systematics and Population Studies. Academic Press, Sydney.410pp.

Rohlf, F.J. (1998). NTSYSpc. Numerical Taxonomy and Multivariate Analysis System. Version 2.0. Exeter Software, Setauket, NY. 31pp.

Salini, J., Shaklee, J.B. (1988). Genetic structure of barramundi (*Lates calcarifer*) stocks from northern Australia. *Australian Journal of Marine and Freshwater Research* **39**, 317-329.

Shaklee, J.B. (1983). The utilization of isozymes as gene markers in fisheries management and conservation. In 'Isozymes: Current Topics in Biology and Medicine. Vol. 11'. (Ed. M.C. Rattazzi, J.G. Scandalios, and G.S. Whitt) pp. 213-247. (Academic Press: New York).

Shaklee, J.B. (1984). Genetic variation and population structure in the damselfish, *Stegastes fascialatus*, throughout the Hawaiian Archipelago. *Copeia* **1984**, 629-640.

Shaklee, J.B. (2004). Allozyme analysis reveals substantial population divergence in the narrow-barred Spanish mackerel, *Scomberomorus commerson*, throughout its Indo-west Pacific range. (IN PREPARATION).

Shaklee, J.B., Keenan, C.P. (1986). A practical laboratory guide to the techniques and methodology of electrophoresis and its application to fish fillet identification. Report 177, CSIRO, Australia. Marine Laboratories, 59pp.

Shaklee, J.B., Bentzen, P. (1998). Genetic identification of stocks of marine fish and shellfish. *Bulletin of Marine Science* **62**, 589-621.

Shaklee, J.B., Samollow, P.B. (1984). Genetic variation and population structure in a deepwater snapper, *Pristipomoides filamentosus*, in the Hawaiian Archipelago. *NOAA Fishery Bulletin* **82**, 703-713.

Shaklee, J.B., Allendorf, F.W., Morizot, D.C., Whitt, G.S. (1990a). Gene nomenclature for protein-coding loci in fish. *Transactions of the American Fisheries Society* **119**, 2-15.

Shaklee, J.B., Phelps, S.R., Salini, J. (1990b). Analysis of fish stock structure and mixed-stock fisheries by the electrophoretic characterization of allelic isozymes. In 'Application of Electrophoretic and Isoelectric Focusing Techniques in Fisheries Management'. (Ed. D.H. Whitmore) pp. 173-196. (CRC Press: Boca Raton).

Shaklee, J.B., Salini, J., Garrett, R.N. (1993). Electrophoretic characterization of multiple genetic stocks of barramundi perch in Queensland, Australia. *Transactions of the American Fisheries Society* **122**, 685-701.

Shaklee, J.B., Beacham, T.D., Seeb, L., White, B.A. (1999). Managing fisheries using genetic data: Case studies from four species of Pacific salmon. *Fisheries Research* **43**, 45-78.

Shulman, M.J. (1998). What can population genetics tell us about dispersal and biogeographic history of coral-reef fishes? *Australian Journal of Ecology* **23**, 216-225.

Shulman, M.J., Bermingham, E.L. (1995). Early life histories, ocean currents, and the population genetics of Caribbean reef fishes. *Evolution* **49**, 897-910.

Smith, P.J., Francis, R.I.C.C., McVeagh, M. (1991). Loss of genetic diversity due to fishing pressure. *Fisheries Research* **10**, 309-316.

Tobin, A. (2000). Review of Spanish mackerel (*Scomberomorus commerson*) in Queensland waters: life history and biology, historical catch and effort, and recent trends. Queensland Fisheries Service, 33pp.

Utter, F.M., Allendorf, F.W., May, B. (1979). Genetic basis of creatine kinase isozymes in skeletal muscle of salmonid fishes. *Biochemical Genetics* **17**, 1079-1091.

Waples R.S. (1998). Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *Journal of Heredity* **89**, 438-450.

Wright, S. (1943). Isolation by distance. *Genetics* **28**, 114-138.

Wright, S. (1978). 'Evolution and the Genetics of Populations, Vol. 4. Variability Within and Among Natural Populations'. University of Chicago Press, Chicago.

Figure legends

Figure 1. Map showing approximate locations of individual collection sites (#1-18; cf. Table 1) and presumed distributions of the three proposed genetic stocks of narrow-barred Spanish mackerel in the Australian -- Papua New Guinea region. IJ = Irian Jaya; PNG = Papua New Guinea; "??" indicates unknown stock identity/transition zone due to absence of collections from this area. (note that the approximate location of the 200m depth contours is indicated by the dotted lines)

Figure 2. Multidimensional scaling analysis of genetic interrelationships of 18 collections of narrow-barred Spanish mackerel, *Scomberomorus commerson*, from 14 locations in the Australian-southern Papua New Guinea region using Cavalli-Sforza and Edwards chord distances. The stress2 value was 0.29973, indicating a "poor" fit of the plot to the genetic distance data.

Table 1. Information about narrow-barred Spanish mackerel collections in Australia and southern Papua New Guinea.

Collection number	Location	Number of fish	Sex			Dates of collection
			Males	Females	Unknown	
Australia						
1	Moreton Bay, Qld.	127	64	59	4	January - April, 1983
2	Moreton Bay, Qld.	124	62	62	0	January - March, 1984
3	Moreton Bay, Qld.	103	41	62	0	January - February, 1985
4	Cairns, Qld.	111	68	30	13	October, 1981
5	Cairns, Qld.	179	76	62	41	November - December, 1982
6	Mornington Island, Qld.	162	43	116	3	13-30 July, 1983
7	Cape Croker (Arnhem Land) N.T.	118	6	11	101	January - April, 1984
8	Holothuria Banks, W.A.	102	47	53	2	17-18 August, 1983
9	Lord Mayor Shoal, W.A.	156	51	87	18	25-26 July, 1983
10	north of Broome, W.A.	129	57	32	40	20-21 June, 1983
11	Bedout Isl. (north of Port Hedland, W.A.)	154	65	85	4	9-10 July, 1983
12	Gloma Shoal, W.A.	159	27	125	7	28-30 June, 1983
13	Monte Bello Islands, W.A.	118	26	66	26	August - September, 1982
14	Cape Cuvier	171	77	90	4	6-28 June, 1985
Torres Strait						
15	Torres Strait #1 (Bramble Cay, Qld)	152	80	69	3	4-7 October, 1983
16	Torres Strait #2 (Bramble Cay, Qld)	110	42	68	0	August - September, 1984
Papua New Guinea						
17	Port Moresby, PNG	79	37	41	1	November, 1984 - March,1985
18	Tufi, PNG	109	58	51	0	9-18 October, 1982

Table 2. Common alleles in *S. commerson* and observed genotypes of known-species reference samples and unknown specimens assigned to species based on their multi-locus genotypes.
(allele codes as in Appendix 1; "+" = A (100) allele; "??" = uncertain genotype; "--" no data)

	Locus																				
Fish #	ADA1	CK-A	CK-C	EST-4	G3PDH-2	GPI-A	GPI-B	sIDHP	LDH-A	mMDH	sMDH-A	sMDH-B	MEP	PEPB	PEPD-1	PEPD-2	PGDH	PGM	sSOD		collection number and locality
known, reference specimens:																				known species	
	+ F D	**+ B**	**+**	**+ D**	**+**	**+ C**	**+**	**+**	**+**	**+**	**+**	**+ C**	**+ D B**	**+**	**+**	**+**	**+ D E**	**+**	**+**	***S. commerson***	**composite**[1]
2210	DD	BB	++	CC	DD	BB	++	++	++	++	++	++	BC	++	DD	EE	++	++	++	*S. queenslandicus*	6 Mornington Isl
2211	DD	BB	++	AC	DD	BB	++	++	++	++	++	++	BB	++	DD	EE	++	++	++	*S. queenslandicus*	6 Mornington Isl
2212	FF	BB	++	++	DD	BB	++	++	++	++	++	++	BB	++	DD	EE	++	++	++	*S. queenslandicus*	6 Mornington Isl
2213	DD	BB	++	++	DD	BB	++	++	++	++	++	++	BB	++	DD	EF	++	++	++	*S. queenslandicus*	6 Mornington Isl
2046	HJ	DD	BB	BB	GG	BE	EE	--	EE	GG	DD	++	GG	++	--	GG	--	OO	++	*S. semifasciatus*	6 Mornington Isl
2047	HH	DD	BB	BB	GG	BB	EE	++	EE	GG	DD	++	BG	++	--	GG	--	OO	++	*S. semifasciatus*	6 Mornington Isl
2505	--	BB	++	DD	FF	BB	++	GG	FF	++	DD	FF	GG	FF	II	GG	HH	DD	JJ	*S. monroi*	7 Cape Croker
2506	--	BB	++	DD	FF	BB	++	GG	FF	++	DD	FF	GG	FF	II	GG	HH	DN	JJ	*S. monroi*	7 Cape Croker
2515	--	BB	++	DE	FF	BB	++	GG	FF	++	DD	FF	GG	FF	II	GG	HH	DD	JJ	*S. monroi*	7 Cape Croker
2516	--	BB	++	DD	FF	BB	++	GG	FF	++	DD	FF	GG	FF	II	GG	HH	DD	JJ	*S. monroi*	7 Cape Croker
2521	--	BB	++	DD	FF	BG	++	GG	FF	++	DD	FF	GG	FF	II	GG	HI	DD	JJ	*S. monroi*	7 Cape Croker
2507	DD	BB	++	++	HH	BB	++	++	++	++	++	(GG)	CC	++	DD	GG	++	LL	++	*S. guttatus*	7 Cape Croker
358	??	CC	--	GG	FF	FF	GG	BB	BB	II	EE	DD	FI	DG	HH	FF	JJ	MQ	FF	*Acanthocybium solandri*	13 Monte Bello Isl
unknowns:																				assigned species	
223	DD	BB	++	++	DD	BB	++	++	++	++	++	++	BB	--	DD	EE	++	++	++	*S. queenslandicus*	13 Monte Bello Isl
224	DF	BB	++	++	DD	BB	++	++	++	++	++	++	BB	++	DD	EE	++	++	++	*S. queenslandicus*	13 Monte Bello Isl
252	DD	BB	++	++	AD	BB	++	++	++	++	++	++	BC	++	++	EE	++	++	++	*S. queenslandicus*	13 Monte Bello Isl
253	DD	BB	++	AC	DD	BB	++	AD	++	++	++	++	BB	++	++	EE	++	++	++	*S. queenslandicus*	13 Monte Bello Isl
254	DF	BB	++	++	DD	BB	++	++	++	++	++	++	BB	++	DD	EE	++	AP	++	*S. queenslandicus*	13 Monte Bello Isl
255	DD	BB	++	++	DD	BB	++	++	++	++	++	++	BB	++	--	--	++	++	++	*S. queenslandicus*	13 Monte Bello Isl
286	DD	BB	++	++	AD	BB	++	++	++	++	++	++	BB	++	DD	EH	++	++	++	*S. queenslandicus*	13 Monte Bello Isl
301	DD	BB	++	++	DD	BB	++	++	++	++	++	++	BB	++	DD	EE	++	++	++	*S. queenslandicus*	13 Monte Bello Isl
319	DD	BB	--	++	DD	BB	++	++	++	++	++	++	AD	++	--	EE	++	++	++	*S. queenslandicus*	13 Monte Bello Isl
320	DD	BB	--	++	DD	BB	++	++	++	++	++	++	BB	++	DD	EE	++	++	++	*S. queenslandicus*	13 Monte Bello Isl
338	DD	BB	--	++	DD	BB	++	++	++	++	++	++	BB	++	DD	EE	++	++	++	*S. queenslandicus*	13 Monte Bello Isl
344	DD	BB	--	++	DD	BB	++	++	++	++	++	++	BB	AE	DD	EE	++	++	++	*S. queenslandicus*	13 Monte Bello Isl
345	DD	BB	--	++	DD	BB	++	++	++	++	++	++	BC	++	DD	EE	++	++	++	*S. queenslandicus*	13 Monte Bello Isl
346	DD	BB	--	++	DD	BB	++	++	++	++	++	++	BC	++	DD	EE	++	++	++	*S. queenslandicus*	13 Monte Bello Isl
347	DD	BB	--	++	DD	BB	++	++	++	++	++	++	BB	++	CD	EE	++	++	++	*S. queenslandicus*	13 Monte Bello Isl
348	DD	BB	--	++	DD	BB	++	++	++	++	++	++	BB	++	--	EE	++	++	++	*S. queenslandicus*	13 Monte Bello Isl
352	DD	BB	--	AD	DD	BB	++	++	++	++	++	++	BB	++	CD	EF	++	++	++	*S. queenslandicus*	13 Monte Bello Isl
353	DD	BB	--	++	DD	BB	++	++	++	++	++	++	BB	AF	DD	EF	++	++	++	*S. queenslandicus*	13 Monte Bello Isl
1078	DD	BB	--	--	DD	BB	++	++	++	++	++	--	BB	++	DD	EE	++	++	++	*S. queenslandicus*	13 north of Broome
2183	DD	BB	++	++	AD	BB	++	++	++	++	++	++	BB	++	??	EE	++	++	++	*S. queenslandicus*	6 Mornington Isl
1538	HJ	DD	BB	BB	GG	BB	EE	++	EE	GG	DD	++	GG	++	--	GG	BB	OO	++	*S. semifasciatus*	9 Lord Mayor Shoal
2282	KK	BB	??	AD	FF	BB	++	GG	--	++	DD	FF	GG	FF	II	GG	--	DD	JJ	*S. monroi*	17 Port Moresby

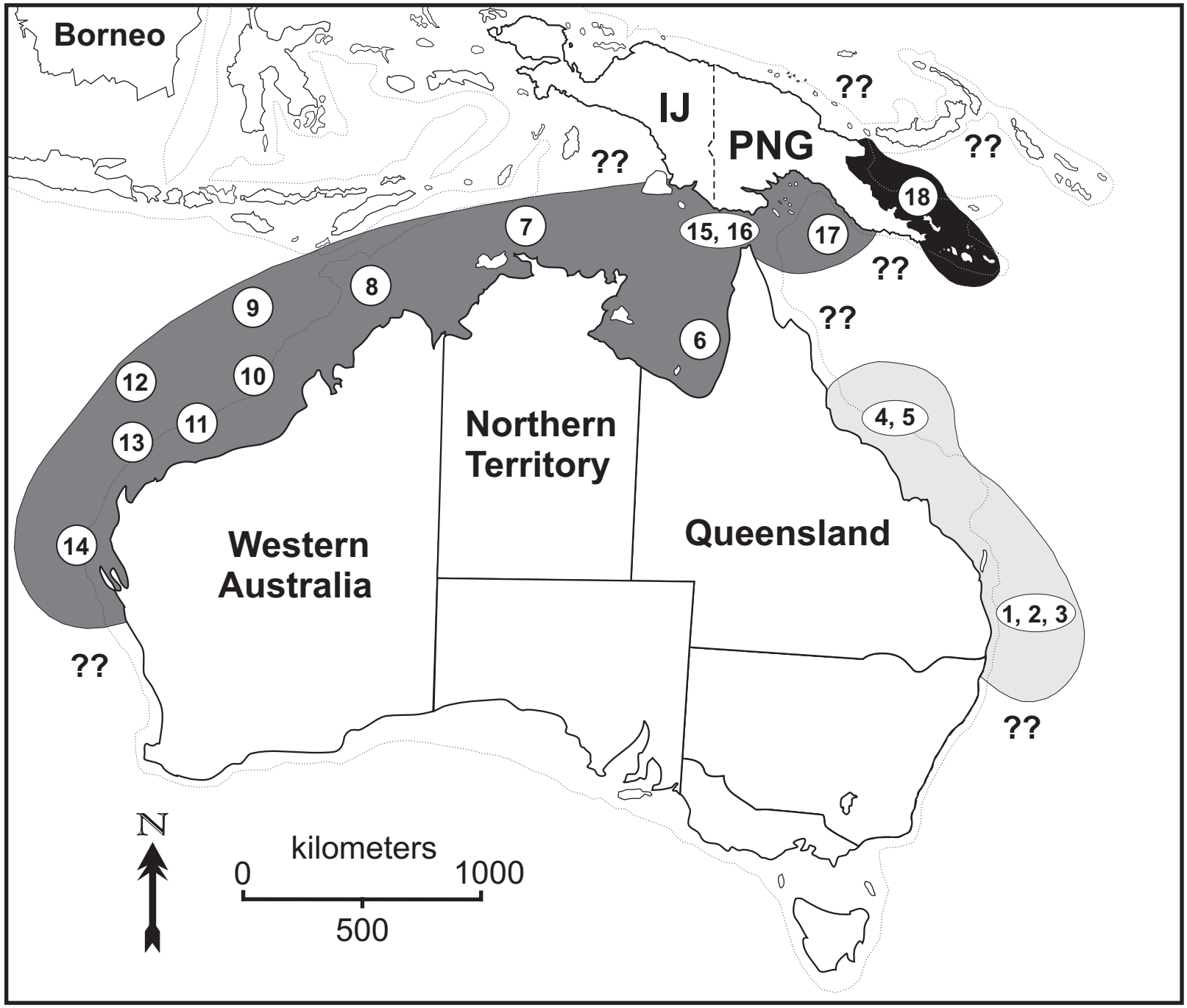
[1] = range-wide (JBS; unpublished data) listing of all alleles occurring at frequencies of at least 0.1 in at least one population of *S. commerson* (listed from highest to lowest frequency)

Table 3. Observed (from FSTAT) and expected (from MSA) heterozygosities at 28 loci in entire 14 population data set.

Locus	Ho	He
mAAT	0.0230	0.0185
ACP	0.5470	0.5309
ADAl	0.4500	0.4668
ADH	0.2140	0.2330
sAH	0.2000	0.1999
CK-A	0.1450	0.1660
CK-B	0.0030	0.0026
EST-4	0.1740	0.1736
GPI-A	0.2670	0.2650
GPI-B	0.0020	0.0034
G3PDH-2	0.0050	0.0055
mIDHP	0.0030	0.0027
sIDHP	0.1100	0.1114
LDH-A	0.0010	0.0009
LDH-B	0.0150	0.0148
LDH-C	0.0110	0.0102
sMDH-A	0.0010	0.0013
sMDH-B	0.0040	0.0055
mMDH	0.0230	0.0231
MEP	0.0220	0.0256
MPI	0.0120	0.0110
PEPB	0.0140	0.0141
PEPD-1	0.0320	0.0328
PEPD-2	0.0490	0.0506
PGDH	0.5760	0.5616
PGM	0.0420	0.0406
sSOD	0.0100	0.0076
mSOD	0.0230	0.0243
average	0.1064	0.1073

Table 4. Tests of population differentiation and pairwise genetic distances for 14 populations of Spanish mackerel. The unadjusted p-values from genotypic tests of population differentiation (FSTAT), with significant values in bold, and significant loci (GENEPOP) are shown above diagonal. The Cavalli-Sforza & Edwards (1967) chord distances (MSA) are shown below diagonal. The number of fish in each population is shown in parentheses below the population name.

	Moreton (354)	Cairns (290)	Torres Strait (262)	Mornington (161)	Croker (118)	Holothuria (102)	Lord Mayor (156)	Broome (128)	Bedout (154)	Gloma (159)	Monte Bello (118)	Cuvier (171)	Moresby (76)	Tufi (109)
Moreton Bay	-	0.30095	0.00071 sAH	0.00767	**0.00001** sAH	**0.00034**	**0.00002** sAH	**0.00003** sAH	0.00082 sAH	**0.00001** sAH	**0.00001** sAH	**0.00001** ACP	0.12115	**0.00001** mAAT sAH CK-A
Cairns	0.02901	-	0.01156	0.00832	**0.00003**	**0.00001**	**0.00001** CK-A	**0.00001**	**0.0001**	**0.00001** sAH	**0.00001** sAH	**0.00001** ACP sAH	0.03893	**0.00001** sAH CK-A
Torres Strait	0.03722	0.03746	-	0.75371	0.03009	0.01911	**0.00004**	0.05165	0.7736	0.00532	0.14165	0.00125 ACP	0.98989	**0.00001** sAH CK-A
Mornington	0.04111	0.03913	0.03952	-	0.30236	0.98992	0.8126	0.43909	0.40937	0.19697	0.01166	0.00063	0.80893	**0.00001** sAH
Croker	0.04855	0.04430	0.04436	0.04252	-	0.22778	0.01593	0.03435	0.21213	0.02226	0.04052	0.00152 ACP	0.08653	**0.00002** sAH
Holothuria	0.04860	0.04834	0.04883	0.03770	0.04280	-	0.47468	0.47586	0.82838	0.48384	**0.00044**	0.00658	0.05399	**0.00014** sAH
Lord Mayor	0.04038	0.04602	0.04943	0.03939	0.04846	0.03986	-	0.26469	0.40527	0.05215	**0.00001**	**0.00001** ACP	0.09834	**0.00001** sAH
Broome	0.04685	0.04564	0.04339	0.04451	0.04589	0.04654	0.04560	-	0.63842	0.2093	0.0221	0.01231	0.57779	**0.00001**
Bedout	0.03888	0.04139	0.03785	0.03928	0.04195	0.04573	0.04476	0.04611	-	0.10448	0.16381	0.05279	0.92308	**0.00009** sAH CK-A
Gloma	0.04853	0.04948	0.05096	0.04514	0.04869	0.04427	0.04602	0.04808	0.04612	-	0.12936	**0.00045** ACP	0.69665	**0.00022** CK-A
Monte Bello	0.04959	0.04670	0.04728	0.05094	0.04462	0.05744	0.05679	0.05483	0.05132	0.04971	-	0.3594	0.38431	**0.00001** CK-A
Cuvier	0.04418	0.04626	0.04450	0.04770	0.04790	0.05119	0.05426	0.05130	0.04578	0.05277	0.04382	-	0.03358	**0.00001** sAH CK-A
Moresby	0.04640	0.04523	0.04335	0.04520	0.04694	0.05545	0.05314	0.05130	0.04819	0.05467	0.04856	0.05278	-	0.00071
Tufi	0.06116	0.06274	0.05844	0.05774	0.05212	0.05335	0.06338	0.06282	0.05734	0.05915	0.05690	0.06465	0.05745	-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Queensland
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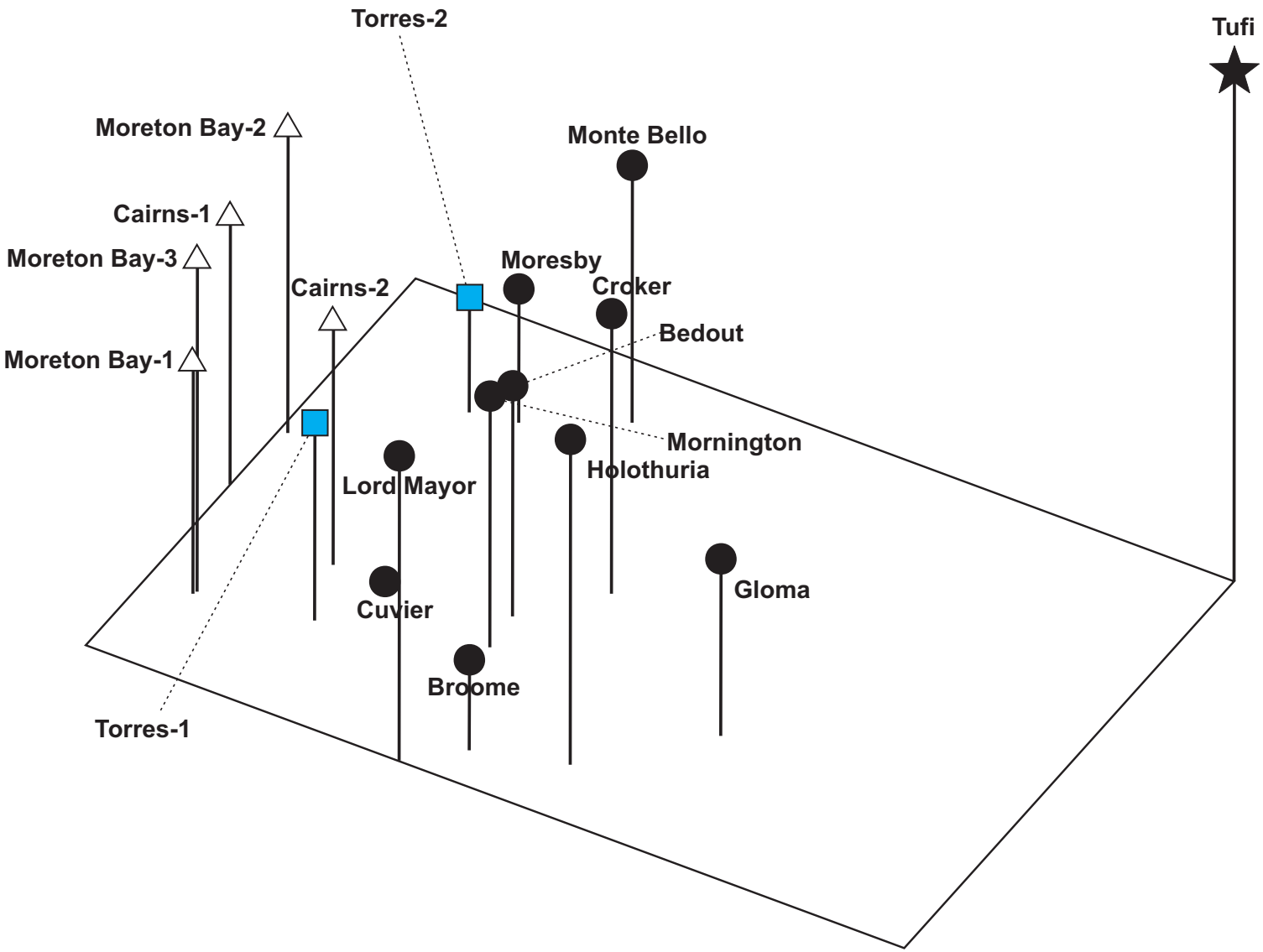
Torres-2
Tufi
Moreton Bay-2
Monte Bello
Cairns-1
Moreton Bay-3
Moresby
Cairns-2
Croker
Bedout
Moreton Bay-1
Mornington
Holothuria
Lord Mayor
Gloma
Cuvier
Broome
Torres-1

Appendix 1. Loci, tissues, buffers, and allele mobilities for the Australian Spanish mackerel study of Shaklee (2004).

Genetic status: P = polymorphic at the $P_{0.95}$ level, V = polymorphic at the $P_{0.99}$ level, R = rare variation (not variable at $P_{0.99}$ level), M = monomorphic in this study, nl = new locus (only screened in a few fish), us = unreliable scoring (no data available); Tissues: M = muscle, E = eye, and L = liver; Buffers described in Shaklee and Keenan 1986 (CAM[E] = CAAPM, CAD = CAEA, CAT = TRIC), bold type indicates buffer used to determine relative mobilities; Subunit structure: M = monomer, D = dimer, T = tetramer

Locus[B]	Genetic status	Tissue(s)	Buffer(s)	Presumed subunit structure	Numeric & Alphabetic Allele Codes[A] 1 A	2 B	3 C	4 D	5 E	6 F	7 G	8 H	9 I	10 J	11 K	12 L	13 M	14 N	15 O	16 P	17 Q	Number of alleles
mAAT	V	L	CAD*7	D	-100	-132	-118	-83	-104	-90												6
(sAAT-1)	V; nl	M, L	**CAD*7**; CAME 6.8; CAT 7.2	D	100	50																2
(sAAT-2)	R; nl	M	**CAD*7**; CAME 6.8	D	100	130																2
(sAAT-3)	P; nl	L, E	**CAT 7.2**; CAD*7	D	100	137	130	126	70	106												6
ACP	P	L	**CAM 6.0**; CAD	D	100	300	80	40	-20	-90	-250	-350	-200									9
(ADA)	P; us	M	CAD*7; Tris-Gly; EBT	M																		
ADAl	P	L	CAD*7	D	-100	-116	-87	-69	-58	-50	-79	-32	-20	30	0							11
ADH	P	L	LiOH-RW	D	-100	-280	40	-180	-140													5
sAH	P	L	CAT 7.2	M	100	108	96	90	80	104	76	120										8
(AK)	M	M	CAD*7	M	100	263																2
CK-A[C]	P	M	Poulik	D[C]	100	87	64	120														4
CK-B	R	E	EBT	D	100	96	93	103														4
(CK-C)	R	E	EBT	D	100	142	53															3
(ESTD)	R	E	CAT 7.2	D	100	83	120															3
(EST-3)	V	E	**EBT**; Poulik	M	100	94	103	107	91													5
EST-4	P	E	**EBT**; Poulik	M	100	105	103	95	88	110	107											7
(FDHG)	V, nl	L, M	Tris-Gly	D	100	143	190	57														4
(FH)	R	M	TC-4	T	100	125	143	49														4
(GAPDH)	M	E	CAME6.8; EBT	T	100																	1
(GDA)	P, us	M	CAD*7	D																		
GPI-A	P	M	Poulik	D	100	110	91	78	124	61	116											7
GPI-B[D]	R	M	Poulik; **Tris-Gly**	D	100	137	35	124	115	81	72											7
(G3PDH-1)	R	L	**CAT 7.2; TC-1**	D	100	168	140	131	90	78	-70											7
G3PDH-2	V	M	**CAD*7**; TC-1	D	-100	176	-120	82	70	-94	-85	-87										8
(IDDH)	R	L	**LiOH-RW**; Tris-Gly	T	100	156																2
mIDHP	R	M	TC-1	D	100	130	73	85														4
sIDHP	P	L	**TC-1;** CAD*7; CAT 7.2	D	100	120	82	73	116	62	118											7
LDH-A	R	M, E	Poulik	T	100	225	125	360	400	50												6
LDH-B	V	M, E	Poulik; TC-1; **CAT 7.2**	T	100	146	156	136	116	14												6
LDH-C	V	E	**EBT**; Poulik	T	100	105	103	93	97													5
sMDH-A	V	L	CAD*7	D	100	60	125	134	92													5
sMDH-B	R	M, E	**CAD*7**; TC-1	D	100	115		80	57	92	(105)											6
mMDH	V	M	**CAD*7**; TC-1; TC-4	D	100	260	115	60	5	230	200	50	163									9
MEP	V	M	TC-4; **TC-1**	T	100	104	108	92	84	76	112	98	81									9
MPI	V	M	Tris-Gly; **TC-1**	M	100	120	112	89	82													5
PEPB	V	M	CAD*7	M	100	111	92	82	86	116	96											7
PEPD-1	V	M	CAD*7	D	100	118	76	91	112	106	86	110	49									9
PEPD-2	V	M	CAD*7	D	100	118	111	91	83	70	86	52										8
PGDH	P	L	**CAD*7**; TC-1	D	100	108	95	85	79	65	50	110	116	98								10
PGM[E]	V	M	CAD*7	M	-100	-118	-84	-84	-100	-112	-82	-133	-121	-135	-112	-88	-147	-97	-88	-112	-133	
PGM[E]	V	M	Poulik	M	100	73	123	100	124	81	100	48	100	60	78	100	48	120	123	73	75	
PGM[E]	V	M	**composite**[E]	M	-100	-118	-84	-86	-102	-112	-70	-133	-121	-137	-110	-88	-147	-97	-90	-108	-135	17
sSOD	V	L, M	**LiOH-RW**; Poulik	D	100	112	93	16	148	160	7	24	10	98								10
mSOD	V	L, M	**LiOH-RW**; Poulik	T	100	180	38	5														4
(TPI-1)	V, nl	E, L, M	EBT; **Tris-Gly**	D	100	104	92															3
(TPI-2)	R, nl	M, L, E	EBT; **Tris-Gly**	D	-100	-230																2

[A] = alleles preceeded with a dash (-) encoded allozymes with cathodal mobilities; underlined alleles observed only in other species (not*S. commerson*)

[B] = loci in parentheses were not used for any analyses of *S. commerson* (although G3PDH-2 was useful for identifying other species)

[C] = although creatine kinase is known to be a dimer, monomeric patterns of variation were observed for CK-A (see text)

[D] = the relative mobilities reported for GPI-B were based on the relative mobilities of interlocus heterodimers between GPI-A and GPI-B

[E] = final PGM genotypes were derived from mobilities on both Poulik and CAD*7 gels with genotype codes identical or similar to the designations on CAD*7

Accessory Publication:

Details of allozyme variation in narrow-barred Spanish mackerel (*Scomberomorus commerson*) in the Australia – Torres Strait region

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Abstract – from Shaklee (2004). Patterns of allozyme variation were used to evaluate genetic aspects of stock structure in *Scomberomorus commerson* in the Australia - Papua New Guinea region. Over 2,300 fish from eighteen collections were characterized using 28 variable loci (22 of which were polymorphic at the $\mathbf{P}_{0.99}$ level). Allele frequencies at the three locations sampled more than once were stable over a two- to three-year period. Despite relatively high levels of genetic variation in this species, only modest population subdivision was revealed (e.g., average pairwise $F_{ST} = 0.002$). Explicit pairwise tests of population differentiation and MDS analyses indicated the existence of three genetic groups in the region: one in northeastern Papua New Guinea (Tufi), a second along the east coast of Queensland (Moreton Bay – Cairns), and the third ranging from the Port Moresby region of southern Papua New Guinea, through the Torres Strait, and across northern Australia (from the Gulf of Carpentaria in Queensland to Cape Cuvier in Western Australia). The results suggest that *S. commerson* harvested in the Torres Strait are part of a stock shared by Australia, Irian Jaya, and Papua New Guinea. Management and conservation of the *S. commerson* fisheries on this stock, and allocation of catch, should reflect its apparent shared nature.

This Accessory Publication provides details of the locus screening, allele scoring and allele pooling in Shaklee (2004), and documents allele frequencies at 18 loci for the 18 individual collections and three pooled localities reported in that study. It also provides data for observed and expected heterozygosities at the 12 most variable loci in the 18 collections. This publication also includes a table of allele frequencies at 10 loci for five areas in Australia and the Torres Strait that were characterized in 1981-1985 by Shaklee (2004) and were independently characterized in 1998 and 1999 by Ovenden and Street (unpublished data). Shaklee and Ovenden made a substantial effort to standardize allele scoring for these loci in their respective data sets. The data presented in this table demonstrate substantial allele frequency stability for these loci and locations over this 15-18 year period.

Tables

Table 1. Allele frequencies in *Scomberomorus commerson* at ten loci in five areas of Australia sampled from 1981 to 1999. Data in columns headed "Shaklee" are from Shaklee, J.B. 2004. Patterns of allozyme variation in narrow-barred Spanish mackerel (*Scomberomorus commerson*) reveal population subdivision in the Australia – Torres Strait – New Guinea region. Marine Freshwater Research [submitted], data in columns headed "Ovenden & Street" are unpublished data of J.R. Ovenden and R. Street (Southern Fisheries Research Centre, Queensland Department of Primary Industries, Deception Bay, Queensland, 4508 Australia).

Figure Legends

Figure 1. Isozyme banding patterns and genotypes for four enzyme systems screened by Shaklee (2004) in narrow-barred Spanish mackerel, *Scomberomorus commerson*. Gels with anode toward top, cathode toward bottom, sample origin indicated by arrow. *** = other species CK-A: muscle, Poulik buffer, all samples from collection 12; ACP: liver, CAM 6.0 buffer, selected phenotypes; ADAl: liver, CAD*7 buffer, samples #1-16 from collection 6 and samples #17-28 from collection 17; PGM: muscle, CAD*7 buffer, selected heterozygotes (genotypes highlighted with * are distinctive on Poulik buffer).

Figure 2. Isozyme banding patterns and genotypes for three enzyme systems screened by Shaklee (2004) in narrow-barred Spanish mackerel, *Scomberomorus commerson*. Gels with anode toward top, cathode toward bottom, sample origin indicated by arrow; *** = other species. EST-4: eye, EBT, samples #1-14 from collection 5 and samples #15-28 from collection 1; ADH: liver, LiOH buffer, samples #1-17 from collection 12 and samples #18-28 from collection 2; mSOD & sSOD: liver, LiOH buffer, selected phenotypes.

List of Appendixes

Appendix 1. Loci, tissues, buffers, and allele mobilities for the Australian Spanish mackerel study of Shaklee (2004).

Appendix 2. Pooling of rare alleles in the study of Shaklee (2004).

Appendix 3. Allele frequencies at 18 loci polymorphic at the $P_{0.95}$ level for the 18 individual collections and three localities of narrow-barred Spanish mackerel in Shaklee (2004).

Appendix 4. Observed and expected heterozygosities (from MSA) and P-values of Hardy-Weinberg tests (from GENEPOP) at the twelve most variable loci in Shaklee (2004).

Table 1. Allele frequencies in *Scomberomorus commerson* at ten loci in five areas of Australia sampled from 1981 to 1999.

Data in columns headed "Shaklee" are from Shaklee, J.B. 2004. Patterns of allozyme variation in narrow-barred Spanish mackerel (Scomberomorus commerson) reveal population subdivision in the Australia – Torres Strait – New Guinea region. Marine Freshwater Research [submitted].

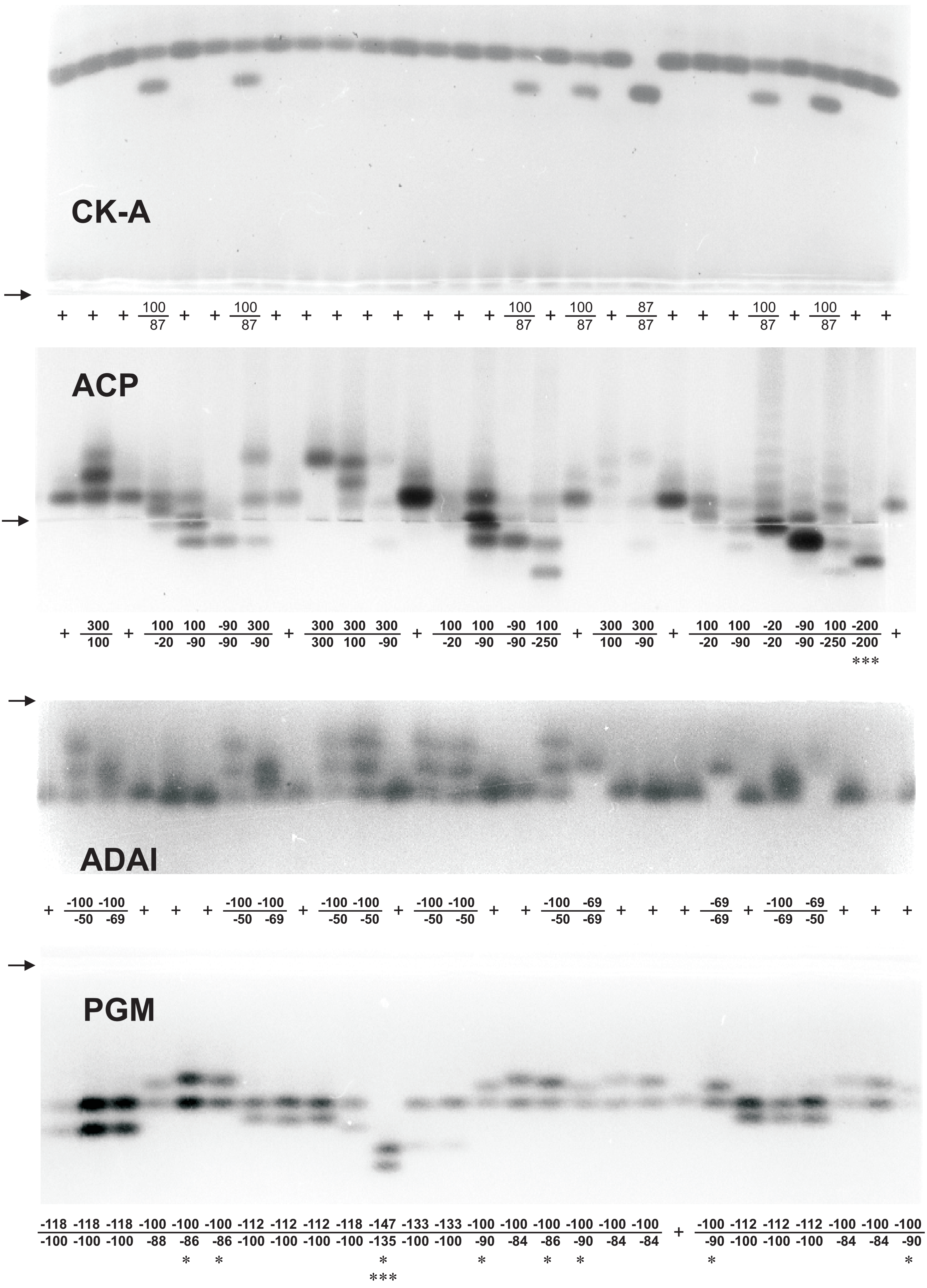
Data in columns headed "Ovenden & Street" are unpublished data of J.R. Ovenden and R. Street (Southern Fisheries Research Centre, Queensland Department of Primary Industries, Deception Bay, Queensland, 4508 Australia).

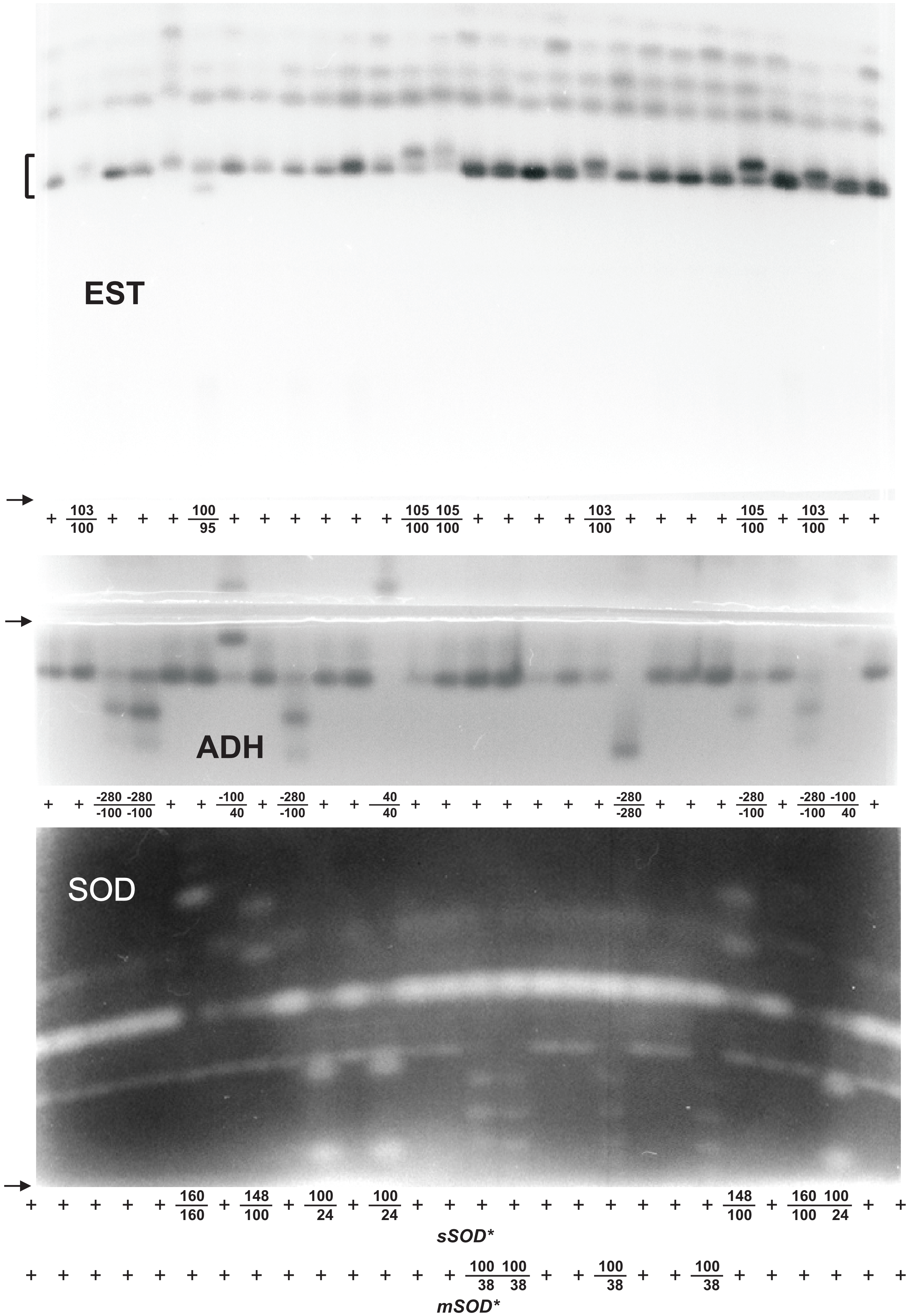
			Shaklee		Ovenden & Street		Shaklee		Ovenden & Street		Shaklee	Ovenden & Street		Shaklee	Ovenden & Street	Shaklee		Ovenden & Street	
Locus	alleles		Moreton Bay	Cairns	Townsville		Torres Strait (Bramble Cay)				Cape Croker	Bathurst and Melville Isl.		Holothuria Banks	West of Holothuria reefs	Monte Bello Isl.	Cape Cuvier	Shark Bay, Exmouth & Dampier	
			1983, 1984, &	1981+ 1982	1998	1999	1983	1984	1998	1999	1984	1998	1999	1983	1998	1982	1985	1998	1999
	Shaklee	Ovenden & Street	1985																
mAAT	-100+	-100	1.000	0.998	1.000	1.000	0.990	0.982	0.984	0.992	0.987	0.990	0.996	0.995	1.000	0.983	0.988	0.980	1.000
	-132	-132	-	0.002	-	-	0.010	0.009	0.012	-	0.013	-	0.004	0.005	-	0.017	0.012	0.020	-
	-83+	-83	-	-	-	-	-	0.010	-	-	-	-	-	-	-	-	-	-	-
		-33	-	-	-	-	-	-	-	0.008	-	0.010	-	-	-	-	-	-	-
		40	-	-	-	-	-	-	0.004	-	-	-	-	-	-	-	-	-	-
	(n)		(354)	(290)	(111)	(81)	(152)	(110)	(123)	(121)	(118)	(104)	(131)	(102)	(86)	(118)	(171)	(51)	(99)
ACP	100+	100	0.612	0.600	0.605	0.530	0.628	0.591	0.645	0.619	0.665	0.645	0.616	0.649	0.631	0.581	0.602	0.600	0.574
	300	200	0.055	0.062	0.064	0.071	0.049	0.055	0.066	0.047	0.034	0.040	0.065	0.059	0.074	0.072	0.059	0.040	0.064
	-20+		0.004	0.003	-	-	0.007	-	-	-	0.013	-	-	0.005	-	-	0.012	-	-
	-90	20	0.327	0.331	0.332	0.399	0.313	0.355	0.289	0.335	0.288	0.315	0.319	0.287	0.295	0.343	0.326	0.360	0.362
	-250+		0.001	0.003	-	-	0.003	-	-	-	-	-	-	-	-	0.004	-	-	-
	(n)		(352)	(290)	(110)	(84)	(152)	(110)	(121)	(118)	(118)	(100)	(138)	(101)	(88)	(118)	(170)	(50)	(94)
ADH	-100	-100	0.883	0.894	0.927	0.942	0.911	0.886	0.904	0.955	0.843	0.859	0.918	0.853	0.892	0.886	0.830	0.890	0.894
	-280+	-280	0.096	0.088	0.064	0.052	0.062	0.082	0.076	0.045	0.127	0.112	0.075	0.108	0.091	0.081	0.143	0.090	0.086
	40	40	0.021	0.018	0.009	0.006	0.027	0.032	0.020	-	0.030	0.029	0.007	0.039	0.017	0.034	0.026	0.020	0.020
	(n)		(349)	(278)	(110)	(86)	(146)	(110)	(125)	(122)	(118)	(103)	(140)	(102)	(88)	(118)	(171)	(50)	(99)
CK-A	100	100	0.887	0.878	0.851	0.865	0.873	0.895	0.917	0.856	0.945	0.879	0.913	0.955	0.903	0.890	0.889	0.907	0.895
	87	87	0.113	0.122	0.140	0.135	0.127	0.105	0.083	0.144	0.055	0.121	0.087	0.045	0.097	0.110	0.111	0.093	0.105
		91	-	-	0.009	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	(n)		(354)	(290)	(111)	(85)	(150)	(110)	(126)	(101)	(118)	(107)	(138)	(101)	(93)	(118)	(171)	(54)	(100)
EST-4	100	100	0.916	0.909	0.846	0.910	0.872	0.893	0.898	0.891	0.915	0.953	0.871	0.916	0.904	0.911	0.915	0.902	0.897
	105+	105	0.072	0.072	0.145	0.072	0.095	0.084	0.094	0.097	0.055	0.042	0.058	0.054	0.056	0.064	0.061	0.078	0.088
	103	103	0.010	0.012	-	-	0.020	0.019	-	-	0.021	-	0.036	0.025	0.006	0.021	0.018	0.010	-
	95	95	0.001	-	0.005	0.018	0.010	0.005	0.008	0.008	0.008	0.005	0.018	0.005	0.034	0.004	0.006	0.010	0.005
	88	88	-	0.007	0.005	-	0.003	-	-	0.004	-	-	0.018	-	-	-	-	-	0.005
		91	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.005
	(n)		(353)	(286)	(107)	(83)	(152)	(107)	(127)	(119)	(118)	(106)	(139)	(101)	(89)	(118)	(171)	(51)	(97)
GPI-A	100	100	0.866	0.852	0.851	0.801	0.841	0.855	0.821	0.847	0.814	0.832	0.841	0.837	0.801	0.835	0.822	0.824	0.810
	110	110	0.011	0.010	0.018	0.042	0.013	0.014	0.012	0.010	0.030	0.009	0.011	0.010	0.032	0.004	0.009	0.029	0.015
	91	91	0.120	0.130	0.131	0.151	0.146	0.123	0.155	0.134	0.148	0.154	0.148	0.144	0.145	0.157	0.167	0.147	0.155
	78	78	0.003	0.007	-	0.006	-	0.009	0.012	0.010	0.008	0.005	-	0.010	0.022	0.004	0.003	-	0.020
	(n)		(354)	(288)	(111)	(83)	(151)	(110)	(126)	(101)	(118)	(107)	(135)	(101)	(93)	(118)	(171)	(51)	(100)
sIDHP	100	100	0.939	0.939	0.927	0.929	0.961	0.940	0.956	0.912	0.942	0.947	0.954	0.944	0.983	0.941	0.947	0.931	0.970
	120	131	0.007	0.007	-	0.006	-	0.005	-	-	0.009	-	-	-	-	0.008	0.006	-	-
	116	120[1]	0.040	0.043	0.050	0.060	0.023	0.046	0.024	0.063	0.035	0.034	0.043	0.046	0.011	0.021	0.023	0.029	0.020
	82		0.001	-	-	-	0.007	-	0.012	-	-	0.005	-	-	-	-	-	0.010	-
	73	89[1]	0.013	0.011	0.023	0.006	0.010	0.009	0.004	-	0.013	0.005	0.004	0.010	0.006	0.030	0.023	0.020	0.010
		85[1]	-	-	-	-	-	-	0.004	0.025	-	0.010	-	-	-	-	-	0.010	-
	(n)		(354)	(278)	(109)	(84)	(152)	(108)	(126)	(119)	(113)	(104)	(140)	(98)	(89)	(118)	(171)	(51)	(99)
LDH-B	100	100	0.987	0.995	0.995	1.000	0.993	0.991	0.992	1.000	0.987	1.000	0.996	1.000	0.989	0.996	0.997	1.000	0.990
	146+	149	0.012	0.005	0.005	-	0.007	0.009	0.008	-	0.013	-	0.004	-	0.011	0.004	0.003	-	0.010
	(n)		(353)	(289)	(111)	(86)	(152)	(110)	(127)	(100)	(117)	(108)	(138)	(102)	(93)	(118)	(170)	(54)	(100)
PGDH	100	100	0.663	0.691	0.696	0.708	0.641	0.623	0.650	0.626	0.557	0.676	0.570	0.564	0.592	0.610	0.626	0.647	0.660
	108	108	0.024	0.028	0.007	0.013	0.023	0.036	0.028	0.033	0.030	0.028	0.015	0.029	0.033	0.021	0.029	0.039	0.046
	95		0.004	0.002	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	85	95[1]	0.188	0.176	0.138	0.253	0.181	0.132	0.213	0.171	0.226	0.134	0.267	0.245	0.245	0.199	0.221	0.216	0.165
	79	85[1]	0.111	0.090	0.145	0.019	0.115	0.182	0.079	0.150	0.135	0.139	0.141	0.123	0.120	0.140	0.091	0.078	0.113
	65+	79[1]	0.010	0.014	0.014	0.006	0.039	0.027	0.031	0.020	0.052	0.023	0.007	0.039	0.011	0.030	0.032	0.020	0.015
	(n)		(352)	(290)	(69)	(77)	(152)	(110)	(127)	(123)	(115)	(108)	(135)	(102)	(92)	(118)	(170)	(51)	(97)
PGM[2]	100	100	0.985[3]	0.983	0.971	0.976	0.993[3]	0.972	0.988	0.970	0.992	0.990	0.989	0.985[3]	0.984	0.962	0.980	0.981	0.980
F	123+124+120+123	119	0.001	0.010	0.005	-	-	0.014	0.008	0.005	-	-	-	0.005	0.005	0.008	0.003	-	0.005
S	73+81+48+60+78	76+85+87+47	0.014	0.007	0.024	0.024	0.007	0.014	0.004	0.025	0.008	0.010	0.011	0.010	0.011	0.030	0.018	0.019	0.015
	(n)		(352)	(290)	(111)	(85)	(152)	(110)	(126)	(101)	(118)	(109)	(137)	(101)	(93)	(118)	(170)	(54)	(100)

[1] = frequencies of Ovenden and Street alleles were shifted to minimize differences with frequencies observed by Shaklee

[2] = PGM alleles were based only on Poulik buffer results (Ovenden and Street did not screen this locus on CAD*7 buffer); individual presumed alleles were pooled into two classes - fast (F) and slow (S) to ensure consistency of scoring between studies

[3] = differences in allele frequencies between this table and Appendix 3 were due to the failure to detect certain PGM alleles (*86 and *88) on Poulik buffer [see Appendix 1]


CK-A
+ + + 100/87 + + 100/87 + + + + + + + 100/87 + 100/87 + 87/87 + + + 100/87 + 100/87 + +
ACP
+ 300/100 + 100/-20 100/-90 -90/-90 300/-90 + 300/300 300/100 300/-90 + 100/-20 100/-90 -90/-90 100/-250 + 300/100 300/-90 + 100/-20 100/-90 -20/-20 -90/-90 100/-250 -200/-200 *** +
ADAI
+ -100/-50 -100/-69 + + + -100/-50 -100/-69 + -100/-50 -100/-50 + -100/-50 -100/-50 + + -100/-50 -69/-69 + + + -69/-69 + -100/-69 -69/-50 + + +
PGM
-118/-100 -118/-100 -118/-100 -100/-88 -100/-86 * -100/-86 * -112/-100 -112/-100 -112/-100 -118/-100 -147/-135 * *** -133/-100 -133/-100 -100/-90 * -100/-84 -100/-86 * -100/-90 * -100/-84 -100/-84 + -100/-90 * -112/-100 -112/-100 -112/-100 -100/-84 -100/-84 -100/-90 *


EST
+ 103/100 + + + 100/95 + + + + + + 105/100 105/100 + + + + 103/100 + + + + 105/100 + 103/100 + +
ADH
+ + -280/-100 -280/-100 + + -100/40 + -280/-100 + + 40/40 + + + + + + + -280/-280 + + + -280/-100 + -280/-100 -100/40 +
SOD
+ + + + + 160/160 + 148/100 + 100/24 + 100/24 + + + + + + + + + + 148/100 + 160/100 100/24 + +
sSOD*
+ + + + + + + + + + + + + + 100/38 100/38 + + 100/38 + + 100/38 + + + + + +
mSOD*

Appendix 1. Loci, tissues, buffers, and allele mobilities for the Australian Spanish mackerel study of Shaklee (2004).

Genetic status: P = polymorphic at the $P_{0.95}$ level, V = polymorphic at the $P_{0.99}$ level, R = rare variation (not variable at $P_{0.99}$ level), M = monomorphic in this study, nl = new locus (only screened in a few fish), us = unreliable scoring (no data available); Tissues: M = muscle, E = eye, and L = liver; Buffers described in Shaklee and Keenan 1986 (CAM[E] = CAAPM, CAD = CAEA, CAT = TRIC), bold type indicates buffer used to determine relative mobilities; Subunit structure: M = monomer, D = dimer, T = tetramer

Locus[B]	Genetic status	Tissue(s)	Buffer(s)	Presumed subunit structure	Numeric & Alphabetic Allele Codes[A] 1 A	2 B	3 C	4 D	5 E	6 F	7 G	8 H	9 I	10 J	11 K	12 L	13 M	14 N	15 O	16 P	17 Q	Number of alleles
mAAT	V	L	CAD*7	D	-100	-132	-118	-83	-104	-90												6
(sAAT-1)	V; nl	M, L	**CAD*7**; CAME 6.8; CAT 7.2	D	100	50																2
(sAAT-2)	R; nl	M	**CAD*7**; CAME 6.8	D	100	130																2
(sAAT-3)	P; nl	L, E	**CAT 7.2**; CAD*7	D	100	137	130	126	70	106												6
ACP	P	L	**CAM 6.0**; CAD	D	100	300	80	40	-20	-90	-250	-350	-200									9
(ADA)	P; us	M	CAD*7; Tris-Gly; EBT	M																		
ADAl	P	L	CAD*7	D	-100	-116	-87	-69	-58	-50	-79	-32	-20	30	0							11
ADH	P	L	LiOH-RW	D	-100	-280	40	-180	-140													5
sAH	P	L	CAT 7.2	M	100	108	96	90	80	104	76	120										8
(AK)	M	M	CAD*7	M	100	263																2
CK-A[C]	P	M	Poulik	D[C]	100	87	64	120														4
CK-B	R	E	EBT	D	100	96	93	103														4
(CK-C)	R	E	EBT	D	100	142	53															3
(ESTD)	R	E	CAT 7.2	D	100	83	120															3
(EST-3)	V	E	**EBT**; Poulik	M	100	94	103	107	91													5
EST-4	P	E	**EBT**; Poulik	M	100	105	103	95	88	110	107											7
(FDHG)	V, nl	L, M	Tris-Gly	D	100	143	190	57														4
(FH)	R	M	TC-4	T	100	125	143	49														4
(GAPDH)	M	E	CAME6.8; EBT	T	100																	1
(GDA)	P, us	M	CAD*7	D																		
GPI-A	P	M	Poulik	D	100	110	91	78	124	61	116											7
GPI-B[D]	R	M	Poulik; **Tris-Gly**	D	100	137	35	124	115	81	72											7
(G3PDH-1)	R	L	**CAT 7.2; TC-1**	D	100	168	140	131	90	78	-70											7
G3PDH-2	V	M	**CAD*7**; TC-1	D	-100	176	-120	82	70	-94	-85	-87										8
(IDDH)	R	L	**LiOH-RW**; Tris-Gly	T	100	156																2
mIDHP	R	M	TC-1	D	100	130	73	85														4
sIDHP	P	L	**TC-1;** CAD*7; CAT 7.2	D	100	120	82	73	116	62	118											7
LDH-A	R	M, E	Poulik	T	100	225	125	360	400	50												6
LDH-B	V	M, E	Poulik; TC-1; **CAT 7.2**	T	100	146	156	136	116	14												6
LDH-C	V	E	**EBT**; Poulik	T	100	105	103	93	97													5
sMDH-A	V	L	CAD*7	D	100	60	125	134	92													5
sMDH-B	R	M, E	**CAD*7**; TC-1	D	100	115		80	57	92	(105)											6
mMDH	V	M	**CAD*7**; TC-1; TC-4	D	100	260	115	60	5	230	200	50	163									9
MEP	V	M	TC-4; **TC-1**	T	100	104	108	92	84	76	112	98	81									9
MPI	V	M	Tris-Gly; **TC-1**	M	100	120	112	89	82													5
PEPB	V	M	CAD*7	M	100	111	92	82	86	116	96											7
PEPD-1	V	M	CAD*7	D	100	118	76	91	112	106	86	110	49									9
PEPD-2	V	M	CAD*7	D	100	118	111	91	83	70	86	52										8
PGDH	P	L	**CAD*7**; TC-1	D	100	108	95	85	79	65	50	110	116	98								10
PGM[E]	V	M	CAD*7	M	-100	-118	-84	-84	-100	-112	-82	-133	-121	-135	-112	-88	-147	-97	-88	-112	-133	
PGM[E]	V	M	Poulik	M	100	73	123	100	124	81	100	48	100	60	78	100	48	120	123	73	75	
PGM[E]	V	M	**composite**[E]	M	-100	-118	-84	-86	-102	-112	-70	-133	-121	-137	-110	-88	-147	-97	-90	-108	-135	17
sSOD	V	L, M	**LiOH-RW**; Poulik	D	100	112	93	16	148	160	7	24	10	98								10
mSOD	V	L, M	**LiOH-RW**; Poulik	T	100	180	38	5														4
(TPI-1)	V, nl	E, L, M	EBT; **Tris-Gly**	D	100	104	92															3
(TPI-2)	R, nl	M, L, E	EBT; **Tris-Gly**	D	-100	-230																2

[A] = alleles preceeded with a dash (-) encoded allozymes with cathodal mobilities; underlined alleles observed only in other species (not S. commerson)

[B] = loci in parentheses were not used for any analyses of S. commerson (although G3PDH-2 was useful for identifying other species)

[C] = although creatine kinase is known to be a dimer, monomeric patterns of variation were observed for CK-A (see text)

[D] = the relative mobilities reported for GPI-B were based on the relative mobilities of interlocus heterodimers between GPI-A and GPI-B

[E] = final PGM genotypes were derived from mobilities on both Poulik and CAD*7 gels with genotype codes identical or similar to the designations on CAD*7

Appendix 2. Pooling of rare alleles in the study of Shaklee (2004).

Locus	Allelic class	Unpooled allele	Total number of alleles pooled	Collection[s] (and number[s] if > 1)
mAAT	-100	-104	1	C12
	-83	-90	1	C16
ACP	100	80	?[A]	C14 (N = 7) + ?
	-20	40	?[A]	C14 (N = 4) + ?
	-250	-350	1	C13
ADAl	-69	-79	2	C11, C12
ADH	-280	-180	4	C6 (N = 2), C12, C18;
	-280	-140	1	C11
sAH	100	104	4	C2, C4, C11, C18
	80	76	2	C12 (N = 2)
EST-1	105	110	1	C16
LDH-B	146	157	1	C1
	146	116	1	C1
	146	14	1	C11
mMDH	115	230	3	C7, C10, C12
	60	50	1	C2
MEP	100	98	1	C9
	108	112	1	C9
	92	84	1	C14
PEPD-1	83	70	5	C7, C8, C11, C14, C16
PEPD-2	100	106	1	C12
	86	91	1	C11
	86	76	1	C14
PGDH	65	50	1	C11
PGM	-118	-137	1	C7
	-112	-102	3	C4 C5, C13
	-112	-133	2	C4 (N = 2)
	-112	-121	1	C15
	-112	-110	1	C13
	-86	-88	2	C1, C3
	-86	-90	4	C4, C12, C13, C16
	-84	-70	2	C18 (N = 2)
mSOD	38	5	2	C5, C9
sSOD	112	148	2	C11, C13
	112	160	1	C13
	16	24	2	C18 (N = 2)
	16	7	1	C13

[A] = allele only recognized at end of study (in Cape Cuvier collection - C14); probably not consistently resolved throughout study

Appendix 3. Allele frequencies at 18 loci polymorphic at the $P_{0.95}$ level for the 18 individual collections and three localities of narrow-barred Spanish mackerel in Shaklee (2004). C# = collection number (cf. Table 1); (n) = number of fish successfully scored for each locus; + = one or more additional, rare alleles pooled with this allelic class.

	Moreton Bay			Cairns		Mornington	Cape	Holothuria	Lord Mayor
Locus	1983	1984	1985	1981	1982	Island	Croker	Banks	Shoal
alleles	C1	C2	C3	C4	C5	C6	C7	C8	C9
mAAT									
-100 +	1.000	1.000	1.000	1.000	0.997	0.994	0.987	0.995	1.000
-132	-	-	-	-	0.003	0.006	0.013	0.005	-
-83 +	-	-	-	-	-	-	-	-	-
(n)	(127)	(124)	(103)	(111)	(179)	(161)	(118)	(102)	(156)
ACP									
100 +	0.579	0.625	0.637	0.590	0.606	0.623	0.665	0.649	0.577
300	0.067	0.048	0.049	0.063	0.061	0.062	0.034	0.059	0.080
-20 +	0.008	-	0.005	0.005	0.003	0.009	0.013	0.005	0.022
-90	0.345	0.323	0.309	0.342	0.324	0.306	0.288	0.287	0.321
-250 +	-	0.004	-	-	0.006	-	-	-	-
(n)	(126)	(124)	(102)	(111)	(179)	(162)	(118)	(101)	(156)
ADAl									
-100	0.661	0.706	0.699	0.676	0.665	0.685	0.712	0.667	0.692
-116	-	-	-	-	-	-	-	-	-
-69 +	0.240	0.226	0.228	0.239	0.223	0.210	0.199	0.225	0.231
-50	0.098	0.069	0.073	0.086	0.112	0.105	0.089	0.108	0.077
(n)	(127)	(124)	(103)	(111)	(179)	(162)	(118)	(102)	(156)
ADH									
-100	0.878	0.854	0.922	0.900	0.890	0.889	0.843	0.853	0.845
-280 +	0.106	0.114	0.063	0.091	0.086	0.093	0.127	0.108	0.110
40	0.016	0.033	0.015	0.009	0.024	0.019	0.030	0.039	0.045
(n)	(123)	(123)	(103)	(110)	(168)	(162)	(118)	(102)	(155)
sAH									
100 +	0.937	0.923	0.908	0.924	0.912	0.886	0.915	0.897	0.881
108	0.059	0.073	0.087	0.072	0.074	0.080	0.042	0.074	0.074
96	-	0.004	-	-	-	0.003	-	-	-
90	0.004	-	0.005	0.005	0.014	0.028	0.042	0.025	0.042
80 +	-	-	-	-	-	0.003	-	0.005	0.003
(n)	(127)	(124)	(103)	(111)	(176)	(162)	(118)	(102)	(155)
CK-A									
100	0.902	0.891	0.864	0.892	0.869	0.919	0.945	0.955	0.952
87	0.098	0.109	0.136	0.108	0.131	0.081	0.055	0.045	0.048
(n)	(127)	(124)	(103)	(111)	(179)	(160)	(118)	(101)	(156)

Appendix 3. (cont.)

	Moreton Bay			Cairns		Mornington	Cape	Holothuria	Lord Mayor
Locus	1983	1984	1985	1981	1982	Island	Croker	Banks	Shoal
alleles	C1	C2	C3	C4	C5	C6	C7	C8	C9
EST-4									
100	0.929	0.931	0.883	0.937	0.891	0.913	0.915	0.916	0.939
105 +	0.060	0.065	0.097	0.050	0.086	0.062	0.055	0.054	0.042
103	0.012	0.004	0.015	-	0.020	0.016	0.021	0.025	0.016
95	-	-	0.005	-	-	0.009	0.008	0.005	0.003
88	-	-	-	0.014	0.003	-	-	-	-
(n)	(126)	(124)	(103)	(111)	(175)	(161)	(118)	(101)	(155)
GPI-A									
100	0.850	0.899	0.845	0.838	0.862	0.873	0.814	0.837	0.840
110	0.016	0.008	0.010	0.009	0.011	0.015	0.030	0.010	0.019
91	0.134	0.089	0.141	0.144	0.121	0.102	0.148	0.144	0.135
78	-	0.004	0.005	0.009	0.006	0.009	0.008	0.010	0.006
(n)	(127)	(124)	(103)	(111)	(177)	(162)	(118)	(101)	(156)
sIDHP									
100	0.953	0.944	0.917	0.964	0.922	0.938	0.943	0.944	0.929
120	-	0.016	0.005	0.009	0.006	0.016	0.008	-	0.022
116	0.031	0.028	0.063	0.027	0.054	0.040	0.036	0.046	0.035
82	-	-	0.005	-	-	-	-	-	0.003
73	0.016	0.012	0.010	-	0.018	0.006	0.014	0.010	0.010
(n)	(127)	(124)	(103)	(111)	(167)	(161)	(113)	(98)	(155)
LDH-B									
100	0.984	0.984	0.995	0.991	0.997	1.000	0.987	1.000	1.000
146 +	0.016	0.016	0.005	0.009	0.003	-	0.013	-	-
(n)	(127)	(124)	(102)	(111)	(178)	(162)	(117)	(102)	(156)
mMDH									
100	0.984	0.996	0.995	0.995	0.986	0.991	0.975	0.985	0.984
260	0.004	-	-	0.005	-	0.003	-	0.010	0.013
115 +	-	-	-	-	-	-	0.008	-	-
60 +	-	0.004	-	-	0.008	-	0.017	-	-
5	0.012	-	0.005	-	0.006	0.006	-	0.005	0.003
(n)	(127)	(124)	(103)	(111)	(179)	(162)	(118)	(101)	(156)
MEP									
100 +	0.984	0.976	1.000	0.982	0.986	0.988	1.000	0.995	0.984
108 +	-	-	-	-	-	0.003	-	-	0.003
104	0.004	0.016	-	0.018	0.014	0.009	-	-	0.010
92 +	0.004	-	-	-	-	-	-	0.005	0.003
76	0.008	0.008	-	-	-	-	-	-	-
(n)	(126)	(124)	(103)	(111)	(179)	(162)	(118)	(102)	(156)

Appendix 3. (cont.)

	Moreton Bay			Cairns		Mornington	Cape	Holothuria	Lord Mayor
Locus	1983	1984	1985	1981	1982	Island	Croker	Banks	Shoal
alleles	C1	C2	C3	C4	C5	C6	C7	C8	C9
PEPD-1									
100 +	0.988	0.976	0.990	0.986	0.989	0.984	0.992	0.985	0.984
118	0.012	0.004	0.010	0.014	0.008	0.003	0.004	-	-
112	-	0.020	-	-	0.003	0.009	-	0.015	0.016
86 +	-	-	-	-	-	0.003	0.004	-	-
(n)	(127)	(124)	(103)	(110)	(179)	(160)	(118)	(100)	(155)
PEPD-2									
100	0.988	0.964	0.995	0.964	0.969	0.981	0.979	0.980	0.984
111	-	-	-	-	0.003	-	-	-	0.003
91	0.004	-	-	-	0.003	-	-	-	-
83 +	0.008	0.036	0.005	0.036	0.025	0.019	0.021	0.020	0.013
(n)	(127)	(124)	(103)	(111)	(179)	(162)	(118)	(101)	(156)
PGDH									
100	0.680	0.661	0.646	0.703	0.684	0.577	0.557	0.564	0.564
108	0.032	0.032	0.005	0.023	0.031	0.034	0.030	0.029	0.048
95	0.004	-	0.010	-	0.003	-	-	-	-
85	0.152	0.202	0.214	0.171	0.179	0.219	0.226	0.245	0.196
79	0.120	0.101	0.112	0.090	0.089	0.136	0.135	0.123	0.167
65 +	0.012	0.004	0.015	0.014	0.014	0.034	0.052	0.039	0.026
(n)	(125)	(124)	(103)	(111)	(179)	(162)	(115)	(102)	(156)
PGM									
-100	0.984	0.972	0.990	0.986	0.980	0.985	0.992	0.960	0.984
-118 +	0.008	0.028	-	0.005	0.003	0.009	0.008	0.010	0.003
-112 +	-	-	-	0.005	0.009	-	-	-	0.006
-86 +	0.004	-	0.010	0.005	-	0.003	-	0.025	0.003
-84 +	0.004	-	-	-	0.008	0.003	-	0.005	0.003
(n)	(127)	(124)	(101)	(111)	(179)	(162)	(118)	(101)	(156)
mSOD									
100	0.976	0.988	0.995	0.991	0.975	0.988	0.996	0.980	0.984
180	0.004	-	-	-	-	-	-	-	0.006
38 +	0.020	0.012	0.005	0.009	0.025	0.012	0.004	0.020	0.009
(n)	(126)	(124)	(103)	(111)	(179)	(162)	(118)	(102)	(156)
sSOD									
100	1.000	1.000	1.000	1.000	1.000	0.991	1.000	1.000	1.000
112 +	-	-	-	-	-	0.006	-	-	-
16 +	-	-	-	-	-	0.003	-	-	-
(n)	(127)	(124)	(103)	(111)	(179)	(162)	(118)	(102)	(156)

Appendix 3. (cont.)

Locus *alleles*	Broome C10	Bedout Island C11	Gloma Shoal C12	Monte Bello Islands C13	Cape Cuvier C14	Torres Strait 1983 C15	Torres Strait 1984 C16	Port Moresby C17	Tufi PNG C18
mAAT									
-100 +	0.980	0.990	0.987	0.983	0.988	0.990	0.982	0.987	0.968
-132	0.012	0.010	0.009	0.017	0.012	0.010	0.009	0.013	0.032
-83 +	0.008	-	0.003	-	-	-	0.010	-	-
(n)	(128)	(154)	(159)	(118)	(171)	(152)	(110)	(76)	(109)
ACP									
100 +	0.570	0.605	0.550	0.581	0.602	0.628	0.591	0.558	0.583
300	0.078	0.056	0.072	0.072	0.059	0.049	0.055	0.096	0.046
-20 +	0.012	0.013	0.022	-	0.012	0.007	-	0.006	0.018
-90	0.332	0.324	0.355	0.343	0.326	0.313	0.355	0.340	0.344
-250 +	0.008	0.003	-	0.004	-	0.003	-	-	0.009
(n)	(128)	(153)	(159)	(118)	(170)	(152)	(110)	(78)	(109)
ADAl									
-100	0.686	0.714	0.704	0.729	0.655	0.694	0.700	0.679	0.722
-116	0.008	0.003	-	-	-	-	0.009	0.006	0.005
-69 +	0.236	0.221	0.195	0.203	0.272	0.217	0.209	0.244	0.165
-50	0.070	0.062	0.101	0.068	0.073	0.089	0.082	0.071	0.108
(n)	(129)	(154)	(159)	(118)	(171)	(152)	(110)	(78)	(106)
ADH									
-100	0.849	0.870	0.866	0.886	0.830	0.911	0.886	0.880	0.808
-280 +	0.119	0.101	0.099	0.081	0.143	0.062	0.082	0.080	0.144
40	0.032	0.029	0.035	0.034	0.026	0.027	0.032	0.040	0.048
(n)	(126)	(154)	(157)	(118)	(171)	(146)	(110)	(50^{A})	(104)
sAH									
100 +	0.898	0.873	0.881	0.869	0.915	0.861	0.886	0.895	0.791
108	0.055	0.084	0.057	0.059	0.048	0.096	0.082	0.079	0.057
96	0.004	-	0.003	-	-	-	-	-	0.014
90	0.039	0.039	0.053	0.072	0.033	0.033	0.032	0.026	0.138
80 +	0.004	0.003	0.006	-	0.003	0.010	-	-	-
(n)	(127)	(154)	(159)	(118)	(165)	(151)	(110)	(57^{A})	(105)
CK-A									
100	0.938	0.906	0.899	0.890	0.889	0.873	0.895	0.928	0.986
87	0.062	0.094	0.101	0.110	0.111	0.127	0.105	0.072	0.014
(n)	(129)	(154)	(159)	(118)	(171)	(150)	(110)	(76)	(109)

Appendix 3. (cont.)

Locus *alleles*	Broome C10	Bedout Island C11	Gloma Shoal C12	Monte Bello Islands C13	Cape Cuvier C14	Torres Strait 1983 C15	Torres Strait 1984 C16	Port Moresby C17	Tufi PNG C18
EST-4									
100	0.922	0.897	0.890	0.911	0.915	0.872	0.893	0.864	0.882
105 +	0.055	0.064	0.060	0.064	0.061	0.095	0.084	0.110	0.071
103	0.023	0.028	0.044	0.021	0.018	0.020	0.019	0.026	0.033
95	-	0.007	0.006	0.004	0.006	0.010	0.005	-	0.014
88	-	0.004	-	-	-	0.003	-	-	-
(n)	(128)	(141)	(159)	(118)	(171)	(152)	(107)	(77)	(106)
GPI-A									
100	0.822	0.841	0.824	0.835	0.822	0.841	0.855	0.897	0.876
110	0.031	0.010	0.031	0.004	0.009	0.013	0.014	0.013	-
91	0.140	0.143	0.135	0.157	0.167	0.146	0.123	0.077	0.119
78	0.008	0.006	0.009	0.004	0.003	-	0.009	0.013	0.005
(n)	(129)	(154)	(159)	(118)	(171)	(151)	(110)	(78)	(109)
sIDHP									
100	0.926	0.940	0.949	0.941	0.947	0.961	0.940	0.929	0.966
120	-	0.010	0.013	0.008	0.006	-	0.005	0.010	0.005
116	0.050	0.040	0.025	0.021	0.023	0.023	0.046	0.031	0.024
82	-	-	-	-	-	0.007	-	-	-
73	0.023	0.010	0.013	0.030	0.023	0.010	0.009	0.031	0.005
(n)	(128)	(151)	(157)	(118)	(171)	(152)	(108)	(49^{A})	(103)
LDH-B									
100	0.996	0.987	1.000	0.996	0.997	0.993	0.991	0.986	0.968
146 +	0.004	0.013	-	0.004	0.003	0.007	0.009	0.014	0.032
(n)	(129)	(154)	(159)	(118)	(170)	(152)	(110)	(74)	(109)
mMDH									
100	0.981	0.990	0.984	0.996	0.980	0.993	0.986	1.000	1.000
260	0.008	0.003	0.006	0.004	0.015	-	-	-	-
115 +	-	0.003	0.003	-	0.003	-	0.009	-	-
60 +	0.008	0.003	0.006	-	0.003	-	-	-	-
5	0.004	-	-	-	-	0.007	0.005	-	-
(n)	(129)	(154)	(159)	(118)	(171)	(152)	(110)	(78)	(109)
MEP									
100 +	0.969	0.987	0.991	1.000	0.991	0.970	0.991	0.987	1.000
108 +	0.008	-	-	-	-	0.003	0.005	0.006	-
104	0.008	0.003	0.003	-	0.006	0.013	0.005	0.006	-
92 +	0.016	0.010	0.006	-	0.003	0.010	-	-	-
76	-	-	-	-	-	0.003	-	-	-
(n)	(128)	(154)	(159)	(118)	(171)	(152)	(110)	(78)	(109)

Appendix 3. (cont.)

Locus *alleles*	Broome C10	Bedout Island C11	Gloma Shoal C12	Monte Bello Islands C13	Cape Cuvier C14	Torres Strait 1983 C15	Torres Strait 1984 C16	Port Moresby C17	Tufi PNG C18
PEPD-1									
100 +	0.984	0.977	0.978	0.975	0.980	0.987	0.977	0.981	0.995
118	0.008	0.010	0.003	0.025	0.018	0.013	0.005	-	-
112	0.008	0.010	0.019	-	-	-	0.009	0.013	0.005
86 +	-	0.003	-	-	0.003	-	0.009	0.006	-
(n)	(129)	(154)	(159)	(118)	(171)	(150)	(110)	(79)	(107)
PEPD-2									
100	0.981	0.981	0.975	0.966	0.965	0.970	0.964	0.962	0.963
111	-	-	-	-	-	0.007	-	-	-
91	-	-	-	-	0.003	0.003	-	0.006	-
83 +	0.020	0.019	0.025	0.034	0.032	0.020	0.037	0.032	0.037
(n)	(129)	(154)	(159)	(118)	(171)	(150)	(110)	(79)	(107)
PGDH									
100	0.579	0.555	0.619	0.610	0.626	0.641	0.623	0.627	0.595
108	0.028	0.039	0.013	0.021	0.029	0.023	0.036	0.018	0.019
95	-	-	-	-	-	-	-	-	0.010
85	0.236	0.247	0.201	0.199	0.221	0.181	0.132	0.164	0.195
79	0.130	0.140	0.135	0.140	0.091	0.115	0.182	0.191	0.138
65 +	0.028	0.019	0.031	0.030	0.032	0.039	0.027	-	0.043
(n)	(127)	(154)	(159)	(118)	(170)	(152)	(110)	(55^{A})	(105)
PGM									
-100	0.988	0.968	0.981	0.962	0.979	0.990	0.973	0.994	0.959
-118 +	0.008	0.019	0.009	0.025	0.015	0.003	0.014	0.006	0.028
-112 +	-	-	-	0.008	-	0.003	-	-	-
-86 +	-	0.003	0.009	0.004	0.003	0.003	0.005	-	0.005
-84 +	0.004	0.010	-	-	0.003	-	0.009	-	0.009
(n)	(128)	(154)	(159)	(118)	(170)	(152)	(110)	(79)	(109)
mSOD									
100	0.977	0.994	0.981	1.000	0.991	0.990	1.000	0.994	0.991
180	0.004	-	-	-	-	-	-	-	-
38 +	0.019	0.006	0.019	-	0.009	0.010	-	0.006	0.009
(n)	(129)	(154)	(159)	(118)	(169)	(152)	(110)	(77)	(109)
sSOD									
100	0.988	1.000	1.000	0.979	0.994	0.997	1.000	0.994	0.986
112 +	0.012	-	-	0.016	0.006	0.003	-	-	0.005
16 +	-	-	-	0.004	-	-	-	0.006	0.009
(n)	(129)	(154)	(159)	(118)	(171)	(152)	(110)	(77)	(109)

Appendix 3. (cont.)

Locus *alleles*		Moreton Bay C1+2+3	Cairns 4+5	Torres Strait C15+16
mAAT				
-100	+	1.000	0.998	0.987
-132		-	0.002	0.010
-83	+	-	-	0.004
(n)		(354)	(290)	(262)
ACP				
100	+	0.612	0.600	0.613
300		0.055	0.062	0.052
-20	+	0.004	0.003	0.004
-90		0.327	0.331	0.330
-250	+	0.001	0.003	0.002
(n)		(352)	(290)	(262)
ADAl				
-100		0.688	0.669	0.697
-116		-	-	0.004
-69	+	0.232	0.229	0.214
-50		0.081	0.102	0.086
(n)		(354)	(290)	(262)
ADH				
-100		0.883	0.894	0.900
-280	+	0.096	0.088	0.070
40		0.021	0.018	0.029
(n)		(349)	(278)	(256)
sAH				
100	+	0.923	0.917	0.872
108		0.072	0.073	0.090
96		0.001	-	-
90		0.003	0.010	0.033
80	+	-	-	0.006
(n)		(354)	(287)	(261)
CK-A				
100		0.887	0.878	0.883
87		0.113	0.122	0.117
(n)		(354)	(290)	(260)

Appendix 3. (cont.)

Locus *alleles*	Moreton Bay C1+2+3	Cairns 4+5	Torres Strait C15+16
EST-4			
100	0.916	0.909	0.880
105 +	0.072	0.072	0.091
103	0.010	0.012	0.019
95	0.001	-	0.008
88	-	0.007	0.002
(n)	(353)	(286)	(259)
GPI-A			
100	0.866	0.852	0.847
110	0.011	0.010	0.013
91	0.120	0.130	0.136
78	0.003	0.007	0.004
(n)	(354)	(288)	(261)
sIDHP			
100	0.939	0.939	0.952
120	0.007	0.007	0.002
116	0.040	0.043	0.032
82	0.001	-	0.004
73	0.013	0.010	0.010
(n)	(354)	(278)	(260)
LDH-B			
100	0.987	0.995	0.992
146 +	0.012	0.005	0.008
(n)	(353)	(289)	(262)
mMDH			
100	0.992	0.990	0.990
260	0.001	0.002	-
115 +	-	-	0.004
60 +	0.001	0.005	-
5	0.006	0.003	0.006
(n)	(354)	(290)	(262)
MEP			
100 +	0.986	0.984	0.979
108 +	-	-	0.004
104	0.007	0.016	0.010
92 +	0.001	-	0.006
76	0.006	-	0.002
(n)	(353)	(290)	(262)

Appendix 3. (cont.)

Locus *alleles*		Moreton Bay C1+2+3	Cairns 4+5	Torres Strait C15+16
PEPD-1				
100	+	0.984	0.988	0.983
118		0.008	0.010	0.010
112		0.007	0.002	0.004
86	+	-	-	0.004
(n)		(354)	(289)	(260)
PEPD-2				
100		0.982	0.967	0.967
111		-	0.002	0.004
91		0.001	0.002	0.002
83	+	0.017	0.029	0.027
(n)		(354)	(290)	(260)
PGDH				
100		0.663	0.691	0.634
108		0.024	0.028	0.029
95		0.004	0.002	-
85		0.188	0.176	0.160
79		0.111	0.090	0.143
65	+	0.010	0.014	0.034
(n)		(352)	(290)	(262)
PGM				
-100		0.982	0.983	0.983
-118	+	0.013	0.003	0.008
-112	+	-	0.006	0.002
-86	+	0.004	0.002	0.004
-84	+	0.001	0.005	0.004
(n)		(352)	(290)	(262)
mSOD				
100		0.986	0.981	0.994
180		0.001	-	-
38	+	0.013	0.019	0.006
(n)		(353)	(290)	(262)
sSOD				
100		1.000	1.000	0.998
112	+	-	-	0.002
16	+	-	-	-
(n)		(354)	(290)	(262)

[A] = two separate collections from this locality; no data for fish in one entire collection due to poor sample quality

Appendix 4. Observed and expected heterozygosities (from MSA) and P-values of Hardy-Weinberg tests (from GENEPOP) at the twelve most variable loci in Shaklee (2004). Significant deviations highlighted in bold. (note that the adjusted alpha value for the 18 tests at each locus is 0.0028)

	ACP			ADAl			ADH			sAH			CK-A			EST-4		
Collection	Ho	He	P-value	Ho	He	P-value	Ho	He	P-value	Ho	He	P-value	Ho	He	P-value	Ho	He	P-value
1 Moreton Bay-1	0.548	0.543	0.852	0.535	0.497	0.449	0.211	0.218	0.770	0.126	0.119	1.000	0.197	0.178	0.607	0.111	0.135	0.121
2 Moreton Bay-2	0.565	0.505	0.150	0.460	0.448	0.171	0.187	0.258	0.003	0.129	0.150	0.248	0.202	0.195	1.000	0.137	0.129	1.000
3 Moreton Bay-3	0.559	0.499	0.380	0.495	0.456	0.248	0.155	0.146	1.000	0.184	0.169	1.000	0.214	0.236	0.390	0.175	0.211	0.192
4 Cairns-1	0.486	0.533	0.648	0.514	0.481	0.173	0.164	0.182	0.007	0.162	0.151	1.000	0.198	0.194	1.000	0.126	0.120	1.000
5 Cairns-2	0.503	0.525	0.556	0.514	0.497	0.946	0.208	0.201	0.835	0.176	0.163	0.504	0.240	0.229	0.743	0.194	0.198	0.718
6 Mornington Isl	0.549	0.516	0.215	0.475	0.477	0.459	0.185	0.202	0.325	0.198	0.209	0.162	0.138	0.150	0.068	0.161	0.163	0.724
7 Cape Croker	0.517	0.475	0.198	0.407	0.448	0.400	0.229	0.273	0.073	0.153	0.159	0.375	0.110	0.105	1.000	0.169	0.159	1.000
8 Holothuria Banks	0.574	0.496	0.257	0.480	0.496	0.219	0.196	0.262	0.016	0.176	0.190	0.334	0.089	0.086	1.000	0.168	0.158	0.520
9 Lord Mayor Shoal	0.641	0.559	0.244	0.429	0.463	0.542	0.258	0.273	0.182	0.200	0.218	0.094	0.058	0.092	0.000	0.097	0.117	0.001
10 north of Broome	0.594	0.560	0.389	0.426	0.470	0.285	0.254	0.266	0.542	0.197	0.190	0.753	0.109	0.117	0.391	0.141	0.147	0.553
11 Bedout Isl	0.503	0.528	0.603	0.442	0.440	0.102	0.208	0.233	0.064	0.221	0.235	0.514	0.175	0.171	1.000	0.206	0.191	1.000
12 Gloma Shoal	0.572	0.567	0.038	0.484	0.458	0.248	0.248	0.240	0.238	0.208	0.219	0.368	0.176	0.182	0.658	0.208	0.203	0.748
13 Monte Bello Isls	0.542	0.542	0.979	0.407	0.425	0.177	0.178	0.209	0.008	0.212	0.238	0.154	0.186	0.197	0.355	0.178	0.166	1.000
14 Cape Cuvier	0.524	0.558	0.003	0.450	0.493	0.261	0.257	0.290	0.130	0.145	0.160	0.178	0.199	0.198	1.000	0.158	0.159	0.526
15 Torres Strait-1	0.500	0.507	0.832	0.447	0.465	0.113	0.137	0.166	0.025	0.265	0.249	0.197	0.187	0.222	0.063	0.197	0.231	0.062
16 Torres Strait-2	0.564	0.525	0.575	0.518	0.462	0.010	0.209	0.208	0.134	0.191	0.208	0.314	0.191	0.188	1.000	0.196	0.198	0.740
17 Port Moresby	0.538	0.568	0.915	0.359	0.477	0.052	0.120	0.220	0.002	0.193	0.194	0.270	0.145	0.135	1.000	0.247	0.243	1.000
18 Tufi, PNG	0.523	0.542	0.974	0.453	0.442	0.480	0.317	0.327	0.193	0.352	0.362	0.130	0.028	0.027	1.000	0.198	0.217	0.043
		X^2 =	42.7		X^2 =	56.2		X^2 =	92.5		X^2 =	40.1		X^2 =	38.3		X^2 =	39.8
		df =	36		df =	36		df =	36		df =	36		df =	36		df =	36
		P =	0.205		P =	0.017		P =	0.000		P =	0.295		P =	0.366		P =	0.306

	GPI-A			sIDHP			PEPD-1			PEPD-2			PGDH			PGM		
	Ho	He	P-value	Ho	He	P-value	Ho	He	P-value	Ho	He	P-value	Ho	He	P-value	Ho	He	P-value
1 Moreton Bay-1	0.252	0.260	0.838	0.094	0.091	1.000	0.024	0.023	1.000	0.024	0.023	1.000	0.464	0.501	0.681	0.031	0.031	1.000
2 Moreton Bay-2	0.185	0.184	1.000	0.097	0.109	0.071	0.048	0.048	1.000	0.073	0.070	1.000	0.516	0.513	0.999	0.040	0.055	0.083
3 Moreton Bay-3	0.204	0.268	0.036	0.165	0.155	1.000	0.019	0.019	1.000	0.010	0.010	-	0.534	0.527	0.333	0.020	0.020	1.000
4 Cairns-1	0.315	0.278	0.143	0.072	0.070	1.000	0.027	0.027	1.000	0.072	0.070	1.000	0.414	0.470	0.309	0.027	0.027	1.000
5 Cairns-2	0.266	0.243	0.775	0.150	0.147	0.464	0.022	0.022	1.000	0.061	0.060	1.000	0.508	0.492	0.460	0.039	0.039	1.000
6 Mornington Isl	0.216	0.227	0.113	0.118	0.119	0.320	0.031	0.031	1.000	0.025	0.036	0.046	0.562	0.600	0.722	0.031	0.031	1.000
7 Cape Croker	0.314	0.317	0.393	0.106	0.111	0.210	0.017	0.017	1.000	0.042	0.042	1.000	0.704	0.620	0.300	0.017	0.017	1.000
8 Holothuria Banks	0.257	0.281	0.293	0.092	0.107	0.257	0.030	0.030	1.000	0.040	0.039	1.000	0.598	0.608	0.563	0.079	0.077	1.000
9 Lord Mayor Shoal	0.288	0.277	0.859	0.135	0.135	0.546	0.019	0.032	0.032	0.019	0.032	0.032	0.628	0.615	0.947	0.032	0.032	1.000
10 north of Broome	0.287	0.305	0.287	0.148	0.140	1.000	0.031	0.031	1.000	0.023	0.038	0.016	0.591	0.593	0.104	0.023	0.023	1.000
11 Bedout Isl	0.266	0.273	0.268	0.099	0.114	0.096	0.045	0.045	1.000	0.039	0.038	1.000	0.578	0.612	0.368	0.058	0.064	0.140
12 Gloma Shoal	0.283	0.303	0.130	0.096	0.099	0.180	0.044	0.049	0.005	0.050	0.049	1.000	0.560	0.558	0.581	0.038	0.037	1.000
13 Monte Bello Isls	0.314	0.280	0.505	0.119	0.114	1.000	0.051	0.050	1.000	0.068	0.066	1.000	0.602	0.570	0.748	0.076	0.074	1.000
14 Cape Cuvier	0.310	0.298	0.901	0.099	0.102	0.376	0.041	0.040	1.000	0.070	0.068	1.000	0.535	0.550	0.008	0.041	0.041	1.000
15 Torres Strait-1	0.305	0.272	0.029	0.079	0.077	1.000	0.027	0.026	1.000	0.047	0.059	0.007	0.553	0.542	0.033	0.020	0.020	1.000
16 Torres Strait-2	0.273	0.256	1.000	0.102	0.115	0.319	0.045	0.045	1.000	0.073	0.071	1.000	0.527	0.562	0.413	0.055	0.054	1.000
17 Port Moresby	0.205	0.190	1.000	0.143	0.137	1.000	0.038	0.038	1.000	0.076	0.074	1.000	0.636	0.548	0.611	0.013	0.013	-
18 Tufi, PNG	0.211	0.219	0.689	0.068	0.066	1.000	0.009	0.009	-	0.075	0.072	1.000	0.552	0.589	0.446	0.083	0.080	1.000
		X^2 =	39		X^2 =	28.5		X^2 =	17.4		X^2 =	31.2		X^2 =	40.3		X^2 =	8.900
		df =	36		df =	36		df =	34		df =	34		df =	36		df =	34
		P =	0.336		P =	0.809		P =	0.992		P =	0.605		P =	0.287		P =	1.000