FISHING & FISHERIES RESEARCH CENTRE TECHNICAL REPORT No. 10

# DEFINING THE STOCK STRUCTURE OF NORTHERN AUSTRALIA'S THREADFIN SALMON SPECIES

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FRDC Project No. 2007/032

Australian Government
Fisheries Research and Development Corporation

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F&F

James Cook University
AUSTRALIA

Northern Territory Government

Department of Fisheries
Government of Western Australia

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**Fisheries Research & Development Corporation Project No. 2007/032**

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# Acronyms used throughout this report

**ABARE**.....Australian Bureau of Agricultural and Resource Economics
**ABI**.....Applied Biosystems Incorporated
**AC**.....Arthur's Creek
**AMOVA**.....Analysis of Molecular Variance
**ANOVA**.....Analysis of Variance
**ANCOVA**.....Analysis of Co-variance
**AL**.....Albert River
**AR**.....Archer River
**BMB**.....Blue Mud Bay
**CB**.....Cleveland Bay
**CH**.....Chambers Bay
**cm**.....centimetre
**CV**.....Coefficient of variation
**DEEDI**.....Department of Employment, Economic Development and Innovation
**EC**.....East Coast
**EMB**.....Eighty Mile Beach
**FL**.....Fork Length
**FLR**.....Flinders River
**FR**.....Fitzroy River
**FRDC**.....Fisheries Research & Development Corporation
**GoC**.....Gulf of Carpentaria
**GulfMAC**.....Gulf of Carpentaria Management Advisory Committee
**IBD**.....Isolation-by-distance analysis
**JCU**.....James Cook University
**KB**.....Keppel Bay
**KR**.....Kendall River
**$L_F$**.....Fork length
**LR**.....Love River
**mm**.....millimetre
**MAC**.....Management Advisory Committee
**MANOVA**.....Multivariate Analysis of Variance
**mtDNA**.....mitochondrial DNA
**MTSRF**.....Marine and Tropical Sciences Research Facility
**NT**.....Northern Territory
**NTDoR**.....Northern Territory Department of Resources
**PA**.....Port Alma

**PERMANOVA** .....Permutational Analysis of Variance
**QLD**.....Queensland
**QDPI&F**.....Queensland Department of Primary Industries and Fisheries (now DEEDI)
**QFIRAC**.....Queensland Fishing Industry Research Advisory Committee
**QPIF** .....Queensland Primary Industries and Fisheries (Department of Employment, Economic Development and Innovation)
**QSIA** .....Queensland Seafood Industry Association
**RB** .....Roebuck Bay
**RR** .....Roper River
**t** .....Tonne
**TSV** .....Townsville
**UK** .....United Kingdom
**VB** .....von Vertalanffy
**VBGF** .....von Bertalanffy growth function
**WA** .....Western Australia
**WR** .....Walker River

# Non-Technical Summary

**2007/032 Defining the stock structure of northern Australia's threadfin salmon species**

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**OBJECTIVES:**

1. To determine the stock structure of king and blue threadfin salmon across northern Australia.
2. To use these findings to define the appropriate cross-jurisdictional management framework for sustainable use of king and blue threadfin resources in northern Australia.

**OUTCOMES ACHIEVED TO DATE:**

**The project has determined that the appropriate spatial scale for management of blue and king threadfin fisheries is likely to be by state/territory and at local scales within these jurisdictions.** The project identified that both king and blue threadfins show limited adult and larval movement between localised stocks separated by as little as several tens of kilometres. This means that the likelihood of straddling stocks of either species in northern Australia is unlikely; however there is evidence for the exchange of genetic material among adjacent stocks within the Gulf of Carpentaria. This information will greatly assist compliance with the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* for net fisheries in northern Australia by providing the necessary basis for robust assessment of the status of threadfin stocks, thereby helping to deliver sustainable their harvest.

**The project provides the spatial framework for monitoring and stock assessment of threadfins in northern Australia.** Fine spatial scale stock separation will dictate the scale at which future monitoring and stock assessments for threadfins need to be carried out. Also, although not central to the project, the estimation of life history parameters for many of the stocks identified provide critical input parameters that will improve the certainty in future stock assessments. These parameters include growth and size at sex change. This has delivered considerable value to the project and further enhances management outcomes.

**The project has delivered on threadfin research identified as a high priority.** This study had been identified as a high priority for some time by the Fisheries Research Advisory Boards (FRAB) to the FRDC in both Queensland and the Northern Territory. It was also deemed a high priority by commercial, recreational and fisheries management agencies in Western Australia, the Northern Territory and Queensland.

**The project provided further evidence for the utility of using a holistic approach in stock structure studies.** The approach taken in this study followed that of recent FRDC studies in using multiple techniques simultaneously to identify fish stocks, and confirmed the value in adopting such an approach in providing greater certainty in the scale of stock separation and in interpreting the possible mechanisms that influence this separation.

**The project provided significant human capital development opportunities.** The samples collected during the project provided material for two PhD student projects to be carried out on the life history characteristics of both blue threadfin (Aaron Ballagh, James Cook University) and king threadfin (Bradley Moore, James Cook University / University of Queensland). These significantly value-added to the stock structure study here (Chapters 5 and 9) and to the outcomes of the project. The analyses of parasites have also contributed to the PhD carried out by Brad Moore (Chapters 2 and 6).

**The project further enhanced links between research, industry and management.** Due to the inter-jurisdictional nature of this project fisheries managers from Western Australia, the Northern Territory and Queensland were formally consulted and included on the project team to facilitate the timely management uptake of research outcomes. This meant that contact with each of these managers was regular and they were kept reliably informed of project results. Sample collections were also largely reliant on the willing assistance of commercial fishers and required the establishment and maintenance of good relationships between the project team and industry.

**SUMMARY:**

The requirement for Queensland, Northern Territory and Western Australian jurisdictions to ensure sustainable harvest of fish resources relies on robust information on the resource status. In northern Australia management of inshore fisheries that target blue threadfin (*Eleutheronema tetradactylum*) and king threadfin (*Polydactylus macrochir*) is independent for each of these jurisdictions. However, the lack of information on the stock structure and biology of threadfins means that the appropriate spatial scale of management is not known and assessment of the resource status is not possible. Establishing the stock structure of blue and king threadfin would also immensely improve the relevance of future resource assessments for fishery management of threadfins across northern Australia. This highlighted the urgent need for stock structure information for this species.

The impetus for this project came from unsuccessful attempts in Queensland to conduct stock assessments for the king and blue threadfin resource, research that indicated the potential for localised stock structure, and the assessment that blue and king threadfin in Western Australia were fully and over-exploited respectively. The project objectives were to determine the stock structure of blue and king threadfin across their northern Australian range, and use this information to define management units and their appropriate spatial scales.

We used multiple techniques concurrently to determine the stock structure of each species, including: genetic analyses (mitochondrial DNA and microsatellite DNA), otolith (ear bones) stable isotope ratios, parasite abundances, and life history parameters (growth and size at sex change). This holistic approach to stock identification gave the advantage of using techniques that were informative about the fish's life history at different spatial and temporal scales, increasing the likelihood of detecting different stocks where they existed and providing greater certainty in the signals given by the data. Genetics can inform about the evolutionary patterns as well as rates of mixing of fish from adjacent areas, while parasites and otolith microchemistry are directly influenced by the environment and so will inform about the patterns of movement during the fishes lifetime. Life history characteristics are influenced by both genetic and environmental factors.

We adopted a phased sampling approach whereby sampling was carried out at broad spatial scales in the first year at locations along the east coast, within the Gulf of Carpentaria (GoC), and the Western Australian coastline. Using each of the techniques to compare fish samples collected from each of these locations we tested the null hypothesis for each species that they were comprised of a single homogeneous population across northern Australia. The null hypothesis was rejected after the first year leading us to re-sample the first year locations to test for temporal stability in stock structure, and to assess stock structure at finer spatial scales by sampling at other locations as well.

Blue threadfin showed strong site fidelity with localised stock structuring evident and adjacent stocks separated by only tens of kilometres. This was found even where continuous habitat was present along coastlines with no obvious barriers to mixing. This was shown by clear and consistent signals of differences between fish from different locations including genetic differences. Blue threadfins also show what is called 'isolation by distance' whereby the farther apart stocks are from one another the greater the genetic differences between them. There was also extreme variability found in the life history characteristics among the different stocks.

Similarly, king threadfin also showed fine scale stock structure with limited mixing between adjacent stocks separated by tens to hundreds of kilometres. Where there was sufficient distances separating them, or bio-geographical barriers such as headlands separating adjacent stocks, king threadfin were also genetically distinct. King threadfin also exhibited 'isolation by distance' though the pattern was not

as strong as in blue threadfin. King threadfins also show a high degree of variation in their life history characteristics among the different stocks identified. Further, in the eastern Gulf of Carpentaria evidence of overfishing of king threadfin was evident in the truncation of size and age structures compared with samples taken over a decade ago, and the presence of females much smaller than found elsewhere or reported from the same region previously.

The management implications of these results indicate the need for management of threadfin fisheries in Australia to be carried out on regional scales much finer than are currently in place. Given the fine spatial scale stock structure evident for both threadfin species management at local scales may not be pragmatic. At the very least management should consider these spatial dynamics by implementing monitoring and assessment of threadfin fisheries guided by the stocks identified in this study, and by the likely spatial scale of stocks indicated by these results. We also encourage the assessment of the threadfin resource status for the major fishery region in northern Australia. We recommend that the signals of overfishing detected for king threadfin in the Gulf of Carpentaria need to be investigated to assess the status of the stocks present in that region.

**KEYWORDS:**
Blue threadfin, King threadfin, *Eleutheronema tetradactylum*, *Polydactylus macrochir*, stock structure, spatial dynamics, otolith isotope ratios, population genetics, parasites, fisheries, management.

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# Section 1
# Introduction

# Chapter 1: Introduction

## 1.1 Overview

Prior to this study very little was known about the spatial dynamics or biology of king threadfin (*Polydactylus macrochir*) and blue threadfin (*Eleutheronema tetradactylum*) in Australia. This was despite the fact they are targeted as commercial and recreational species across northern Australia in inshore fisheries. Early work on blue threadfin biology was by Stanger (1974) and Garrett (1997), and more recently by Pember *et al.* (2005). Garrett (1997) found evidence of fine spatial scale stock structure for blue threadfin in the Gulf of Carpentaria using genetics and more recently Zischke *et al.* (2009) came to the same conclusions using parasites and tag-recapture data on the east coast. The earliest study on the biology of king threadfin was by Garrett (1997) where they also found evidence of fine scale genetic stock structure within the Gulf of Carpentaria. Further work on king threadfin biology was carried out recently in Western Australia (Pember *et al.* 2005). In 2002 stock assessments for both species were attempted in Queensland but fitting data to models was not possible and so estimation of stock status is unknown (Welch *et al.* 2002). The paucity of knowledge for informed management of and the range of issues contributing to concerns for threadfin fisheries in northern Australia were highlighted by Welch *et al.* (2005).

In this study we used a holistic approach using several complementary techniques for identifying stocks of blue and king threadfin simultaneously across all fishery jurisdictions in northern Australia (Begg and Waldman 1999). The techniques used were genetics (distribution of mitochondrial DNA and microsatellite DNA (blues only) genotypes), parasites (species and abundance), and otolith chemistry (stable isotope ratios). Variability in life history parameters was also used as a stock identification methodology for each species and provided significant value-adding by two PhD students who separately estimated life history parameters for each species and, in doing so, provided important parameter estimates for stocks identified for both species. The project therefore set out to determine the appropriate spatial scale for monitoring, assessment and management of threadfin fisheries across northern Australia and to provide information on life history characteristics for each stock identified, further informing management of these stocks. This was important given the multi-jurisdictional coverage of threadfin fisheries and also in the disparate management strategies currently used for these species across these jurisdictions.

In this report we present the results of the techniques separately for each species in individual chapters. In the current chapter we cover background material presented in the original project description and proposal (Section 1). We then deal with the results of blue threadfin analyses (Section 2) and then with king threadfin analyses (Section 3), followed by a final section that presents the overall findings by

integrating all results for each species (Section 4). Within each of the species sections (Sections 2 and 3) we first present results of analyses of parasite data as an independent method to assess stock structure at fine spatial and temporal scales (Chapters 2 and 6). This is followed by results of genetic data derived from mitochondrial DNA (Chapters 3 and 7) and microsatellite DNA loci (Chapter 3). Otolith stable isotope ratio results also provide stock structure information across fine spatial and temporal scales compared to genetic data and are presented in Chapters 4 and 8. Estimates of growth and size at sex change parameters were also used to infer stock structure for each species and are presented in Chapters 5 and 9. Finally we integrate results from all techniques and for both species in Chapter 10 and discuss management implications. The final chapter (Chapter 11) provides the final conclusions and recommendations arising from the project.

## 1.2 Background

King threadfin salmon (*Polydactylus macrochir*, previously *P. sheridani*; Motomura *et al.* 2001) and blue threadfin salmon (*Eleutheronema tetradactylum*) form the second most important target species group for northern Australia's inshore net fisheries after the prized barramundi and across northern Australia are worth in excess of $6 million annually. The northern Australia threadfin catch was approximately 1,300t in 2004 and in excess of 1,000t in 2005, and adds value to the significant barramundi inshore commercial fisheries worth in excess of $12 million per year (ABARE 2008; QDPI&F CFISH database; Newman *et al.* 2005; Northern Territory Government 2009; Pember *et al.* 2005). Threadfins also form an important component of coastal recreational fisheries in Queensland, the Northern Territory, and Western Australia with estimates of 185,000 fish taken in 2000/2001 and a further 118,000 released (Henry and Lyle, 2003). This equates to crude estimates of recreational catch by weight for threadfins of 200-450t. Despite their fishery importance, surprisingly little is known about the stock structure and basic biology of either threadfin species in northern Australian waters, due to the greater research focus on barramundi and other fishery species over the years. The recent FRDC project report (No. 2002/003) filled in some of the very important information gaps on the biology of each of the two threadfin species in Western Australia (Pember *et al.* 2005), and also identified the vulnerability of both species to over-harvest. Despite relatively modest levels of harvest compared to other regions of Australia, they concluded that WA blue threadfins are currently fully exploited while king threadfins were considered to be over-exploited.

In 2002 Queensland's Department of Primary Industries and Fisheries (QDPI&F) undertook an initial stock assessment of king and blue threadfin on the east coast and the Gulf of Carpentaria but due to data limitations were not able to estimate the status of either species (Welch *et al.* 2002). Fishery observer data and anecdotal information from fishers indicated high levels of uncertainty due to factors such as unvalidated catch and effort data, high levels of discarding, and effort creep in the fishery (Welch *et al.* 2005). Further, this assessment was predicated on the assumption of single stocks in

Queensland Gulf of Carpentaria and East Coast waters, an assumption that was unfounded. A recent collaborative study between James Cook University, the University of Queensland and Queensland's Primary Industries and Fisheries found evidence that there is little or no mixing between populations of blue threadfins separated by as few as approximately 100 km on the east coast (Zischke *et al.* 2009). Despite the evidence for separate stocks, management arrangements in Queensland, Western Australia and the Northern Territory are currently independent of each other and differ greatly in the respective jurisdictions. For example, king threadfin minimum legal sizes are 60 cm in Queensland, are not applied in the Northern Territory, and are 45 cm in Western Australia. Where stocks may be shared between jurisdictions, effective and cooperative management can only be founded on an understanding of stock structure. With the introduction of the Commonwealth's *Environment Protection and Biodiversity Conservation* (EPBC) *Act* there is now a legislated mandate for a whole of ecosystem approach to fisheries management in which the ecological sustainability of all harvested species must be assured. Consequently, establishing appropriate management units for blue and king threadfins was identified as a research priority by Queensland's Gulf Management Advisory Committee (GulfMAC) and East Coast Inshore Finfish MAC. Threadfins have also been given a high research priority by QDPI&F, the Queensland Seafood Industry Association (QSIA), Sunfish, Fisheries WA, the WA Fishing Industry Council (WAFIC), and NTDoR, and are a high priority species group for the Queensland Fishing Industry Research Advisory Committee (QFIRAC) and the Northern Territory's Fisheries Research Advisory Body (NT FRAB).

At the 1997 Australian Society for Fish Biology (ASFB) workshop "Taking stock: defining and managing shared resources", a clear message was that comprehensive studies of stock structure should include different complementary methods (Hancock 1998). In this study we propose to use three complementary methods to determine stock structure: genetics, parasite loading, and otolith microchemistry. This stock id 'toolkit' proved to be a powerful combination of techniques in two recent FRDC projects determining stock structure of grey mackerel (*Scomberomorus semifasciatus*) (FRDC Project No. 2005/010; Welch *et al.* 2009) and Spanish mackerel (*Scomberomorus commerson*) across northern Australia (FRDC Project No. 1998/159; Buckworth *et al.* 2007; Moore *et al.* 2003). Genetic analyses typically identify differences on large spatial and temporal scales, where gene flow is minimal. Parasite species composition and loadings and otolith microchemistry reflect residence and movement of fish in different ways at smaller temporal scales and may be used to resolve discrete units of adult fish from genetically homogenous populations. This project will test this stock identification 'toolkit' against threadfins, very different species types to the mackerels it has proven effective on. Further, ANSA tag-and-release efforts on king threadfins in Western Australia suggests that while smaller male fish tend to be resident fish, the larger female fish often move large distances. The use of the otolith microchemistry and parasite methods will enable this hypothesis to be tested.

## 1.3 Need

Threadfins form an important component of barramundi fisheries and are likely to play a significant ecological role in northern Australian inshore habitats. Despite their importance and the expansion of coastal fisheries, the status of the threadfin resource in most parts is unknown (Welch *et al.* 2002) whereas in Western Australia they are considered fully or over-exploited (Pember *et al.* 2005). This uncertainty arises from the limited understanding of threadfin biology, stock structure and a lack of available data on resource exploitation (Welch *et al.* 2005). The inshore net fisheries across northern Australia are currently managed separately and under vastly different management regimes. However, without knowledge of threadfin stock structure, the appropriate spatial scales of management are not known. In August 2003, the Northern Australian Fisheries Management Forum (NAFM) signalled its intention to move from single jurisdiction-based fishery assessment and management towards a more integrated approach that reflected the management needs of species across their northern Australian range. Elucidation of threadfin stock structure is vital for their management at an appropriate ecosystem scale. Attending to these critical issues for threadfins will also provide a framework for addressing management of other inshore species that are fished in adjacent State and Territory waters. Consequently, this project addresses research priorities outlined by QFIRAC, the NT FRAB, Fisheries WA and Sunfish.

Industry has also expressed major concerns for the sustainability of the threadfin fisheries. These concerns are based on fishers' personal experiences, whereby large concentrations of king threadfins usually associated with inshore fishing grounds, especially in the south-east Gulf of Carpentaria, are now being encountered much less frequently, and their movement on and off the grounds is considered much more erratic than previous years (G. Ward, Gulf of Carpentaria commercial net fisher, pers. comm.).

## 1.4 Objectives

1. To determine the stock structure of king and blue threadfin salmon across northern Australia.
2. To use these findings to define the appropriate cross-jurisdictional management framework for sustainable use of king and blue threadfin resources in northern Australia.

## 1.5 Methods

This section provides an overview of the general sampling approach and techniques used during this study. For details of the individual techniques and their data analyses readers are directed to individual chapters.

The sampling approach was driven by the need to determine the stock structure of threadfins for fisheries management purposes. Since the majority of catch of both species is taken by the commercial sector, sampling was dictated primarily by the availability of samples provided by this sector and therefore locations sampled were determined by effort within the commercial fishery. We also adopted a phased approach to sampling since stock structure was uncertain so that early detection of evidence for a single stock prevented unnecessary sampling effort (Abaunza *et al.* 2008). This type of approach has been successfully adopted for recent stock structure studies in northern Australia (Buckworth *et al.* 2007; Welch *et al.* 2009). This phased approach can be described by three major elements:

**ELEMENT 1** (Year 1): Broad spatial scale genetic and environmental differences in king and blue threadfin populations were established across their Queensland, Northern Territory and Western Australia range using the Spanish mackerel stock identification toolkit as recommended by Ward and Rogers (2003). If the first year results do not support the notion of separate stocks, then the project would cease after Element 1.

**ELEMENT 2** (Year 2): Finer spatial and short-term (inter-annual) temporal scale resolution of king and blue threadfin stocks was investigated at an increased number of locations.

**ELEMENT 3** (Year 3): Project results were finalised and the management units for king and blue threadfin in Queensland, the Northern Territory and Western Australia defined. This information was presented to stakeholder groups and management agencies via a final workshop and presentations to management committees (e.g. Queensland Inshore fishery Scientific Advisory Group).

The project used four basic techniques to examine king and blue threadfin stock structure: 1) mitochondrial DNA genetic analyses (microsatellite DNA was used also for blue threadfins); 2) whole otolith solution based microchemistry; 3) parasite incidence; and 4) life history parameters. In the first year of the project, these techniques were applied to establish if broad spatial scale structural variation existed across the major fishing grounds, through the collection of samples from four primary regions in Queensland (east coast and Gulf of Carpentaria), the Northern Territory, and Western Australia (Broome area). The samples were collected primarily by fisheries-dependent sampling with some fisheries-independent sampling also conducted. These samples were used to provide material for genetic, otolith, parasite and biological analyses. Being largely dependent on commercial fishing activities, the timing and exact location of sampling was fairly opportunistic. Further measurements and biological samples will be collected and population parameters will be established for each population sampled through an externally funded student project. The use of life history parameters was a value-adding exercise and not part of the initial FRDC project. The determination of these parameters for both species at different spatial scales provided not only an additional and important measure for identifying different stocks, but this information informs the choice of appropriate management strategies for each stock while also providing information critical as input parameters for future stock assessments.

Since the first year results supported the notion of separate stocks of both king and blue threadfin, we extended the sampling program in the second year to describe finer spatial scale population structure and temporal (inter-annual) variability in the short-term. This included additional king threadfin samples collected from the Brisbane River primarily with the help of recreational anglers. This resulted in sample collections that extended from the western and eastern limits of the known Australian range of blue and king threadfins covering approximately 14,000 km of coastline, and providing a total of 11 locations sampled for blue threadfin (Figure 1.1) and 10 locations sampled for king threadfin (Figure 1.2). In summary for blue threadfin, two locations were sampled on the north-western Australian coast, six locations were sampled in the Gulf of Carpentaria, and three locations were sampled on the east coast. For king threadfins, two locations were sampled on the north-western Australian coast, one location was sampled on the mid-Northern Territory coast, four locations were sampled in the Gulf of Carpentaria, and three locations were sampled on the east coast. Details of the numbers of samples used from each location for the different techniques are provided in the respective chapters.

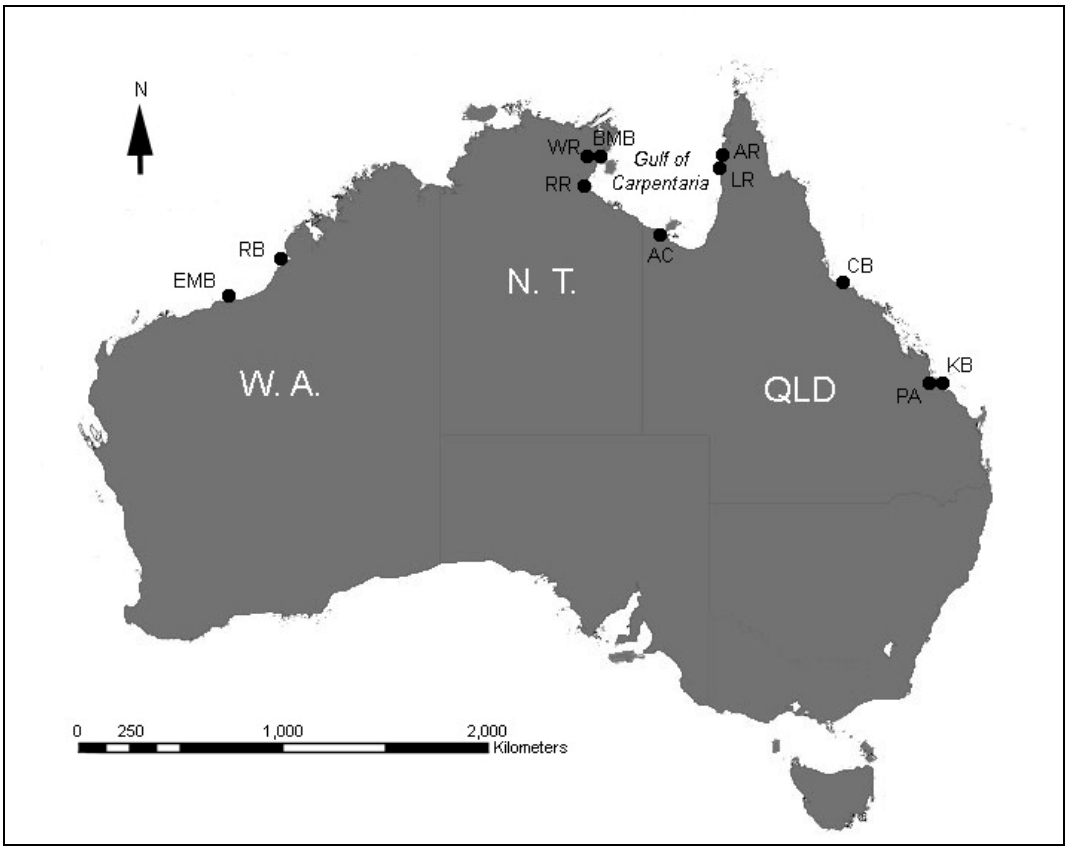



**Figure 1.1:** Locations from which samples of blue threadfin were sourced during the project. EMB = Eighty Mile Beach; RB = Roebuck Bay; WR = Walker River; BMB = Blue Mud Bay; RR = Roper River; AC = Arthur's Creek; LR = Love River; AR = Archer River; CB = Cleveland Bay; KB = Keppel Bay; PA = Port Alma.

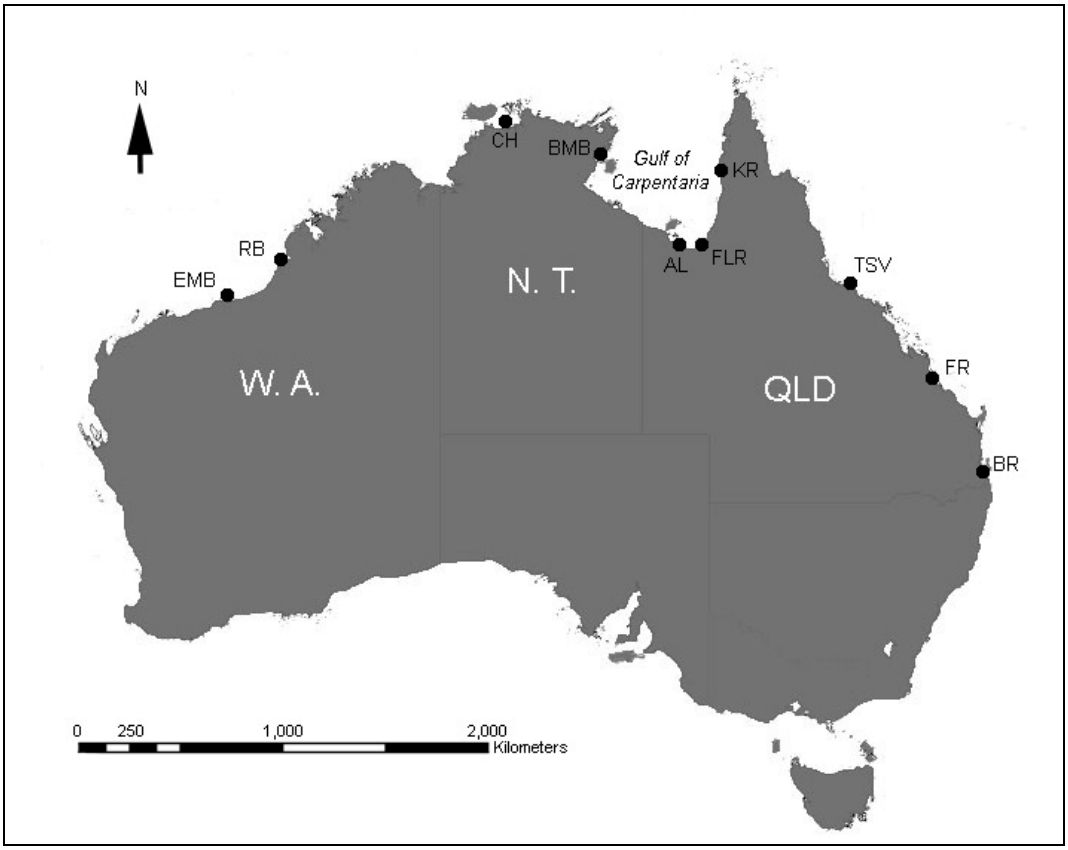
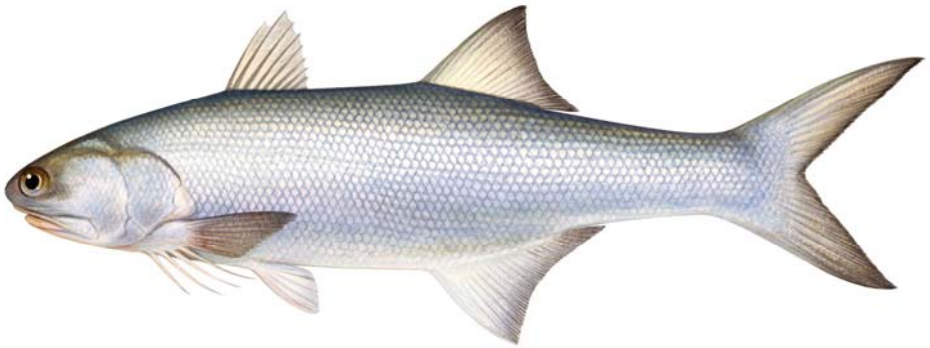



**Figure 1.2:** Locations from which samples of king threadfin were sourced during the project. EMB = Eighty Mile Beach; RB = Roebuck Bay; CH = Chambers Bay; BMB = Blue Mud Bay; AL = Albert River; FLR = Flinders River; KR = Kendall River; TSV = Townsville; FR = Fitzroy River; BR = Brisbane River.

At the completion of each major project element project workshops were held to assess the approach and project progress. A final workshop was held over two days in March 2010 in Townsville. On the first day the project team members gathered to present the results of analyses of each technique for both species. The overall results were discussed and interpreted by the project team as to the stock structure of each species and the implications for management. On the second day workshop participants included fisheries managers from Western Australia, Northern Territory and Queensland, as well as a Queensland recreational fishing representative (Sunfish) and a stock assessment expert. Representatives for commercial fishing interests were also invited but were unable to attend. On this day the project team presented the project findings to the workshop highlighting the management implications.

# Section 2

# Blue Threadfin



Blue threadfin salmon, *Eleutheronema tetradactylum*

Illustration © R. Swainston http://www.anima.net.au

# Chapter 2: Stock structure of blue threadfin, *Eleutheronema tetradactylum*, as indicated by parasites

**Brad R. Moore, Jason M. Stapley, Quentin Allsop, Stephen J. Newman, Aaron C. Ballagh, David J. Welch and Robert J. G Lester**

## 2.1 Introduction

The blue threadfin, *Eleutheronema tetradactylum* Shaw 1804 (Polynemidae), inhabits estuarine and turbid coastal foreshores from the Persian Gulf through southern and southeast Asia to Papua New Guinea and northern Australia (Motomura *et al.* 2002). The species is an important component of commercial and artisanal fisheries across its distribution (Kailola *et al.* 1993; Motomura *et al.* 2002).

*Eleutheronema tetradactylum* is commercially exploited within the multispecies inshore net fisheries of northern Australia, which principally target barramundi (*Lates calcarifer*). These fisheries are divided into four sectors based on state and administrative boundaries: Western Australia, the Northern Territory, Queensland Gulf of Carpentaria and the Queensland east coast. The species is also an important component of the recreational catch across northern Australia. However, despite their importance to fisheries, the stock structure and movements of blue threadfin across northern Australia are undefined, so it is unknown if fishing in any one region impacts on the sustainability of harvest elsewhere. As the species' distribution crosses state and territorial boundaries, a need for interstate cooperative management may exist. Knowledge of the stock structure and movement of blue threadfin across Australia's north is therefore crucial to ensure sustainable harvest of the resource.

Parasites have been widely used to provide information about their commercially exploitable hosts for almost a century. Parasite loadings have been used to discern the stock structure and movements of a variety of fish species (e.g. Lester *et al.* 1988; Speare 1995; Moore *et al.* 2003; Campbell *et al.* 2007). The basic premise underlying the use of parasites as biological tags from which host movements and stock structure can be deduced is that naturally occurring parasites have a discontinuous distribution compared to that of their hosts (Lester 1990). Consequently, organisms only become infected with a certain parasite only when they come within the endemic area of that parasite (MacKenzie and Abaunza 1998). A parasite's endemic area is the geographical location in which conditions are suitable for transmission, and in which the parasite occurs naturally. As a fish moves into a parasite's endemic area, they become infected, and as they move out, they carry a legacy of their occupancy within the area (Lester 1990). Consequently, the spatial relationships between host populations may be deduced

by analysing parasite faunas of individuals from different regions. Where the parasite fauna is different, the history of the fish is different according to the time scale of the parasite counted: recent history for temporary parasites, long-term history for permanent parasites (Lester 1990).

Zischke *et al.* (2009) conducted a preliminary analysis of the movements and subsequent stock structure of blue threadfin along the east coast of Queensland, Australia. Their results, based on parasite faunas and tagging data, suggest that blue threadfin undergo limited mixing and therefore form multiple stocks along Queensland's east coast. However, little is known on the stock structure of blue threadfin across the species' greater Australian distribution. In this chapter, parasites were used to provide information on the movements and subsequent stock structure of *E. tetradactylum* across northern Australia. Here, the term 'stock' refers to non-mixing post-juvenile fish populations that therefore comprise an independent management unit with respect to exploitation.

## 2.2 Methods

### Sample Collection

Fish were collected from commercial fishers and state fisheries agencies from ten sites across northern Australia between winter 2007 and winter 2009 (Figure 2.1, Table 2.1). One site (Roebuck Bay in Western Australia) was sampled twice in 2008 (austral autumn and winter) to examine temporal patterns in abundance of the parasites encountered. Sites were separated by tens to hundreds of kilometres and were centred on the important commercial and recreational fishing areas for *E. tetradactylum* across northern Australia. For each fish collected, the site, date of capture, sex, maturity stage and caudal fork length ($L_F$) was recorded. Heads and viscera, or gills and viscera, were frozen in individually labelled plastic bags for later laboratory examination. Sagittal otoliths (hereafter referred to as otoliths) were removed from all fish for later aging.

In the laboratory, samples were defrosted in water, and the gill opercula and viscera were examined for metazoan parasites, including those encysted in or outside of the stomach wall. Parasites encountered were extracted, identified, enumerated, and categorised as 'permanent' or 'temporary', based on their probable life span in or on the fish.

To facilitate parasite identification, unfrozen samples were taken from Cleveland Bay and the Fitzroy River. Trypanorhynchs were placed in fresh water to facilitate tentacle eversion. Representative specimens were stained in Mayer's haematoxylin, dehydrated in ethanol, cleared using methyl salicylate and mounted in Canada balsam. Once identified, the morphology of the scolex, bothridia and blastocyst were considered adequate to separate trypanorhynch species. All parasites were identified according to descriptions in Podder (1937), Cannon (1977), Pillai (1985), Campbell and Beveridge (1996), Amin *et al.* (2003) and Palm (2004).

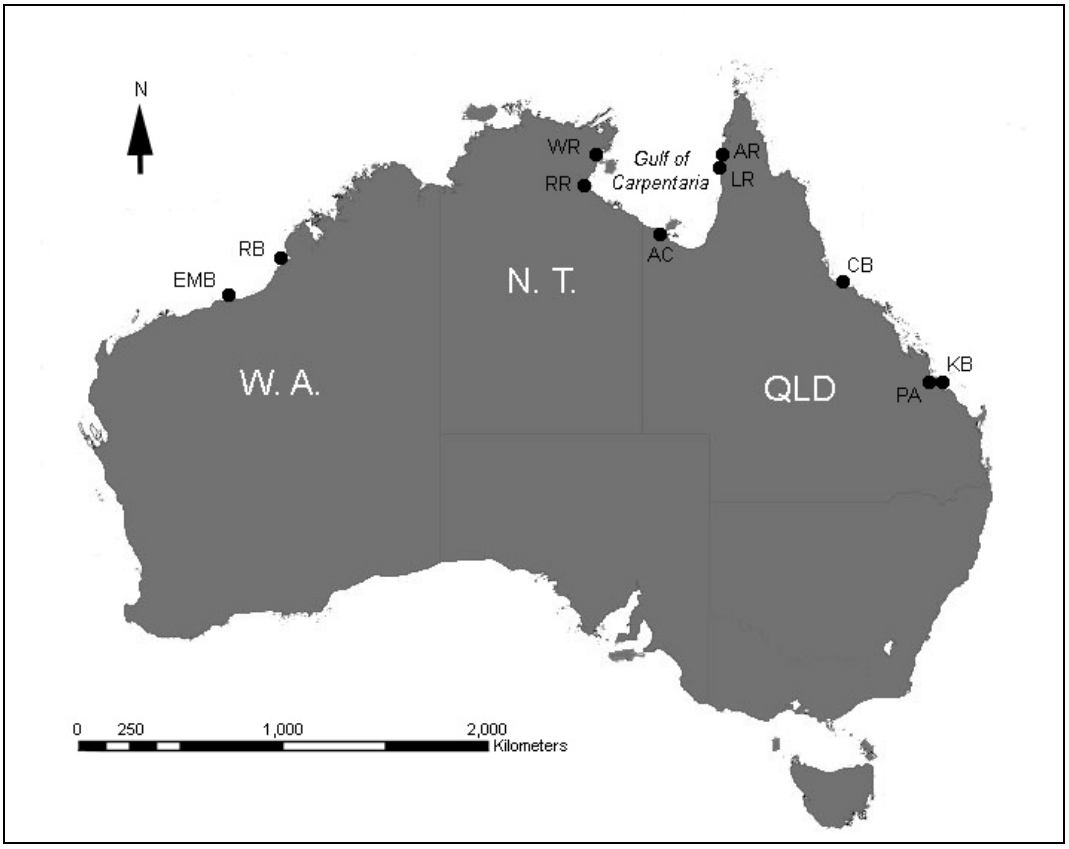



**Figure 2.1:** The ten sample sites used in this study. EMB = Eighty Mile Beach; RB = Roebuck Bay; WR = Walker River; RR = Roper River; AC = Arthur's Creek; LR = Love River; AR = Archer River; CB = Cleveland Bay; KB = Keppel Bay; PA = Port Alma.

Ages of *E. tetradactylum* were estimated by the number of opaque bands, determined to be annuli by Pember *et al.* (2005) and an assessment of the marginal index of the whole otolith. Each otolith was read twice, without knowledge of the fish length or date of capture. When the two counts of the annuli did not agree, a third reading was taken, and the two concurrent readings were accepted as the number of annuli. When all three counts differed, the otolith was rejected from further analysis. An extra half or full year was added to the age of the fish if the width of the final annuli and otolith margin was observed to be half, or equal that, of the penultimate band, respectively. All otoliths were read by the same reader (A. Ballagh).

### Data Analysis

Summary statistics including mean parasite abundance (number of a particular parasite per host examined including uninfected hosts) and prevalence (number of hosts infected with a particular parasite) were compiled for each parasite species, following Bush *et al.* (1997). Only parasite species with a prevalence $\geq$ 10 % in at least one of the samples were used in the analysis (component species; Bush *et al.* 1990). A high variance:mean ratio for all parasite species indicated an over-dispersed

distribution. The natural log of the parasite + 1 (Ln[x + 1]) was used to normalise the data. This transformed data was used throughout the analyses.

Long-lived parasites tend to accumulate with host age (Rohde 1982) which may obscure differences in parasite fauna between areas if samples contain hosts of different ages. To reduce this effect, parasite species were examined for correlation with host age and $L_F$. Where significant, the numbers of the correlated species were adjusted to the mean host age or $L_F$. No adjustment was made if the parasite abundance was zero.

One-way ANOVA was applied to identify differences in abundance of the individual parasite species deemed as being 'permanent' in or on the fish across the eleven sample groups. Significant results were examined using Tukey-Kramer post-hoc pair-wise comparisons (Sokal and Rohlf 1995).

Differences between sites in permanent parasite community assemblages based on the abundance data of the permanent parasite species were examined by multiple analysis of variance (MANOVA), followed by canonical discriminant function analysis using *Statistica* v7.0. The results of the discriminant function analyses were displayed as graphs of the first and second canonical axes. Confidence levels of 95% are given as shaded circles around the mean canonical score with the radius equal to the square root of 5.99/number of fish in the sample. Partial Wilk's lambda values were used to indicate the discriminatory power of the individual parasites, ranging from 0 (total discriminatory power) to 1 (no discriminatory power).

## 2.3 Results

Ten parasite species were considered as suitable markers to investigate movement of *E. tetradactylum* and were therefore counted in all fish (Table 2.1). The parasites encountered were classified as 'permanent' or 'temporary', based on their probable life span in or on the fish. Parasites deemed to be permanent in the fish included the nematodes *Anisakis* sp. and *Terranova* (type II), the cestodes *Otobothrium australe, Pterobothrium pearsoni*, *Callitetrarhynchus gracilis*, *Paranybelinia* sp. and *Nybelinia* sp., and the acanthocephalan *Pomphorhynchus* sp. Parasites classified as temporary included the copepod *Thysanote eleutheronemi* and the acanthocephalan *Neoechinorhynchus topseyi*. Representative specimens of each parasite species were stored in the Marine Parasitology laboratory at the University of Queensland. As we sought to determine the long-term movements of *E. tetradactylum*, higher statistical analyses were only conducted on permanent parasite species.

The eight permanent parasite species infecting *E. tetradactylum* were examined for correlation with host age and $L_F$. *Callitetrarhynchus gracilis*, *Nybelinia* sp. and *Otobothrium australe* were found to be significantly correlated with age and were thus adjusted to the mean age of the samples (2.84 years

old). *Terranova* sp. was found to be more significantly correlated with host $L_F$ than age, and was thus adjusted to the mean $L_F$ of 414 mm.

One-way ANOVA indicated that the abundance of all parasite species exhibited a significant difference across the eleven samples. Tukey-Kramer pair-wise comparisons of the abundance of the eight permanent parasites gave an indication of the similarity between the temporally and spatially distinct samples (Table 2.2). In terms of temporal stability, the Roebuck Bay replicates appeared homogeneous, with the abundance of only *Paranybelinia* sp. being significantly different. The Roebuck Bay samples appeared similar to Eighty Mile Beach samples, with only *Paranybelinia* sp. abundance being significantly different. In the western Gulf of Carpentaria, samples from the Walker and Roper rivers appeared homogeneous, differing only in *O. australe* abundance. The relatively low abundances of *P. pearsoni* and high abundances of *O. australe* in fish from these sites suggest they are distinct from the other Gulf sites. In the eastern Gulf, samples from the Archer and Love Rivers appeared homogeneous, with no significant differences in any parasite species apparent. These sites also appeared similar to Arthur's Creek samples, differing only in *P. pearsoni* abundance. Cleveland Bay fish appeared distinct to all other sites. Keppel Bay fish appeared similar to those from Port Alma, with only the abundance of *C. gracilis* being significantly different. Fish from these sites appeared distinct to all other sites.

Multivariate analysis of the permanent parasite fauna of blue threadfin revealed significant differences in the parasite community assemblages across the sites (MANOVA, d.f. 80, 3211, $P<0.05$). Discriminant function analysis, based on the abundances of the eight permanent species produced clear separation between western, Gulf of Carpentaria and east coast sites (Figure 2.2). The replicate samples from Roebuck Bay (location IDs RB 'a' and RB 'b') grouped together at all axes, suggesting a common history and that the parasite faunas were stable across time. Samples from Eighty Mile Beach (EMB) separated from the other samples on the third canonical axes, indicative of an isolated population. In the western Gulf of Carpentaria, Walker River (WR) and Roper River (RR) fish each appeared distinct from all other samples. In the eastern Gulf, fish from the Archer and Love rivers (AR and LR) showed strong similarities and grouped together at all axes. Fish from Arthur's Creek (AC) in the south-eastern Gulf appeared distinct to all other samples. On Queensland's east coast, Cleveland Bay fish (CB) appeared significantly different from fish collected from Keppel Bay and Port Alma (KB and PA). Keppel Bay and Port Alma fish grouped together at all axes, suggesting homogeneity. Thus the permanent parasite data suggest at least eight isolated populations of blue threadfin across northern Australia.

**Table 2.1:** Average numbers of parasites per fish in blue threadfin, *Eleutheronema tetradactylum*, sampled from ten sites across northern Australia (untransformed data). Number of fish infected shown in parentheses.

| **Management jurisdiction** | **Western Australia** | | | **Northern Territory** | | **Queensland Gulf of Carpentaria** | | | **Queensland east coast** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Site** | **Eighty Mile Beach** | **Roebuck Bay 'a'** | **Roebuck Bay 'b'** | **Walker River** | **Roper River** | **Arthur's Creek** | **Love River** | **Archer River** | **Cleveland Bay** | **Keppel Bay** | **Port Alma** |
| **Sampling season (year)** | **Autumn (2009)** | **Autumn (2008)** | **Winter (2008)** | **Spring (2007)** | **Autumn (2009)** | **Winter (2008)** | **Winter (2008)** | **Spring (2007)** | **Winter (2008)** | **Winter (2007)** | **Spring (2008)** |
| **Site ID** | **EMB** | **RBa** | **RBb** | **WR** | **RR** | **AC** | **LR** | **AR** | **CB** | **KB** | **PA** |
| **Sample size** | 47 | 42 | 48 | 47 | 36 | 51 | 48 | 50 | 51 | 49 | 50 |
| **Mean FL (mm)** | 324 | 435 | 393 | 373 | 350 | 449 | 396 | 427 | 449 | 427 | 497 |
| **Mean age** | 1.9 | 3.1 | 2.8 | 3.2 | 1.9 | 3.1 | 2.8 | 2.8 | 2.9 | 2.8 | 3.6 |
| **Temporary parasites** | | | | | | | | | | | |
| *Thysanote eleutheronemi* | 0.0 (2) | - | - | - | 0.0 (0) | 1.3 (25) | 0.5 (14) | 0.3 (3) | 0.3 (11) | 2.0 (26) | 0.6 (19) |
| *Neoechinorhynchus topseyi* | 0.1 (4) | 0.3 (6) | 1.0 (19) | 1.5 (16) | 0.7 (11) | 0.1 (3) | 0.7 (13) | 117 (14) | 0.7 (21) | 1.0 (15) | 0.4 (12) |
| **Permanent parasites** | | | | | | | | | | | |
| *Pterobothrium pearsoni* | 0.6 (14) | 0.2 (4) | 0.2 (11) | 0.3 (7) | 0.1 (2) | 2.9 (43) | 6.5 (46) | 8.6 (48) | 0.4 (16) | 1.1 (21) | 0.9 (19) |
| *Callitetrarhynchus gracilis* | 0.1 (6) | 0.6 (16) | 0.8 (24) | 0.2 (7) | 0.4 (12) | 0.7 (15) | 0.6 (15) | 1.2 (25) | 0.3 (13) | 3.0 (41) | 2.7 (30) |
| *Paranybelinia* sp. | 3.3 (11) | 15.8 (32) | 6.4 (26) | 0.0 (0) | 1.0 (6) | 1.0 (8) | 0.3 (3) | 0.4 (7) | 0.1 (5) | 0.6 (11) | 0.4 (9) |
| *Nybelinia* sp. | 0.0 (1) | 0.0 (1) | 0.0 (0) | 0.2 (8) | 0.0 (1) | 1.2 (25) | 1.6 (26) | 0.6 (18) | 0.6 (15) | 2.3 (24) | 3.1 (27) |
| *Anisakis* sp. | 0.0 (0) | 0.0 (2) | 0.0 (1) | 0.0 (0) | 0.0 (0) | 0.0 (1) | 0.0 (0) | 0.0 (2) | 0.3 (10) | 0.4 (10) | 0.2 (4) |
| *Terranova* (type II) | 4.0 (31) | 3.0 (19) | 2.5 (17) | 0.1 (5) | 0.3 (6) | 0.1 (15) | 0.3 (7) | 0.2 (8) | 0.4 (12) | 0.6 (19) | 0.8 (13) |
| *Pomphorhynchus* sp. | 0.0 (0) | 0.0 (0) | 0.0 (0) | 0.0 (2) | 0.1 (2) | 0.2 (3) | 0.2 (6) | 0.2 (6) | 0.0 (0) | 0.1 (3) | 0.0 (2) |
| *Otobothrium australe* | 116.5 (47) | 105.6 (42) | 120.1 (48) | 106.6 (47) | 70.6 (34) | 57.3 (51) | 61.9 (48) | 55.3 (50) | 3.5 (33) | 0.6 (6) | 0.4 (5) |

**Table 2.2:** Comparison of abundance of eight permanent parasite species infecting *Eleutheronema tetradactylum* from the eleven samples (ten sites) across northern Australia. The number in the table corresponds to the parasite species that is significantly different at the 95% level of confidence based on Tukey-Kramer pair-wise comparison (natural log of parasite abundance).

| Site | EMB | RBa | RBb | WR | RR | AC | LR | AR | CB | KB | PA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **EMB** | | 3 | 3 | 6 | 6, 8 | 1, 4, 6, 8 | 1, 4, 6, 8 | 1, 2, 6, 8 | 5, 6, 8 | 2, 4, 5, 6, 8 | 2, 4, 6, 8 |
| **RBa** | | | 3 | 3, 6 | 3, 6, 8 | 1, 3, 4, 8 | 1, 3, 4, 6, 8 | 1, 3, 6, 8 | 3, 6, 8 | 1, 2, 3, 4, 5, 8 | 2, 3, 4, 6, 8 |
| **RBb** | | | | 3, 6 | 3, 8 | 1, 3, 4, 8 | 1, 3, 4, 6, 8 | 1, 3, 6, 8 | 3, 6, 8 | 2, 3, 4, 5, 8 | 3, 4, 8 |
| **WR** | | | | | 8 | 1, 4, 8 | 1, 4, 8 | 1, 2, 8 | 5, 8 | 2, 4, 5, 8 | 2, 4, 8 |
| **RR** | | | | | | 1, 4 | 1, 4 | 1 | 8 | 1, 2, 4, 5, 8 | 2, 4, 8 |
| **AC** | | | | | | | 1 | 1 | 1, 8 | 1, 2, 5, 8 | 1, 2, 8 |
| **LR** | | | | | | | | | 1, 4, 5, 7, 8 | 1, 2, 5, 8 | 1, 2, 8 |
| **AR** | | | | | | | | | 1, 2, 8 | 1, 2, 4, 5, 8 | 1, 4, 8 |
| **CB** | | | | | | | | | | 2, 4, 8 | 2, 4, 8 |
| **KB** | | | | | | | | | | | 2 |
| **PA** | | | | | | | | | | | |

**Key to species:**

1 = *Pterobothrium pearsoni* 2 = *Callitetrarhynchus gracilis* 3 = *Paranybelinia* sp. 4 = *Nybelinia* sp.

5 = *Anisakis* sp. 6 = *Terranova* (type II) 7 = *Pomphorhynchus* sp. 8 = *Otobothrium australe*

All parasites, with the exception of *Pomphorhynchus* sp. were capable of discrimination ($P<0.05$). In the analysis, *O. australe* had the most discriminating power (Partial Wilk's lambda = 0.22), followed by (in order of greatest to least discriminating power), *P. pearsoni* (0.47), *Paranybelinia* sp. (0.76), *C. gracilis* (0.87), *Terranova* (type II) (0.89), *Nybelinia* sp. (0.92), and *Anisakis* sp. (0.96). The first two axes (displayed) accounted for 92% of the variation in area-to-area differences in parasite numbers. The third axis accounted for a further four percent of the variability.

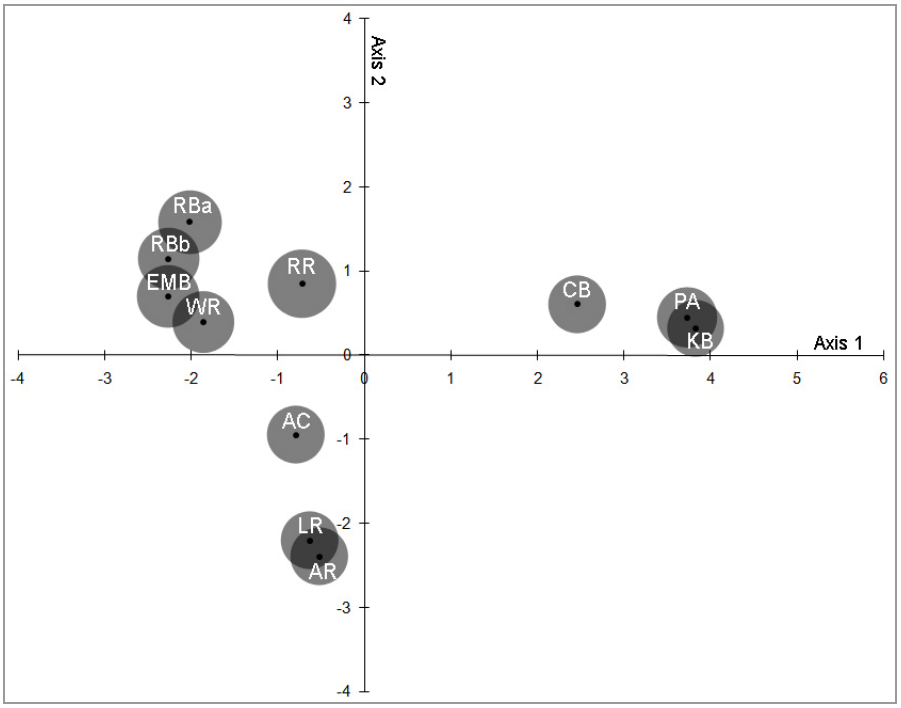
## 2.4 Discussion

The ability to discern host movements and consequently separate fish stocks on the basis of their parasite fauna relies on differences in distribution or infection intensity of the parasite species under investigation (MacKenzie 1983). Such differences may arise from environmental variation, such as temperature and salinity profiles, or variability in the distribution and abundance of definitive or intermediate hosts. In this study, all analysed parasite species exhibited a difference in distribution or abundance across the study sites.

Abundances of four of the parasite species (*Callitetrarhynchus gracilis*, *Nybelinia* sp., *Otobothrium australe*, and *Terranova* (type II)), were positively correlated with fish age (or length). The positive correlation of the abundances of these four parasites species and fish age indicates that these parasites are picked up continuously throughout the life of the fish. In contrast, abundances of *Pterobothrium pearsoni*, *Paranybelinia* sp., *Pomphorhynchus* sp. and *Anisakis* sp. showed no correlation in their abundance with that of the age or length of the fish. These four species were thought to be long-lived as no degenerating cysts were found. This suggests that these species may accumulate in juvenile *E. tetradactylum* before they have entered the fishery.

As long-lived parasites tend to accumulate with host age, differences in parasite fauna between sites may have been obscured between samples that contained fish of different ages. To reduce this effect, parasite species were examined for correlation with host age (or length), and adjusted where significant. This adjustment was based on parasite data pooled across all sites. However, anecdotal evidence suggests that parasite accumulation rates are not constant across all sites, and may be more suitable on a site-specific basis. Although further refinement of this technique is warranted, it is unlikely to affect the results, given the considerable difference in parasite abundances apparent in the raw (unadjusted) parasite data (Table 2.1).

**A.**




**B.**

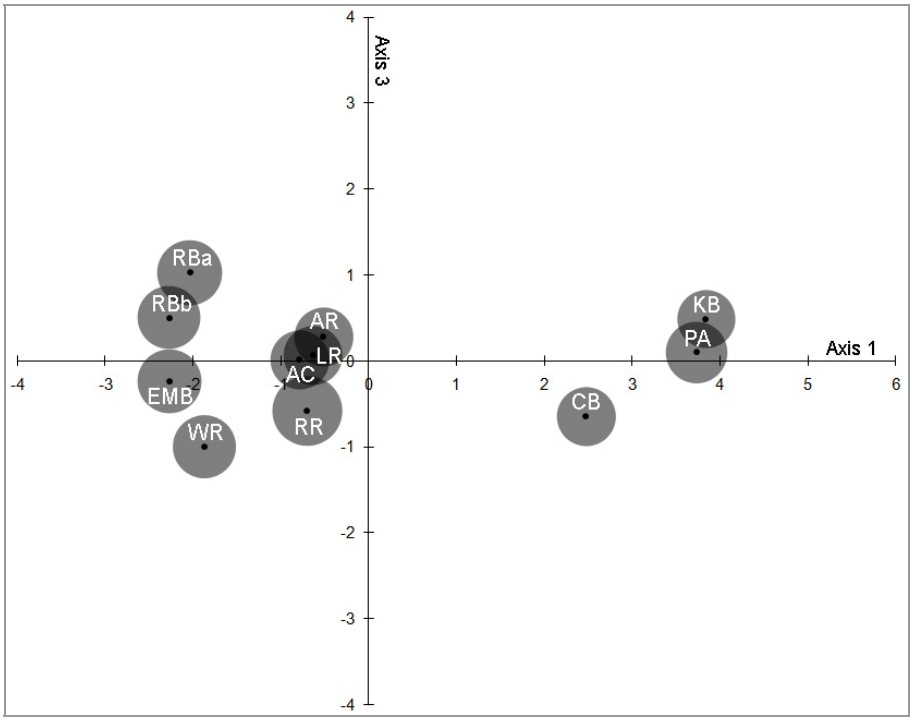



**Figure 2.2:** Results of canonical discriminant function analysis of permanent parasite species from blue threadfin from the ten sites across northern Australia (see Figure 2.1, Table 2.1). A = Axis 1 vs. 2; B = Axis 1 vs. 3. Shaded circles represent 95% confidence intervals.

The utility of parasites as a natural marker of fish movement and stock structure is determined to an extent by the temporal stability of the parasites used, particularly when samples have been collected at different times. It was not possible to examine the temporal stability of the parasites infecting *E. tetradactylum* at all sites in the present study due to the remoteness of the sampling sites and the variable nature of fishing activities. As some sites were sampled in different years it is possible that the observed patterns between sites may be an artefact of temporal differences in the abundances of the parasites encountered. We consider this to be unlikely for a number of reasons. First, although ectoparasites have been demonstrated to exhibit considerable temporal variation (e.g. Timi *et al.* 2009), encysted parasite such as the larval cestodes and nematodes used here have been shown to be relatively stable, at least over the timeframes of the current study (Campbell *et al.* 2007). Second, few differences in parasite abundance were observed between the temporally replicated samples of this study, with only the abundance of the trypanorhynch *Paranybelinia* sp. differing between the replicates. Third, a concurrent study into the movements and stock structure of king threadfin, *Polydactylus macrochir*, which used seven of the eight parasite species used here, collected from a number of identical sites, suggest that these parasites are relatively stable across time (Moore *et al.* Chapter 6).

Zischke *et al.* (2009) hypothesised that the fine scale stock structure observed for *Eleutheronema tetradactylum* in Queensland's east coast waters may be in part due to the prominent rocky headlands and peninsulas and associated clear waters of this coast acting as a barrier to movement. We found evidence for a number of distinct stocks in the waters of the Gulf of Carpentaria, a largely continuous stretch of estuarine and coastal habitat characterised by turbid waters and sandy sediments (Somers 1994; Somers and Long 1994). However, the results of the present study suggest that adult *E. tetradactylum* are highly resident to a particular location, even when adjacent habitat seems conducive to longshore movement.

***Implications for fisheries management and future directions***

The results of this study provide evidence of limited connectivity of *E. tetradactylum* between the main fishing areas across northern Australia. We found evidence for at least eight isolated stocks of blue threadfin across northern Australia: a combined stock from the Archer and Love Rivers in the eastern Gulf of Carpentaria, a combined stock from Keppel Bay and Port Alma on Queensland's east coast, and isolated stocks from the Eighty Mile Beach, Roebuck Bay, Walker River, Roper River, Arthur's Creek and Cleveland Bay areas. It is likely that further sampling on a finer scale would detect a greater number of stocks, based on the highly resident nature of *E. tetradactylum* observed here.

Populations of *E. tetradactylum* across northern Australia are currently managed separately by state-based management agencies. Our findings suggest that post-recruitment populations of *E. tetradactylum* are highly sedentary, with little mixing between sites. As such, the long-term effects of fishing are likely to be highly localised within the current administrative boundaries, implying little need

for interstate cooperative management. The site-specific nature of adult *E. tetradactylum* observed in the current study renders the species vulnerable to serial depletion. As such, the development of harvest strategies and establishment of suitable fishery regulations should be conducted in a way that recognises the highly resident nature of adult *E. tetradactylum* in Australian waters.

# Chapter 3: Isolation by distance governs population genetic patterns of the four-fingered threadfin *Eleutheronema tetradactylum* (Polynemidae) in Australian waters

**John B. Horne, Paolo Momigliano, David J. Welch, Stephen J. Newman and Lynne van Herwerden**

## 3.1 Introduction

Population connectivity and the dispersal tendencies of the pelagic larval propagules of demersal marine organisms is, at present, a rigorously studied area of marine biology. In recent years the level of interest and attention to this area has grown dramatically due to its direct relevance to the effective design of marine reserves, conservation efforts and sustainable fisheries (Palumbi, 2003; Jones *et al.* 2009). Traditionally, marine population paradigms have been thought of in terms of openness or closedness, referring to the prevalence of either external or internal recruitment respectively (Sale, 1991; Cowen, 2002). More recently, however, it has been suggested that both external and internal maintenance of benthic marine populations are simultaneously important processes (Jones *et al.* 2009).

In recent years various genetic tools have become a standard in studies relating to the spatial dynamics of marine fish populations, which are logistically difficult to observe by other means. Oceanic fishes, such as coral reef specialists, often show little genetic population structure at large spatial scales (e.g. Bay *et al.* 2004; Craig *et al.* 2007; Shultz *et al.* 2007; Horne *et al.* 2008). In contrast coastal, inshore fishes typically exhibit highly structured and fragmented populations (e.g. Chenoweth and Hughes, 2003; Durand *et al.* 2005; Hickerson and Cunningham, 2005; Bradbury *et al.* 2008). The implications of this pattern are that inshore fisheries need to be managed at a fine spatial scale, and details of the geographic boundaries of breeding stocks are critical to maximising the longevity and efficiency of the fishery. Additionally, notwithstanding the importance of inshore fisheries, our knowledge of coastal and estuarine fishes is disproportionate to those of other marine biomes (Blaber, 2002).

*Eleutheronema tetradactylum* is a large carnivorous fish (average TL 50cm) that inhabits tropical estuaries and other shallow near-shore environments and is an important food fish of northern Australia. At present, the stock structure and population genetics of this species are unknown. The purpose of this study was to evaluate the genetic connectivity of *E. tetradactylum* populations from the east Queensland coast, the Gulf of Carpentaria and Western Australia using mitochondrial-DNA. During

the course of this work five microsatellite loci were also developed for this species and applied to a subset of populations to complement the mitochondrial analyses. Data from both the mitochondrial and nuclear genomes will provide robust information about the population dynamics of this species essential for proper management.

## 3.2 Methods

### Sampling

*E. tetradactylum* was sampled from eleven locations across the northern coast of Australia between 2007 and 2009 (Figure 3.1). Samples were collected using monofilament gillnets usually from commercial fishing activities and supplied by fishers. Fin clips were taken from individual samples from each location and in one location (Roebuck Bay) re-sampling was conducted at least six months apart to assess temporal stability in genetic structure.

### Laboratory procedures

Total genomic DNA was extracted from fin clips using the chelex extraction protocol outlined by (Walsh *et al.* 1991). We amplified the mitochondrial gene cytochrome oxidase subunit 1 (CO1) of *E. tetradactylum* using universal primers (FishF1; 5'-TCA ACC AAC CAC AAA GAC ATT GGC AC3' and FishR1; 5'-TAG ACT TCT GGG TGG CCA AAG AAT CA3') furnished by Ward *et al.* (2005). PCR reactions were conducted as follows: 20 µL PCR reactions containing 2.5 mM Tris–Cl (pH 8.7), 5 mM KCL, 5 mM (NH4)2SO4, 200 µM each dNTP, 2.5-3.5 mM MgCl2, 10µM each primer and 1 U of Taq Polymerase (Qiagen Ltd.). Thermocycling was carried out with an initial denaturation of 94°C for two minutes, 35 cycles of denaturation, annealing and extension (94°C for 30 s, 55°C for 30 s, and 72°C for 90 s) and a final extension of 72°C for ten minutes. PCR products were confirmed by gel electrophoresis on 1.5% agarose gels and purified by either a standard isopropanol purification or an ammonium acetate ethanol clean-up. PCR products were sequenced with the FishF1 primer using ABI (Applied Biosystems Incorporated) technologies at Macrogen sequencing service Seoul, South Korea. GenBank, Accession Numbers for all sequences are XXX-XXX.

We also developed five microsatellite loci using the magnetic bead capture protocol of Glenn and Schable (2005). Tissue was digested with proteinase K and DNA extracted using a salt-chloroform method (Sambrook *et al.* 1989). Total genomic DNA was digested into 300-1000 bp fragments using the restriction enzymes Rsa I and Hae III separately. Double stranded SNX linkers were ligated to both sides of the blunt ended fragments using T4 DNA ligase.

Linker ligated DNA fragments were PCR amplified using the super SNX-24 primer and subsequently annealed to two different combinations of biotinylated oligo probes of di- and tetra-nucleotide motifs [$(AG)_{16}$ + $(AC)_{16}$, $(AAAC)_6$ + $(AATT)_8$]. Reaction mixtures consisted of 25 µL 2x hyb solution, 10 µL

mixed oligos (1 µM each), 10 µL linker ligated DNA, 5 µL nuclease free $H_2O$ for a total volume of 50 µL. Thermocycler temperatures for the annealing of probes were as follows: 95°C for five minutes, 70°C for five seconds followed by 99 five-second incremental step downs of 0.2°C and 50°C for 10 minutes followed by 20 five-second incremental step downs of 0.5°C.

50 µL of Streptavidin bonded magnetic beads (Dynabeads, Invitrogen Dynal, Olso, Norway) were washed twice in 250 µL of TE buffer, twice in 1x hyb solution and suspended in a final volume of 150 µL 1x hyb solution. The hybridised DNA-oligo fragments were added to the magnetic bead solution and captured with a magnetic particle separator, while the supernatant and miscellaneous DNA was discarded.

Microsatellite enriched DNA was again PCR amplified using the super SNX-24 primer and ligated into the pCR®2.1-TOPO vector (Invitrogen) as per the manufacturer's instructions. Cloned inserts were sent to the Australian Genome Research Facility at the University of Queensland, Brisbane, for bacterial transformation and sequencing with universal M13 primers. The resulting DNA sequences were screened, microsatellite loci identified and primers designed using the program Msatcommander (Faircloth, 2008). A total of twenty loci were detected and the nine best (those with the longest number of repeats) were chosen for further screening.

Sample DNA was PCR amplified using a tailed-primer approach as described by Boutin-Ganache *et al.* (2001). Forward or reverse primers were tailed with the sequence CAGTCGGGCGTCATCA in Msatcommander (Faircloth, 2008) and third primers of the same sequence were fluoro-labelled with HEX, TET or FAM. PCR amplifications of microsatellite loci were carried out in 10 µL reactions with the following reagents: 1 µL 10x Pfu buffer, 200 µM of each dNTP, 15 µL each of forward and reverse primers and 3 µL of flurolabeled primer (10 µM), 0.1 U *Pfu* DNA polymerase (Promega, Madison, Wisconsin), 7.42 µL of $H_2O$ and 1 µL of chelex extracted DNA template. PCR products were purified using an ethanol and ammonium acetate precipitation and read using Amersham MegaBACE instrumentation at the James Cook University Genetics Analysis Facility.

Five microsatellite loci were chosen for the final analysis (Table 3.1) and the sequences for each may be found in Genbank, accession numbers: XXX-XXX. Primers of locus Etet1 were unable to be designed with tails, for this reason Etet1-f was fluoro-labelled with HEX and PCR amplified with two primers with similar parameters as stated above. Samples from six locations (n = 288) were genotyped: Roebuck Bay (1$^{st}$ sample), Eighty Mile Beach, Archer River, Love River, Cleveland Bay and Port Alma to ensure that the sampling was hierarchical and represented two samples from each of the three geographic regions examined.

**Table 3.1:** Description of five novel microsatellite loci for the four finger threadfin (*Eleutheronema tetradactylum*). Data generated from the analysis of 288 individuals from six putative populations: name of locus, repetitive sequence motif, forward and reverse primer sequences, annealing temperature ($T_a$ °C), number of alleles ($N_a$), size range of resulting fragment in units of base pairs.

| locus | Repeat motif | Primer Sequences (5'-3') | $T_a$ (°C) | $N_a$ | Size range (bp) |
|---|---|---|---|---|---|
| Etet1 | $(AAAAC)_{32}$ | F 5'-CCCAGAAGGCAGCGTGAG<br>R 5'-TCCCATGAGTGATAGCTTTTGC | 58° | 33 | 128-264 |
| Etet2 | $(GT)_{15}$ | F 5'-TTGGGCATGGTGGCTTTTG<br>R 5'-GTCAGATGGACCAGATTAACTTCC | 51-58° | 19 | 206-241 |
| Etet3 | $(GTTT)_6$ | F 5'-GTGCAACGAGGTCATCAGC<br>R 5'-TGCACACCTTCTCCAGCTC | 51-58° | 8 | 194-215 |
| Etet4 | $(GT)_{16}$ | F 5'-GCACAGCTCGGTTTTCTGG<br>R 5'-TACAACCTCCCTGCTGGAC | 51-58° | 19 | 155-191 |
| Etet5 | $(GT)_{14}(GAGT)_4$ | F 5'-CACCGTGTGCTTGTGCAG<br>R 5'-TAGTCCAGGTTCGCTCCAG | 51-58° | 25 | 202-260 |

### *Population genetic analysis*

Minimum spanning networks of COI haplotypes were constructed in TCS (Clement *et al.* 2000). Molecular diversity indices, haplotype diversity ($h$) and nucleotide diversity ($\pi$) of COI haplotypes were calculated in DNAsp (Rozas *et al.* 2003). Analysis of molecular variance (AMOVA) was performed in *Arlequin* v3.1 (Excoffier *et al.* 2005). Overall AMOVA and pair wise *F*st values were computed for COI and microsatellite datasets using 10,000 permutations. Fu's *F*s test for population expansion (Fu, 1997) and mismatch distribution (Rogers and Harpending, 1992) were computed for COI in *Arlequin*. Shoreline distances between sampled populations were estimated in kilometres using Google™ Earth v4.3 and compared to genetic distance (pairwise *F*st) in isolation-by-distance analysis (IBD), performed online using IBD web service (Jensen *et al.* 2005).

### *Hardy-Weinberg equilibrium and linkage*

Tests of Hardy-Weinberg equilibrium were performed in *GenePop* (Rousset, 2008) as well as in *Arlequin* 3.1. Tests were run for the recommended 10,000 permutations. Tests of linkage disequilibrium were also performed in both programs at default settings.

### *Assignment tests*

The genetic structure of populations of *E. tetradactylum* was estimated by using the clustering method based on Bayesian inference implemented in the software *STRUCTURE* v2.3.3 (Falush *et al.* 2003; Pritchard *et al.* 2000). The number of genetic clusters (parameter K) was estimated using the method proposed by Evanno (2005). Twenty independent runs were performed for each possible number of K (1 to 6). The appropriate run length was determined by comparing multiple runs at each number of K,

and by checking that key parameters reached equilibrium before the end of the burning phase. Each iteration was run for 10,000,000 generations, of which 200,000 were the burning phase and 800,000 generations were sampled. All runs were performed using the admixture model with default settings. The number of K was selected by looking at L(K), L'(K), L''|K| and ΔK values (Evanno *et al.* 2005). Another, longer run was performed using the selected K value (K = 2; 1,000,000 generation burning, 4,000,000 sampled generations).

## 3.3 Results

633 bp of the mitochondrial COI region were amplified for 485 *E. tetradactylum* individuals. There were a total of 119 polymorphic sites, of which 18 were parsimony informative (101 singletons) and 48 individual haplotypes were identified (Figure 3.1). The large proportion of singleton mutations is characteristic of demographic expansion. In conjunction, Fu's *F*s value was strong and significantly negative (*F*s = -26.602, $p < 0.0001$). This signal of expansion in the data is probably due to recent range expansions since the end of the Pleistocene associated with an increase in available habitat as rising sea levels bathed the fringes of the Australian continental shelf (Voris, 2000). Overall, haplotype and nucleotide diversity were 0.79 and 0.003 respectively. Genetic diversities of individual populations are reported in Table 3.2 for COI and Table 3.3 for microsatellite data. At least four out of six populations conformed to expectations of Hardy-Weinberg equilibrium for all microsatellite loci tested. No consistent evidence of linkage between any two loci was detected.

### Population genetics

Pair wise *F*st values for each population are respectively reported in Tables 3.4 and 3.5 for COI and microsatellite data. Most pair wise structuring was significant, suggesting a limited amount of population connectivity in this species as has also been observed in other coastal in-shore fishes. The difference in magnitude of structuring between the COI marker and microsatellite loci is not unexpected, given that maternally inherited mtDNA should have a small effective population size relative to the total population sampled using microsatellites that are from the nuclear genome (Birky *et al.* 1989). Furthermore, hyper-variable microsatellite loci have smaller maximum *F*st values than mtDNA (Hedrick, 1999). Some populations, such as Archer River and Love River; Keppel Bay and Port Alma, have a strong and significant population structure in the mtDNA but not in the microsatellite loci. These populations share a close geographic proximity and in both cases one population was sampled a year before the other, therefore they may be considered temporal samples of the same location.

**Table 3.2:** Sample sizes (*n*), number of haplotypes (*n* haplotypes), haplotype diversity (*h*), nucleotide diversities ($\pi$) of COI for all regions and populations of *Eleutheronema tetradactylum*.

| | ***n*** | ***n* haplotypes** | ***h*** | ***π*** |
|---|---|---|---|---|
| **WA** | **132** | **8** | **0.18** | **0.0003** |
| Roebuck Bay 1 | 38 | 1 | 0.00 | 0.0000 |
| Eighty Mile Beach | 47 | 7 | 0.43 | 0.0007 |
| Roebuck Bay 2 | 47 | 2 | 0.04 | 0.0001 |
| **NT** | **112** | **19** | **0.52** | **0.0011** |
| Walker River | 27 | 9 | 0.51 | 0.0012 |
| Blue Mud Bay | 39 | 6 | 0.53 | 0.0009 |
| Roper River | 46 | 9 | 0.50 | 0.0011 |
| **QLD GoC** | **124** | **14** | **0.59** | **0.0012** |
| Archer River | 39 | 11 | 0.74 | 0.0018 |
| Arthur's Creek | 45 | 5 | 0.53 | 0.0010 |
| Love River | 40 | 5 | 0.45 | 0.0007 |
| **QLD EC** | **117** | **19** | **0.83** | **0.0024** |
| Cleveland Bay | 30 | 7 | 0.77 | 0.0021 |
| Keppel Bay | 41 | 10 | 0.84 | 0.0021 |
| Port Alma | 46 | 7 | 0.62 | 0.0011 |

**Table 3.3:** Sample sizes (*n*), observed heterozygosity and expected heterzygosity ($H_O/H_E$) and calculated probability of departure from Hardy-Weinberg equilibrium over five microsatellite loci. No evidence for linkage between loci was detected.

| | ***n*** | **Etet1** | **Etet2** | **Etet3** | **Etet4** | **Etet5** |
|---|---|---|---|---|---|---|
| **WA** | **96** | **0.958/0.923, p= 0.17** | **0.641/0.691, p= 0.21** | **0.597/0.718, p= 0.09** | **0.750/733, p= 0.70** | **0.578/0.606, p= 0.29** |
| Roebuck Bay | 48 | 0.951/0.929, p= 0.25 | 0.583/0.701, p= 0.08 | 0.600/0.715, p= 0.21 | 0.738/0.736, p= 0.51 | 0.434/0.635, p= 0.0002 |
| Eighty Mile Beach | 48 | 0.973/0.920, p= 0.3 | 0.729/0.694, p= 0.47 | 0.595/0.736, p= 0.18 | 0.720/0.750, p= 0.53 | 0.708/0.618, p=0.18 |
| **QLD GoC** | **96** | **0.772/0.932, p = 0.00** | **0.787/0.882, p= 0.02** | **0.710/0.741, p= 0.35** | **0.933/0.897, p= 0.80** | **0.756/0.904, p= 0.03** |
| Archer River | 48 | 0.772/0.927, p= 0.01 | 0.739/0.888, p= 0.002 | 0.625/0.720, p= 0.23 | 0.975/0.911, p= 0.96 | 0.682/0.899, p= 0.002 |
| Love River | 48 | 0.760/0.9388, p=0.00 | 0.772/0.886, p= 0.07 | 0.659/0.734, p= 0.12 | 0.904/0.883, p= 0.74 | 0.837/0.908, p= 0.55 |
| **QLD EC** | **96** | **0.903/0.938, p= 0.37** | **0.740/0.854, p= 0.51** | **0.722/0.705, p= 0.61** | **0.853/0.821, p= 0.57** | **0.740/0.843, p= 0.22** |
| Cleveland Bay | 48 | 0.875/0.930, p= 0.36 | 0.717/0.867, p= 0.31 | 0.666/0.667, p= 0.73 | 0.847/0.849, p=0.44 | 0.868/0.876, p= 0.51 |
| Port Alma | 48 | 0.911/0.921, p= 0.73 | 0.756/0.824, p= 0.513 | 0.808/0.744, p= 0.48 | 0.808/0.773, p=0.25 | 0.545/0.691, p= 0.051 |

Overall population structure across all sampled populations was conspicuous and significant in both sets of data: $\Phi_{st}$ = 0.62, p < 0.0001 (COI) and $\Phi_{st}$ = 0.073, p < 0.0001 (microsatellites). The dominant genetic structure in the data is the partition between Western Australia and the rest of the sampled range as evidenced by the deep pair wise *F*st values. A biomodal mismatch distribution (Figure 3.2) may be an indication of population contraction/fragmentation, as has been suggested for other organisms (e.g. Strasburg *et al.* 2007), and most likely reflects an east-west split between the Western Australian populations and all others.

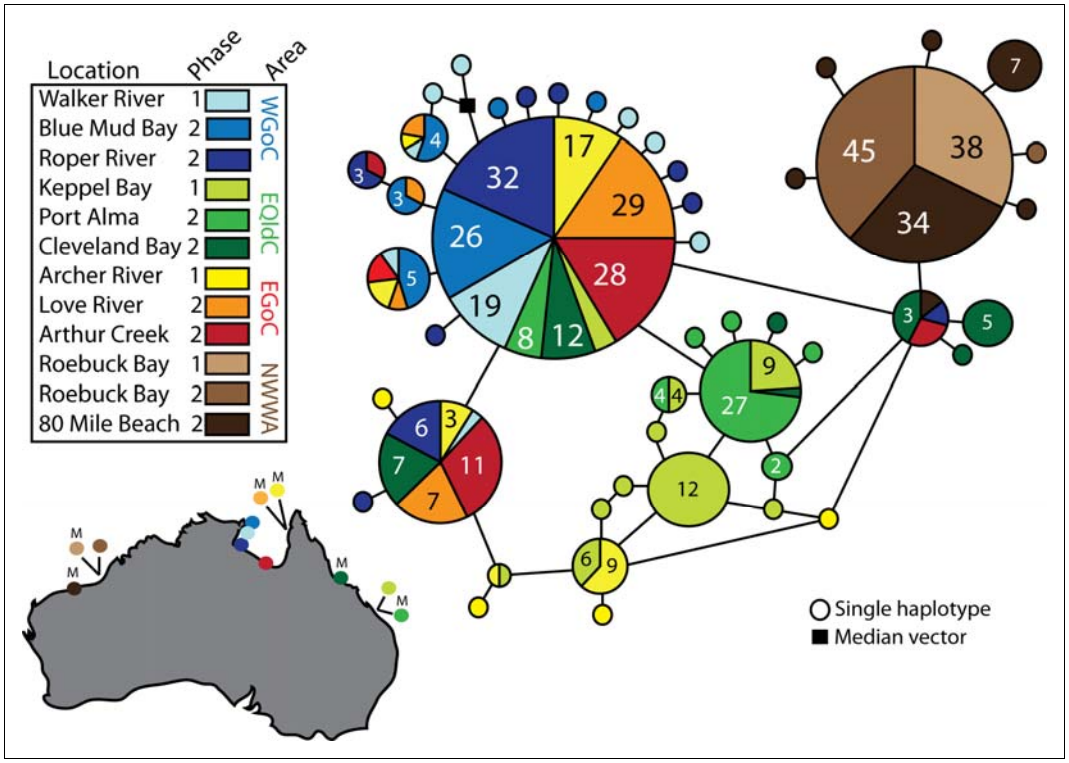



**Figure 4.1:** Sample collections of *E. tetradactylum* across Northern Australia. Some locations are sampled in both phase 1 and 2 as temporal samples. Populations marked with 'M' were also analysed for five microsatellite loci. Also shown, minimum spanning network of 485 *E. tetradactylum* COI haplotypes.

**Table 3.4:** Pairwise population structures ($F_{st}$) generated from 633 bp of the mitochondrial COI region, for twelve sampled *E. tetradactylum* populations (n = 485). Significant values are highlighted in bold.

| | AR | AC | LR | KB | CB | PA | WR | BMB | RR | RB1 | EMB |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **AR** | | | | | | | | | | | |
| **AC** | **0.107** | | | | | | | | | | |
| **LR** | **0.111** | *-0.005* | | | | | | | | | |
| **KB** | **0.298** | **0.460** | **0.471** | | | | | | | | |
| **CB** | **0.134** | **0.074** | **0.110** | **0.385** | | | | | | | |
| **PA** | **0.469** | **0.509** | **0.532** | **0.250** | **0.420** | | | | | | |
| **WR** | **0.104** | **0.055** | *0.021* | **0.416** | **0.116** | **0.475** | | | | | |
| **BMB** | **0.135** | **0.092** | **0.054** | **0.455** | **0.162** | **0.508** | *0.007* | | | | |
| **RR** | **0.106** | *-0.000* | *-0.008* | **0.451** | **0.093** | **0.496** | *0.023* | **0.057** | | | |
| **RB1** | **0.782** | **0.840** | **0.888** | **0.797** | **0.724** | **0.863** | **0.858** | **0.865** | **0.838** | | |
| **EMB** | **0.724** | **0.769** | **0.804** | **0.754** | **0.651** | **0.807** | **0.768** | **0.785** | **0.768** | **0.068** | |
| **RB2** | **0.792** | **0.845** | **0.889** | **0.807** | **0.737** | **0.867** | **0.862** | **0.868** | **0.842** | *-0.004* | **0.071** |

**Table 3.5:** Pairwise population structures ($F_{st}$) generated from 5 microsatellite loci for 6 sampled. *E. tetradactylum* populations (n=288). Significant values are highlighted in bold.

| | AR | LR | CB | PA | RB | EMB |
|---|---|---|---|---|---|---|
| **AR** | | | | | | |
| **LR** | 0.0005 | | | | | |
| **CB** | **0.0154** | **0.0153** | | | | |
| **PA** | **0.0435** | **0.0312** | **0.0321** | | | |
| **RB** | **0.1098** | **0.1054** | **0.1259** | **0.1538** | | |
| **EMB** | **0.0974** | **0.0979** | **0.1147** | **0.1536** | 0.0003 | |

### Assignment tests

The mean posterior probability (L(K)), the rate of change of posterior probability (L'(K)), the second order rate of change of the posterior probability (|L''(K)|), and the ΔK values all strongly indicate the presence of two distinct genetic clusters (Figure 3.3(a)). Populations from eastern Australia and the Gulf of Carpentaria belong to the same genetic cluster, while the two populations from Western Australia belong to a second genetic cluster (Table 3.6 and Figure 3.3(b)). However, a small fraction of individuals in each population belongs to the opposite genetic cluster, indicating some degree of connectivity over the evolutionary time scales measured when using genetic markers that are passed down from generation to generation. Expected heterozygosity was high in both populations (cluster 1 = 0.8647, cluster 2 = 0.7536).

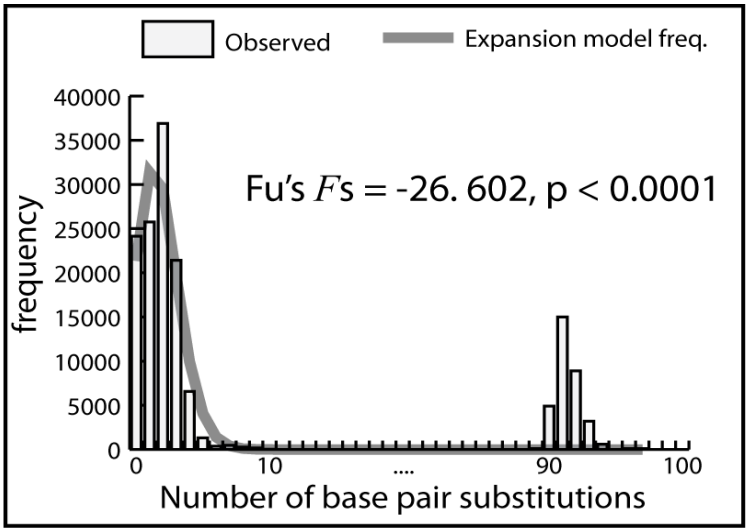



**Figure 3.2:** Mismatch distribution for COI haplotypes and expansion model frequency. The right hand peak has a mode of 300 observations but been exaggerated in order to make it visible. Fu's *F*s and corresponding p value.

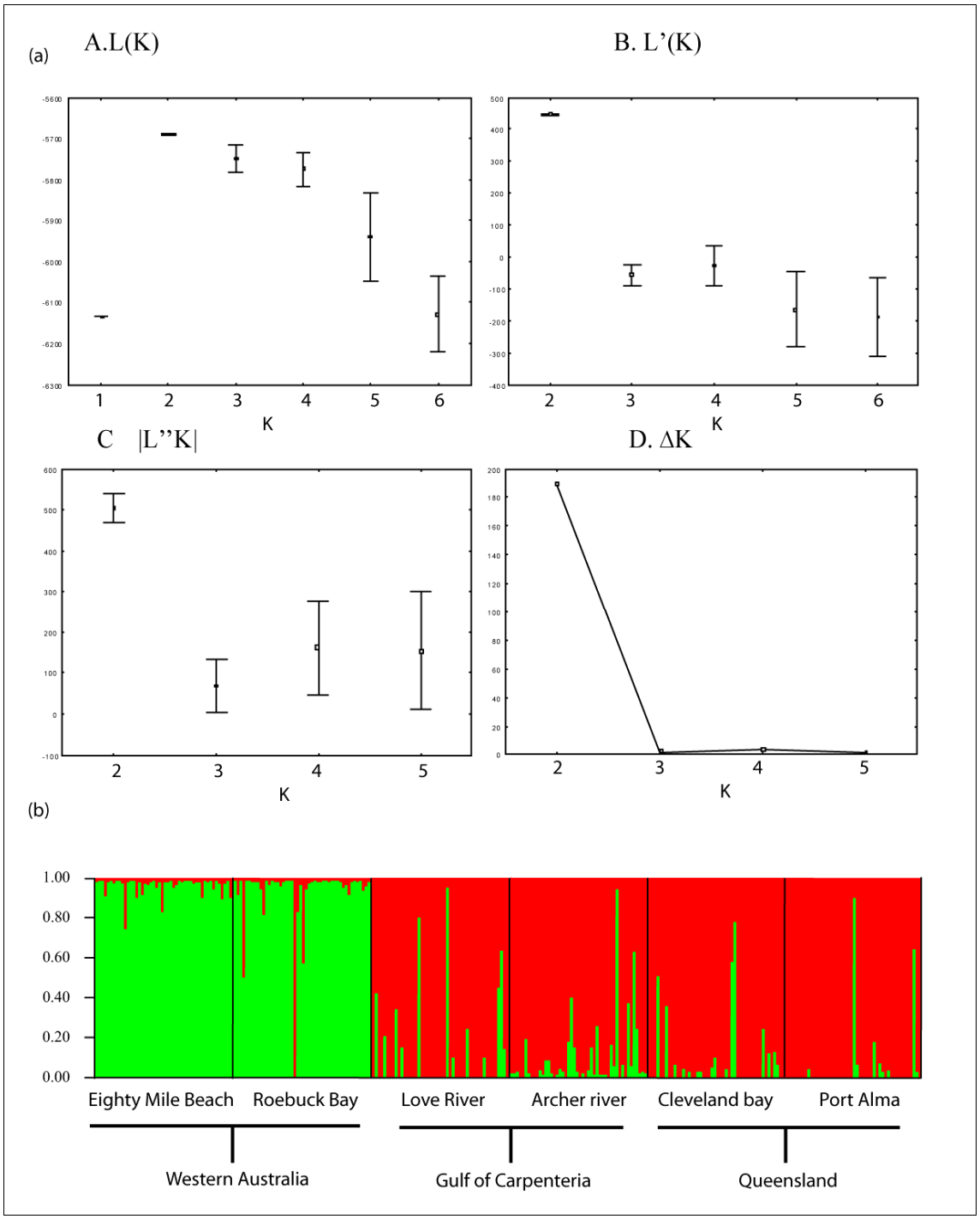



**Figure 3.3:** Determination of the true number of K using the method from Evanno *et al.* (2005). (a). (A) mean L(K)± sd for 20 independent runs. (B) Likelihood distribution rate of change (L'(K)= L(K)-(L(K-1)). (C) Second order rate of change (absolute values) of likelihood distribution |L''K|=|L'(K+1)-L'(K)|. (D) ΔK= m|L''(K)|/s[L(K)]. According to this methods K = 2 clearly stands out as the true number of genetic clusters. (b). Bayesian population assignment test inferred from five microsatellite loci. Western Australian populations are colored green, while all other populations are shown in red. Vertical lines represent individuals and the likelihood of that individual belonging to a specific cluster. Vertical black lines represent the geographic boundary between populations.

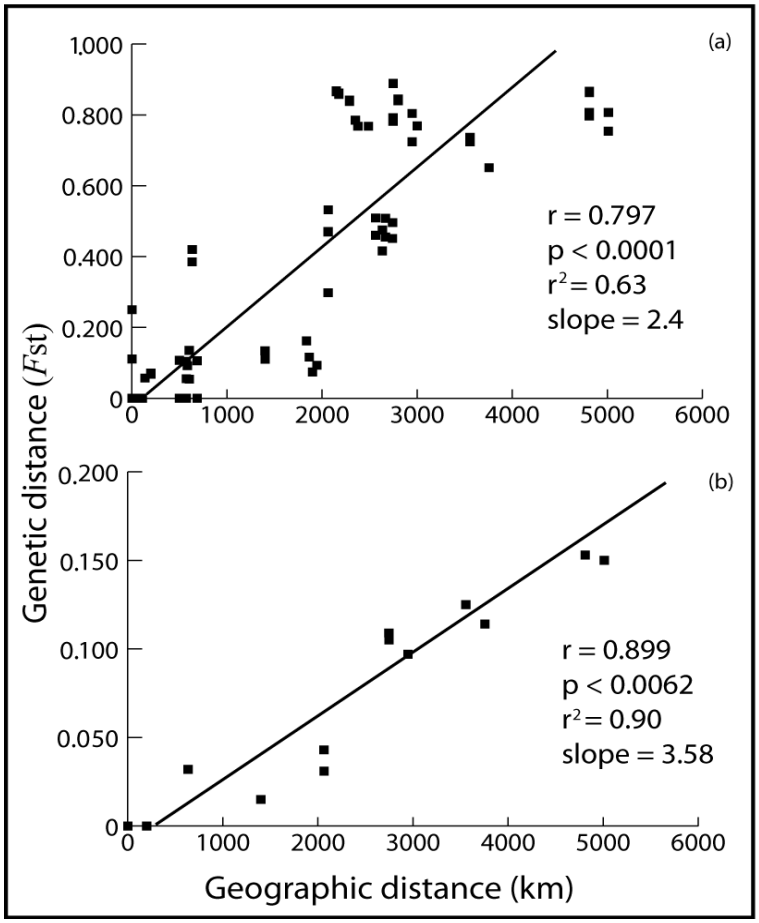



**Figure 3.4:** Isolation-by-distance analysis generated from 10,000 mantel test randomisations. (a) genetic distance (mitochondrial COI) *F*st against geographic distance and corresponding correlation coefficient (r), p value (p), coefficient of determination ($r^2$) and slope of the regression line. (b) genetic distance (microsatellite loci) *F*st against geographic distance (km) and corresponding data.

**Table 3.6:** Proportion of membership of each population to the inferred genetic clusters.

| Population | Inferred Genetic Clusters | | Sample size |
|---|---|---|---|
| | 1 | 2 | |
| PA | 0.946 | 0.054 | 48 |
| CB | 0.921 | 0.079 | 48 |
| LR | 0.886 | 0.114 | 48 |
| AR | 0.901 | 0.099 | 48 |
| RB | 0.067 | 0.933 | 48 |
| EMB | 0.036 | 0.964 | 48 |

### Isolation by distance

Plots of geographic versus genetic distance are shown in Figure 3.4. For both sets of data distance appears to be a significant feature of the observed genetic patterns. For the microsatellite data, the relationship between geographic and genetic distance was positive and nearly linear ($r = 0.948$, $p < 0.0062$) with a regression line slope of 3.587. Geographic distance accounted for approximately 90% of the genetic variance in the microsatellite data ($r^2 = 0.899$). In the mitochondrial COI marker the relationship between geographic and genetic distance was also strong but comparatively less than in the microsatellite loci ($r = 0.797$, $p < 0.0001$, slope = 2.4). Overall, geographic distance accounted for less than 65% of the observed variance ($r^2 = 0.635$).

Excluding comparisons between populations: Love River, Archer River, Keppel Bay and Port Alma from the COI analysis did not yield a notably altered result ($r = 0.8$, $p < 0.0001$, slope = 2.4, $r^2 = 0.65$), suggesting that the unusual structuring observed at these locations is not producing a significant bias. Also, to test for biases in sample size, the COI data set was scaled down to include only the populations for which microsatellite data was obtained. When analysing this subset of COI data, the results were: $r = 0.84$, $p < 0.05$, $r^2 = 0.7$, slope = 1.983. Although the reduction in populations appeared to slightly enhance the signal of isolation-by-distance, the outputs are still considerably less than observed in the microsatellite data. The original analysis of samples from the 11 populations, containing the most data, is therefore preferred.

## 3.4 Discussion

The genetic patterns exhibited by *E. tetradactylum* populations of Northern Australia are best described in terms of four factors: (1) subdivision between Western Australia and all other sampled populations; (2) significant genetic structuring between 49 out of 55 population pairs examined, including most (but not all) population pairs within the three geographic regions sampled here; (3) significant correlation between genetic and geographic distances (i.e. isolation by distance); and (4) an unusual temporal instability of the mitochondrial DNA but not the nuclear genome.

### East-West Separation

The unique genetic identity of the Western Australia populations compared to the rest of the sampled range was detected by multiple analyses and appears to be unambiguous. Isolation-by-distance alone does not satisfactorily explain this distinction because, based on our generalised coastline measurements, the populations of the Western Gulf of Carpentaria are nearly equidistant to the east and west limits of sampling. However, the Gulf of Carpentaria is an ephemeral body of water and was dry at 75 metres below present-day sea level (Voris, 2000). The genetic affinity of the gulf populations to the east Queensland coast could be due to recent colonisations from that area since the last glacial maximum within the last 10kya. Furthermore, the closure of the Torres Strait, which is <25 metres deep

(Saint-Cast and Condie, 2006), would have extended the coastline distance between east and west Australia by including New Guinea. Alternatively, there may be a current barrier to dispersal between Roebuck Bay and the Gulf of Carpentaria, the nature of which can only be speculated at present. However, because assignment tests show a small amount of genetic exchange it seems best to conclude that limited dispersal is maintaining pre-existing genetic signatures.

### Isolation-by-distance

Little is known about the movements of *E. tetradactylum* but the strong correlation between geographic distance and genetic distance suggests that, (1) the pelagic larvae of this species have a tendency to recruit close to where they were spawned; and (2) the adults have limited home range sizes and may exhibit spawning site fidelity.

Retention of pelagic eggs and larvae may be assisted by coastal eddies and other physical oceanographic features (Cowen, 2002). Increasing evidence also suggests that pelagic fish larvae have highly developed sensory organs that aid in natal homing (Lecchini *et al.* 2005; Gerlach *et al.* 2007) and that larvae determine their own dispersal trajectories through active behaviour (Leis *et al.* 2007). *E. tetradactylum* larvae are reported to recruit to mangrove areas (Manson *et al.* 2005), which probably give off chemical cues that are detectable at some distance. Leis *et al.* (2009) observed that larvae of *E. tetradactylum*, from aquaculture stocks, when released into the ocean initially swam in circles; a behaviour that may be an attempt to remain within local olfactory plumes, as has been documented for some reef fish larvae, but not others (Gerlach *et al.* 2007). Apart from swimming in circles, *E. tetradactylum* larvae were shown to have a significant sense of directionality and a tendency to favour shallow water. In general, the behaviours of *E. tetradactylum* larvae appear to be consistent with natal homing.

Adult *E. tetradactylum* commonly attain sizes greater than fifty centimetres and should be capable of along-shore migration. However, because isolation by distance was so highly significant it is likely that these fish have limited home range sizes, or if individuals do roam great distances, they return to the same spawning areas to mate. Zischke *et al.* (2009) showed, by mark and recapture studies, that time at liberty was not significantly correlated with the distance from the original capture site. Thus, limited home range sizes and site attachment are consistent with both genetic and recapture data.

### Temporal instability

Temporally discrete population structuring is common in many marine organisms, even when there is a prevailing lack of spatial population structure. Sometimes this pattern is called chaotic patchiness and is frequently observed in inter-tidal organisms (see Hellberg *et al.* 2002). Such patterns are also present in fish and in some cases this may be due to seasonally shifting ocean currents (Selkoe *et al.* 2006). Although our temporal data is limited to only two samples, it is possible that sweepstakes reproductive

success, possibly mediated by currents, is the underlying process of this pattern. However, this conclusion is objectionable because, notwithstanding some populations were temporally sampled, no attempt was made to collect age cohorts. So, unless samples were composed mainly of kinship aggregations, sweepstakes reproductive success seems unlikely. Furthermore, such a notion would intuitively contradict the high degree of spatial structuring of the data and does not explain why only the mtDNA shows this pattern.

Another explanation is that the number of breeding females in the population is relatively small compared to males. If only a few females contribute to the mitochondrial gene pool each year, then the ability for maternally inherited genes to maintain specific frequencies in the population are going to be far less than nuclear genes. If mature female *E. tetradactylum* possess a harem of immature males, similar to what has been described in monandric hermaphroditic fishes (Walker and McCormick, 2004; McBride and Johnson, 2007), then a matriarchal social hierarchy may be responsible for patchiness in mitochondrial gene frequencies.

As an illustration, suppose that a population has a dominant female, which is often the largest individual in such mating systems and the larger female has been shown to produce many more eggs than other smaller females (e.g. the stripey snapper, Evans *et al.* 2008), then a small number of females may have an overwhelming reproductive advantage. Also suppose that the dominant female is replaced every year by a female with a different mtDNA haplotype. Lastly, suppose that there are three haplotypes in the population. In other words, each haplotype takes turns occupying the dominant spot. Under this scenario, any given haplotype only has the opportunity to be passed on every three years, while bi-parentally inherited nuclear DNA is passed on during every spawning cycle.

Without corroboration this explanation is largely conjecture. Nevertheless, isolation-by distance analysis indicated that geographic distance accounted for about 90% of the genetic variation of the microsatellites, while only slightly more than 60% of the variation in COI. Part of the difference between the two data sets may be due to a disparity in population number but because of the maternally inherited nature of the mitochondrial genome it is probable that some sort of sex bias is responsible for the remaining variance.

## Conclusions

Apart from a deep genetic partition between the populations of Western Australia and the Gulf of Carpentaria, which is probably residual from low-sea-level land barriers, the predominant feature of *E. tetradactylum* populations is Isolation by distance. This indicates that small home range sizes of adult fish and natal homing in larval fish are highly probable. Also, instability in the frequencies of mtDNA haplotypes between phases of this research may be evidence for a female social hierarchy. It is therefore recommended that management strategies for this fishery be implemented on a local scale,

particularly if these genetic findings are corroborated by other approaches that measure population differentiation at ecological timeframes (e.g. parasite composition, population demography and otolith chemistry).

# Chapter 4: The stock structure of blue threadfin, *Eleutheronema tetradactylum*, across northern Australia as inferred from stable isotopes in sagittal otolith carbonate

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## 4.1 Introduction

The blue threadfin *Eleutheronema tetradactylum*, is an inshore tropical polynemid fish that has a wide distribution ranging from the Persian Gulf in the western Indian Ocean, through to China and southern Japan in the east and south to southern New Guinea and Australia (Saleem *et al.* 1989; Janekitkarn *et al.* 1999; Motomura *et al.* 2001). In Australian waters, *E. tetradactylum* ranges from the Ashburton River in Western Australia to Sandy Cape in Queensland. It is an epipelagic, neritic, coastal species that typically occupy shallow, turbid inshore waters (Newman *et al.* 2003; Pember, 2006). The available biological data on *E. tetradactylum* in Australian waters indicate that size is variable among locations; in the Gulf of Carpentaria they attain a maximum size of at least 1,000 mm and 10 kg (Bibby and McPherson, 1997), whereas in Western Australian waters they attain a maximum size of at least 800 mm and 5 kg (Pember *et al.* 2005; Pember, 2006), they exhibit rapid growth and reach at least six years of age (Pember *et al.* 2005; Pember, 2006).

Throughout its distribution, *E. tetradactylum* provides an important source of food for local populations and forms the basis of substantial commercial, recreational and artisanal fisheries (Gopalakrishnan, 1972; Iwatsuki *et al.* 2000; Motomura *et al.* 2002). Among the indigenous people of northern Australia, *E. tetradactylum* is an important food species, having significant economic, health and social value (Pember, 2006; Moore *et al.* 2009). Across northern Australia, *E. tetradactylum* is an important commercial and recreational fish species. In 2005, the total landings of *E. tetradactylum* from the State of Queensland (Gulf of Carpentaria and east coast) were over 203 metric tons with smaller landings from the commercial fisheries of the Northern Territory and Western Australia. A national recreational fishing survey undertaken in 2000/2001 reported that the Australia wide catch of all threadfins was over 185,000 individual fish (Henry and Lyle, 2003). Despite its economic importance to communities across

northern Australia little is known about its stock structure and movement. Information on stock structure is vital for determining the appropriate spatial scale at which a species should be managed to ensure the sustainable harvest and management of the resource particularly if a species is highly targeted and/or heavily exploited and/or is being managed as a single homogeneous stock or management unit across a wide spatial area. A study on the Queensland east coast found that that blue threadfin exhibit limited movement and population sub-units may be isolated by physical barriers such as headlands (Zischke *et al.* 2009).

A population management unit capable of independent exploitation or fishable stock can be considered to be a spatially defined group of fish, possibly a breeding population, which exhibits no significant mixing with neighbouring individuals for a temporal period. When using stable isotopes in sagittal otolith carbonates this temporal period is defined as the longevity of the fish (the entire life of the individual fish) as the whole otolith is utilised. Stable isotopes are also advantageous to studies examining population structure, as they are neutral, non-radioactive variants of an element and, as a result of their slightly different atomic masses, their relative incorporation into fish otoliths can be modified by environmental conditions or biological activity (Campana 1999).

As stable isotope values of $\delta^{18}O$ and $\delta^{13}C$ from individual fish can differ with latitude and location (Campana, 1999; Høie *et al.* 2003; LeGrande and Schmidt, 2006; Solomon *et al.* 2006), these variations in stable isotope composition serve as natural tags that are characteristics of different locations and as such different population management units (e.g. Gao *et al.* 2001). Thus, the presence of stable isotopes in sagittal otoliths and their variation with environmental conditions provide an opportunity to use them as natural markers to explore the spatial structure of fish populations (e.g. Edmonds and Fletcher, 1997; Kennedy *et al.* 1997; Schwarcz *et al.* 1998; Edmonds *et al.* 1999; Newman *et al.* 2000; Gao *et al.* 2001; Gao and Beamish, 2003; Gao *et al.* 2004; Newman *et al.* 2009a). In theory, if the stable isotopes of $\delta^{18}O$ and $\delta^{13}C$ in the sagittal otolith carbonate from two groups of fish are similar they are likely to have had a common environmental life history. In contrast, if the stable isotopes of $\delta^{18}O$ and $\delta^{13}C$ in the sagittal otolith carbonate of one group of fish is significantly different from that of another group of fish they are likely to have had a different environmental life history. Similar logic has been applied to the use of trace elemental concentrations in otolith carbonates as a methodology to discriminate among fish stocks (e.g. Campana *et al.* 1994).

In this study we used otolith stable isotope signatures to determine the stock structure of *E. tetradactylum* across northern Australia. This study formed part of a major research effort that aimed to determine whether it is appropriate to manage stocks of *E. tetradactylum* independently in the various management jurisdictions across northern Australia. Populations of *E. tetradactylum* are currently managed either as separate fisheries or as part of mixed species inshore net fisheries by state/territory-based management agencies in Western Australia, the Northern Territory and Queensland (Gulf of

Carpentaria and east coast). The appropriateness of managing these fisheries separately, as under existing arrangements, depends on the degree of mixing between management areas.

## 4.2 Methods

### Sampling design

Samples of *E. tetradactylum* were collected using monofilament gillnet fishing methods at each location across northern Australia. Otoliths were collected from fish at eleven locations extending from Western Australia across northern Australian waters, throughout the Gulf of Carpentaria (GoC) to sites on the Queensland east coast of Australia (Figure 4.1), covering a coastline length of approximately 14,000 kilometres. Locations sampled were Eighty Mile Beach, Western Australia (EMB), Roebuck Bay, WA, (RB), Blue Mud Bay, Northern Territory, (BMB), Walker River, NT (WR), Roper River, NT (RR), Arthur's Creek, QLD (AC), Archer River, QLD (AR), Love River, QLD (LR), Cleveland Bay, QLD (CB), Keppel Bay, QLD (KB), and Port Alma, QLD (PA). A target total of forty pairs of sagittal otolith samples were collected from each location. Where possible, samples were collected on two separate occasions, a minimum of six months apart. A summary of the sampling data, and summary results from the stable isotope analyses of the sagittal otolith carbonate of *E. tetradactylum* from across northern Australia are detailed in Appendix 4.1.

### Otolith preparation

Sagittae were allowed to dry in envelopes prior to processing. One sagitta from each fish was selected at random and cleaned by scrubbing with a nylon brush under ultrapure water, air-dried (50°C) and crushed to powder in an agate mortar and pestle. Powdered sagittae were cleaned of organic matter (contaminants) by treatment with hydrogen peroxide and deproteinated by dissolving and extracting protein using a centrifuge. The sagittae were deproteinated to remove proteins contained in the otolith carbonate matrix. These proteins could potentially degrade to form $CO_2$ on analysis of the carbon and oxygen stable isotopes and therefore potentially interfere with the measurement of the carbon and oxygen stable isotopes.

Powdered sagittae were then analysed for oxygen ($^{18}O/^{16}O$) and carbon ($^{13}C/^{12}C$) stable isotopes by standard mass spectrometric techniques after the carbonate was decomposed to $CO_2$ with 100% phosphoric acid (Newman *et al.* 2009a). A laboratory reference sample was run every thirty samples. The laboratory reference sample, consisting of a batch solution of digested otolith reference material, was used to monitor measurement precision across sample batches, and was subsequently used to normalise sample batches to a constant reference value. Stable isotopes are reported using the international standard delta ($\delta$) notation relative to the PDB-1 standard for carbonates (i.e. $\delta^{18}O$ and $\delta^{13}C$).

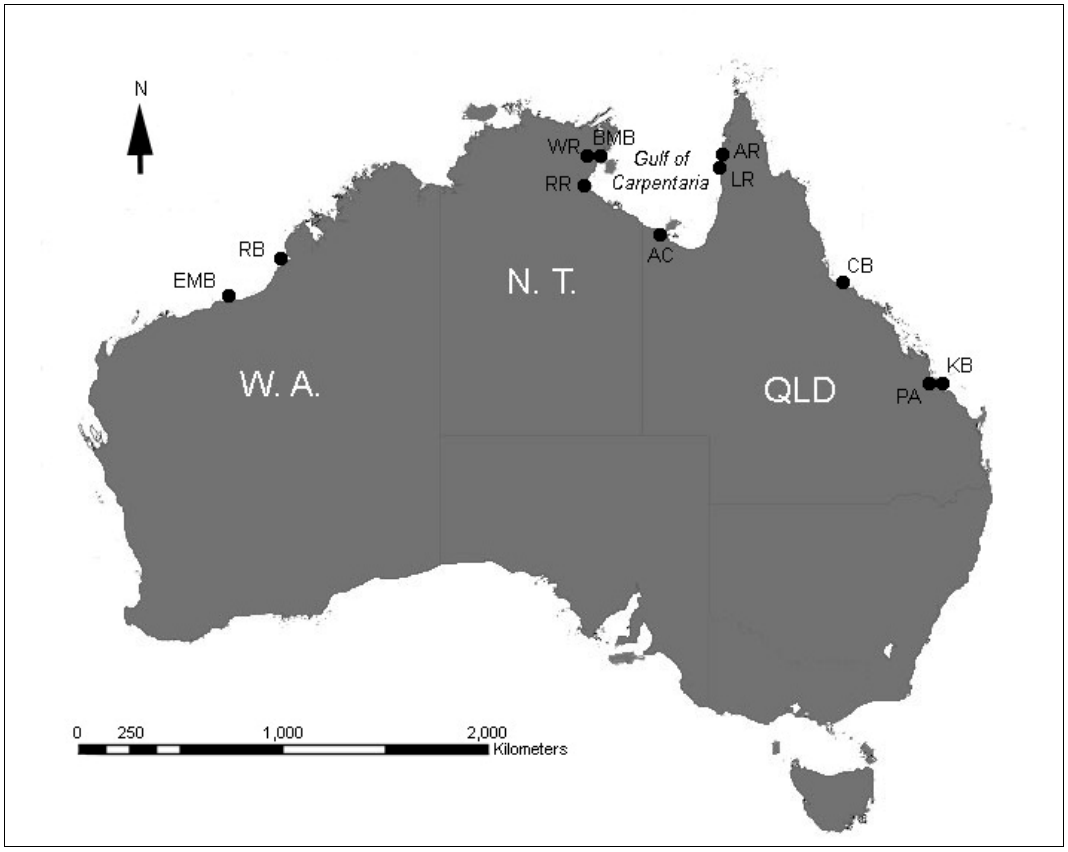



**Figure 4.1:** Locations of the sampling sites for *E. tetradactylum* across northern Australia (EMB – Eighty Mile Beach; RB – Roebuck Bay; BMB – Blue Mud Bay; WR – Walker River, RR – Roper River; AC – Arthur's Creek; LR – Love River; AR – Archer River; CB – Cleveland Bay; PA – Port Alma; and KB – Keppel Bay).

### Statistical analysis

Univariate and multivariate analyses were undertaken to assess the variability in stable isotope signatures of *E. tetradactylum* among locations. The temporal variability in the stable isotopes of $\delta^{18}O$ and $\delta^{13}C$ was assessed for Roebuck Bay, sampled in March 2008 and August 2008. Analysis of covariance (ANCOVA) was used to determine the effect of sampling period (fixed factor, Type III sum of squares) with otolith weight as a covariate; the interaction term was also tested (Zar, 1999). Linear regression was also used to explore the relationship between stable isotopes of $\delta^{18}O$ and $\delta^{13}C$ and otolith weight within each sampling period (Zar, 1999).

The spatial variation in stable isotopes ($\delta^{18}O$ and $\delta^{13}C$) was evaluated using ANCOVA to test for differences among locations (fixed factor, Type III sum of squares) with otolith weight as a covariate including assessment of the interaction effect (Zar, 1999). A one-way ANOVA was further used to test the effect of location, *a posteriori* multiple comparison of means were carried out using Student-Newman-Kuels tests to evaluate differences.

A non-parametric multivariate analysis of variance (Permutational ANOVA (PERMANOVA): Anderson, 2001; Anderson and Gorley, 2007) was used to test for effects of location on both $\delta^{18}O$ and $\delta^{13}C$ to determine whether there was greater separation of sites when both stable isotopes are considered simultaneously in a multivariate analysis. The $\delta^{18}O$ and $\delta^{13}C$ values were first normalised to make them scale independent with the resemblance matrix based on Euclidean distance. PERMANOVA was based on a single fixed factor; location, with Type III sums of squares and unrestricted permutations. *A posteriori* multiple comparisons of means were undertaken using the pairwise tests available within the PERMANOVA routine (Anderson and Gorley, 2007). To visualise the data and evaluate whether there were latitudinal or longitudinal trends in the stable isotope signatures of the locations, the means and standard errors were calculated for each isotope at each location. Locations were ranked from 1 to 11, with 0.5 added to the rank of Roebuck Bay I (March 2008) for Roebuck Bay II (August 2008) with these ranks included as 'factors' in the dataset. Means were normalised and the Euclidean distances calculated. Non-multidimensional scaling was used to visualise the sites with a trajectory linking each by either latitude or longitude.

## 4.3 Results

### Temporal comparison

Sampling period had a significant effect on both $\delta^{18}O$ and $\delta^{13}C$ in Roebuck Bay, as did the covariate, otolith weight. For $\delta^{13}C$, there was no interaction between otolith weight and sampling period ($p = 0.284$) but both otolith weight and sampling period were significant, accounting for approximately 53% of the variation in $\delta^{13}C$ (Table 4.1). Sampling period had a much greater effect on $\delta^{13}C$ than did otolith weight. The exclusion of otolith weight as a covariate resulted in sampling period accounting for approximately 40% of the variation in $\delta^{13}C$. Linear regressions relating $\delta^{13}C$ and otolith weight for each sampling period in Roebuck Bay (March 2008 and August 2008) were significant ($p=0.0006$ and $p=0.02$), but only accounted for 26% and 11% of the variation in $\delta^{13}C$ (Figure 4.2).

The same pattern occurred for $\delta^{18}O$ with the interaction of otolith weight and sampling period again not significant ($p = 0.725$). However, both sampling period and otolith weight comprised approximately 59% of the variation in $\delta^{18}O$ (Table 4.1). Again, sampling period had a greater effect on $\delta^{18}O$ than otolith weight. With the removal of the covariate, sampling period alone accounted for approximately 39% of the variation in $\delta^{18}O$. Linear regressions relating $\delta^{18}O$ and otolith weight for each sampling period in Roebuck Bay (March 2008 and August 2008) were significant ($p <0.0001$) and accounted for 32% and 37% of the variation in $\delta^{18}O$ in each sampling period (Figure 4.2).

### Spatial comparison

This study examined populations of *E. tetradactylum* sampled at eleven locations (Appendix 4.1). However, as Roebuck Bay showed significantly different isotope signatures between the two sampling periods, these two sampling periods were treated further as 'locations'. ANCOVA indicated that both $\delta^{18}O$ and $\delta^{13}C$ were significantly different for the factors location and otolith weight, with a significant interaction between location and otolith weight (Table 4.2). However, in both cases, the variance explained by location far exceeded the variance explained by either otolith weight or the interaction. The exclusion of otolith weight and the interaction of both factors reduced the amount of variation explained in $\delta^{18}O$ and $\delta^{13}C$ by only two and four percent, respectively (Table 4.2). As such, variation in both $\delta^{18}O$ and $\delta^{13}C$ were significantly different among locations.

**Table 4.1:** ANCOVA of the $\delta^{13}C$ and $\delta^{18}O$ values of the sagittal carbonate of *Eleutheronema tetradactylum* in Roebuck Bay (Western Australia) to determine the effect of sampling period (fixed factor, Type III sum of squares) with otolith weight as a covariate.

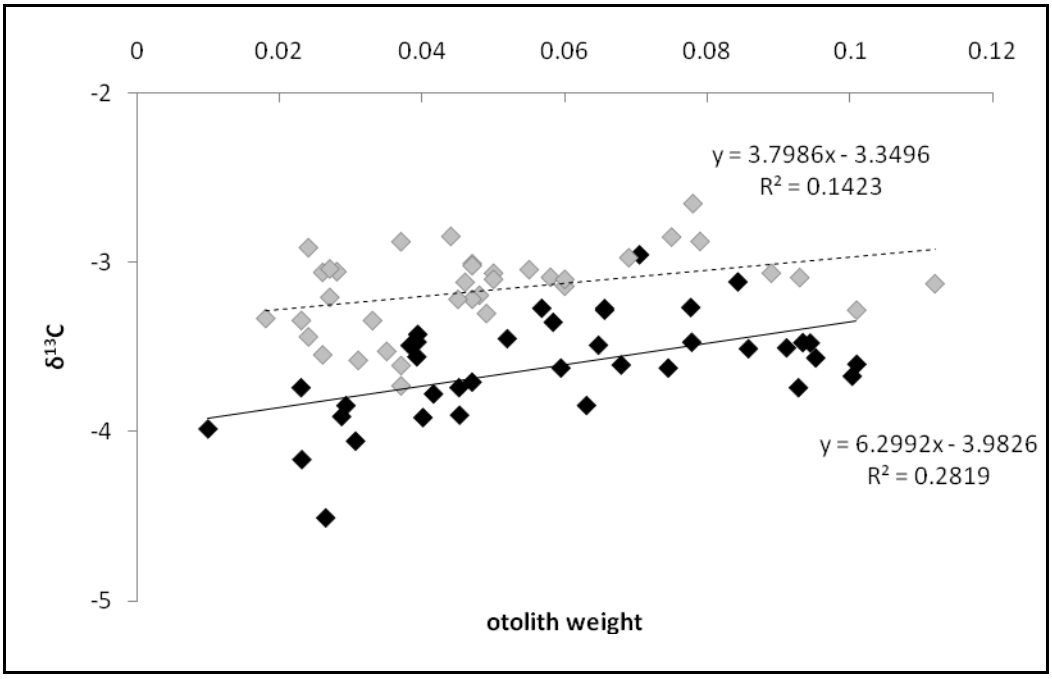
**Dependent Variable: $\delta^{13}C$**

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | 4.961[a] | 2 | 2.481 | 42.699 | .000 |
| Sampling period | 4.489 | 1 | 4.489 | 77.276 | .000 |
| Otolith weight | 1.154 | 1 | 1.154 | 19.855 | .000 |
| Error | 4.183 | 72 | .058 | | |
| Corrected Total | 9.144 | 74 | | | |

**Dependent Variable: $\delta^{18}O$**

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | 1.691[a] | 2 | .845 | 54.766 | .000 |
| Sampling period | 1.367 | 1 | 1.367 | 88.564 | .000 |
| Otolith weight | .607 | 1 | .607 | 39.322 | .000 |
| Error | 1.111 | 72 | .015 | | |
| Corrected Total | 2.802 | 74 | | | |

**A.**




**B.**

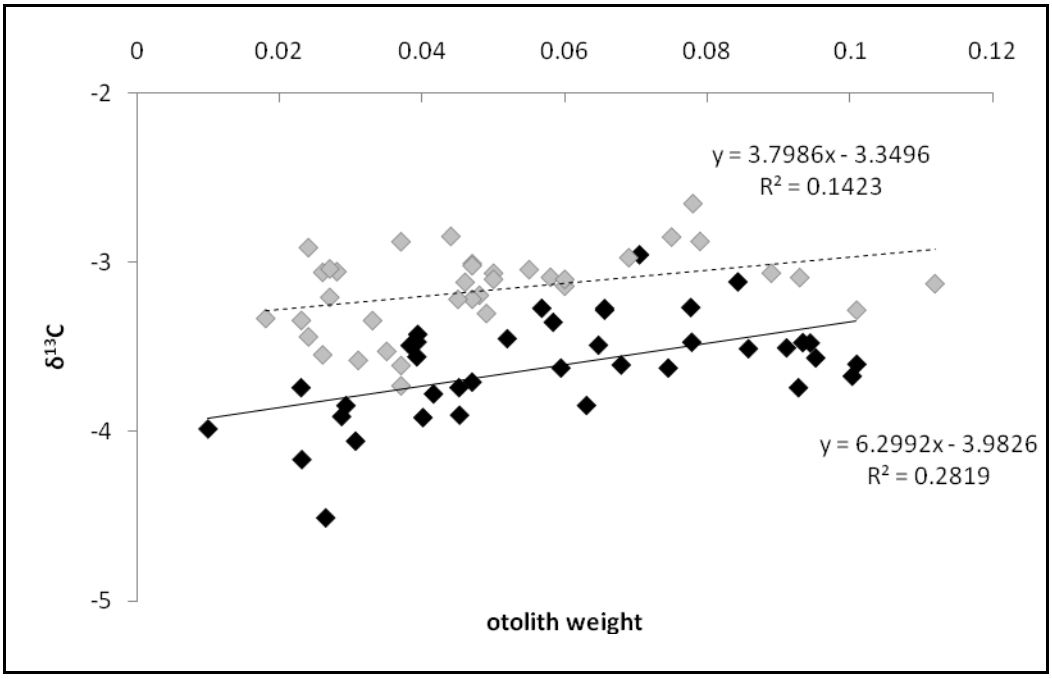



**Figure 4.2:** Relationship between otolith weight and $\delta^{13}C$ (upper) and $\delta^{18}O$ (lower) for Roebuck Bay sampling period I (black; solid line) and Roebuck Bay sampling period II (grey; dashed line). All regression equations significant at $p < 0.05$.

Locations were significantly discriminated by $\delta^{18}O$ and $\delta^{13}C$ values based on SNK tests (Table 4.3). The $\delta^{13}C$ stable isotope separated locations into seven groups with a high degree of overlap between Archer River, Love River, Blue Mud Bay and Arthur's Creek, as well as among Cleveland Bay, Eighty Mile Beach and Roebuck Bay II (Table 4.3). The locations listed above tended to occur across a gradient of groups, with a given location belonging to two to three groups (Figure 4.3a). Locations separated more clearly based on $\delta^{18}O$ stable isotope values with eight distinct groups and with no location belonging to more than one group (Table 4.3). Overall, all locations were significantly different except, (1) Archer River and Love River which were not significantly different; and (2) Walker River and Blue Mud Bay which were not significantly different (Table 4.3). Cleveland Bay was not significantly different to Eighty Mile Beach, but these locations are readily separated by longitude and locations in between (Figure 4.3b). Note the Roebuck Bay I and Roebuck Bay II locations were significantly different to each other and to all other locations indicating fine scale spatial separation within that Bay. Consequently, the samples in Roebuck Bay represent different subpopulations of fish.

The PERMANOVA, combining both $\delta^{18}O$ and $\delta^{13}C$ indicated that locations were significantly different and discriminates the locations more clearly than ANOVA based on each stable isotope in isolation (Table 4.4; Figures 4.4 and 4.5). *A posteriori* pairwise tests indicated that all sites were significantly different from each other at P (Perm<0.05) except for: Cleveland Bay and Eighty Mile Beach (P (perm) = 0.223) and Archer River, Love River and Walker River (P (perm) = 0.156 to 0.629); Walker River and Blue Mud Bay were also similar with a P (perm) = 0.068, but with little overlap suggesting limited mixing. Roper River was excluded from the nMDS, as it was such an extreme value (Figure 4.5) that differences among the remaining sites could not be visualised. On its exclusion (Figure 4.4), differences and similarities among sites were clearly represented and followed a trajectory along latitude. Roebuck Bay I and II were significantly different from each other, as demonstrated by the temporal analysis, but in the context of overall spatial variation, were relatively close to each other, compared to the high and low latitude sites.

In summary, all locations are significantly different except for Archer River and Love River. While Cleveland Bay was not significantly different to Eighty Mile Beach, these locations are readily separated by longitude and all locations in between (Figure 4.1). The lack of difference between Archer River and Love River suggests either mixing between these two groups of fish or a similarity in environmental conditions at these locations.

**Table 4.3:** *A posteriori* multiple comparison of means (Student-Newman-Kuels tests) from the one factor analysis of variance to test the effect of location on $\delta^{18}O$ and $\delta^{13}C$ stable isotope values.

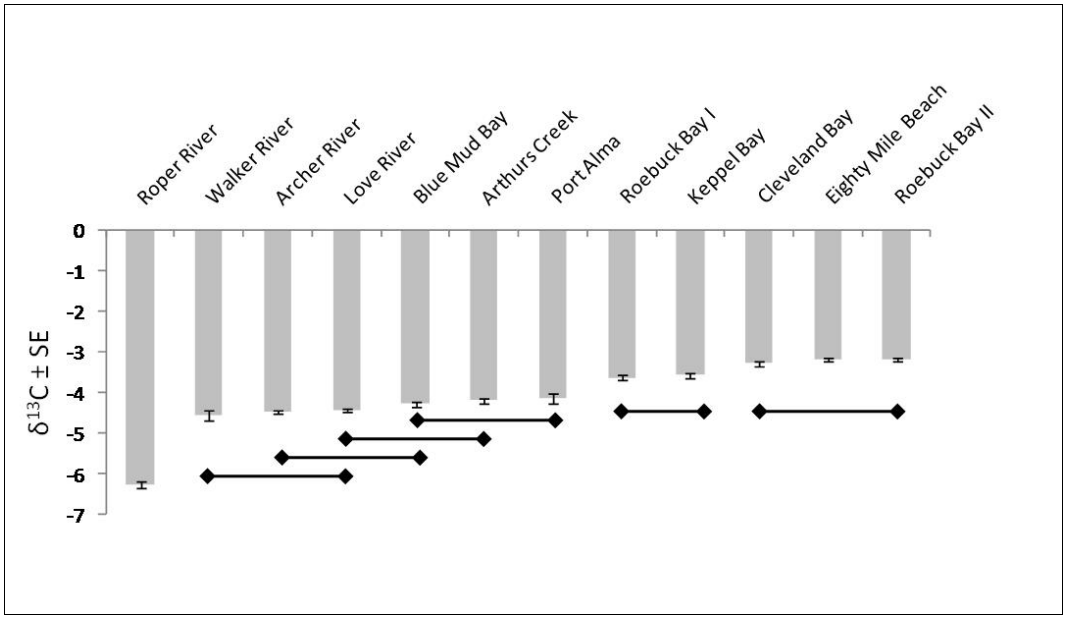
**SNK - $\delta^{18}O$**

| Location | N | Subset | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Roper River | 40 | -4.35 | | | | | | | |
| Arthur's Creek | 39 | | -2.18 | | | | | | |
| Archer River | 40 | | | -1.92 | | | | | |
| Love River | 40 | | | -1.90 | | | | | |
| Walker River | 35 | | | | -1.72 | | | | |
| Blue Mud Bay | 40 | | | | -1.63 | | | | |
| Eighty Mile Beach | 39 | | | | | -1.26 | | | |
| Cleveland Bay | 39 | | | | | -1.19 | | | |
| Roebuck Bay I | 38 | | | | | -1.13 | | | |
| Roebuck Bay II | 40 | | | | | | -.89 | | |
| Port Alma | 40 | | | | | | | -.59 | |
| Keppel Bay | 40 | | | | | | | | -.38 |
| **Sig.** | | **1.000** | **1.000** | **.784** | **.230** | **.159** | **1.000** | **1.000** | **1.000** |

**SNK - $\delta^{13}C$**

| Location | N | Subset | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Roper River | 40 | -6.24 | | | | | | |
| Walker River | 35 | | -4.54 | | | | | |
| Archer River | 40 | | -4.47 | -4.47 | | | | |
| Love River | 40 | | -4.42 | -4.42 | -4.42 | | | |
| Blue Mud Bay | 40 | | | -4.27 | -4.27 | -4.27 | | |
| Arthur's Creek | 39 | | | | -4.19 | -4.19 | | |
| Port Alma | 40 | | | | | -4.13 | | |
| Roebuck Bay I | 38 | | | | | | -3.61 | |
| Keppel Bay | 40 | | | | | | -3.56 | |
| Cleveland Bay | 39 | | | | | | | -3.26 |
| Eighty Mile Beach | 39 | | | | | | | -3.18 |
| Roebuck Bay II | 40 | | | | | | | -3.17 |
| **Sig.** | | **1.000** | **.482** | **.130** | **.060** | **.354** | **.609** | **.646** |

**A.**




**B.**

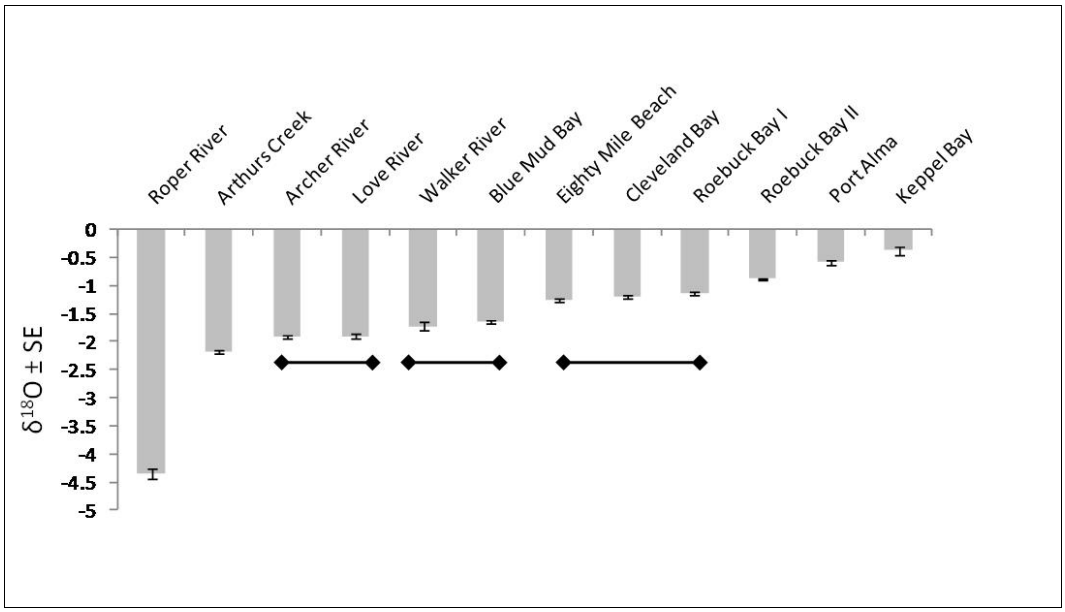



**Figure 4.3:** Summary of the results from the *a posteriori* multiple comparison of means (SNK tests) from the one factor analysis of variance to test the effect of location on (a) $\delta^{13}C$ and (b) $\delta^{18}O$ stable isotope values.

**Table 4.4:** Summary of PERMANOVA results across all locations with fixed factors based on Type III sums of squares with unrestricted permutations of the raw data.

PERMANOVA **($\delta^{18}$O and $\delta^{13}$C)**

| Source | df | SS | MS | Pseudo-F | P (perm) | Unique perms |
|---|---|---|---|---|---|---|
| Locations | 11 | 779.15 | 70.832 | 215.57 | 0.001 | 997 |
| Residual | 453 | 148.85 | 0.32859 | | | |
| **Total** | **464** | **928** | | | | |

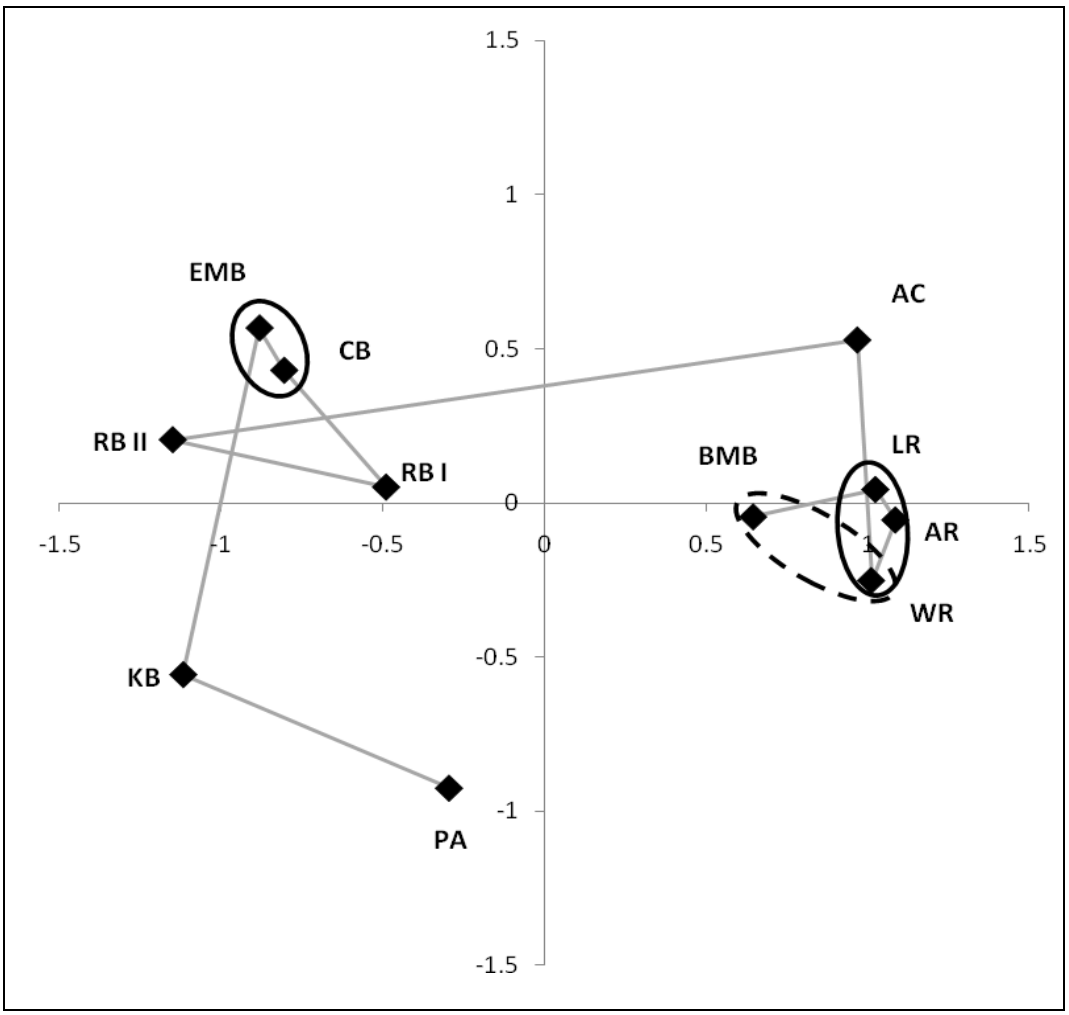



**Figure 4.4:** nMDS of the mean $\delta^{18}$O and $\delta^{13}$C values by site, excluding Roper River. The line connects the locations by order of latitude (BMB = highest latitude; KB = lowest latitude) and the ellipses group sites that were not significantly different from each other from the PERMANOVA. Locations are EMB = Eighty Mile Beach; RB I = Roebuck Bay sampling period I; RB II = Roebuck Bay sampling period II; BMB = Blue Mud Bay; WR = Walker River; AC = Arthur's Creek; AR = Archer River; LR = Love River; CB = Cleveland Bay; KB = Keppel Bay and PA = Port Alma.

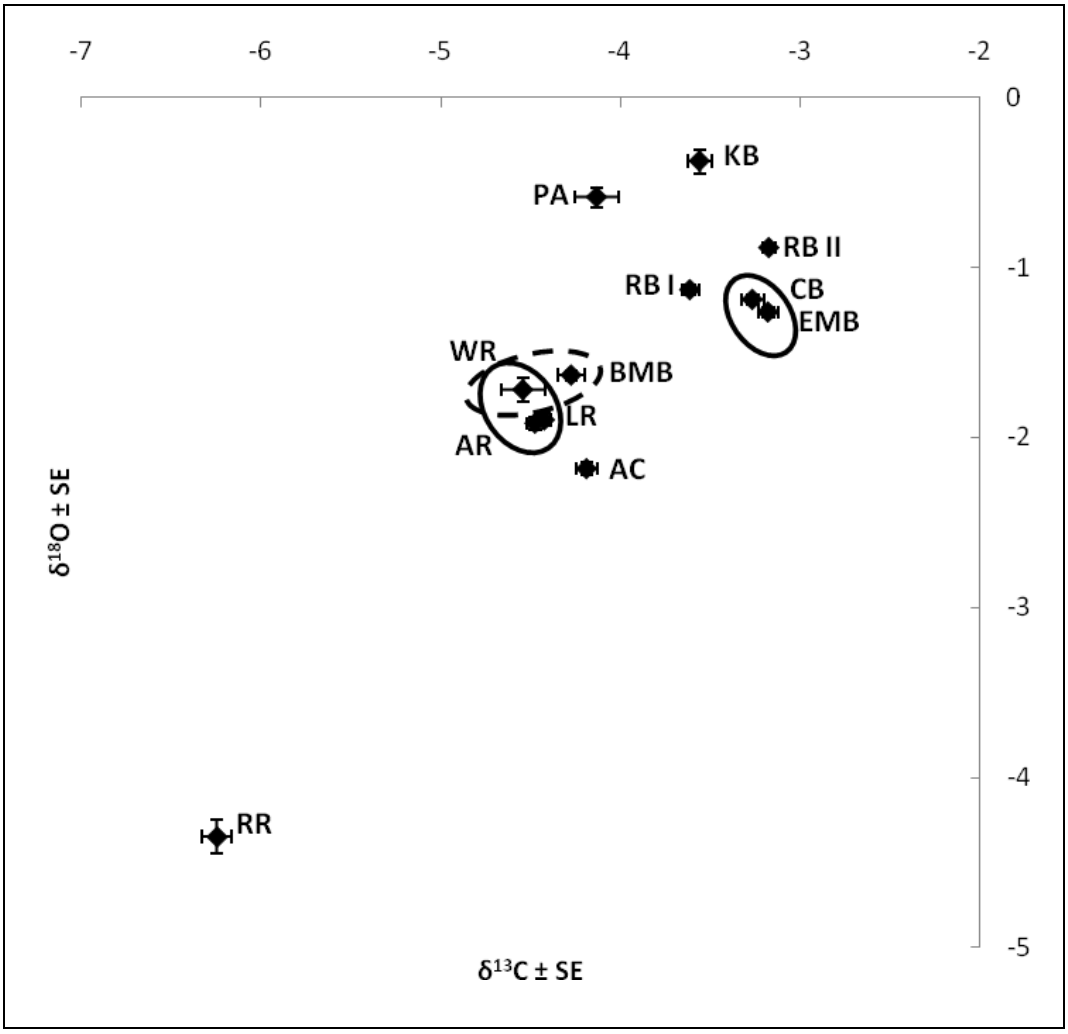



**Figure 4.5:** Mean $\delta^{18}O$ values (± standard error) versus mean $\delta^{13}C$ values (± standard error) of *E. tetradactylum* sagittal otolith carbonate for each location. Ellipses include those sites not statistically different from each other from the PERMANOVA.

## 4.4 Discussion

The use of stable isotopes in a whole otolith analysis provides information on the recent history of individual fish and the environments that they have occupied, whereas genetic studies relate to variation over an evolutionary time scale. As the use of stable isotopes related specifically to the life history of the individual fish, differences among sampled populations have direct relevance to fisheries management. The results of this study indicate the likely existence of at least eleven separate stocks or management units of *E. tetradactylum* across northern Australia.

The extent of population subdivision reported from this study is limited to the sampling locations examined. Given the fine scale spatial structuring of *E. tetradactylum* populations, it is hypothesised that this species is likely to comprise an extensive number of independent populations or management units throughout their distributional range. On the east coast of Australia there appear to be a minimum of at least three stocks, with separation of populations in close proximity to each other (e.g. Keppel Bay and Port Alma). The Gulf of Carpentaria region appears to comprise of a minimum of at least five stocks, none of which cross State/Territory boundaries. In Western Australia there appears to a minimum of at least three stocks, with partitioning evident at a very fine spatial scale within Roebuck Bay.

The two sites that could not be separated in this study (Archer River and Love River) are in close proximity and their similarity may have resulted from a lack of contrasting environmental variables between these locations. The fact that no significant difference was detected does not imply that no difference exits. It indicates the need for additional related studies. Moore *et al.* (2003) used parasites to determine the stock structure of *S. commerson* and were able to detect differences among sites that could not be resolved from the stable isotope study of Newman *et al.* (2009a) on the same species. Overall, the fine scale stock structure of *S. commerson* from parasite data was in general agreement with those of the otolith stable isotope analysis. However, the results of both studies highlight the value of using multiple techniques when assessing the structure of fish stocks. Moreover, the power to detect change is extended when using multiple techniques across areas with similar environmental attributes.

The separation of temporal samples within Roebuck Bay provides evidence for fine scale spatial partitioning within the Bay. Samples from Roebuck Bay were collected from a commercial fisher that operates throughout the Bay over an area in excess of twenty kilometres. Samples were collected six months apart and from different vessel skippers. Liaison with these skippers indicates that samples were probably derived from different areas within the Bay at the different time periods. Regardless of the exact sampling locations, the data clearly indicate fine scale partitioning with Roebuck Bay (Figures 4.2-4.5). This fine scale population partitioning has profound fisheries management implications.

Furthermore, there is the need to impose a caveat for future stock structure studies. It is vitally important that the collection of material for the purposes of indentifying stock structure must be undertaken with observers aboard all vessels in order to determine the exact spatial locations from which samples are derived. It must be assumed that fish exhibit some degree of population subdivision in the absence of any knowledge or data to the contrary. In addition, from a pragmatic fisheries management viewpoint, population subdivision must be assumed until proven otherwise in order to limit the potential for localised depletion of fish populations. This study further indicates that analysis of the stable isotopic composition of fish otoliths provide excellent proxies for stock structure studies in fisheries management.

For species such as *E. tetradactylum* to maintain independent fine scale populations they must exhibit restricted migration or movement among locations. On the scale examined in this study movement in some locations appears restricted at a spatial scale of less than twenty kilometres. This is clearly demonstrated in the significant differences in the temporal samples within Roebuck Bay, which infers fine scale within Bay population partitioning. This finding is consistent with those of Zischke *et al.* (2009) who also found an indication of fine spatial scale stock structure for *E. tetradactylum* on the Queensland east coast using parasites and conventional tagging data. They proposed that headlands may act as natural barriers to movement and mixing of blue threadfin populations therefore isolating populations into discrete units at small spatial scales. In the present study we also found fine spatial scale separation however this was not determined by discontinuity in habitat or geographical barriers. We therefore postulate that these populations are maintaining site fidelity influenced by local inlets, creeks, tidal tributaries, rivers and/or wetlands over the duration of their life history.

In terms of overall abundance, the Polynemidae are characteristic components of the unvegetated nearshore waters and mangroves in north-western Australia (Pember 2006) and the shallow coastal waters of other parts of the tropical Indo-West Pacific region (Blaber, 2000). Polynemid species are typically associated with turbid nearshore waters (Blaber *et al.* 1995). Within this nearshore environment the abundance and species composition of the Polynemidae is variable among locations (Pember, 2006). This study has demonstrated that adult assemblages of *E. tetradactylum* are significantly different among locations and that populations are maintaining site fidelity in these nearshore turbid waters possibly associated with inlets, creeks, tidal tributaries, rivers and/or wetlands over the duration of their life history. The mechanisms that underpin the fine scale spatial stock structure remain to be resolved.

The population subdivision of *E. tetradactylum* is evident along expansive stretches of open beach systems and coastal embayments with no physical barriers such as headlands. These results indicate that optimal fisheries management will require a review of the current spatial arrangements, particularly the potential for localised depletion of stocks on small spatial scales. Evidence from this study indicates

that the stock structure of *E. tetradactylum* across northern Australia clearly separates all the jurisdictions (Western Australia, Northern territory and Queensland), thus discriminating west, north and east coast populations and further indicate a significant number of smaller subdivisions are present within these jurisdictions. The results imply that localised depletions are the foremost concern for the future sustainability of fisheries for *E. tetradactylum*.

The results of this study signify the importance of stock structure studies to fisheries management. It is vitally important for fisheries managers to have sufficient and, most importantly, detailed knowledge of the underlying stock structure of fish populations, particularly of adult assemblages for determining the appropriate spatial scale of management to ensure the sustainability of fisheries. Stock assessments of many fish populations are often restrained to arbitrarily defined geographical management areas such as State and/or Territory boundaries or intra-state bioregions that have little relation to the accurate spatial stock structure of a fish population. The fine scale population subdivision of *E. tetradactylum* populations indicates that if fishing effort is not subject to sufficiently rigorous regulation then there is a genuine risk of localised depletion and potential loss of adult assemblages. These data need to be explicitly incorporated into the fisheries management planning and decision-making processes within fisheries management to ensure the sustainability of *E. tetradactylum* fisheries into the future. It is recommended that for management purposes, fisheries managers consider the fine scale spatial stock structure of this species in determining the appropriate spatial scale for the management, monitoring and stock assessment of *E. tetradactylum* populations.

**Appendix 4.1:** Summary of the sampling data, and results from the stable isotope analyses of the sagittal otolith carbonate of *Eleutheronema tetradactylum* from locations across northern Australia (WA = Western Australia; NT = Northern Territory; QLD = Queensland; GoC = Gulf of Carpentaria; East Coast = EC). More specific locations details are listed in the methods section.

| Location (code) | Position (latitude, longitude) | Date of sampling | N | Length Range (FL, mm) mean (range) | Otolith weight (mg) mean (range) | (‰ PDB) mean (range) | $\delta^{18}O$ (‰ PDB) mean (range) |
|---|---|---|---|---|---|---|---|
| Eighty Mile Beach, WA | 19°34'S, 121°25'E | April 2009 | 39 | 334 (261-482) | 35.7 (21.0-64.5) | -3.18 (-3.64 to -2.13) | -1.26 (-1.59 to -0.77) |
| Roebuck Bay, WA | 18°05'S, 122°17'E | March 2008 | 38 | 432 (203-600) | 58.8 (9.9-100.9) | -3.61 (-4.51 to -2.95) | -1.13 (-1.37 to -0.76) |
| Blue Mud Bay, NT GoC | 13°26'S, 136°03'E | September 2008 | 40 | 328 (259-399) | 40.8 (20.2-86.8) | -4.27 (-5.08 to -3.15) | -1.63 (-1.97 to -1.26) |
| Walker River, NT GoC | 13°35'S, 135°50'E | September 2007 | 35 | 381 (260-610) | 60.1 (18.8-144.5) | -4.54 (-6.05 to -2.93) | -1.72 (-2.71 to -1.13) |
| Roper River, NT GoC | 14°45'S, 135°23'E | March 2009 | 40 | 360 (298-540) | 40.1 (20.7-94.8) | -6.24 (-7.23 to -4.89) | -4.35 (-5.34 to -2.28) |
| Arthur's Creek, QLD GoC | 16°55'S, 138°58'E | August 2008 | 39 | 441 (315-565) | 61.6 (35.0-104.3) | -4.19 (-5.19 to -3.29) | -2.18 (-2.68 to -1.88) |
| Archer River, QLD GoC | 13°30'S, 141°33'E | September 2007 | 40 | 414 (250-540) | 54.4 (20.1-87.7) | -4.47 (-5.21 to -4.04) | -1.92 (-2.26 to -1.41) |
| Love River, QLD GoC | 13°30'S, 141°33'E | August 2008 | 40 | 396 (330-470) | 63.6 (35.0-84.0) | -4.42 (-4.88 to -4.01) | -1.90 (-2.62 to -1.53) |
| Cleveland Bay, QLD EC | 19°13'S, 146°55'E | July 2008 | 39 | 447 (334-547) | 58.1 (36.0-92.0) | -3.26 (-4.37 to -2.29) | -1.19 (-1.63 to -0.85) |
| Keppel Bay, QLD EC | 23°23'S, 150°54'E | July 2007 | 40 | 435 (336-615) | 57.8 (30.9-118.3) | -3.56 (-4.84 to -2.64) | -0.38 (-2.02 to 0.32) |
| Port Alma, QLD EC | 23°34'S, 150°52'E | October 2008 | 40 | 507 (323-815) | 74.6 (31.0-187.0) | -4.13 (-5.94 to -2.84) | -0.59 (-1.81 to 0.14) |

# Chapter 5: Comparison of life-history characteristics of blue threadfin, *Eleutheronema tetradactylum*, across Northern Australia

**Aaron C. Ballagh, David J. Welch, Quentin Allsop, Jason M. Stapley and Stephen J. Newman**

## 5.1 Introduction

Understanding of the life-history characteristics of exploited fish species is fundamental to the premise of sustainable fisheries management (Beverton and Holt 1957). The life-history characteristics of fish populations determine important biological attributes such as productivity, which significantly affect vulnerability to exploitation and responses to fishing (Jennings *et al.* 1998; Bianchi *et al.* 2000). Estimates of age and growth rate are two of the most influential life-history characteristics controlling the productivity of fish populations (Campana and Thorrold 2001), and a recent estimate of growth rate coupled with age at first maturity are minimum requirements for effective single-species fisheries management (Sale 1982). It has also been shown that life histories of fish can be plastic and can change in response to fishing pressure (Rochet 1998). As such, it is imperative that management strategies be based on the best available life-history parameter estimates that are relevant to the population being managed.

A major goal of modern fisheries research is to acquire knowledge on spatial and temporal variation, to provide information on stock subdivision, genetic depletion, and the capacity of populations to cope with environmental changes (Palsbøll *et al.* 2007; Schwartz *et al.* 2007; Chopelet *et al.* 2009). As well as the life-history characteristics, the identification of the stock structure of exploited fish species is fundamental to fisheries management and provides the basis for the determination of appropriate spatial management units (Begg and Waldman 1999). Hilborn and Walters (1992) define stocks as self-reproducing groups of fish having similar life-history characteristics, however many definitions have been postulated in the literature and the term ‘stock’ is often used inter-changeably with the term ‘population’. Geographical differences in life-history characteristics, such as growth, have been used as evidence of separate stocks and suggest the need to consider the spatial scale of assessment and management (Begg *et al.* 1999; Rahikainen and Stephenson 2004; Begg 2005). As such, an understanding of life-history characteristics is fundamental where separate stock is identified (Abaunza *et al.* 2008).

Life-history parameters have been used in stock identification studies because they reflect both the genotypic and environmental influences on a stock, and therefore differences in life-history parameters are likely to reflect geographically and/or reproductively isolated populations (Begg and Sellin 1998; Begg *et al.* 1999). Estimates of life-history parameters can not only help elucidate different fish stocks, but are crucial to determining the biological attributes of each stock on which management is based (Begg *et al.* 1999).

Blue threadfin, *Eleutheronema tetradactylum*, are an important inshore fish species that form a large component of the inshore fin fish fishery in northern Australia (Anon, 2007). In Australia, blue threadfin are harvested in the inshore net fishery which primarily targets barramundi. The species occurs right around the northern coastline of Australia from Gladstone on the east coast of Queensland to Exmouth on the west coast of Western Australia. On the Queensland east coast, over 200 tonnes of threadfin is harvested annually with blue threadfin making up the second highest catch species by weight within the Barramundi inshore net fishery (Anon, 2007). Threadfin are also an important component of inshore recreational catch across northern Australia (Pember *et al.* 2005), with recent figures of the recreational take of blue threadfin on the Queensland east coast at over 55 tonnes (Anon, 2007). Despite their importance however, little information is available about the population biology and stock structure across northern Australia, with the exception of the Pilbara and Kimberly regions in Western Australia (Pember *et al.* 2005; Pember 2006) and a preliminary study on the east coast (Zischke *et al.* 2009).

Blue threadfin are protandrous hermaphrodites maturing first as a male and later changing to female (Pember *et al.* 2005). In Western Australia, the species typically reaches sexual maturity as males at the end of their first year of life when their lengths are ca. 200 mm, and change from male to female at ca. 400 mm when they are some two years old (Pember *et al.* 2005).

A recent assessment of the status of Queensland's threadfin fishery identified a distinct lack of suitable information with which to parameterise stock assessment models (Welch *et al.* 2002). In particular, insufficient data on blue threadfin made formal assessment difficult, with the only life-history parameters available for Queensland fish coming from the Gulf of Carpentaria (Welch *et al.* 2002). This becomes a bigger problem for management as previous work has identified different genetic stocks in Queensland between the east coast and the Gulf of Carpentaria (Garrett 1997). Further, recent research using parasites and tag-recapture data of blue threadfin on the Queensland east coast suggests the possibility of separate sub-stocks (Zischke *et al.* 2009).

In this study, the stock structure of blue threadfin across northern Australian was inferred by comparing life-history parameter estimates from different locations. Fish collected from several locations around Australia were used to estimate life-history parameters, which are presented as a basis for understanding the species' biology on a regional scale. Morphology was also used as an indicator of

stock structure by comparing morphometric relationships between locations, and morphological relationships are also presented.

## 5.2 Methods

### Sample collection

Blue threadfin samples were collected by commercial fishing operations from several locations around the northern coast of Australia from July 2007 to August 2009 (Figure 6.1, Table 6.1). Samples were collected in two phases to provide for temporal comparisons, with Phase I samples collected in 2007-2008 and Phase II samples collected in 2008-2009 (see Chapter 1 for project sampling summary).

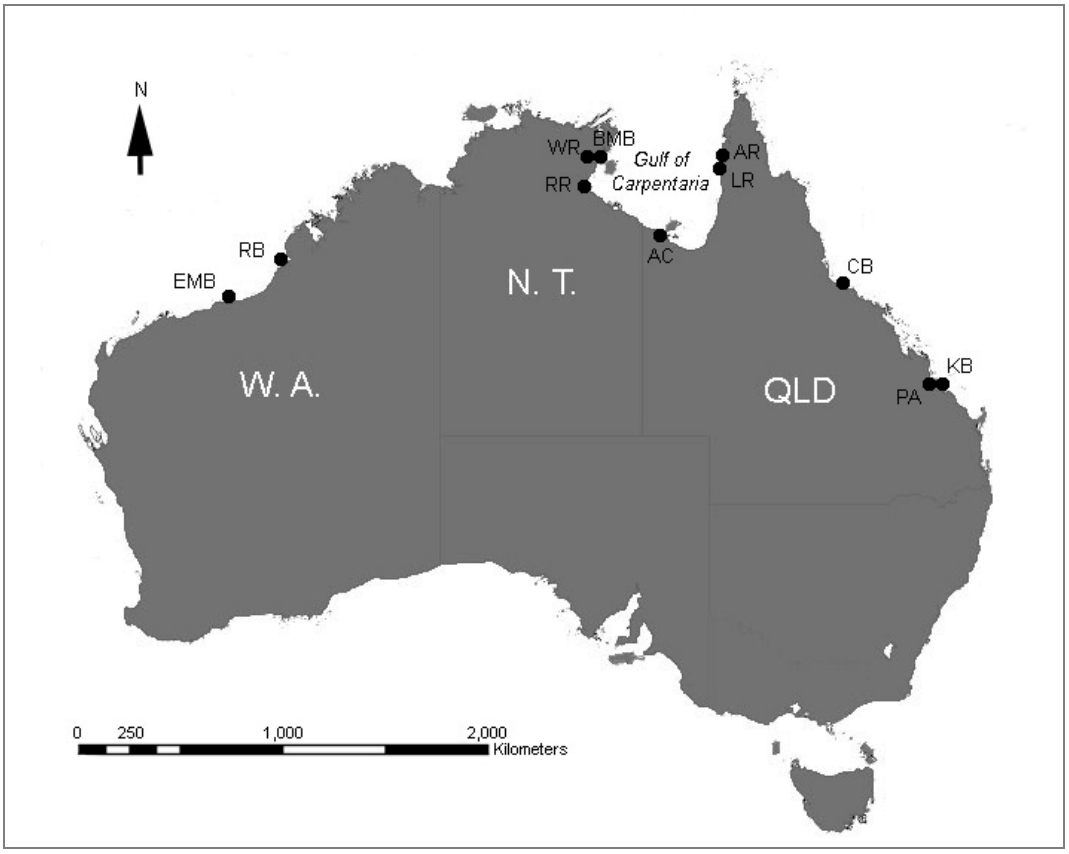



**Figure 5.1:** Sample locations across northern Australia. EMB = Eighty Mile Beach; RB = Roebuck Bay; WR = Walker River; BMB = Blue Mud Bay; AC = Arthur's Creek; LR = Love River; AR = Archer River; CB = Cleveland Bay; KB = Keppel Bay; PA = Port Alma.

**Table 5.1:** Locations and numbers of blue threadfin samples used for estimation of age and growth, and the mean, median and range of fork lengths (FL) and ages (s.e. = standard error, F = Female, M = Male, Tr = Transitional and U = Unknown).

| Location | Sample Numbers | | | | Mean FL (mm ± s.e.) | Mean Age (years ± s.e.) | Median Age | Range | |
|---|---|---|---|---|---|---|---|---|---|
| | F | M | Tr | U | | | | FL (mm) | Age (years) |
| **EMB** | 46 | 13 | 17 | | 353 ± 9.6 | 2.24 ± 0.11 | 1.5 | 261 - 549 | 1.5 - 4.5 |
| **RB1** | 45 | 9 | 3 | | 424 ± 12.2 | 3.04 ± 0.14 | 3.5 | 203 - 600 | 0.5 - 5.5 |
| **RB2** | 60 | 6 | 8 | | 394 ± 9.6 | 2.79 ± 0.10 | 3 | 254 - 622 | 1 - 4.5 |
| **WR** | 62 | 11 | | | 383 ± 7.6 | 3.36 ± 0.16 | 3 | 260 - 610 | 1 - 6.5 |
| **BMB** | 57 | | | 1 | 325 ± 4.3 | 2.03 ± 0.13 | 1.5 | 259 - 399 | 0.5 - 4.5 |
| **RR** | 33 | 15 | 3 | | 359 ± 8 | 2.02 ± 0.14 | 1.5 | 298 - 540 | 0.5 - 4.5 |
| **AC** | 43 | | | 3 | 449 ± 8.5 | 3.12 ± 0.12 | 3 | 315 - 585 | 1.5 - 5.5 |
| **LR** | 78 | 2 | 1 | 1 | 396 ± 5.8 | 2.69 ± 0.08 | 3 | 300 - 545 | 1.5 - 4 |
| **AR** | 64 | | | | 431 ± 7.6 | 2.84 ± 0.09 | 3 | 250 - 615 | 1 - 6 |
| **CB** | 55 | 14 | 10 | 1 | 445 ± 5.9 | 2.95 ± 0.09 | 3 | 329 - 581 | 1.5 - 5 |
| **KB** | 22 | 48 | 4 | 2 | 436 ± 11.5 | 2.89 ± 0.12 | 2.5 | 297 - 723 | 1.5 - 6 |
| **PA** | 29 | 34 | 4 | 1 | 468 ± 15.4 | 3.29 ± 0.15 | 2.5 | 323 - 815 | 1.5 - 6.5 |

Morphometric measurements (mm) taken included fork length (FL), total length (TL), head length (HL) - measured from the tip of the nose to the furthest edge of the pre-operculum, and upper jaw length (UJL) - measured from the front edge of the upper jaw to the rear edge (Figure 6.2), and total weight where possible (grams). Each fish was macroscopically sexed and staged using the staging system of Pember *et al.* (2005). Sagittal otoliths were removed, cleaned and stored dry in vials or envelopes.

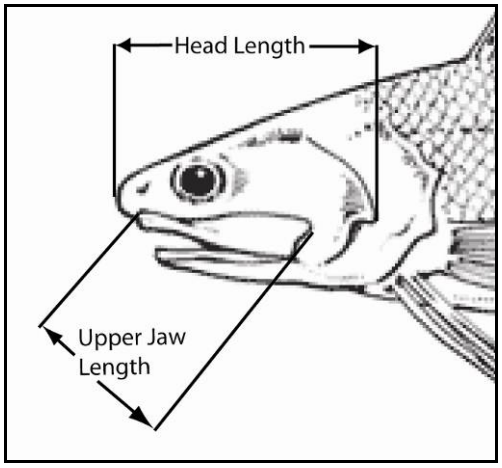



**Figure 5.2:** Head length and upper jaw length measurements.

### Age estimation

Whole sagittal otoliths were immersed in mineral oil and viewed against a black background through a stereo-dissection microscope (10× to 40× magnification). Otoliths were aged whole rather than taking transverse sections as age estimates from whole otoliths have been found to be an accurate method of aging for blue threadfin (Pember *et al.* 2005). Otoliths were aged through the 'read' area (Figure 6.3) from the posterior end on the proximal surface. Complete annual increments (annuli - one translucent and one opaque zone), were counted from the nucleus to the outer edge of the otolith. All otoliths were read at least twice. If annuli counts from the first two reads agreed, the count was accepted as the agreed age, otherwise the otolith was read a third time and any two agreed counts were accepted as the agreed age.

### Back-calculation

Digital still images of all otoliths were taken using an image analysis system (Diagnostic Instruments digital camera connected to an Olympus SZX9 stereo-dissection microscope and the *Image Pro* 6.2 image analysis software). Images were checked against agreed age estimates to ensure that all the annuli were clearly distinguishable. Otoliths were measured through the 'read' area (Figure 6.3). Measurements were taken from the nucleus to the furthest point from the nucleus on the posterior end of the proximal side of the otolith (Figure 6.3). This provided a reference axis that was consistent across all otoliths. In cases where annuli along the reference axis were ambiguous, a second axis was measured from the nucleus to the edge of the otolith within the read area where the annuli were clearer (Figure 6.3). This second line was called the measurement axis. The length of the reference axis and measurement axis (where present) were then measured, as was the distance from the nucleus to the outer edge of the opaque band for each annuli. Standardising the annuli distances taken on the measurement axis to the reference axis was done using a conversion ratio:

$$A_R = \frac{R_A}{M_A} \times A_M \qquad (1)$$

where: $R_A$ and $M_A$ are the lengths of the reference and measurement axes respectively, $A_R$ is the distance from the nucleus to the annulus measured along the reference axis and $A_M$ is the distance from the nucleus to the annulus measured along the measurement axis.

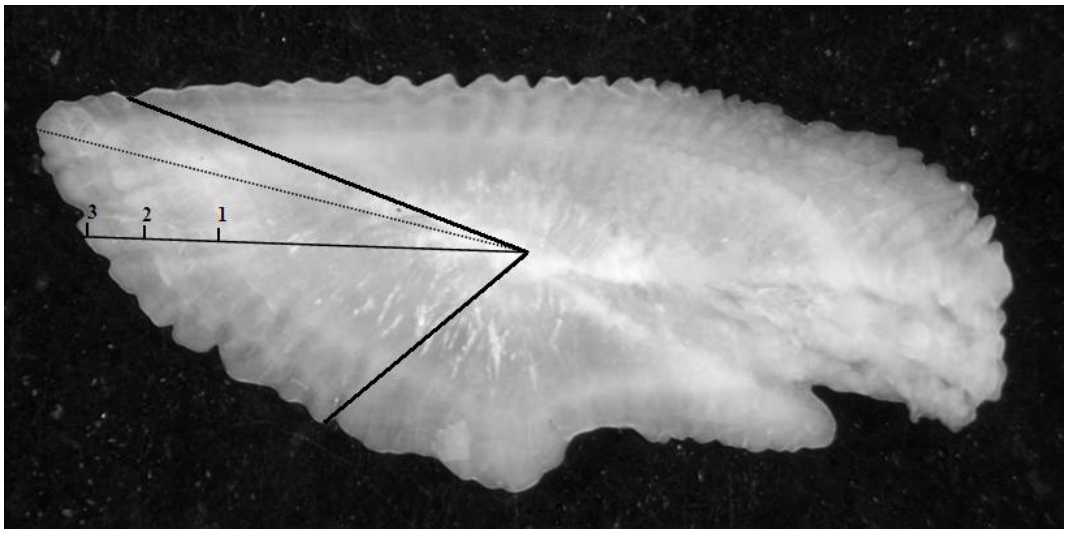



**Figure 5.3:** The read area on the posterior end of the proximal surface of a three year-old blue threadfin sagittal otolith, with the reference axis (dashed line) and the measurement axis with check marks at the outer edge of each annuli.

To determine the form of the relationship between otolith radius (reference axis) and the fish's fork length, regression analysis was used based on data from all locations combined. Linear and non-linear regression of fork length on otolith radius resulted in a slightly better fit ($R^2$-value) for a non-linear power relationship of the form:

$$FL = a \times O^b \quad (2)$$

where *FL* is the fork length, *O* is the otolith radius, *a* is the coefficient of the power function and *b* is the exponent. It was therefore decided to linearise the fork length to otolith radius relationship using a log transformation of the data, giving a relationship of the form:

$$LogFL = Loga + bLogO \quad (3)$$

The relationship of log otolith radius and log fork length was tested between locations using Analysis of Covariance (ANCOVA, (Bartlett *et al.* 1984). This relationship was then determined for each sample location using geometric mean regression (GMR, (Ricker 1992). Back-calculated length at age was determined using the body proportional hypothesis (BPH) of (Francis 1990) combined with the GMR of log otolith radius to log fork length. The BPH, which assumes the ratio of average fish length to individual fish length is constant for any given otolith radius, was modified to account for the use of log transformed data and is described by the equation:

$$LogL_t = ((c + dLogO_t)/(c + dLogO_c))LogL_c \quad (4)$$

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

### Length at Sex Change and Maturity

All samples which had a sex and fork length recorded, whether aged or not, were used to estimate length at sex change. Although the reproductive stage of gonads was also recorded, insufficient immature individuals were collected from any of the locations to reliably estimate size at maturity.

A logistic function was fitted to the proportion of females (relative to males and transitional individuals) in each 25mm length class to estimate the length at which blue threadfin change sex. The length at sex change was estimated for each location using the logistic equation:

$$P_S = (1 + e^{-\ln 19(S - S_{50})/(S_{95} - S_{50})})^{-1} \quad (5)$$

where $P_S$ is the proportion of females in each length class s, and $S_{50}$ and $S_{95}$ are the length at which 50% and 95% of the population are females. Likelihood ratio tests were used to test for differences in the length at sex change among locations.

### Morphometrics

The relationship between fork length and weight was described by a power function of the form:

$$W = a \times FL^b \quad (6)$$

where *W* is the weight, *FL* is the fork length, *a* is the coefficient of the power function and *b* is the exponent. This relationship was compared among locations using ANCOVA with fork length the covariate of weight. Fork length and weight data were log-transformed for the analysis to satisfy the assumption of linearity.

The relationship between fork length (*FL*) and upper jaw length (*UJL*), fork length and head length (*HL*) and fork length and total length (*TL*) were all described by a linear function. These relationships were also compared among locations using ANCOVA with fork length the covariate.

### Data analysis

The von Bertalanffy growth function (VBGF, Beverton and Holt 1957) was used to describe the growth of blue threadfin for back-calculated length at age data:

$$L_t = L_\infty(1-e^{(-K(t-t_0))}) \qquad (7)$$

where $L_t$ is the length at age $t$, $L_\infty$ is the theoretical maximum length, $K$ is the growth coefficient or the rate at which $L_\infty$ is asymptotically reached, and $t_0$ is the theoretical age where length is equal to zero.

Comparisons between the same locations collected in each Phase were made to test for temporal stability of samples. Growth was compared between the two Phases for each location using likelihood ratio tests (Kimura 1980). The slope of the relationship between log fork length and log otolith radius between Phase I and II was also tested using the methods for comparing two slopes as outlined in (Zar 1999). Data from locations which did not differ significantly between phases were pooled for all other analyses.

Likelihood ratio tests were used to test for differences in the growth of blue threadfin between locations. A Bonferroni adjustment was used to account for multiple comparisons of the likelihood ratio test by adjusting the significance level:

$$\alpha_{Adj} = \frac{\alpha}{n} \qquad (8)$$

where $\alpha$ is the significance level, $\alpha_{Adj}$ is the adjusted significance level and $n$ is the number of multiple comparisons. All likelihood ratio tests used a common age or length range for each comparison to assure the validity of the comparisons (Haddon 2001).

Analysis of variance (ANOVA) and multiple comparisons were also used to test for differences in back-calculated length-at-age between locations. A full factorial ANOVA using length as the dependent and age and location as fixed factors was initially done to test for differences among locations. One-way ANOVA and Tukey HSD multiple comparisons were then used to test for differences among and between locations for ages 1-5 years.

### Integration

An approach to integrating and synthesising the results of the different tests for differences in regional growth and length at sex-change was adapted from Ballagh *et al.* 2009. For each location comparison, each significant multiple comparison test result was assigned a value of one and added to the results of

other significant tests for each location combination. That is, for the likelihood ratio tests (growth and sex-change), each significant multiple comparison result was assigned a value of one and for ANOVA, each significantly different multiple comparison result for each age class was also assigned a value of one. The scores for each location comparison were then combined and divided by the number of tests to produce a matrix of difference indices for each location combination. These difference indices, in effect, correspond to the percentage of multiple comparison tests which were significantly different.

## 5.3 Results

### Otolith radius to fork length relationship

It was concluded that the power relationship described the fork length to otolith radius relationship better than a linear relationship on the basis of fit ($R^2$-value) and because of the negative fork length values estimated for smaller otolith radius lengths from the linear relationship (Figure 5.4). Fork length and otolith radius data was therefore log transformed to linearise the relationship, which is demonstrated by the linear regression of log fork length on log otolith radius (Figure 5.5).

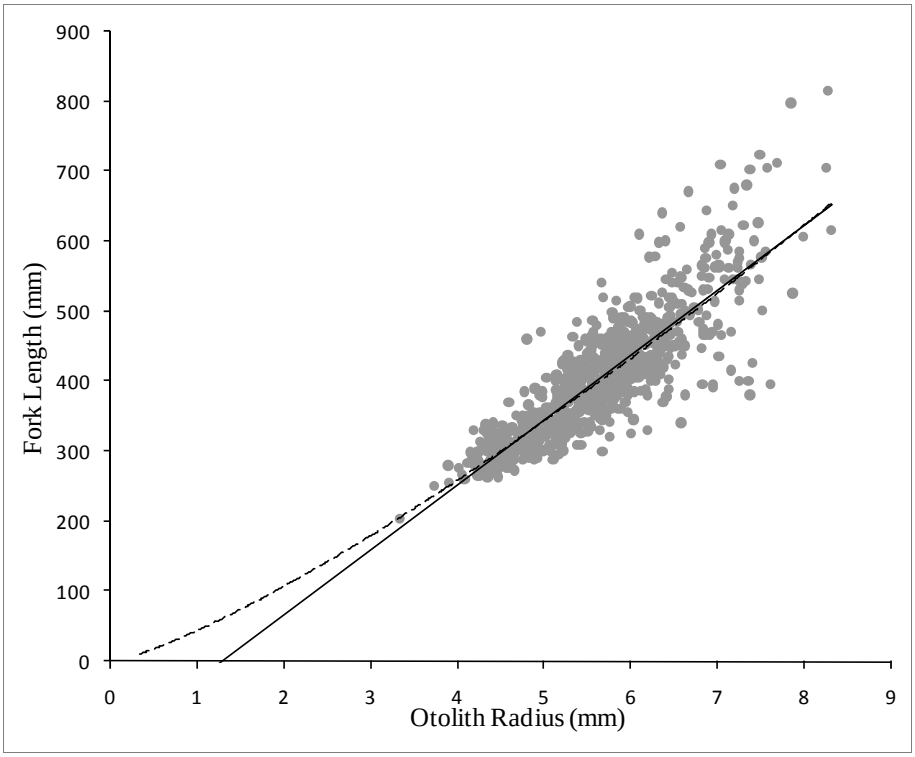



**Figure 5.4:** Plot of fork length on otolith radius and the linear ($y$ = 92.813$x$ - 119.7, $R^2$ = 0.713) and power ($y$ = 44.78$x^{1.27}$, $R^2$ = 0.747) relationships.

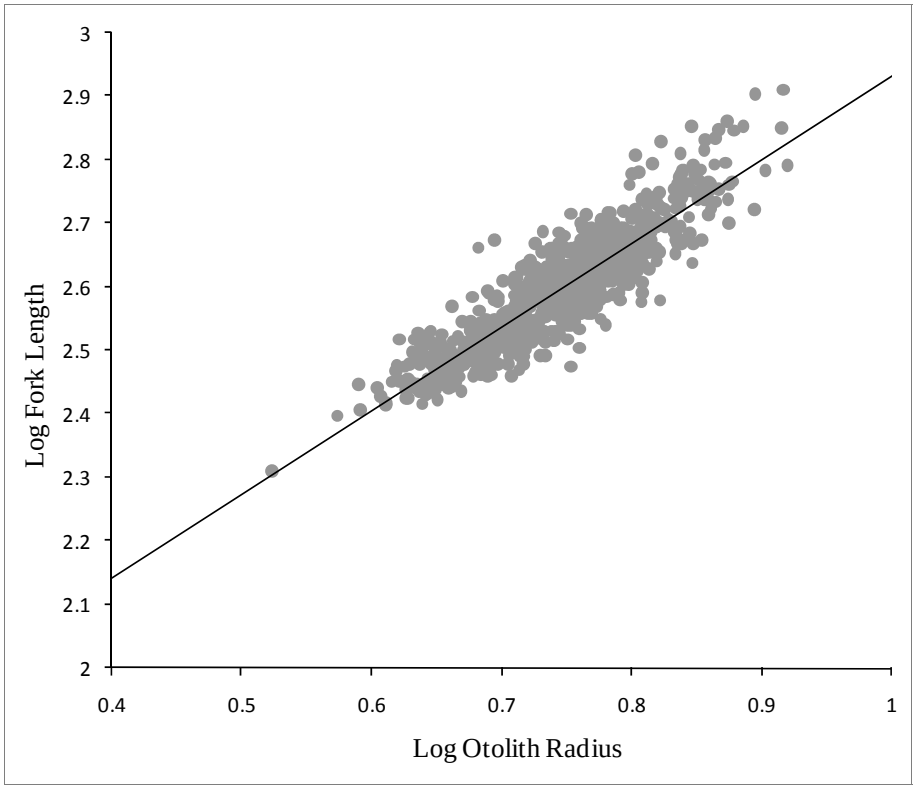



**Figure 5.5:** Plot of log fork length on log otolith radius and the linear relationship ($y$ = 1.318$x$ + 1.613, $R^2$ = 0.79).

The relationship of log fork length to log otolith radius was found to be significantly different between locations from ANCOVA ($F_{9, 789}$ = 9.949, $P$ < 0.0001). Geometric mean regression was then used to determine the parameters of the relationship for each location for use in the back-calculation model (Table 5.3).

### Phase Comparisons

In each sampling phase (Phase I = 2007/08; Phase II = 2008/09) re-sampling of exactly the same location was not possible largely due to the reliance on opportunistic samples provided by fishers. For the purpose of assessing the temporal stability in populations, in this study we assumed that the same population was being sampled for inter-annual collections that were within approximately 50 km of one another. These locations were Roebuck Bay (I and II), Walker River (I) and Blue Mud Bay (II), Archer River (I) and Love River (II), and Keppel Bay (I) and Port Alma (II).

Comparison of the slope of the relationship between log fork length and log otolith radius between locations collected in each phase revealed no significant differences between KB and PA ($t_{140}$ = 0.886, $P$ = 0.377) and the two RB samples ($t_{128}$ = 1.930, $P$ = 0.056) locations. Significant differences in the slope of the relationship were found between the WR and BMB ($t_{123}$ = 3.478, $P$ = 0.001). Similarly,

differences in the slope of the relationship were also found between AR and LR ($t_{142}$ = 2.100, $P$ = 0.037).

Comparisons of growth between the two Phases found no significant differences between KB and PA ($\chi^2$ = 2.337, $P$ = 0.051) and the two RB samples ($\chi^2$ = 7.485, $P$ = 0.058) locations. Significant differences in growth were observed between WR and BMB ($\chi^2$ = 10.703, $P$ = 0.013), and the and between AR and LR ($\chi^2$ = 24.921, $P$ < 0.0001). It was therefore decided to combine the KB and PA samples, and the two RB samples for all further analyses (Table 6.3).

**Table 5.3:** Locations used in all subsequent analyses and the slope and intercept values of the geometric mean regression of log fork length on log otolith radius for each location.

| Location | Slope | Intercept |
|---|---|---|
| Eighty Mile Beach | 1.52 | 1.46 |
| Roebuck Bay | 1.43 | 1.53 |
| Walker River | 1.17 | 1.69 |
| Blue Mud Bay | 0.95 | 1.83 |
| Roper River | 1.29 | 1.66 |
| Arthur's Creek | 1.31 | 1.64 |
| Love River | 1.09 | 1.75 |
| Archer River | 1.21 | 1.7 |
| Cleveland Bay | 1.37 | 1.61 |
| Keppel Bay/Port Alma | 1.66 | 1.36 |

## Growth

A large amount of variation between locations was observed in growth estimates from back-calculated length-at-age (Table 5.4, Figure 5.6). Significant differences were found in the growth of blue threadfin between most locations (91% of pair-wise comparisons) from likelihood ratio rests (Table 5.5).

Significant differences in mean length-at-age were also found between locations. ANOVA revealed a significant interaction between location and age ($F_{5,9}$ = 23, $P$<0.001) for back-calculated length-at-age. Multiple comparisons of length-at-age revealed many significant differences in mean back-calculated length-at-age between locations (Table 5.7).

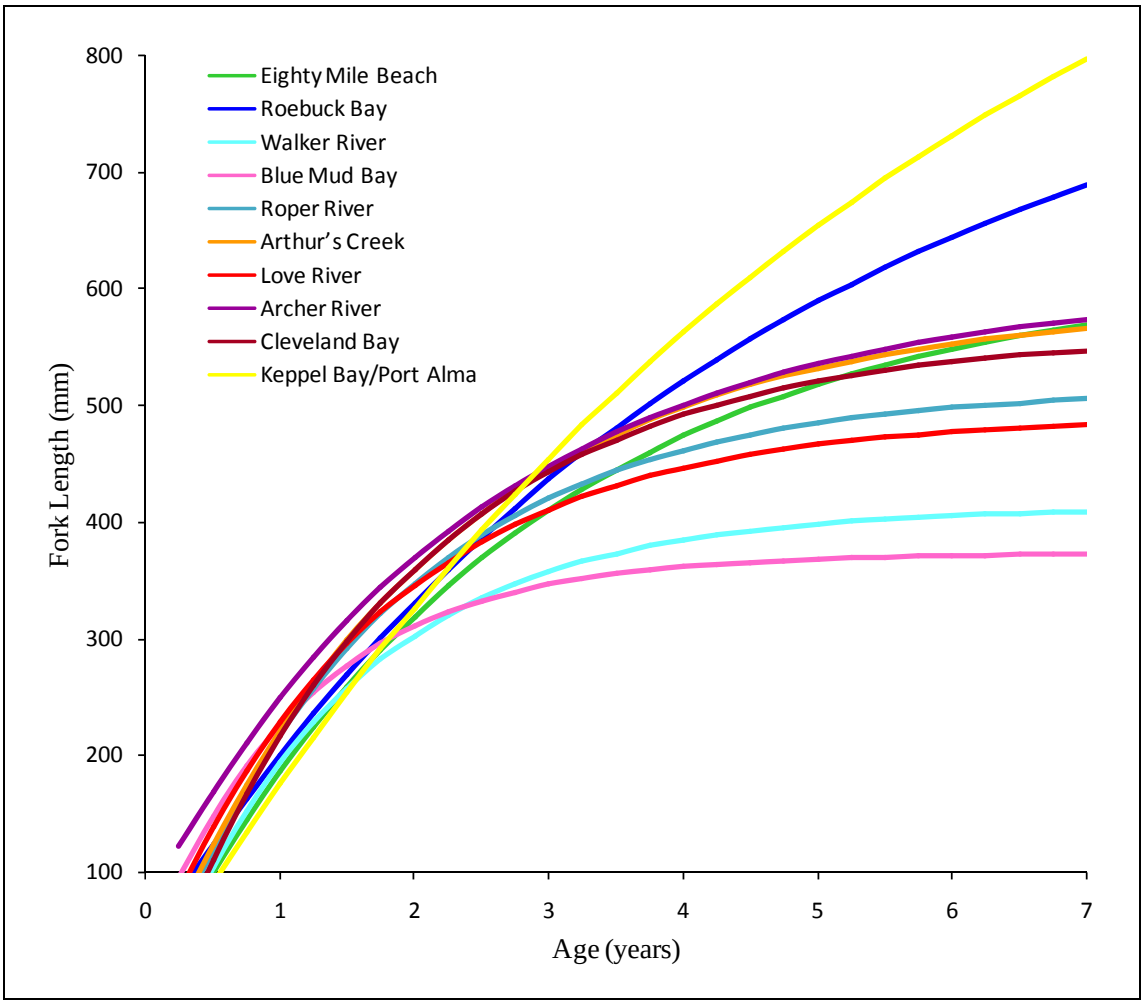



**Figure 5.6:** Back-calculated growth of blue threadfin from different locations around northern Australia.

**Table 5.4:** VBGF parameter estimates for each location from back-calculated length-at-age, age and fork length (FL) ranges (s.e. = standard error).

| Location | *K* (± s.e.) | $L_\infty$(mm ± s.e.) | $t_0$ (years ± s.e.) | Age (years) | FL (mm) |
|---|---|---|---|---|---|
| Eighty Mile Beach | 0.369 (0.089) | 616 (74) | 0.02 (0.11) | 1 - 4 | 114 – 515 |
| Roebuck Bay | 0.217 (0.046) | 871 (111) | -0.2 (0.09) | 1 - 5 | 91 – 583 |
| Walker River | 0.689 (0.101) | 412 (15) | 0.08 (0.11) | 1 - 6 | 85 - 469 |
| Blue Mud Bay | 0.851 (0.352) | 373 (28) | -0.09 (0.34) | 1 - 4 | 123 - 379 |
| Roper River | 0.573 (0.207) | 515 (61) | 0.04 (0.22) | 1 - 4 | 133 - 508 |
| Arthur's Creek | 0.474 (0.090) | 587 (42) | 0.01 (0.12) | 1 - 5 | 134 – 556 |
| Love River | 0.591 (0.127) | 491 (34) | -0.06 (0.14) | 1 - 4 | 138 – 496 |
| Archer River | 0.411 (0.097) | 604 (57) | -0.30 (0.16) | 1 - 5 | 147 - 505 |
| Cleveland Bay | 0.536 (0.075) | 561 (29) | 0.09 (0.08) | 1 - 5 | 140 - 542 |
| Keppel Bay/Port Alma | 0.166 (0.022) | 1161 (103) | 0.02 (0.05) | 1 - 6 | 71 - 769 |

**Table 5.5:** *P*-values from likelihood ratio tests of growth between locations (Bold indicates significant difference, Bonferonni adjusted α = 0.0011).

| Location | BMB | RR | KB/PA | CB | AR | LR | AC | EMB | RB |
|---|---|---|---|---|---|---|---|---|---|
| **WR** | 0.0001 | >0.0001 | >0.0001 | >0.0001 | >0.0001 | >0.0001 | >0.0001 | >0.0001 | >0.0001 |
| **BMB** | | >0.0001 | >0.0001 | >0.0001 | >0.0001 | >0.0001 | >0.0001 | >0.0001 | >0.0001 |
| **RR** | | | >0.0001 | 0.0324 | >0.0001 | 0.3747 | 0.0482 | >0.0001 | 0.0002 |
| **KB/PA** | | | | >0.0001 | >0.0001 | >0.0001 | >0.0001 | >0.0001 | >0.0001 |
| **CB** | | | | | >0.0001 | >0.0001 | 0.8698 | >0.0001 | >0.0001 |
| **AR** | | | | | | >0.0001 | 0.0008 | >0.0001 | >0.0001 |
| **LR** | | | | | | | 0.0002 | >0.0001 | >0.0001 |
| **AC** | | | | | | | | >0.0001 | >0.0001 |
| **EMB** | | | | | | | | | 0.0001 |

## Length at Sex Change

Logistic regression of the proportion of females in 25mm length classes indicates a large variation of lengths at which blue threadfin change sex across locations (Table 5.8, Figure 5.7). It was not possible to estimate length at sex change for the BMB, AR and AC locations as samples from these locations consisted entirely of females suggesting that the length at sex change for these locations was somewhat smaller than the length ranges sampled. From the logistic regressions, it is apparent that blue threadfin from the Queensland East Coast locations (CB and KB/PA) change sex at the largest length (~400-500mm), while the LR location, having only two males and three transitional individuals in the sample, displayed the lowest length at sex change of all locations. Almost all logistic regressions differed significantly among locations except for the WR and EMB locations, and the LR and EMB locations (Table 5.9).

**Table 5.7:** Tukey HSD multiple comparisons of back-calculated length-at-age (Bold indicates significant difference).

| Age | Location | BMB | RR | KB/PA | CB | AR | LR | AC | EMB | RB |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | **WR** | **0.0005** | 0.0729 | 0.0659 | **0.0219** | **<0.0001** | **<0.0001** | **0.013** | 0.9967 | 0.9868 |
| | **BMB** | | 0.9741 | **<0.0001** | 0.9416 | 0.0929 | 0.0929 | 0.9998 | **<0.0001** | **0.0033** |
| | **RR** | | | **<0.0001** | 1.0000 | **0.0017** | 0.8283 | 0.9999 | **0.0037** | 0.3039 |
| | **KB/PA** | | | | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | 0.4963 | **<0.0001** |
| | **CB** | | | | | **0.0001** | 0.6643 | 0.9998 | **0.0004** | 0.1213 |
| | **AR** | | | | | | 0.1008 | **0.0173** | **<0.0001** | **<0.0001** |
| | **LR** | | | | | | | 0.9913 | **<0.0001** | **<0.0001** |
| | **AC** | | | | | | | | **0.0004** | 0.0713 |
| | **EMB** | | | | | | | | | 0.4942 |
| **2** | **WR** | 0.9999 | **0.0038** | **0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | 0.6700 | **<0.0001** |
| | **BMB** | | 0.1398 | 0.3382 | **<0.0001** | **<0.0001** | **0.0044** | **<0.0001** | 0.9969 | 0.2053 |
| | **RR** | | | 0.9362 | 0.7102 | 0.0896 | 1.0000 | 0.7779 | 0.3691 | 0.9865 |
| | **KB/PA** | | | | **<0.0001** | **<0.0001** | 0.1139 | **<0.0001** | 0.7372 | 0.9999 |
| | **CB** | | | | | 0.6506 | 0.2134 | 1.0000 | **<0.0001** | **<0.0001** |
| | **AR** | | | | | | **0.0008** | 0.7634 | **<0.0001** | **<0.0001** |
| | **LR** | | | | | | | 0.4019 | **0.0084** | 0.3917 |
| | **AC** | | | | | | | | **<0.0001** | **0.0003** |
| | **EMB** | | | | | | | | | 0.5123 |
| **3** | **WR** | 0.9997 | **0.0009** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** |
| | **BMB** | | **0.0018** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | 0.0001 | **<0.0001** |
| | **RR** | | | 0.4475 | 0.9109 | 0.7262 | 1.0000 | 0.6534 | 1.0000 | 0.9936 |
| | **KB/PA** | | | | 0.9899 | 1.0000 | **0.0014** | 1.0000 | **0.0010** | 0.2305 |
| | **CB** | | | | | 1.0000 | 0.2302 | 0.9998 | 0.1633 | 0.9954 |
| | **AR** | | | | | | 0.0571 | 1.0000 | **0.0380** | 0.8663 |
| | **LR** | | | | | | | **0.0221** | 1.0000 | 0.5116 |
| | **AC** | | | | | | | | **0.0145** | 0.7248 |
| | **EMB** | | | | | | | | | 0.3856 |
| **4** | **WR** | NA | NA | **<0.0001** | **<0.0001** | NA | NA | **<0.0001** | NA | **<0.0001** |
| | **BMB** | | NA | NA | NA | NA | NA | NA | NA | NA |
| | **RR** | | | NA | NA | NA | NA | NA | NA | NA |
| | **KB/PA** | | | | **0.0069** | NA | NA | **0.0150** | NA | 0.0966 |
| | **CB** | | | | | NA | NA | 0.9982 | NA | 0.5598 |
| | **AR** | | | | | | NA | NA | NA | NA |
| | **LR** | | | | | | | NA | NA | NA |
| | **AC** | | | | | | | | NA | 0.4981 |

| Age | Location | BMB | RR | KB/PA | CB | AR | LR | AC | EMB | RB |
|---|---|---|---|---|---|---|---|---|---|---|
| | **EMB** | | | | | | | | | NA |
| **5** | **WR** | NA | NA | **<0.0001** | NA | NA | NA | NA | NA | NA |
| | **BMB** | | NA | NA | NA | NA | NA | NA | NA | NA |
| | **RR** | | | NA | NA | NA | NA | NA | NA | NA |
| | **KB/PA** | | | | NA | NA | NA | NA | NA | NA |
| | **CB** | | | | | NA | NA | NA | NA | NA |
| | **AR** | | | | | | NA | NA | NA | NA |
| | **LR** | | | | | | | NA | NA | NA |
| | **AC** | | | | | | | | NA | NA |
| | **EMB** | | | | | | | | | NA |

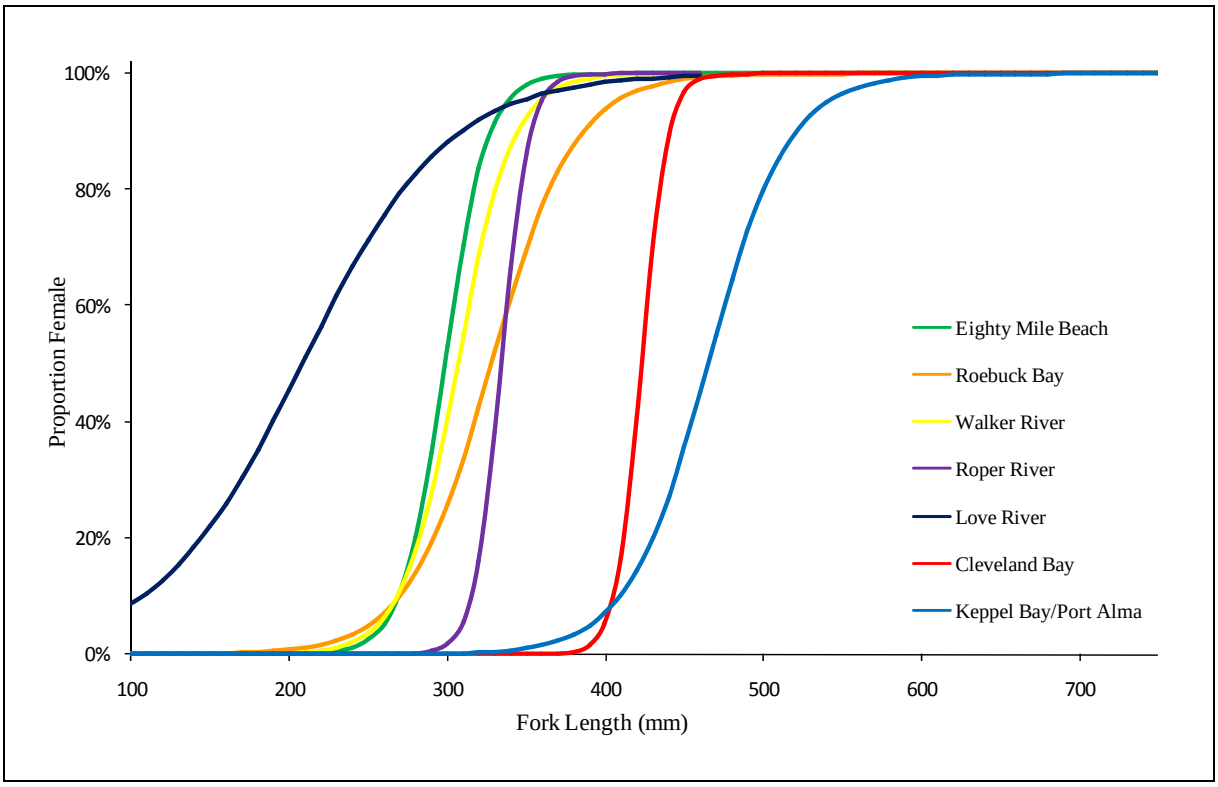



**Figure 5.7:** Logistic regression of the proportion of female blue threadfin in 25mm length classes.

**Table 5.8:** Logistic regression parameters of length at sex change for blue threadfin.

| Location | $S_{50}$ | $S_{95}$ | *n* |
|---|---|---|---|
| Walker River | 307 | 357 | 150 |
| Roper River | 334 | 359 | 52 |
| Keppel Bay/Port Alma | 465 | 540 | 358 |
| Cleveland Bay | 423 | 446 | 127 |
| Love River | 208 | 343 | 188 |
| Eighty Mile Beach | 298 | 337 | 151 |
| Roebuck Bay | 328 | 406 | 312 |

**Table 5.9:** *P*-values from likelihood ratio tests of logistic regressions of length at sex change between locations (Bold indicates significant difference, Bonferonni adjusted α = 0.0024).

| Location | RR | KB/PA | CB | LR | EMB | RB |
|---|---|---|---|---|---|---|
| WR | **>0.0001** | **>0.0001** | **>0.0001** | **0.0005** | 0.0175 | **>0.0001** |
| RR | | **>0.0001** | **>0.0001** | **>0.0001** | **>0.0001** | **>0.0001** |
| KB/PA | | | **>0.0001** | **>0.0001** | **>0.0001** | **>0.0001** |
| CB | | | | **>0.0001** | **>0.0001** | **>0.0001** |
| LR | | | | | 0.0402 | **>0.0001** |
| EMB | | | | | | **>0.0001** |

### Integration

The integration of results from the different location comparisons revealed that all except two location combinations had some level of differences in growth estimates (Figure 5.8). The RR location did not differ significantly with AC, as did the CB and AC locations. Difference indices revealed that the WR and BMB locations differed the most with other locations, but were least different between one another. All other location combinations demonstrated varying levels of difference indices, ranging from 0.20 to 1.00.

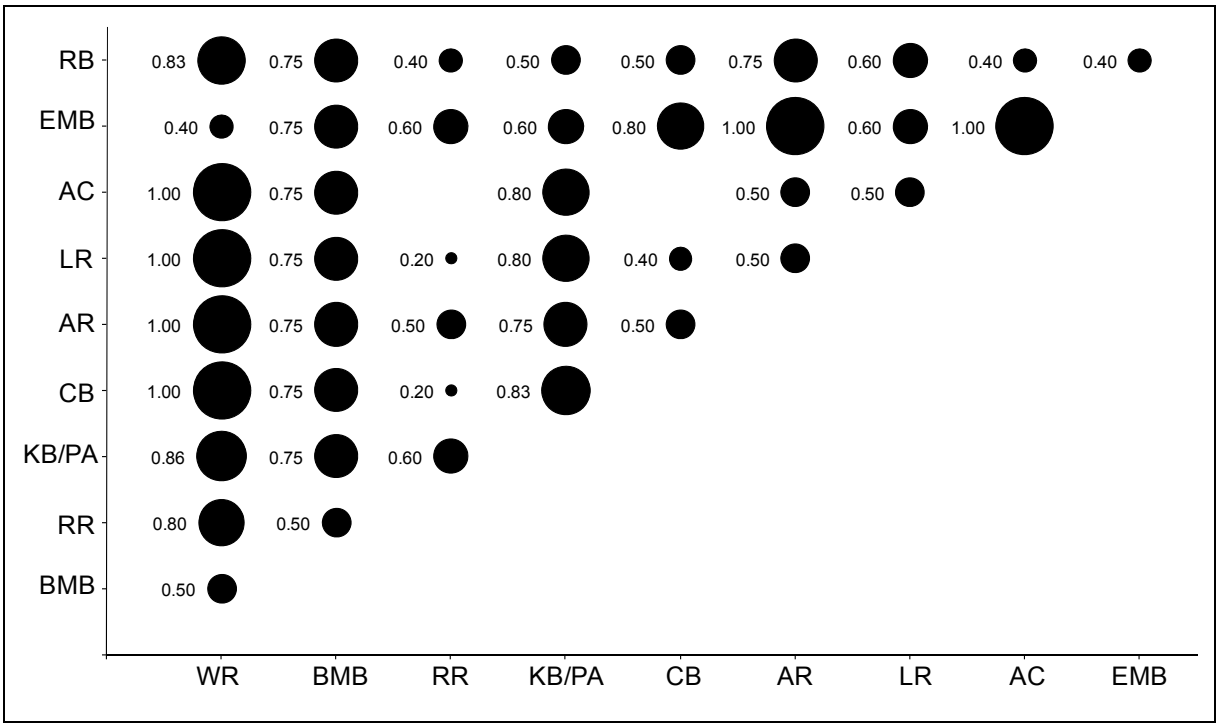



**Figure 5.8:** Indices of difference in blue threadfin growth and length-at-age estimates between locations (numbers indicate difference index value).

### Morphometrics

No significant differences were found among any of the locations for any of the morphometric relationships (Ln*FL*-Ln*W*: $F_{9, 962}$ = 3.384, $P$ < 0.001, *FL-UJL*: $F_{9, 944}$ = 10.018, $P$ < 0.001, *FL-HL*: $F_{9, 943}$ = 6.498, $P$ < 0.001, *FL-TL*: $F_{9, 929}$ = 3.678, $P$ < 0.001). The morphometric relationships were therefore estimated using data from all locations (Figures 5.9 to 5.12).

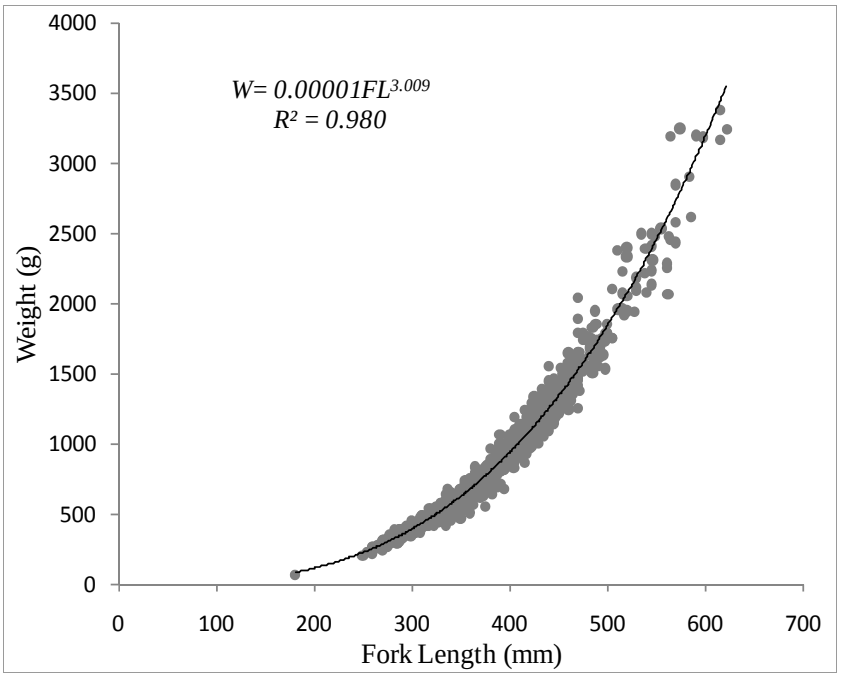



**Figure 5.9:** Blue threadfin fork length to weight relationship.

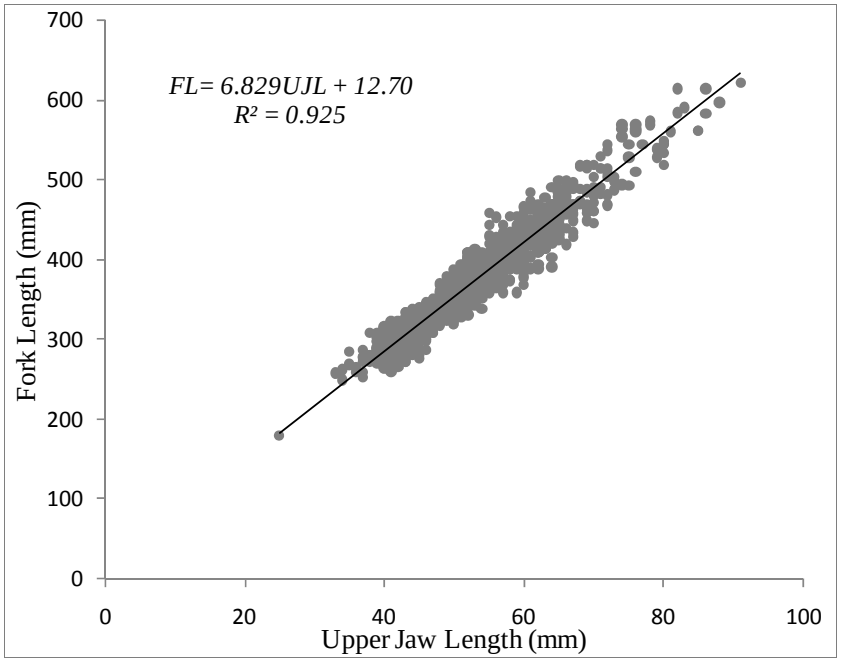



**Figure 5.10:** Blue threadfin fork length to upper jaw length relationship.

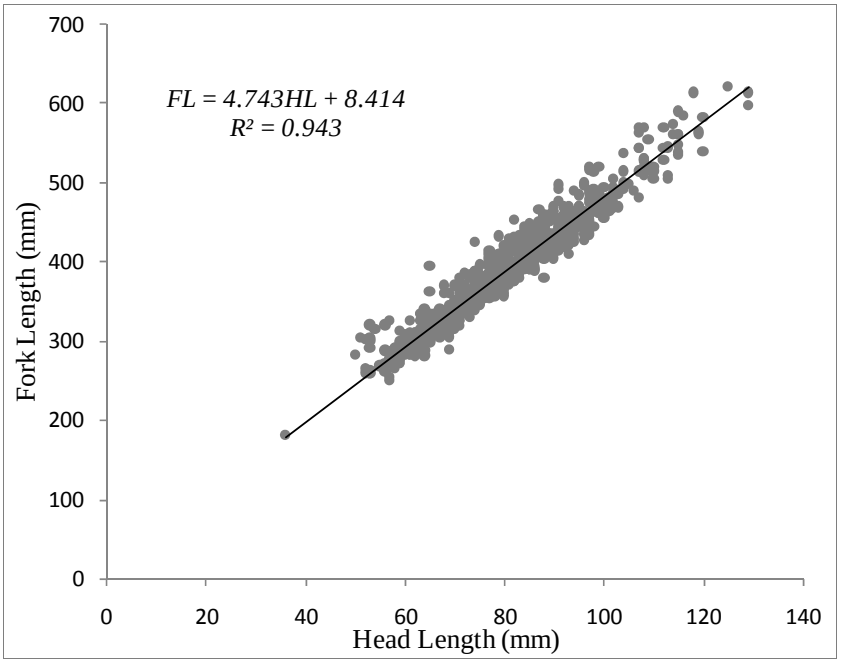



**Figure 5.11:** Blue threadfin fork length to head length relationship.

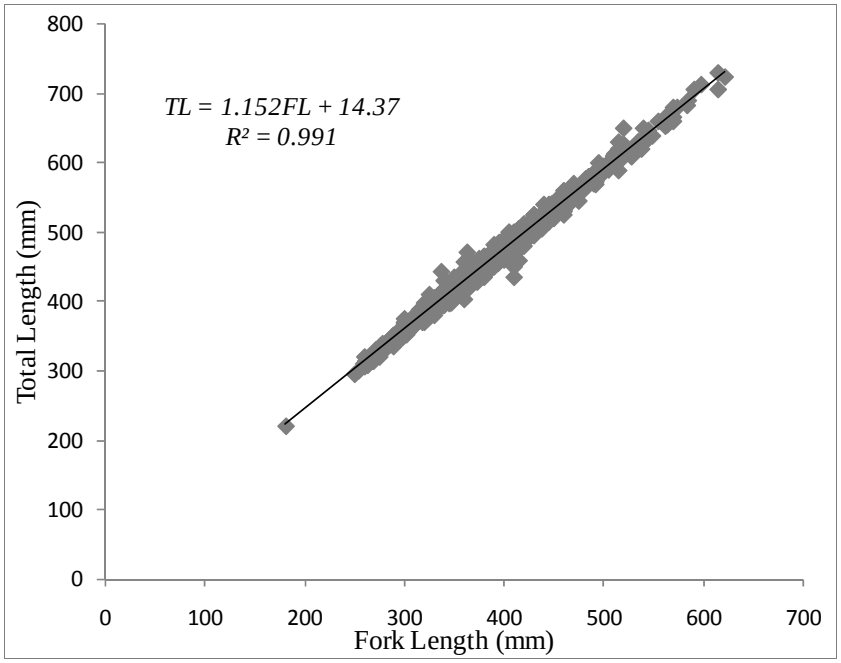



**Figure 5.12:** Blue threadfin fork length to total length relationship.

## 5.4 Discussion

A high degree of variation in life-history characteristics was demonstrated for blue threadfin from different locations around northern Australia, indicating a high degree of spatial population subdivision for the species. More importantly, interpretation of the life-history results here suggests that blue threadfin stocks are localised at small spatial scales suggesting strong fidelity and limited movement. Further, these differences were also shown to be stable over time.

Temporal stability in growth estimates was demonstrated for the two locations that were known to be collected from almost the same location (within 10-20 km) in both Phases (KB/PA and RB). It is not known how near from each other the WR and BMB samples were collected (we know that is likely to be within approx. 150 km) however the temporal comparison suggested it was far enough that they were likely to have come from different populations. The same issue applied to the AR and LR samples, both from the Aurukun region on the eastern Gulf of Carpentaria and separated by less than fifty kilometres. Despite the close proximity of these locations and the lack of any real geographic features separating them, they still displayed differences in growth, length-at-age and otolith radius to fork length relationship. This was consistent with the result of almost all regional comparisons of growth and size at sex change being significantly different, even for adjacent locations. This provides strong evidence that the degree of spatial population subdivision of blue threadfin may be at a very small, localised scale. Evidence for localised population in blue threadfin from the Queensland east coast has been demonstrated previously through parasite analysis (Zischke *et al.* 2009).

While it may be expected to see some evidence of population structure between the Queensland east coast and the Gulf of Carpentaria based on the historical land barrier between Cape York and Papua New Guinea (Chivas *et al.* 2001), currents in the Gulf of Carpentaria (Wolanski *et al.* 1988) and patterns seen in other fish species (Chenoweth *et al.* 1998a, b; Buckworth *et al.* 2007; Welch *et al.* 2009), the level of population structure observed was significantly more substantial than that predicted from historic large-scale ocean basin separation alone. Some studies have demonstrated that a significant component of population structuring in marine fish at a genetic level is related to species' life-history traits (Waples 1987; Doherty *et al.* 1995). Therefore, from the differences in life-history traits seen in blue threadfin, it is likely that the species also displays a high degree of spatial genetic variation.

Another significant finding of this study was the difference in the otolith radius to fork length relationship between locations. Given that no significant changes were found in any of the morphometric relationships between locations, it is surprising that there were differences in the morphology of blue threadfin otoliths between locations. It was noted on examination of the otoliths for ageing, that there seemed to be differences in otolith shape between locations. Given the differences observed in life-history characteristics, blue threadfin otoliths may lend themselves well to otolith shape analysis, which could complement other stock identification techniques.

From our results, it is likely that gear selectivity allows a high proportion of individuals to change sex before being fully selected by the fishing gears. This is because of the small length at sex change seen across most locations. Other studies have demonstrated that commercial gill nets, from which most samples in this study were caught, typically select for a larger length range than other gear types, with the majority of commercially caught fish larger than 375 mm fork length (Pember *et al.* 2005). However because of the small length at sex change seen in some locations, it is likely that catches are sex biased with mostly females being harvested.

Another implication of the observed differences in the length at sex change is the use management tools such as minimum legal size limits. Given the differences seen in the length at sex change between locations, management authorities should be adopting population appropriate minimum legal size limits. Unfortunately, insufficient numbers of small individuals were collected to enable the estimation of length or age at maturity. This was most likely due to the selectivity of the fishing gear used by the commercial fishers from which the samples were sourced. However, given the small lengths at sex change seen from most locations, and the growth rates seen, it is likely that most individuals would mature at a relatively small size in their first or second year as was found in previous studies (Pember *et al.* 2005).

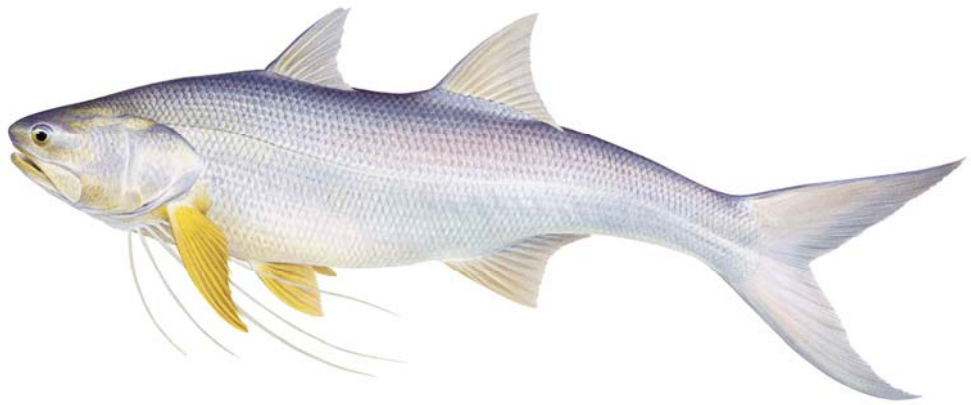
Estimates of growth for blue threadfin from the Western Australia coast differed somewhat to previous estimates of growth in Western Australia from Pember *et al.* (2005). This may be explained by the fact that this study estimated growth from the two Western Australia locations separately, while data from these locations were pooled in Pember *et al.* (2005) to estimate growth. Another reason for the difference could be the differences in methods for estimating length-at-age. Pember *et al.* (2005) used age adjustment which was based on the capture date, the period of annuli formation and the spawning period, while back-calculation was used in this study.

This study utilised a number of methods for statistically comparing growth, as each method on its own does not give a complete overview of the differences in growth as results are not consistent between methods. Likelihood ratio tests can be sensitive to small differences in growth and it has been suggested that comparison of mean length at age be used as well (Wang and Milton 2000). In isolation, the different growth comparison methods can provide inconsistent results, due in part to the assumptions and sensitivities of the analyses and the variable nature of growth. The integration of several methods for comparing growth between locations, produced difference indices that encapsulated all the differences in growth between locations, thus proving to be a useful technique for summarising the results of the different methods in a manner which is easily interpretable. This integration technique might also be applicable to the integration of multiple methods of stock discrimination as well as other life-history characteristics.

This study provides evidence and reasoning for a review of management regimes for blue threadfin across north-eastern Australia. Stock structure of blue threadfin has been shown here to be at fine spatial scales thus providing guidance as to the appropriate spatial scale at which blue threadfin monitoring and assessment should be applied. This study also provides accurate estimates of blue threadfin life history parameters for local populations across a large area of northern Australia which are important input parameters for stock assessments and will further guide the implementation of appropriate management measures.

# Section 3

# King Threadfin



King threadfin salmon, *Polydactylus macrochir*

Illustration © R. Swainston http://www.anima.net.au

# Chapter 6: Stock structure of king threadfin, *Polydactylus macrochir*, as indicated by parasites

**Brad R. Moore, Jason M. Stapley, Quentin Allsop, Stephen J. Newman, David J. Welch & Robert J. G Lester**

## 6.1 Introduction

The king threadfin, *Polydactylus macrochir* Günther 1867, is a large, protandrous Polynemid that inhabits turbid coastal waters, estuaries and mangrove creeks across northern Australia and southern Papua New Guinea (Motomura *et al.* 2000). In Australia, its distribution extends from the Ashburton River in Western Australia across northern Australia to Brisbane in southeast Queensland (Motomura *et al.* 2000).

King threadfin support valuable commercial, recreational and indigenous fisheries across northern Australia and form the second most important target species for northern Australia's commercial inshore net fisheries after the iconic barramundi (*Lates calcarifer*) (Pember *et al.* 2005), with a reported 780 tonnes harvested across Australia in 2005. The bulk of this catch was from the waters of the Queensland Gulf of Carpentaria and Northern Territory (323 and 279 tonnes, respectively), whereas smaller catches were reported in the waters of Western Australia (70 t) and Queensland's east coast (108 t) (Matthews and Grace 2006; DPI&F 2007; Newman *et al.* 2007). The species is also heavily targeted by recreational anglers throughout its distribution, and is highly regarded as both a table and sport fish (Welch *et al.* 2002).

Due largely to a lack of knowledge of king threadfin biology (Welch *et al.* 2005), management and assessment conveniently assumes single stocks exist in the waters of Western Australia, the Northern Territory, Queensland's Gulf of Carpentaria, and Queensland's east coast. The current management arrangements for *P. macrochir* across northern Australia make no allowance for movement of fish between management jurisdictions. As the distribution of king threadfin crosses state and territorial boundaries, a need for cross jurisdictional cooperative management may exist. Conversely, king threadfin may be resident on a small spatial scale, with a number of localised stocks occurring within each jurisdiction. In this case management may be more appropriate on a fine scale. Irrespective of the scenario, knowledge of the stock structure is seen as critical to ensuring sustainable management of the king threadfin across northern Australia.

Parasites have been widely used as biological tags to provide information of movements and stock structure of exploited fish populations. In Australian waters, insight from parasite loadings has been used to discern the stock structure and movements of a variety of fish species (e.g. Lester *et al.* 1988; Speare 1995; Moore *et al.* 2003; Charters *et al.* 2009). The basic premise underlying the use of parasites as biological tags from which host movements and stock structure can be deduced is that naturally occurring parasites have a discontinuous distribution compared to that of their hosts (Lester 1990). Consequently, organisms only become infected with a certain parasite only when they come within the endemic area of that parasite (MacKenzie and Abaunza 1998). A parasite's endemic area is the geographical location in which conditions are suitable for transmission, and in which the parasite occurs naturally. As a fish moves into a parasite's endemic area, they become infected, and as they move out, they carry a legacy of their occupancy within the area (Lester 1990). Consequently, the spatial relationships between host populations may be deduced by analysing parasite faunas of individuals from different locations. Where the parasite fauna is different, the history of the fish is different according to the time scale of the parasite counted: recent history for temporary parasites, long-term history for permanent parasites (Lester 1990).

Zischke *et al.* (2009) conducted a preliminary analysis of the small scale spatial movements of another polynemid species, the blue threadfin, *Eleutheronema tetradactylum*, along the east coast of Queensland, Australia. Their results, based on the parasite fauna of fish collected from four differentially spaced areas, suggest that blue threadfin undergo limiting mixing along Queensland's east coast, indicating multiple stocks in the region. In this study, we used parasites to make inferences about the movements and stock structure of *P. macrochir* across northern Australia. We hypothesised that king threadfin populations form a number of distinct stocks across northern Australia, similar to that reported for *E. tetradactylum* (Zischke *et al.* 2009).

## 6.2 Methods

### Sample Collection

Fish were collected from commercial fishers and state fisheries agencies from nine sites across northern Australia between winter 2007 and winter 2009 (Figure 6.1; Table 6.1). Three sites (Roebuck Bay in Western Australia, Chambers Bay in the Northern Territory and the Fitzroy River on Queensland's east coast) were sampled twice across the sampling period to examine temporal patterns in abundance of the parasites encountered. It was not possible to examine the temporal stability of the parasites infecting *P. macrochir* at all sites due to the remoteness of the sampling sites and the variable nature of fishing activities. Neighbouring sites were separated by tens to hundreds of kilometres and were centred on the important commercial and recreational fishing areas for *P. macrochir* across northern Australia. For each fish collected, the site, date of capture, sex, maturity stage and caudal fork

length (FL) were recorded. Heads and viscera, or gills and viscera, were frozen in individually labelled plastic bags for later laboratory examination. Sagittal otoliths (hereafter referred to as otoliths) were removed from all fish for later aging.

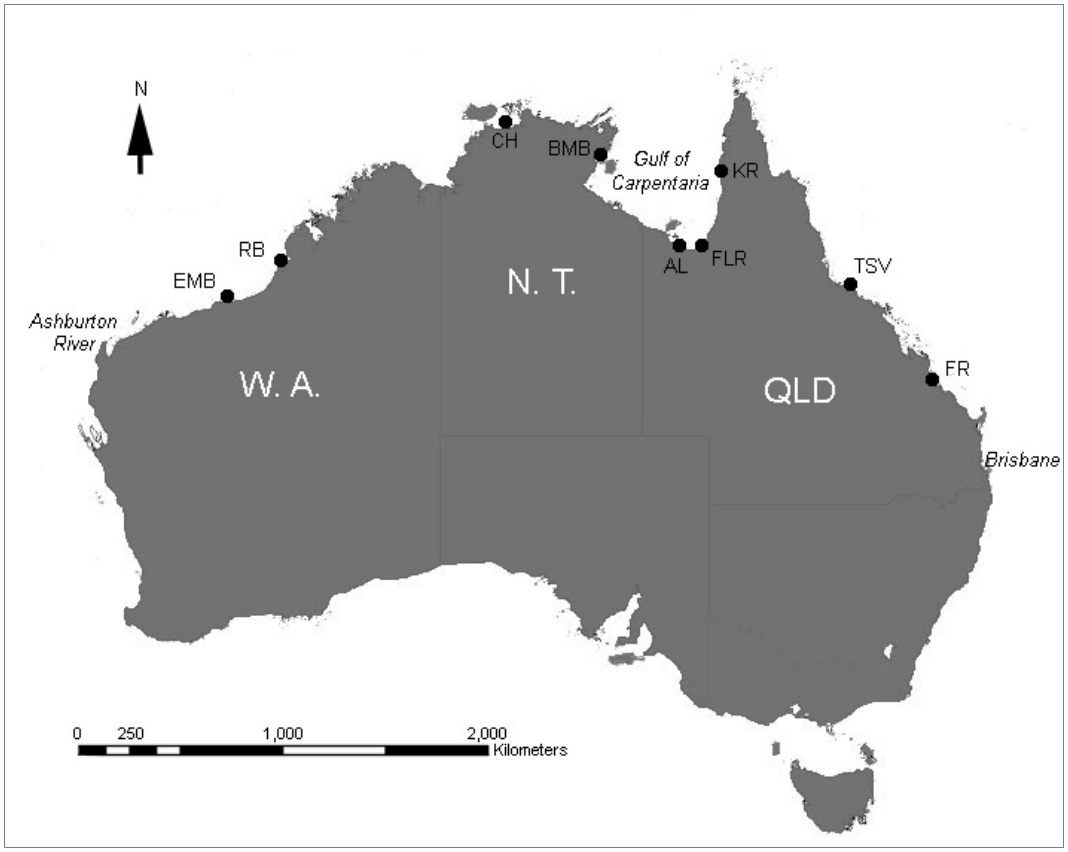



**Figure 6.1:** The sampling sites used in this study. EMB, Eighty Mile Beach; RB, Roebuck Bay; CH, Chambers Bay; BMB, Blue Mud Bay; AL, Albert River; FLR, Flinders River; KR, Kendall River; TSV, Townsville; FR, Fitzroy River.

In the laboratory, samples were defrosted in water, and the gill opercula and viscera were examined for metazoan parasites, including those encysted in or outside of the stomach wall. Parasites encountered were extracted, identified enumerated and categorised as 'permanent' or 'temporary', based on their probable life span in or on the fish.

To facilitate parasite identification, unfrozen samples were taken from the Fitzroy, Mary and Brisbane rivers on Queensland's east coast. Trypanorhynchs were placed in fresh water to facilitate tentacle eversion. Representative specimens were stained in Mayer's haematoxylin, dehydrated in ethanol, cleared using methyl salicylate and mounted in Canada balsam. Once identified, the morphology of the scolex, bothridia and blastocyst were considered adequate to separate trypanorhynch species. Parasites were identified according to descriptions in Podder (1937), Cannon (1977), Pillai (1985), Campbell and Beveridge (1996), Amin *et al.* (2003) and Palm (2004).

Ages of *P. macrochir* were estimated by examining the number of opaque bands (= annuli; Pember *et al.* 2005) and the margin category for whole and sectioned otoliths, as detailed in Chapter 9. Briefly, otoliths in which six or less annuli were counted in the initial read were read whole again, whereas otoliths in which more than six annuli were counted in the initial read were sectioned. Once the method of reading was established each otolith was read twice. When annuli counts between the two reads did not agree, a third reading was taken, and the two concurrent readings being accepted as the number of annuli. When all three counts differed, the otolith was rejected from further analysis. An extra half or full year was added to the age of the fish if the width of the final annuli and otolith margin was observed to be half, or equal that, of the penultimate band, respectively. All otoliths were read by the same reader (B. Moore).

### Data Analysis

Summary statistics including mean parasite abundance (number of a particular parasite per host examined including uninfected hosts) and prevalence (number of hosts infected with a particular parasite) were compiled for each parasite species, following Bush *et al.* (1997). Only parasite species with a prevalence $\geq$ 10% in at least one of the samples were used in the analysis (component species; Bush *et al.* 1990). A high variance:mean ratio for all parasite species indicated an over-dispersed distribution. The natural log of the parasite + 1 (Ln[x + 1]) was used to normalise the data. This transformed data was used throughout the analyses.

Long-lived parasites tend to accumulate with host age (Rohde 1982), which may obscure differences in parasite fauna between areas if samples contain hosts of different ages. To reduce this effect, parasite species were examined for correlation with host age. Where significant, the numbers of the correlated species were adjusted to the mean host age. No adjustment was made if the parasite abundance was zero.

One-way ANOVA was applied to identify differences in abundance of the individual parasite species deemed as being 'permanent' in or on the fish across the twelve sample groups. Significant results were examined using Tukey-Kramer post-hoc pair-wise comparisons (Sokal and Rohlf 1995).

Differences between sites in permanent parasite community assemblages based on the abundance data of the permanent parasite species were examined by multiple analysis of variance (MANOVA), followed by canonical discriminant function analysis (*Statistica* v7.0). The results of the discriminant function analyses were displayed as graphs of the first and second canonical axes. Confidence levels of 95% are given as shaded circles around the mean canonical score with the radius equal to the square root of 5.99/number of fish in the sample (Mardia *et al.* 1979). Partial Wilk's lambda values were used to indicate the discriminatory power of the individual parasites, ranging from 0 (total discriminatory power) to 1 (no discriminatory power).

## 6.3 Results

Ten parasite species (or taxa) were considered as suitable markers to investigate movement of *P. macrochir* and were therefore counted in all fish. Permanent parasites included the nematodes *Anisakis* sp. and *Terranova* (type II), the cestodes *Callitetrarhynchus gracilis*, *Nybelinia* sp., *Otobothrium australe*, *Pterobothrium pearsoni*, *Pterobothrium* sp A and the acanthocephalan *Pomphorhynchus* sp. Temporary parasites included the copepod *Thysanote eleutheronemi* and the acanthocephalan *Neoechinorhynchus topseyi*. Representative specimens of each parasite species were stored in the Marine Parasitology laboratory at the University of Queensland. As we sought to determine the long-term movements of *P. macrochir*, higher statistical analyses were only conducted on permanent parasite species.

A summary of the untransformed parasite data for the sampled locations is presented in Table 6.1. Transformed counts of five species, *C. gracilis*, *O. australe*, *Pterobothrium pearsoni*, *Pomphorhynchus* sp. and *Terranova* (type II) were significantly correlated with host age and were thus adjusted to the mean host age (3.78 years).

**Table 6.1:** Average numbers of parasites per fish in king threadfin, *Polydactylus macrochir*, sampled from nine sites across northern Australia (untransformed data). Number of fish infected shown in parentheses.

| **Management jurisdiction** | **Western Australia** | | | **Northern Territory** | | | **Queensland Gulf of Carpentaria** | | | **Queensland east coast** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Site** | **Eighty Mile Beach** | **Roebuck Bay 'a'** | **Roebuck Bay 'b'** | **Chambers Bay 'a'** | **Chambers Bay 'b'** | **Blue Mud Bay** | **Flinders River** | **Albert River** | **Kendall River** | **Townsville** | **Fitzroy River 'a'** | **Fitzroy River 'b'** |
| **Sampling season (year)** | **Autumn (2008)** | **Autumn (2008)** | **Winter (2008)** | **Summer (2008)** | **Autumn (2009)** | **Spring (2008)** | **Winter (2007)** | **Spring (2008)** | **Spring (2008)** | **Winter (2008)** | **Spring (2007)** | **Spring (2008)** |
| **Site ID** | **EMB** | **RBa** | **RBb** | **CHa** | **CHb** | **BMB** | **FLR** | **AL** | **KR** | **TSV** | **FRa** | **FRb** |
| **Sample size** | 49 | 57 | 60 | 51 | 53 | 70 | 50 | 35 | 63 | 54 | 49 | 50 |
| **Mean FL (mm)** | 635 | 688 | 566 | 910 | 703 | 656 | 639 | 597 | 535 | 687 | 708 | 755 |
| **Mean age** | 4.4 | 3.3 | 2.4 | 9.1 | 3.4 | 2.8 | 3.7 | 3.5 | 2.1 | 3.0 | 4.3 | 4.6 |
| **Temporary parasites** | | | | | | | | | | | | |
| *Thysanote eleutheronemi* | 0.0 (1) | - | - | - | 0.0 (0) | 0.0 (0) | 0.1 (5) | 0.0 (1) | 0.1 (5) | 0.0 (1) | 0.1 (2) | 0.1 (1) |
| *Neoechinorhynchus topseyi* | 5.0 (22) | 20.2 (40) | 13.8 (55) | 0.2 (3) | 14.9 (25) | 0.0 (0) | 0.2 (4) | 0.0 (0) | 0.0 (1) | 0.1 (2) | 0.1 (2) | 0.0 (0) |
| **Permanent parasites** | | | | | | | | | | | | |
| *Pterobothrium pearsoni* | 0.7 (16) | 0.1 (5) | 0.1 (4) | 3.1 (32) | 0.6 (13) | 0.0 (0) | 0.2 (7) | 0.9 (14) | 1.6 (37) | 0.1 (6) | 0.6 (11) | 0.9 (24) |
| *Pterobothrium* sp. A | 0.7 (13) | 0.3 (9) | 0.1 (2) | 1.7 (44) | 0.7 (20) | 1.3 (39) | 7.9 (48) | 8.2 (34) | 3.2 (57) | 5.9 (42) | 4.6 (44) | 4.8 (40) |
| *Callitetrarhynchus gracilis* | 0.9 (16) | 1.2 (24) | 0.5 (12) | 41.5 (49) | 7.4 (53) | 0.2 (13) | 5.1 (45) | 18.5 (35) | 12.1 (57) | 0.9 (29) | 27.4 (49) | 22.7 (50) |
| *Nybelinia* sp. | 0.0 (0) | 0.0 (0) | 0.0 (0) | 0.1 (3) | 0.0 (0) | 0.0 (0) | 0.0 (2) | 0.0 (0) | 0.0 (3) | 0.3 (12) | 1.5 (21) | 2.1 (35) |
| *Anisakis* sp. | 0.1 (3) | 0.0 (0) | 0.0 (0) | 0.3 (9) | 0.3 (13) | 0.1 (5) | 0.0 (0) | 0.0 (0) | 0.0 (0) | 0.0 (2) | 0.2 (5) | 0.1 (3) |
| *Terranova* (type II) | 3.4 (22) | 0.6 (9) | 0.0 (1) | 25.9 (50) | 1.2 (9) | 0.2 (9) | 0.0 (0) | 0.0 (0) | 0.0 (1) | 0.1 (6) | 0.2 (3) | 0.1 (1) |
| *Pomphorhynchus* sp. | 0.0 (0) | 0.0 (0) | 0.0 (0) | 53.3 (50) | 1.9 (7) | 0.1 (2) | 0.2 (6) | 0.0 (0) | 0.1 (5) | 0.0 (1) | 0.1 (4) | 0.2 (4) |
| *Otobothrium australe* | 69.1 (48) | 12.2 (53) | 3.3 (44) | 1017.9 (51) | 259.3 (53) | 0.0 (0) | 0.4 (10) | 0.4 (9) | 0.6 (19) | 0.0 (0) | 0.0 (0) | 0.0 (0) |

One-way ANOVA indicated that the abundance of all parasite species exhibited a significant difference across the twelve samples. Tukey-Kramer pair-wise comparisons of the abundance of the eight permanent parasites gave an indication of the similarity between the temporally and spatially distinct samples (Table 6.2). In terms of temporal stability, the Roebuck Bay replicates appeared homogeneous, with the abundance of only *Otobothrium australe* being significantly different. The Fitzroy River replicates also appeared homogenous, differing only in the abundance of *Nybelinia* sp. In contrast, the Chambers Bay replicates appeared dissimilar, with abundances of five of the eight parasites being significantly different. Roebuck Bay fish differed to those from Eighty Mile Beach with respect to abundances of *Terranova* (type II) and *Otobothrium australe*, suggesting isolation between these samples. Both Chambers Bay replicates appeared distinct from all other sites. In the Gulf of Carpentaria, samples from Blue Mud Bay appeared anomalous, with no less than two parasites being significantly different to other Gulf sites. Fish from the Flinders and Albert Rivers appeared similar, differing only in *C. gracilis* abundance. The Kendall River samples appeared distinct to these sites, differing in abundances of *Pterobothrium pearsoni*, *Pterobothrium* sp. A and *C. gracilis*. Townsville fish appeared distinct from the Fitzroy River replicates, suggesting isolation.

Multivariate analysis indicated that significant differences existed in the permanent parasite fauna of fish across the locations (MANOVA, d.f. 88 and 4088, $p < 0.05$). Discriminant function analysis, based on the abundances of the eight permanent species produced clear separation between Western Australia, Northern Territory and Queensland samples (Figure 6.2). The replicate samples from Roebuck Bay (site IDs RB ‘a’ and RB ‘b’) grouped together at all axes, suggesting that at this site the parasite assemblages were temporally stable. These samples grouped with Eighty Mile Beach (EMB) at all axes, suggesting a common history of fish between these sites. Replicate samples from Chambers Bay (CH ‘a’ and CH ‘b’) appeared distinct from all other areas, and also appeared distinct from each other, possibly suggesting significant temporal variation in the parasites at this site. In the Gulf of Carpentaria, fish from Blue Mud Bay (BMB) appeared distinct from all other sites. Fish from the Albert River (AL) and the Kendall River (KR) appeared homogenous, as did those from the Flinders River (FLR) and the Kendall River. In the southern Gulf, samples from the Flinders River separated from the Albert River on the third canonical axis, suggesting that these fish have a distinct parasite fauna. On Queensland’s east coast, Townsville (TSV) fish appeared significantly different from fish collected from the Fitzroy River (FR). The Fitzroy River replicate samples (FR ‘a’ and FR ‘b’) grouped together at all axes, suggesting that at this location the parasite fauna was stable across time. In the analysis the first two axes accounted for 84% of the variation between areas. The third axis accounted for a further ten percent of the variability.

**Table 6.2:** Comparison of abundance of eight permanent parasite species infecting *Polydactylus macrochir* from the twelve samples (nine sites) across northern Australia. The number in the table corresponds to the parasite species that is significantly different at the 95% level of confidence based on Tukey-Kramer pair-wise comparison (natural log of parasite abundance).

| Site | EMB | RB 'a' | RB 'b' | CH 'a' | CH 'b' | BMB | AL | FLR | KR | TSV | FR 'a' | FR 'b' |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **EMB** | | 6, 8 | 6, 8 | 1, 2, 3, 6, 7, 8 | 2, 6, 8 | 1, 6, 8 | 2, 3, 6, 8 | 2, 3, 6, 8 | 1, 2, 3, 6, 8 | 3, 6, 8 | 2, 3, 4, 6, 8 | 2, 3, 4, 6, 8 |
| **RB 'a'** | | | 8 | 1, 2, 3, 5, 6, 7, 8 | 2, 5, 7, 8 | 3, 8 | 1, 2, 3, 8 | 2, 3, 8 | 1, 2, 3, 8 | 3, 8 | 2, 3, 4, 8 | 1, 2, 3, 4, 8 |
| **RB 'b'** | | | | 1, 2, 3, 5, 6, 7, 8 | 2, 5, 7, 8 | 3, 8 | 1, 2, 3, 8 | 2, 3, 8 | 1, 2, 3, 8 | 3, 8 | 2, 3, 4, 8 | 1, 2, 3, 4, 8 |
| **CH 'a'** | | | | | 1, 3, 6, 7, 8 | 1, 2, 6, 7, 8 | 1, 2, 3, 5, 6, 7, 8 | 1, 2, 3, 5, 6, 7, 8 | 5, 6, 7, 8 | 1, 2, 3, 6, 7, 8 | 1, 2, 3, 4, 6, 7, 8 | 1, 3, 4, 6, 7, 8 |
| **CH 'b'** | | | | | | 1, 2, 5, 8 | 2, 3, 5, 8 | 2, 3, 5, 8 | 1, 3, 5, 8 | 2, 3, 5, 8 | 2, 3, 4, 8 | 3, 4, 8 |
| **BMB** | | | | | | | 1, 2, 3 | 2, 3 | 1, 2, 3, 8 | 3 | 2, 3, 4 | 1, 2, 3, 4 |
| **AL** | | | | | | | | 2 | 1, 2, 3 | 1, 2 | 3, 4 | 2, 3, 4 |
| **FLR** | | | | | | | | | 1, 2, 3 | 2 | 2, 3, 4 | 1, 2, 3, 4 |
| **KR** | | | | | | | | | | 1, 2, 8 | 1, 4, 8 | 1, 4, 8 |
| **TSV** | | | | | | | | | | | 2, 4 | 1, 2, 4 |
| **FR 'a'** | | | | | | | | | | | | 4 |
| **FR 'b'** | | | | | | | | | | | | |

**Key to species:**

1 = *Pterobothrium pearsoni* 2 = *Callitetrarhynchus gracilis* 3 = *Paranybelinia* sp. 4 = *Nybelinia* sp.

5 = *Anisakis* sp. 6 = *Terranova* (type II) 7 = *Pomphorhynchus* sp. 8 = *Otobothrium australe*

All permanent parasite species were capable of spatial discrimination in their own right ($p < 0.05$). In the analysis, *Otobothrium australe* had the most powerful discriminating properties (partial Wilk's lambda = 0.19), followed by (in order of greatest to least discriminating power), *Callitetrarhynchus gracilis* (0.41), *Pomphorhynchus* sp. (0.44), *Nybelinia* sp. (0.66), *Pterobothrium* sp. A (0.69), *Terranova* (type II) (0.77), *Pterobothrium pearsoni* (0.88) and *Anisakis* sp. (0.96).

It was perceived that the vastly distinct Western Australia and Northern Territory samples were having a profound effect on the outcome of the discriminant function analysis, with their large differences swamping the results from the eastern samples. To reduce this effect, a second discriminant function analysis was performed only on data from Queensland's eastern Gulf of Carpentaria and east coast sites. This analysis revealed clear differences in the permanent parasite assemblages across the sites (Figure 6.3). Townsville fish again appeared dissimilar to those of neighbouring sites. Fish from the Flinders River separated from the Albert and Kendall River samples on the first axis, whereas the Kendall River samples separated from the Albert River on the third axis, suggesting that these fish are distinct. The Fitzroy River replicate samples remained grouped together at all axes, suggesting that at this site the abundances of the parasites examined are stable across the study period. Thus the permanent parasite data suggested at least eight isolated populations of king threadfin across northern Australia.

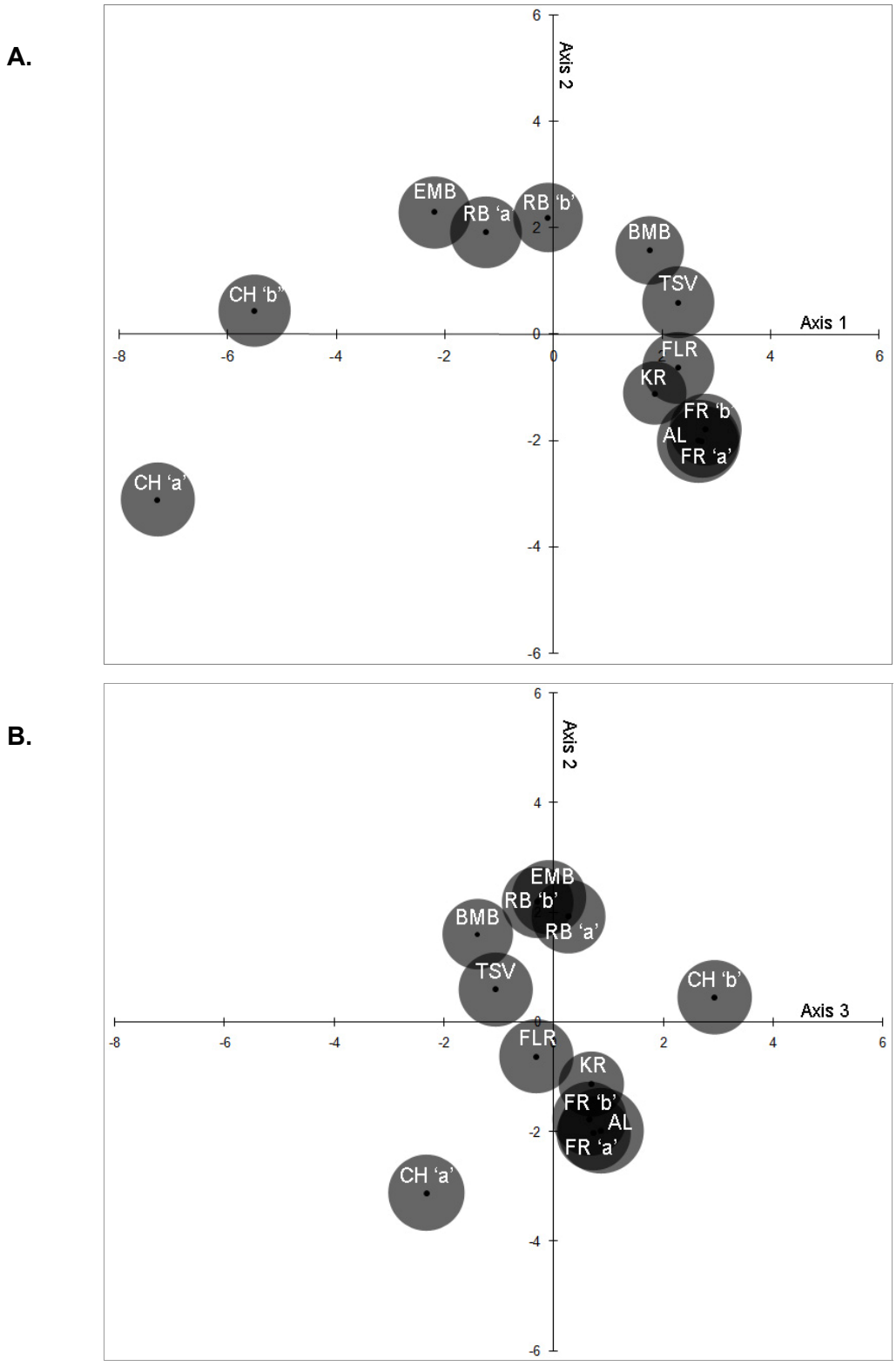
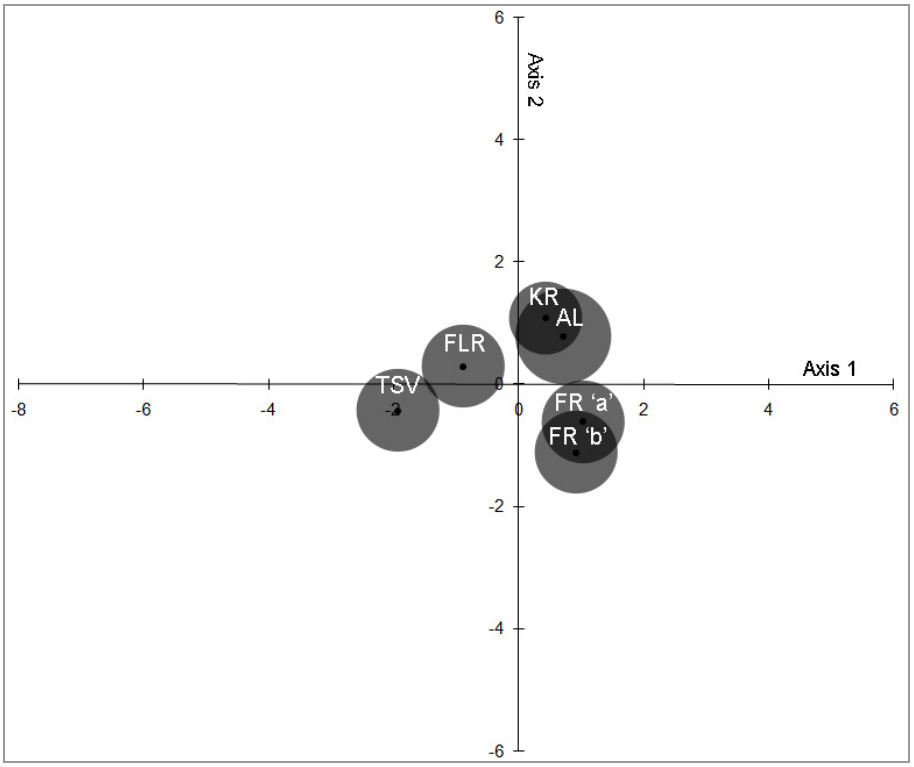



**Figure 6.2:** Results of discriminant function analysis of permanent parasite species from king threadfin from nine sites across northern Australia (see Table 7.1). A = Axis 1 vs. 2; B = Axis 2 vs. 3. Shaded circles represent 95% confidence intervals.

**A.**




**B.**

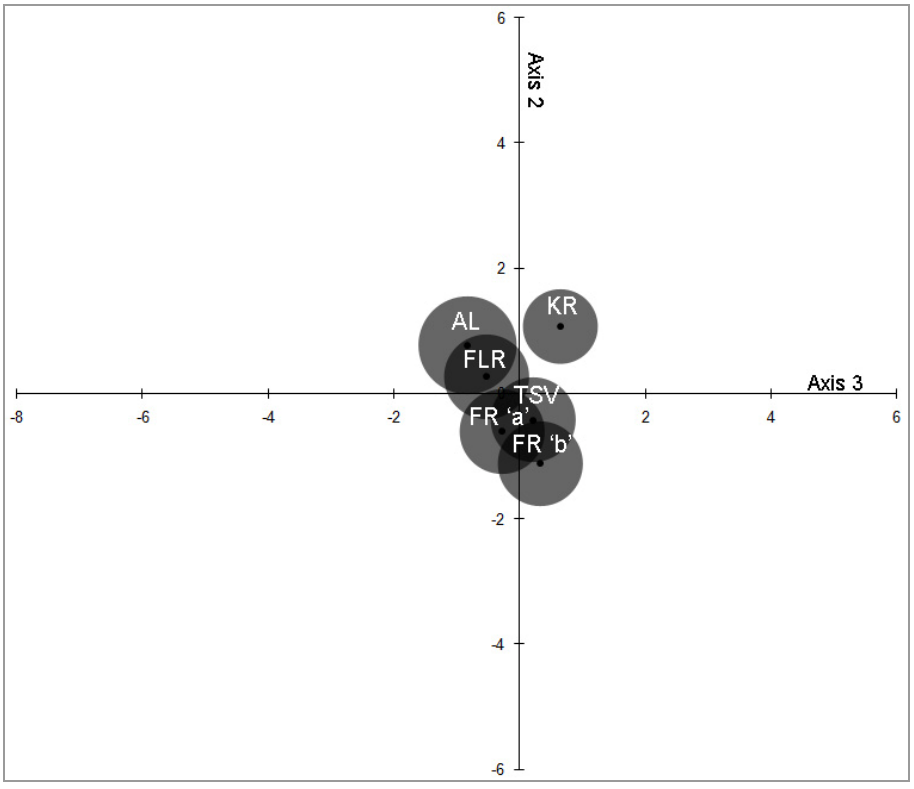



**Figure 6:3:** Results of discriminant function analysis of permanent parasite species from king threadfin from Queensland eastern GoC and east coast sites (see Table 7.1). A = Axis 1 vs. 2; B = Axis 2 vs. 3. Shaded circles represent 95% confidence intervals.

## 6.4 Discussion

The results of this study suggest that king threadfin form a number of isolated, non-mixing stocks across northern Australia. Abundances of eight parasite species deemed to be long-lived in the fish were found to be significantly different between fish across northern Australia at various spatial scales, including between sites separated by as little as tens of kilometres. Such differences suggest that king post-recruitment populations of king threadfin are highly resident and form a number of isolated, non-mixing stocks for the purposes of fisheries management.

The utility of parasites as a natural marker of fish movement and stock structure is determined to an extent by the temporal stability of the parasites used, particularly when samples have been collected at different times. If neighbouring sites are sampled in different years comparisons of parasite fauna between sites may be confounded by temporal patterns in the abundances of the parasites examined (Timi *et al.* 2009). In the present study, the parasite assemblages at two of the three sites in which temporal comparisons were possible showed strong similarities between replicates, suggesting that at these sites the parasite assemblages are temporally stable at least over the timeframes examined. In contrast, the abundances of five parasite species (*Callitetrarhynchus gracilis*, *Otobothrium australe*, *Pterobothrium pearsoni*, *Pomphorhynchus* sp. and *Terranova* (type II)) differed between the replicate samples from Chambers Bay. Although this may reflect temporal variability in the parasite fauna at this site, the differences in parasite abundances may be caused by the vast differences in ages of fish in the two replicates. Although parasite abundances were adjusted for fish age, the adjustment method was conducted on parasite data pooled across all sites, rather than on a site-specific basis. It may be that the discriminating parasites at this site accumulate in the fish at a quicker rate than other sites. Under the current method of adjusting parasite abundances a sample of young fish will have fewer parasites even after adjustment than a natural population of older fish. Further analyses of these samples, including a comparison between fish of similar ages between the two samples, is warranted to better understand the temporal patterns of parasite abundance at this site. Conversely, the differences observed between the two replicates may reflect temporal differences in the local environmental conditions experienced by the fish in the two samples, such as temperature and salinity profiles, or variability in the distribution and abundance of definitive or intermediate hosts (Rohde 1982; Arthur and Albert 1993; MacKenzie and Abaunza 1998; Nagasawa 2001), or may result from fish moving in to Chambers Bay from two different areas. This last scenario is unlikely, given the fine scale spatial structure evident at the other sites analysed. The results highlight the importance of obtaining an understanding of the temporal patterns in parasite assemblages across all regions sampled, particularly when samples from different sites have been collected at different times.

As long-lived parasites tend to accumulate with host age, differences in parasite fauna between sites may have been obscured between samples that contained fish of different ages. To reduce this effect, parasite species were examined for correlation with host age, and adjusted where significant. This

adjustment was based on parasite data pooled across all sites. However, anecdotal evidence suggests that parasite accumulation rates are not constant across all sites, and may be more suitable on a site-specific basis. Although further refinement of this technique is warranted, it is unlikely to affect the results, given the considerable difference in parasite abundances apparent in the raw parasite data (Table 6.1).

In the analysis, three parasite species (*Pterobothrium* sp. A, *Nybelinia* sp. and *Anisakis* sp.) showed no correlation in their abundance with fish age. These species were thought to be long-lived as no degenerating cysts were found. Although the low prevalence and abundance of *Anisakis* sp. observed across all sites prevents any conclusion of the accumulation of the parasite in the fish, the relatively high abundances and lack of correlation with fish age for *Pterobothrium* sp. and *Nybelinia* sp. data suggests that these species may accumulate in juvenile *P. macrochir* before they have entered the fishery. Further sampling, particularly of younger fish, is warranted to further investigate this. In contrast, abundances of *C. gracilis*, *O. australe*, *Pterobothrium pearsoni*, *Pomphorhynchus* sp. and *Terranova* (type II) were positively correlated with fish age, indicating that these parasites are picked up continuously throughout the life of the fish.

The multivariate data suggest a homogenous parasite fauna between fish from Eighty Mile Beach and Roebuck Bay (separated by approximately three hundred kilometres). In contrast, we found significant differences in parasite assemblages of fish from the Albert and Kendall Rivers in the southern Gulf of Carpentaria (separated by approximately one hundred kilometres). This result suggests that fish movement may be less extensive across the north of Australia compared to movements on the west coast, similar to the scenario proposed for the narrow barred Spanish mackerel *Scomberomorus commerson* (Lester *et al.* 2001; Moore *et al.* 2003). Conversely, the similarity in parasite assemblages in fish from Eighty Mile Beach and Roebuck Bay could be explained by a lack of environmental variation between the sites, possibly resulting in a similar parasite fauna between non-mixing fish.

### Implications for fisheries management and future directions

The parasite assemblages examined in the study have shown distinct site-specific signatures which strongly support the hypothesis of fine spatial scale stock structure of *P. macrochir* across northern Australia. We found evidence for at least eight isolated stocks of king threadfin: a combined stock from the waters of Eighty Mile Beach and Roebuck Bay, and isolated stocks from Chambers Bay, Blue Mud Bay, Albert River, Flinders River, Kendall River, Townsville and the Fitzroy River. It is likely that further sampling on a finer scale will detect a greater number of stocks, based on the highly resident nature of *P. macrochir* observed in the present study.

King threadfin are currently managed separately by state-based management agencies in Western Australia, the Northern Territory, and Queensland (separated into Gulf of Carpentaria and east coast

jurisdictions). Little spatial structure is currently assumed in these jurisdictions. However, the findings of the current study suggest that post-recruitment populations of *P. macrochir* are highly sedentary, with little mixing between sites. As such, the long-term effects of fishing are likely to be highly localised within the current jurisdictional boundaries, implying little need to interstate co-operative management. The site-specific nature of adult *P. macrochir* observed in the current study renders the species vulnerable to serial depletion. As such, the development of harvest strategies and establishment of suitable fishery regulations should be conducted in a way that recognises the highly resident nature of adult *P. macrochir* in Australian waters.

# Chapter 7: Stock structure of *Polydactylus macrochir* across northern Australia based on mtDNA

**John B. Horne, Paolo Momigliano, David J. Welch, Stephen J. Newman and Lynne van Herwerden**

## 7.1 Introduction

Chenoweth and Hughes (2003) studied the genetic population structure of *Polynemus sheridani* (now *Polydactylus macrochir*; Motomura *et al.* 2001) using the mitochondrial control region (Dloop). The focus of their study was the putative biogeographic barrier of the Torres Strait, which has been observed in another commercially important fish, *Lates calcarifer* (Chenoweth *et al.* 1998). Sampling took place between the Fitzroy River, Queensland and the Daly River, Northern Territory. Among the findings of Chenoweth and Hughes (2003) were a high degree of population structure and a perplexing inverse relationship between geographic and genetic distance. Specifically, the extreme east and west populations of their sampling design exhibited a strange genetic affinity. In other words, population structure was stronger between populations separated by several hundred kilometres of coastline than by populations separated by greater than 3,000 km. In addition, no evidence of a barrier to gene flow was reported at the Torres Strait for this species.

In the current study, we extended the sample design of Chenoweth and Hughes (2003) collecting from ten populations between the Brisbane River in South Queensland and Eighty Mile Beach in Western Australia. The aims of this study were to assess genetic stock structure of *P. macrochir* at several spatial-scales to inform the basis for fisheries management.

## 7.2 Methods

### Sampling

Sampling was carried out in two phases that took place consecutively in 2008 and 2009. Ten locations were sampled across northern Australia giving a spatial coverage of coastline of approximately 14,000 km (Figure 7.1). Samples were collected using monofilament gillnets usually from commercial fishing activities and supplied by fishers. Fin clips were taken from individual samples from each location and in three locations (Roebuck Bay, Chambers Bay, Fitzroy River) re-sampling was conducted at periods of at least six months apart to assess the temporal stability in genetic structure.

### Laboratory methods

The mitochondrial control region was amplified using the following primer sequences: MT16498H; 5'-CCT GAA GTA GGA ACC AGA TG-3' and Pro-L; 5'-CTA CCT CCA ACT CCC AAA GC-3' (Meyer *et al.* 1990; Palumbi *et al.* 1991) as referenced by Chenoweth and Hughes (2003). PCR was carried out in 20 µL reactions using: 2.5 mM Tris–Cl (pH 8.7), 5 mM KCL, 5 mM (NH4)2SO4, 200 µM each dNTP, 2.5-3.5 mM MgCl2, 10µM each primer and 1 U of Taq Polymerase (Qiagen Ltd.). Thermocycling was carried out with an initial denaturation of 94°C for two minutes, 35 cycles of denaturation, annealing and extension (94°C for 30 s, 55°C for 30 s, 72°C for 90 s) and a final extension of 72°C for ten minutes. PCR products were confirmed by gel electrophoresis on 1.5% agarose gels and purified by either a standard isopropanol purification or an ammonium acetate ethanol clean-up. PCR products were sequenced with the mt16498H primer using ABI (Applied Biosystems Incorporated) technologies at Macrogen sequencing service Seoul, South Korea.

We also sequenced a segment of the mitochondrial Cytochrome Oxidase subunit 1 gene region for a small subset of samples from each region ($n$ = 18) using the universal primers of Ward *et al.* (2005): (FishF1; 5'-TCA ACC AAC CAC AAA GAC ATT GGC AC3' and FishR1; 5'-TAG ACT TCT GGG TGG CCA AAG AAT CA3'). Parameters for PCR and subsequent processing of samples were identical to that described for the control region, including an annealing temperature of 55°C.

### Data analysis

DNA sequences were complied in *Bioedit* v7.0 (Hall, 1999) and aligned using Clustal W (Thompson *et al.* 1994) implemented in *Bioedit*. Sequences were then further aligned by hand. Minimum spanning networks of Dloop haplotypes were constructed in TCS (Clement *et al.* 2000). Molecular diversity indices, haplotype diversity ($h$) and nucleotide diversity ($\pi$) of Dloop haplotypes were calculated in DNAsp (Rozas *et al.* 2003). Analysis of molecular variance (Amova) was performed in *Arlequin* v3.1 (Excoffier *et al.* 2005). Overall AMOVA and pair wise *F*st values were computed using 10,000 permutations. Fu's *F*s test for population expansion (Fu, 1997) and mismatch distribution (Rogers and Harpending, 1992) were calculated in *Arlequin*. Shoreline distances between sampled populations were estimated in kilometres using Google™ Earth v4.3 and compared to genetic distance (pairwise *F*st) in isolation-by-distance analysis (IBD), perfomed online using IBD web service (Jensen *et al.* 2005).

## 7.3 Results

A total of 333 base pairs of the mitochondrial control region were sequenced for 467 individual *P. macrochir* sampled across northern Australia. Out of the 333 base pairs, 65 variable sites were observed. Forty-seven of the variable sites were parsimony informative (found in more than one individual), while 18 were singletons. Haplotype ($h$) and nucleotide ($\pi$) diversities were respectively: 0.874 and 0.00715. Genetic diversity of individual populations is reported in Table 7.1.

**Table 7.1:** Sample sizes (*n*), number of haplotypes (*n* haplotypes), haplotype diversity (*h*), nucleotide diversities ($\pi$) for all regional sampling of *Polydactylus macrochir*.

| | ***n*** | ***n* haplotypes** | ***h*** | ***π*** |
|---|---|---|---|---|
| **WA** | **94** | **11** | **0.65** | **0.0026** |
| Roebuck Bay 1 | 39 | 7 | 0.58 | 0.0022 |
| Eighty Mile Beach | 33 | 7 | 0.76 | 0.0036 |
| Roebuck Bay 2 | 22 | 4 | 0.54 | 0.0018 |
| **NT** | **95** | **34** | **0.93** | **0.0099** |
| Chambers Bay 1 | 41 | 19 | 0.92 | 0.0083 |
| Blue Mud Bay | 33 | 11 | 0.83 | 0.0079 |
| Chambers Bay 2 | 21 | 13 | 0.93 | 0.0113 |
| **QLD GoC** | **116** | **30** | **0.86** | **0.0083** |
| Flinders River | 49 | 21 | 0.91 | 0.0097 |
| Albert River | 21 | 13 | 0.91 | 0.0080 |
| Kendall River | 46 | 16 | 0.87 | 0.0070 |
| **QLD EC** | **137** | **10** | **0.32** | **0.0014** |
| Fitzroy River 1 | 50 | 7 | 0.49 | 0.0020 |
| Townsville | 52 | 1 | 0.00 | 0.0000 |
| Fitzroy River 2 | 35 | 6 | 0.47 | 0.0024 |
| Brisbane River | 25 | 3 | 0.22 | 0.0007 |

Overall, population structure was relatively deep for a marine fish with a pelagic larval phase ($\Phi_{st}$ = 0.306, $p < 0.0001$) and the majority of pair wise population comparisons were significant (Table 7.2). Generally, the populations of *P. macrochir* appear largely insular. In particular, the populations of Western Australia and Brisbane River, the terminal ends of the sampled range, showed a high level of genetic identity compared to the central populations. This may be due to spatial diffusion constraints that arise from being on the periphery of a species range (Ray *et al.* 2003).

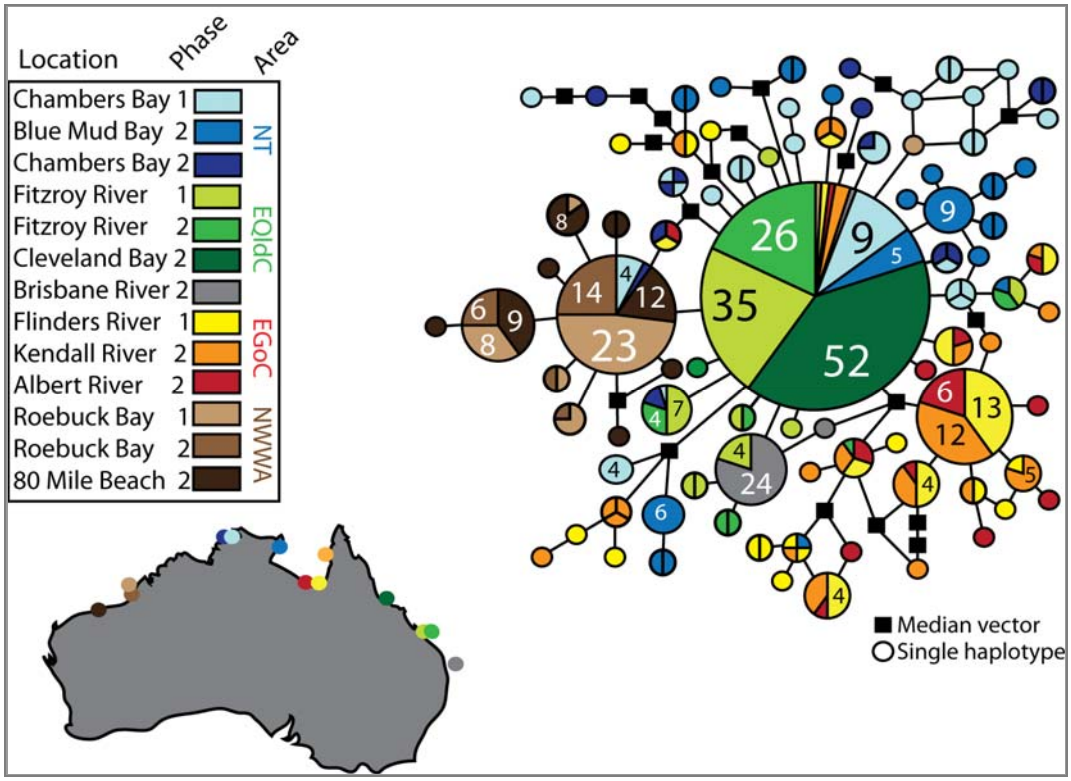



**Figure 7.1:** Map depicting sampling locations of *P. macrochir* across Northern Australia. Minimum spanning network of 467 mitochondrial control region haplotypes.

**Table 7.2:** Pairwise population structures ($F_{st}$) for sampled *P. macrochir* populations significant values are highlighted in bold.

| | BR | FLR | AL | KR | FR1 | TSV | FR2 | CH1 | BMB | CH2 | RB1 | EMB |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BR** | | | | | | | | | | | | |
| **FLR** | **0.368** | | | | | | | | | | | |
| **AL** | **0.494** | -0.015 | | | | | | | | | | |
| **KR** | **0.410** | -0.004 | -0.019 | | | | | | | | | |
| **FR1** | **0.575** | **0.261** | **0.370** | **0.291** | | | | | | | | |
| **TSV** | **0.924** | **0.291** | **0.467** | **0.332** | **0.071** | | | | | | | |
| **FR2** | **0.593** | **0.220** | **0.310** | **0.248** | -0.005 | **0.063** | | | | | | |
| **CH1** | **0.352** | **0.198** | **0.224** | **0.213** | **0.069** | **0.081** | **0.053** | | | | | |
| **BMB** | **0.358** | **0.213** | **0.230** | **0.229** | **0.186** | **0.218** | **0.155** | **0.120** | | | | |
| **CH2** | **0.360** | **0.176** | **0.191** | **0.197** | **0.077** | **0.118** | **0.052** | -0.002 | **0.107** | | | |
| **RB1** | **0.774** | **0.400** | **0.504** | **0.444** | **0.580** | **0.748** | **0.556** | **0.319** | **0.382** | **0.331** | | |
| **EMB** | **0.716** | **0.391** | **0.469** | **0.431** | **0.560** | **0.700** | **0.532** | **0.327** | **0.372** | **0.322** | **0.042** | |
| **RB2** | **0.831** | **0.378** | **0.476** | **0.424** | **0.627** | **0.858** | **0.600** | **0.316** | **0.357** | **0.313** | -0.021 | 0.027 |

Isolation-by-distance analysis was positive and significant ($r = 0.624$, $p < 0.0001$, $r^2 = 0.39$, slope = 1.7) (Figure 7.2), also indicating limited connectivity between adjacent populations. However, this is in contrast with the results of Chenoweth and Hughes (2003), which showed this relationship to be negative. The same genetic affinity between Northern Territory and the Fitzroy River in Queensland (responsible for the negative relationship in the previous study) was also observed in the present study but was unique to these two populations. Another irregularity was profound subdivision between the Brisbane River and Cleveland Bay, which appears to be partly due to reduced genetic diversity in both populations. When these few anomalies are removed from the analysis, the signal of isolation-by-distance was much stronger ($r = 0.786$, $p < 0.001$, $r^2 = 0.62$, slope = 1.5).

The mismatch distribution was a smooth, normally distributed curve, indicative of demographic expansion (Figure 7.3). Fu's *F*s value was significantly negative (-26. 076, $p < 0.0001$) further indicating that demographic expansion has occurred. This strong expansion signal in the data is likely to be recent expansion, given that 18 COI sequences from across the sampled distribution were monomorphic in this species. If polymorphisms are lacking in COI it may mean that sufficient time has not passed for mutations to arise in this more evolutionarily conserved region of the mitochondrial genome. Therefore, present day populations of *P. macrochir* could have recently undergone a genetic bottleneck and become widely distributed in a relatively short period of time.

## 7.4 Discussion

Strong population structure and expansion (recent?) are the two most conspicuous genetic characteristics of the data. However these two features are seemingly incompatible. How can strong genetic structuring occur if expansion was recent? It could be that 18 is an insufficient number of individuals to assess genetic subdivision in the COI marker. However, six of the 18 COI sequences were from Western Australia, a region that shows a high level of genetic heterogeneity in the D-loop region of this species and in the COI region of *Eleutheronema tetradactylum* (Horne *et al.* unpublished data). No polymorphism was seen between any of the Western Australia samples and those from other areas are therefore suspicious in spite of a small sample size.

### Brisbane populations

There has been disagreement by fisherman in the Brisbane area about the status of the local populations of *P. macrochir* (Brad Moore, personal communication). Some believe this species was previously absent from the Brisbane river and that fish now being caught there are due to a range expansion, while others believe that Brisbane populations have always existed but have fluctuated in density. The results of this study support the latter, as Brisbane river samples showed a strong genetic identity compared to other east coast populations. Moreover, the holotype specimen of *P. macrochir* (Günther, 1867) is reportedly from New South Wales (Motomura *et al.* 2001). Thus, the geographic

range of *P. macrochir* extends further south than the Brisbane River, though perhaps this fish is rare at these southerly latitudes. The data of Halliday *et al.* (2008) suggest that year-class strength of *P. macrochir* is highly correlated with rainfall and freshwater output of rivers. If so, perhaps population slumps in the Brisbane area are related to droughts in southern Queensland that have been prolonged over several years. This apparent estuarine dependency and habitat sensitivity may also help explain population structure in this species. Population bottlenecks related to drought cycles may reduce genetic diversity in gene regions such as COI and increase population subdivision (Slatkin, 1977).

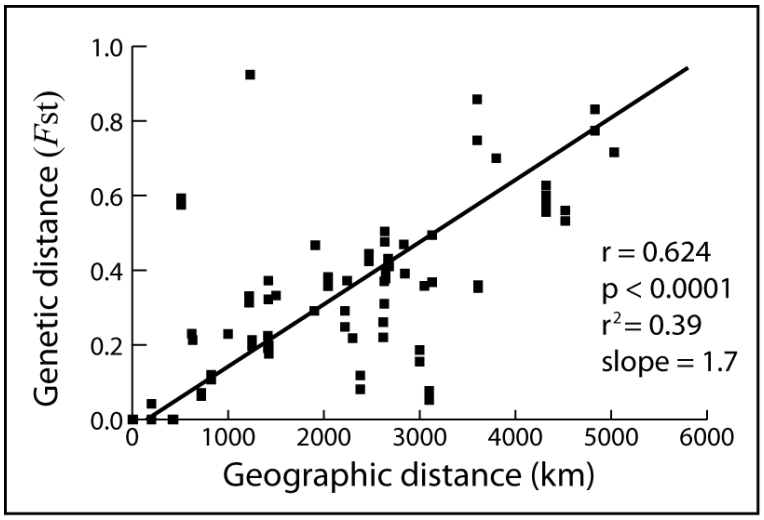



**Figure 7.2:** Mantel test of isolation by distance analysis with the coefficient of correlation (r) and associated p value, coefficient of determination ($r^2$) and slope.

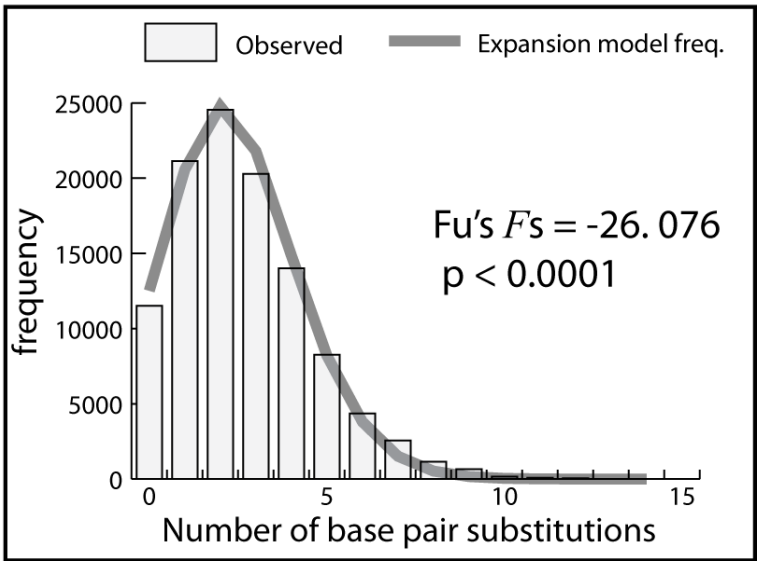



**Figure 7.3:** Mismatch distribution of 467 control region haplotypes and expansion model frequency. Fu's *F*s test of population expansion.

### Comparisons to *Eleutheronema tetradactylum*

The other species of this study, the four finger threadfin *E. tetradactylum*, was reported separately but some comparisons between species are relevant. First, because *E. tetradactylum* exhibited much higher genetic polymorphism in the COI gene region, it could be inferred that the populations of this species are more stable and older than those of *P. macrochir.* It could be that year-class strength of *E. tetradactylum* is not as dependent on rainfall or that *P. macrochir* has stricter habitat requirements that make it sensitive to disturbances. Regardless, based on COI polymorphism, a disparity in age between the two species is a logical conclusion.

Second, in *E. tetradactylum*, some temporal samples from the same location were genetically structured against each other. This pattern was interpreted as a sex-bias in the data, brought about by a year-to-year change in female reproductive success. The same temporal structuring is not observed in *P. macrochir*, suggesting that the reproductive biology of the two species is different, notwithstanding they are both protandrous hermaphrodites. Still, some irregularities in the data are noteworthy, such as the Townsville (TSV) population, in which all haplotypes were identical (Figure 7.1 and Table 7.1). Such a low genetic diversity may be due to a local population bottleneck or it may also reflect a large disequilibrium in reproductive success between individual females.

Third, Western Australian populations of both species appeared to be genetically insular. A congruent east-west split in both species may be a reflection of a biogeographic barrier, which in this case is probably the land bridge that connects Australia and Papua New Guinea during times of low sea level. In both species gulf populations are genetically closer to East Queensland coast samples than to those from Western Australia, suggesting that re-colonisations of the ephemeral body of water known as the Gulf of Carpentaria came from the east.

### Conclusions

Across northern Australia populations of *P. macrochir* are genetically fragmented and highly structured. Isolation-by-distance also seems to be a relevant process in the structuring of populations, with the exception of a few anomalous comparisons, whereby a strong east-west separation is evident with the strongest genetic split between WA and the rest of the sampled range. Thus, population connectivity is low and the probability of migrant exchange between populations decreases with distance. Also, habitat disturbances, particularly related to freshwater outputs, may have drastic consequences on the demographics and genetic diversity of this species. From a policy perspective, fisheries management of *P. macrochir* is recommended at the local scale.

# Chapter 8: Variation in stable isotope ($\delta^{18}O$ and $\delta^{13}C$) signatures in the sagittal otolith carbonate of King threadfin, *Polydactylus macrochir* (Pisces: Polynemidae), across northern Australia reveals multifaceted stock structure

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## 8.1 Introduction

The King threadfin, *Polydactylus macrochir* (Gunther, 1867), is endemic to northern Australia and the southeast coast of Irian Jaya, Indonesia and the southwest coast of Papua New Guinea (Motomura, 2004). This species is distributed from Exmouth Gulf, in Western Australia across northern Australia to the Brisbane River, Queensland, in the east, and from southern coasts of Irian Jaya, Indonesia and Papua New Guinea (Motomura *et al.* 2000; Motomura, 2004; Pember, 2006; Newman *et al.* 2009a). This species inhabits turbid coastal waters, estuaries and mangrove creeks as well as mangrove-lined rivers and is found in depths from the surface to approximately ten metres (Motomura, 2004; Pember, 2006). Life history data on *P. macrochir* in Australian waters indicate that the species has a life span of at least twenty years, an estimated maximum attainable size of approximately 40 kg and 170 cm fork length (Kailola *et al.* 1993).

In northern Australia, *P. macrochir* are a commercially important species from the Eighty Mile Beach area (23°S) north and eastwards across northern Australia and down the east coast to Rockhampton (Kailola *et al.* 1993, Moore *et al.* 2009; Newman, unpublished data). Across northern Australia, the combined annual commercial catch of the polynemids *P. macrochir* and *E. tetradactylum* averages between 800 and 1,200 tonnes and is worth approximately $4 million annually (ABARE, 2005). Fisheries for *P. macrochir* are presently managed across northern Australia by three state-based fishery management agencies, which have separate management arrangements with regional segregation of the inshore mesh met fisheries. All three jurisdictions support highly valuable

commercial net fisheries for *P. macrochir* and therefore the identity of individual component stocks is important for defining fisheries management arrangements.

Current management arrangements make no allowance for migratory fish or overlapping stocks across either state or intra-state fishery boundaries. Consequently, there is a need to identify stocks within these areas in order to assess the impacts of fishing in each area. Therefore, it is important to determine whether post-juvenile populations of *P. macrochir* remain discrete and independent or whether there is evidence of mixing of adult fish among different locations. A fish 'stock' in this paper refers specifically to post-juvenile fish populations that remain discrete and non-mixing (i.e. independent) and therefore comprise a management unit capable of independent exploitation (Newman *et al.* 2000).

Stable isotopes in the sagittal otolith carbonates of fish are increasingly used to understand population structure and movement of adults, to determine nursery habitats of juvenile fish and assess variations in trophic levels (Edmonds and Fletcher, 1997; Kennedy *et al.* 1997; Schwarcz *et al.* 1998; Edmonds *et al.* 1999; Gao and Beamish, 1999; Newman *et al.* 2000; 2009b; 2010; Galvan *et al.* 2010; Gao *et al.* 2010; Gerard and Muhling, 2010). Stable isotopes are beneficial to studies investigating stock structure or population subdivision, as they are neutral, non-radioactive variants of an element and, as a result of their slightly different atomic masses, their relative incorporation into fish otoliths can be modified by environmental conditions or biological activity (Campana, 1999).

The theoretical basis that underpins the use of oxygen stable isotopes is that they are incorporated into sagittal otolith carbonate in, or very close to oxygen isotopic equilibrium with the ambient seawater, and record the environmental changes that an individual fish experiences (e.g. Devereux, 1967; Kalish, 1991; Campana, 1999). Stable carbon isotopes in sagittal otolith carbonate are affected by the metabolism of individual fish (i.e. relative trophic level and changes in metabolic rate over the life of a fish) and dietary shifts (DeNiro and Epstein, 1978; Mulcahy *et al.* 1979; Schwarcz *et al.* 1998; Dufour *et al.* 2005; Huxham *et al.* 2007). In the marine environment temperature and salinity interact to influence the concentrations of stable isotopes. Carbon and oxygen stable isotope values in fish otoliths usually increase with salinity and decrease with increasing temperature (Elsdon and Gillanders, 2002).

The elucidation of stock structure from analysis of the stable isotopic composition of the sagittal otolith carbonate of fish assumes that geographically distinct stocks possess a characteristic isotopic signature that reflects the isotopic composition of the water body in which the fish is resident. However, as noted in earlier works (e.g. Edmonds and Fletcher, 1997; Edmonds *et al.* 1999; Newman *et al.* 2000), knowledge of the causal mechanisms responsible for the stable isotopic composition of teleost otolith carbonate is not necessary for any measured differences to describe stock structure or population subdivision.

The importance of *P. macrochir* as both a commercial and recreational fish species across northern Australia, in association with the fact that it an inshore tropical polynemid that is endemic to Australia and southern central New Guinea, signify that knowledge of its stock structure is vital for determining the spatial scale at which this species should be managed to ensure the development of sustainable harvest strategies for this resource. Therefore, the objective of this study was to analyse the stable isotopic composition of fish otoliths collected from a number of locations across northern Australia in order to determine the stock structure of *P. macrochir*.

## 8.2 Methods

### Sampling design

Samples of *P. macrochir* were collected using monofilament gillnet fishing methods at each location across northern Australia. Otoliths were collected from fish at nine locations extending from Western Australia across northern Australian waters, throughout the Gulf of Carpentaria (GoC) to sites along Queensland on the east coast of Australia (Figure 8.1), covering a coastline length of approximately 14,000 km. Locations sampled were Eighty Mile Beach, WA (EMB), Roebuck Bay, WA (RB), Chambers Bay, NT (CH), Blue Mud Bay, NT (BMB), Albert River, Queensland (AL), Flinders River, Queensland (FLR), Kendall River, Queensland (KR), Townsville, Queensland (TSV) and Fitzroy River, Queensland (FR). A target total of forty pairs of sagittal otolith samples were collected from each location. Where possible, samples were collected on two separate occasions, a minimum of six months apart. A summary of the sampling data, and summary results from the stable isotope analyses of the sagittal otolith carbonate of *P. macrochir* from across northern Australia are detailed in Appendix 8.1.

### Otolith preparation

Sagittae were allowed to dry in envelopes prior to processing. One sagitta from each fish was selected at random and cleaned by scrubbing with a nylon brush under ultrapure water, air-dried (50°C) and crushed to powder in an agate mortar and pestle. Powdered sagittae were cleaned of organic matter (surface contaminants) by treatment with hydrogen peroxide and deproteinated by dissolving and extracting protein using a centrifuge. The otolith carbonate matrix is composed of 96% calcium carbonate (in the form of aragonite), 3% protein and 1% inorganics (Arslan and Paulson, 2003). The sagittae were deproteinated to remove those proteins contained in the otolith carbonate matrix. These proteins could potentially degrade to form $CO_2$ on analysis of the carbon and oxygen stable isotopes and therefore potentially interfere with the measurement of the carbon and oxygen stable isotopes.

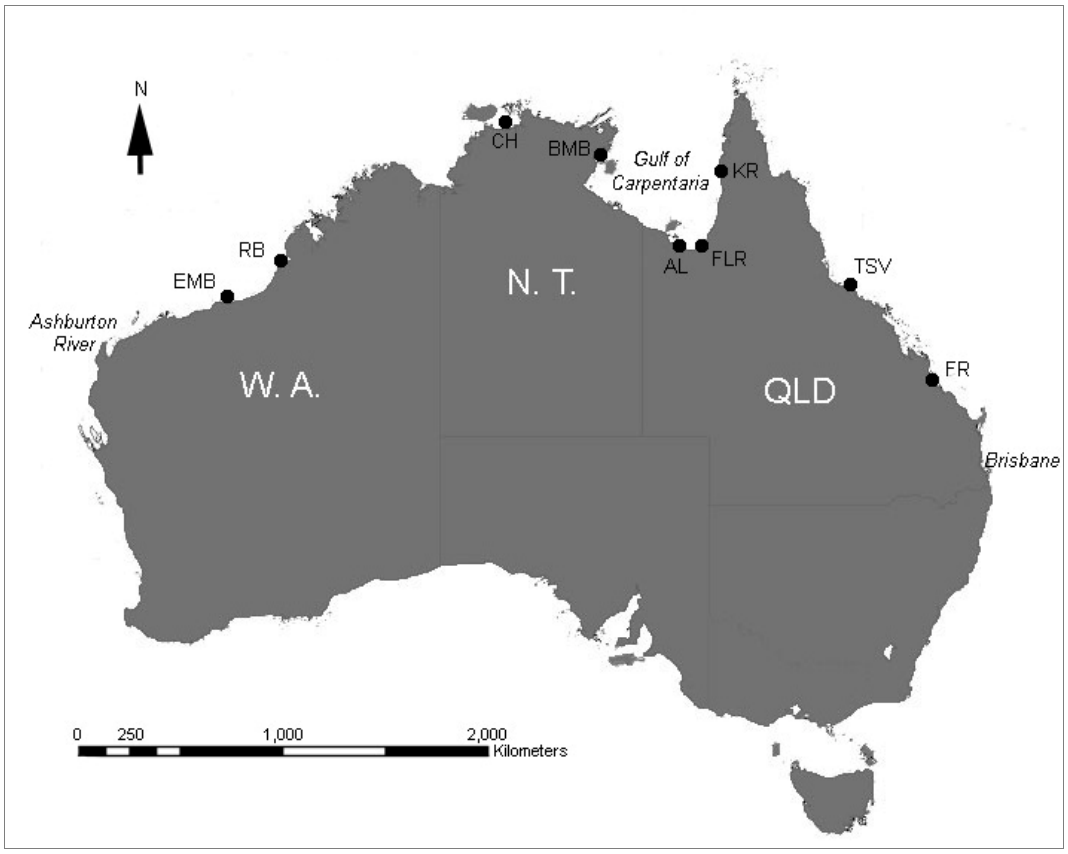


**Figure 8.1:** Locations of the sampling sites for *P. macrochir* across northern Australia (EMB – Eighty Mile Beach; RB – Roebuck Bay; CH – Chambers Bay; BMB – Blue Mud Bay; AL – Albert River, FLR – Flinders River; KR – Kendall River; TSV – Townsville and FR – Fitzroy River).

Powdered sagittae were then analysed for oxygen ($^{18}O/^{16}O$ or $\delta^{18}O$) and carbon ($^{13}C/^{12}C$ or $\delta^{13}C$) stable isotopes by standard mass spectrometric techniques after the carbonate was decomposed to $CO_2$ with 100% phosphoric acid (Newman *et al.* 2009b). A laboratory reference sample was run every thirty samples. The laboratory reference sample, consisting of a batch solution of digested otolith reference material, was used to monitor measurement precision across sample batches, and was subsequently used to normalise sample batches to a constant reference value. Stable isotopes are reported using the international standard delta ($\delta$) notation relative to the PDB-1 standard for carbonates (i.e. $\delta^{18}O$ and $\delta^{13}C$).

### Statistical analysis

The temporal variability in the stable isotopes ($\delta^{18}O$ and $\delta^{13}C$) of *P. macrochir* was assessed for three sites; Roebuck Bay, Chambers Bay and the Fitzroy River that were sampled at least six months apart. Analysis of covariance (ANCOVA) was used to determine the effect of sampling period (fixed factor, Type III sum of squares) with otolith weight as a covariate (which is considered to be proportional to fish age); the interaction term was also tested (Zar, 1999).

The spatial variation in stable isotopes ($\delta^{18}O$ and $\delta^{13}C$) of *P. macrochir* was evaluated using ANCOVA to test for differences among locations (fixed factor, Type III sum of squares) with otolith weight as a covariate including assessment of the interaction effect (Zar, 1999). A one-way ANOVA was further used to test the effect of location, *a posteriori* multiple comparison of means were carried out using Student-Newman-Kuels tests to evaluate differences.

A non-parametric multivariate analysis of variance (Permutational ANOVA (PERMANOVA): Anderson, 2001; Anderson and Gorley, 2007) was used to test for effects of location on both $\delta^{18}O$ and $\delta^{13}C$ to determine whether there was greater separation of sites when both stable isotopes are considered simultaneously in a multivariate analysis. PERMANOVA is a multivariate version of the univariate ANOVA that incorporates experimental design using any distance measure, by means of permutation methods, producing a pseudo F-statistic and significance (*p*) value (Anderson, 2001). A complete description of this method is given in Anderson (2001) and McArdle and Anderson (2001). In the case of a one-way analysis, the PERMANOVA test using permutations assumes only that the observation units are exchangeable. There are no explicit assumptions regarding the distributions of the original variables; there is no normal distribution assumption. The $\delta^{18}O$ and $\delta^{13}C$ values were first normalised to make them scale independent with the resemblance matrix based on Euclidean distance. PERMANOVA was based on a single fixed factor; location, with Type III sums of squares and unrestricted permutations. *A posteriori* multiple comparisons of means were undertaken using the pairwise tests available within the PERMANOVA routine (Anderson and Gorley, 2007). To visualise the data and evaluate whether there were latitudinal or longitudinal trends in the stable isotope signatures of the locations, the means and standard errors were calculated for each isotope at each location. Locations were ranked from 1 to 9, with 0.5 added to the rank of those sites with an additional temporal sample, with these ranks included as 'factors' in the dataset. Means were normalised and the Euclidean distances calculated. Non-multidimensional scaling was used to visualise the sites with a trajectory linking each by either latitude or longitude.

## 8.3 Results

### Temporal comparison

ANCOVA of $\delta^{18}O$ and $\delta^{13}C$ stable isotope values examining the effect of sampling period and otolith weight across the three locations (Roebuck Bay, Chambers Bay and Fitzroy River) that had temporal sample collections exhibited variable effects. ANCOVA results for $\delta^{18}O$ and $\delta^{13}C$ at the Fitzroy River location showed that otolith weight was significantly different ($p = 0.05$ and $p = 0.018$, respectively), but there were no significant differences between sampling periods ($p > 0.25$ and $p > 0.05$, respectively). Similarly, the ANCOVA results for $\delta^{18}O$ and $\delta^{13}C$ at the Roebuck Bay location also showed a similar pattern where otolith weight exhibited a significant effect ($p = 0.032$ and $p < 0.0001$, respectively) and sampling period was not significantly different ($p = 0.587$ and $p = 0.468$, respectively). However, at the

Chambers Bay location, ANCOVA results for $\delta^{18}O$ and $\delta^{13}C$ exhibited significant differences between otolith weight ($p = 0.01$ and $p < 0.0001$). Sampling period was significantly different for $\delta^{13}C$ ($p = 0.0.005$) but not $\delta^{18}O$ ($p = 0.307$). At the Chambers Bay location otolith weight was significantly different due to two separate groups of fish (small fish and large fish, mean otolith weights were significantly different between the two sampling periods) that were coincident with each sampling period. Sampling period was only significantly different for one stable isotope ($\delta^{13}C$) at one location that was related to sampling biases in terms of the size of fish sampled in each sampling period. The date of sampling in each analysis was therefore not significant, thereby providing no evidence of any temporal variation in unbiased $\delta^{13}C$ or $\delta^{18}O$ values.

### Spatial comparison

This study examined populations of *P. macrochir* sampled at nine locations (Appendix 8.1). However, as the temporal factor 'sampling period' was not significantly different, each sampling period at each of the locations was treated as an individual 'location' in subsequent analyses in order to further assess the temporal and spatial variability among all locations. ANCOVA indicated that both $\delta^{18}O$ and $\delta^{13}C$ were significantly different for the factors location and otolith weight, with a significant interaction between location and otolith weight (Table 8.1). However, for both $\delta^{18}O$ and $\delta^{13}C$, the variance explained by location far exceeded the variance explained by either otolith weight or the interaction of both factors. The exclusion of otolith weight and the interaction of both factors reduced the amount of variation explained in $\delta^{18}O$ and $\delta^{13}C$ by only 1% and 4%, respectively (Table 8.1). As a result, variation in both $\delta^{18}O$ and $\delta^{13}C$ were significantly different among locations and this factor accounted for 76% and 70% of the variance.

Locations were significantly discriminated by $\delta^{18}O$ and $\delta^{13}C$ values based on SNK tests (Table 8.2). The $\delta^{13}C$ stable isotope separated locations into five groups with no overlap between groups. Fitzroy River I and II were not significantly different from each other. Blue Mud Bay, Flinders River, Albert River, Kendall River and Chambers Bay II were also not significantly different from each other. Chambers Bay I was significantly different from all other sites as was Townsville. The remaining west coast sites of Roebuck Bay I and II and Eighty Mile Beach were also not significantly different from each other (Figure 8.2a). The locations separated less clearly based on $\delta^{18}O$ stable isotope values with seven groups and a high degree of overlap with some locations belonging to more than one group. Chambers Bay II had the most negative stable isotope signature, followed by Flinders Bay, Chambers Bay I, Kendall River and Albert River which separated in overlapping groups of two (Figure 8.2b). Blue Mud Bay was distinct from all other sites and Townsville grouped with Fitzroy River I and II. Roebuck Bay I and II grouped with Eighty Mile Beach similar to the $\delta^{13}C$ stable isotope result (Figure 8.2).

The PERMANOVA, combining both $\delta^{18}O$ and $\delta^{13}C$ indicated that locations were significantly different and discriminates the locations more clearly than ANOVA based on each stable isotope in isolation (Table 8.3; Figures 8.3 and 8.4). *A posteriori* pairwise tests indicated that all sites were significantly different from each other at P (Perm<0.05) except for: Roebuck Bay I and Eighty Mile Beach (P (perm) = 0.44), Roebuck Bay II and Eighty Mile Beach (P (perm) = 0.071), Fitzroy River I and II (P (perm) = 0.39); Kendall River and Albert River (P (perm) = 0.11) and Kendall River and Flinders River (P (perm) = 0.061), and Flinders River and Chambers Bay II (P(perm) = 0.058). Differences between Chambers Bay I and II were marginal (P (perm) = 0.049) as were differences between Roebuck Bay I and II (P (perm) = 0.024) and Chambers Bay I and Kendall River (P (perm) = 0.047). All other pairwise comparisons were significant at p <0.0001 with the exception of Flinders River and Chambers Bay I (P (perm) = 0.0009).

The nMDS plot clearly showed that differences and similarities among locations followed a trajectory along longitude (Figure 8.3). For the three locations that were sampled across the two temporal sampling periods, the differences within location between each sampling period were relatively small compared to the spatial differences (Figures 8.3 and 8.4), although for both Roebuck Bay and Chambers Bay, the differences were significant (p = 0.024 and p = 0.049).

**Table 8.1:** (A) ANCOVA of the $\delta^{13}C$ and $\delta^{18}O$ values of the sagittal carbonate of *Polydactylus macrochir* across all locations with otolith weight as a covariate and interaction effect and (B) ANOVA of the $\delta^{13}C$ and $\delta^{18}O$ values of the sagittal carbonate of *Polydactylus macrochir* across all locations with the covariate, otolith weight removed from the analysis.

| **A. Tests of Between-Subjects Effects** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Dependent Variable: $\delta^{13}C$** | | | | | **Dependent Variable: $\delta^{18}O$** | | | | |
| **Source** | **Type III Sum of Squares** | **df** | **Mean Square** | **F** | **Sig.** | **Type III Sum of Squares** | **df** | **Mean Square** | **F** | **Sig.** |
| Corrected Model | 703.017[a] | 23 | 30.566 | 57.013 | .000 | 302.911[a] | 23 | 13.170 | 67.876 | .000 |
| Location | 211.455 | 11 | 19.223 | 35.856 | .000 | 47.441 | 11 | 4.313 | 22.228 | .000 |
| Otolith weight (OW) | 1.188 | 1 | 1.188 | 2.215 | .137 | .991 | 1 | .991 | 5.106 | .024 |
| Location * OW | 30.832 | 11 | 2.803 | 5.228 | .000 | 3.720 | 11 | .338 | 1.743 | .062 |
| Error | 237.501 | 443 | .536 | | | 85.956 | 443 | .194 | | |
| Corrected Total | 940.517 | 466 | | | | 388.867 | 466 | | | |
| Adjusted $r^2$ | .734 | | | | | .767 | | | | |
| **B. Tests of Between-Subjects Effects** | | | | | | | | | | |
| | **Dependent Variable: $\delta^{13}C$** | | | | | **Dependent Variable: $\delta^{18}O$** | | | | |
| **Source** | **Type III Sum of Squares** | **df** | **Mean Square** | **F** | **Sig.** | **Type III Sum of Squares** | **df** | **Mean Square** | **F** | **Sig.** |
| Location | 657.519 | 11 | 59.774 | 96.104 | .000 | 295.993 | 11 | 26.908 | 131.828 | .000 |
| Error | 282.998 | 455 | .622 | | | 92.874 | 455 | .204 | | |
| Corrected Total | 940.517 | 466 | | | | 388.867 | 466 | | | |
| Adjusted $r^2$ | .692 | | | | | .755 | | | | |

**Table 8.2:** *A posteriori* multiple comparison of means (Student-Newman-Kuels tests) from the one factor analysis of variance to test the effect of location on $\delta^{18}O$ and $\delta^{13}C$ stable isotope values.

**SNK - $\delta^{18}O$**

| New Site | N | Subset | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Chambers Bay II | 40 | -3.01 | | | | | | |
| Flinders River | 39 | | -2.81 | | | | | |
| Chambers Bay I | 40 | | -2.75 | -2.75 | | | | |
| Kendall River | 40 | | | -2.57 | -2.57 | | | |
| Albert River | 35 | | | | -2.37 | | | |
| Blue Mud Bay | 40 | | | | | -2.02 | | |
| Townsville | 40 | | | | | | -1.53 | |
| Fitzroy River II | 33 | | | | | | -1.48 | |
| Fitzroy River I | 39 | | | | | | -1.33 | |
| Eighty Mile Beach | 41 | | | | | | | -.89 |
| Roebuck Bay II | 40 | | | | | | | -.82 |
| Roebuck Bay I | 40 | | | | | | | -.82 |
| **Sig.** | | **1.000** | **.565** | **.072** | **.056** | **1.000** | **.147** | **.763** |

**SNK - $\delta^{13}C$**

| Location | N | Subset | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| Fitzroy River II | 33 | -6.15 | | | | |
| Fitzroy River I | 39 | -5.87 | | | | |
| Blue Mud Bay | 40 | | -5.51 | | | |
| Flinders River | 39 | | -5.38 | | | |
| Albert River | 35 | | -5.33 | | | |
| Kendall River | 40 | | -5.27 | | | |
| Chambers Bay II | 40 | | -5.04 | | | |
| Chambers Bay I | 40 | | | -4.68 | | |
| Townsville | 40 | | | | -4.07 | |
| Roebuck Bay II | 40 | | | | | -2.89 |
| Roebuck Bay I | 40 | | | | | -2.73 |
| Eighty Mile Beach | 41 | | | | | -2.72 |
| **Sig.** | | **.112** | **.069** | **1.000** | **1.000** | **.598** |

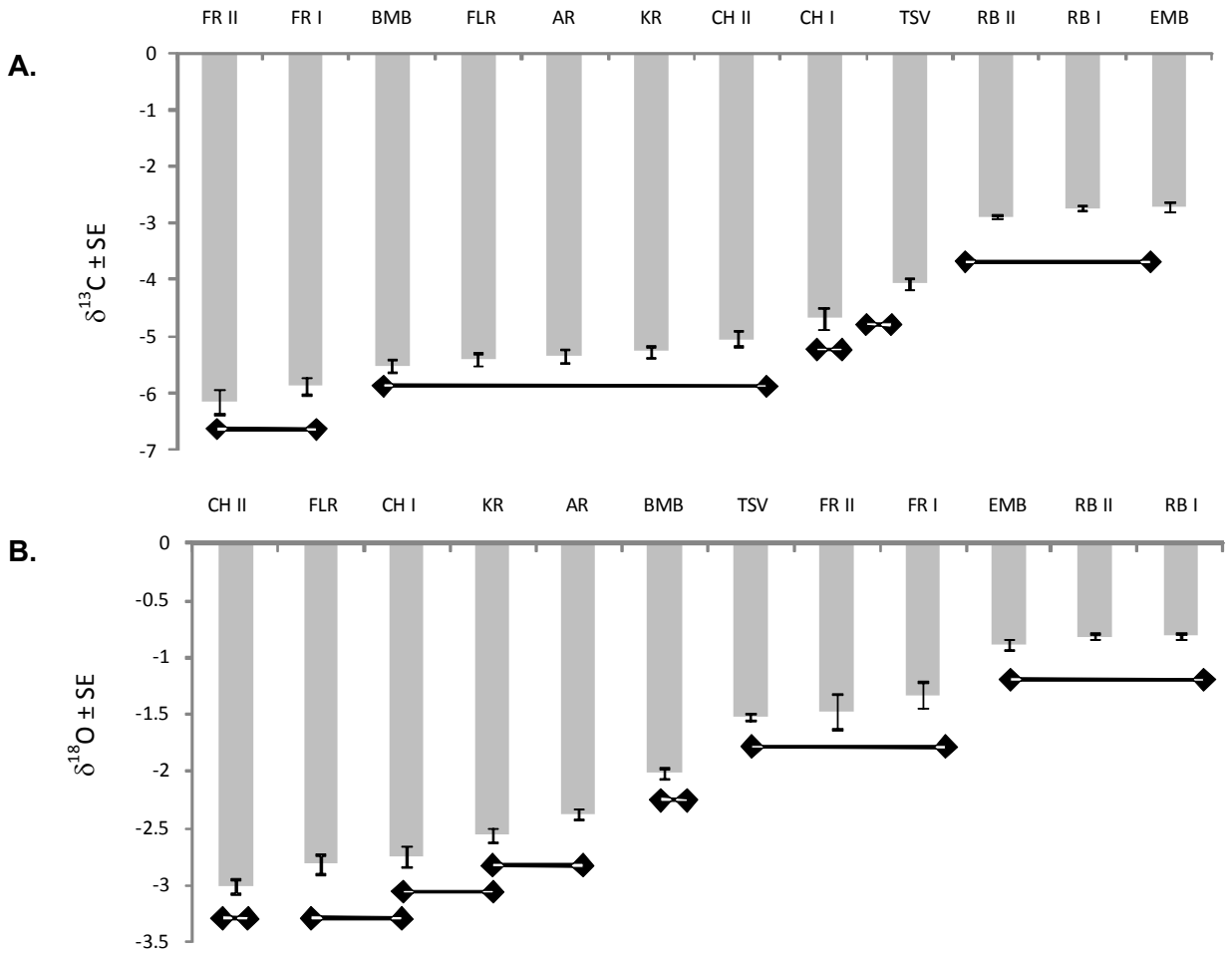


**Figure 8.2:** Summary of the results from the *a posteriori* multiple comparison of means (SNK tests) from the one factor analysis of variance to test the effect of location on (a) $\delta^{13}C$ and (b) $\delta^{18}O$ stable isotope values.

**Table 8.3:** Summary of PERMANOVA results across all locations with fixed factors based on Type III sums of squares with unrestricted permutations of the raw data.

**PERMANOVA ($\delta^{18}O$ and $\delta^{13}C$)**

| Source | df | SS | MS | Pseudo-F | P (perm) | Unique perms |
|---|---|---|---|---|---|---|
| Locations | 11 | 682.35 | 62.031 | 112.4 | 0.001 | 999 |
| Residual | 456 | 251.65 | 0.55187 | | | |
| Total | 467 | 934 | | | | |

## 8.4 Discussion

The stable isotope ratios in the sagittal otolith carbonate of *P. macrochir* populations were significantly different from a number of locations across a coastline length of approximately 14,000 kilometres. These significant and consistent differences in stable isotopic signatures for the fish from the locations that were sampled on separate occasions were persistent through time (Figures 8.3 and 8.4). The Roebuck Bay and Fitzroy River temporal samples were not significantly different, while the Chambers Bay temporal samples were significantly different with the difference related directly to the size of fish sampled during each sampling occasion. The differences between sampling periods from the Chambers Bay were still small compared to the spatial differences between Chambers Bay and all the neighbouring locations (Figures 8.3 and 8.4).

All the locations sampled were significantly different except for the eastern (Queensland) Gulf of Carpentaria sites (Flinders, Kendall and Albert Rivers), which were similar. Overall, there is an east-west longitudinal trajectory in isotope values (Figures 8.3 and 8.4). Six major assemblages or stocks of *P. macrochir* were identified: Western Australia, Northern Territory North Coast, Northern Territory Gulf of Carpentaria, Queensland Gulf of Carpentaria, northern Queensland and southern Queensland.

The stable isotope values represent a mean value integrated over the entire ontogenetic life history of each individual fish, which may range in age from two to possibly 20+ years (Kailola *et al.* 1993). Stable isotope signatures for the locations that were significantly different reflect different environmental conditions, indicating that the adult fish had remained resident in places that offered different environmental regimes. If the adult fish were mixing among locations then isotopic signatures amongst locations would be similar, as was the case for *Pomatomus saltatrix* in mid-western Australia (Edmonds *et al.* 1999). Adult populations of *P.macrochir* exhibit spatial subdivision and those spatially distinct assemblages of fish are non-mixing and can be considered as independent management units or stocks for the purposes of fisheries management.

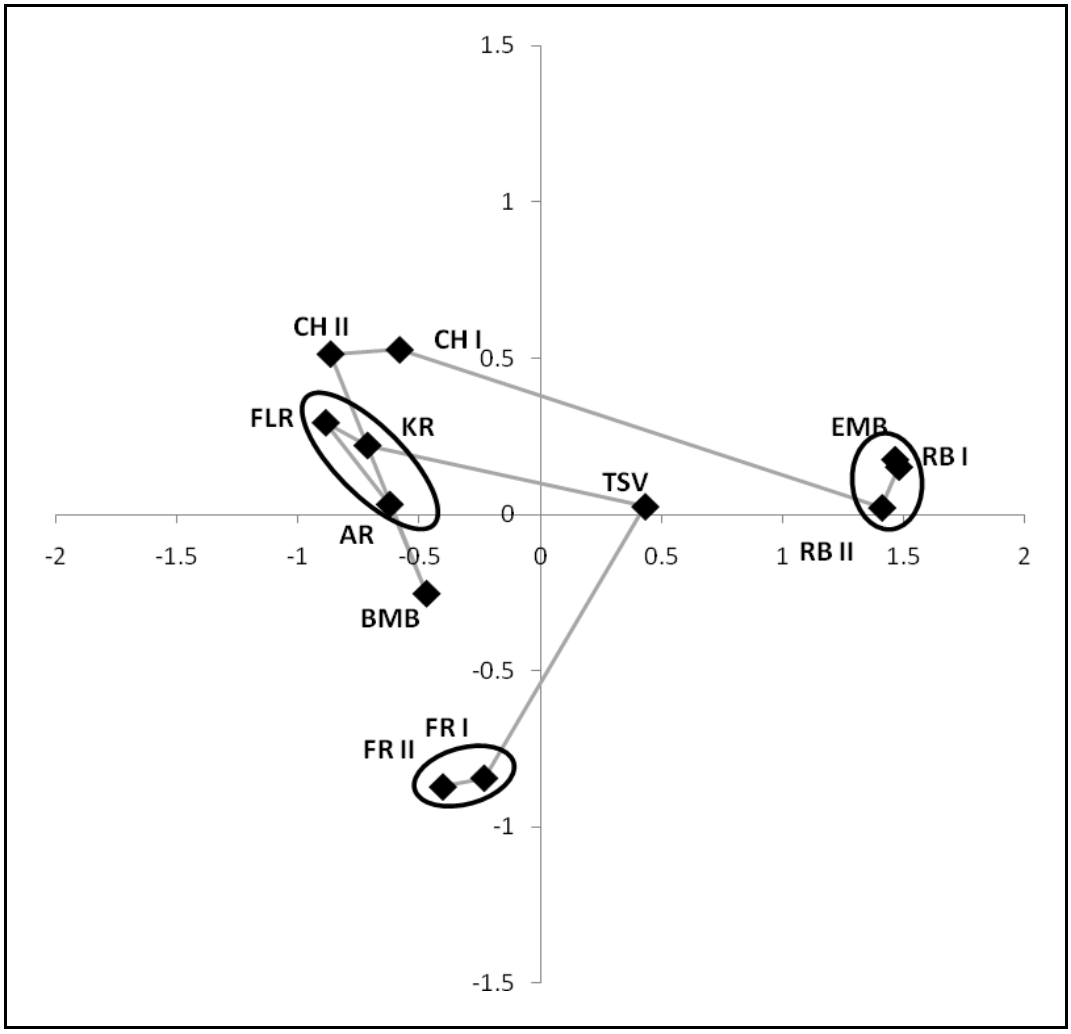


**Figure 8.3:** nMDS of the mean $\delta^{18}O$ and $\delta^{13}C$ values by location. The line connects the locations by order of longitude from Fitzroy River as the eastern most location to Eighty Mile Beach (EMB) as the western most location. The ellipses group sites that were not significantly different from each other from the PERMANOVA (pPerm>0.05). Locations are EMB = Eighty Mile Beach; RB I = Roebuck Bay sampling period I; RB II = Roebuck Bay sampling period II; CH I = Chambers Bay sampling period I; CH II = Chambers Bay sampling period II; BMB = Blue Mud Bay; AL = Albert River, FLR = Flinders River; KR = Kendall River; TSV = Townsville; FR I = Fitzroy River sampling period I; FR II = Fitzroy River sampling period II.

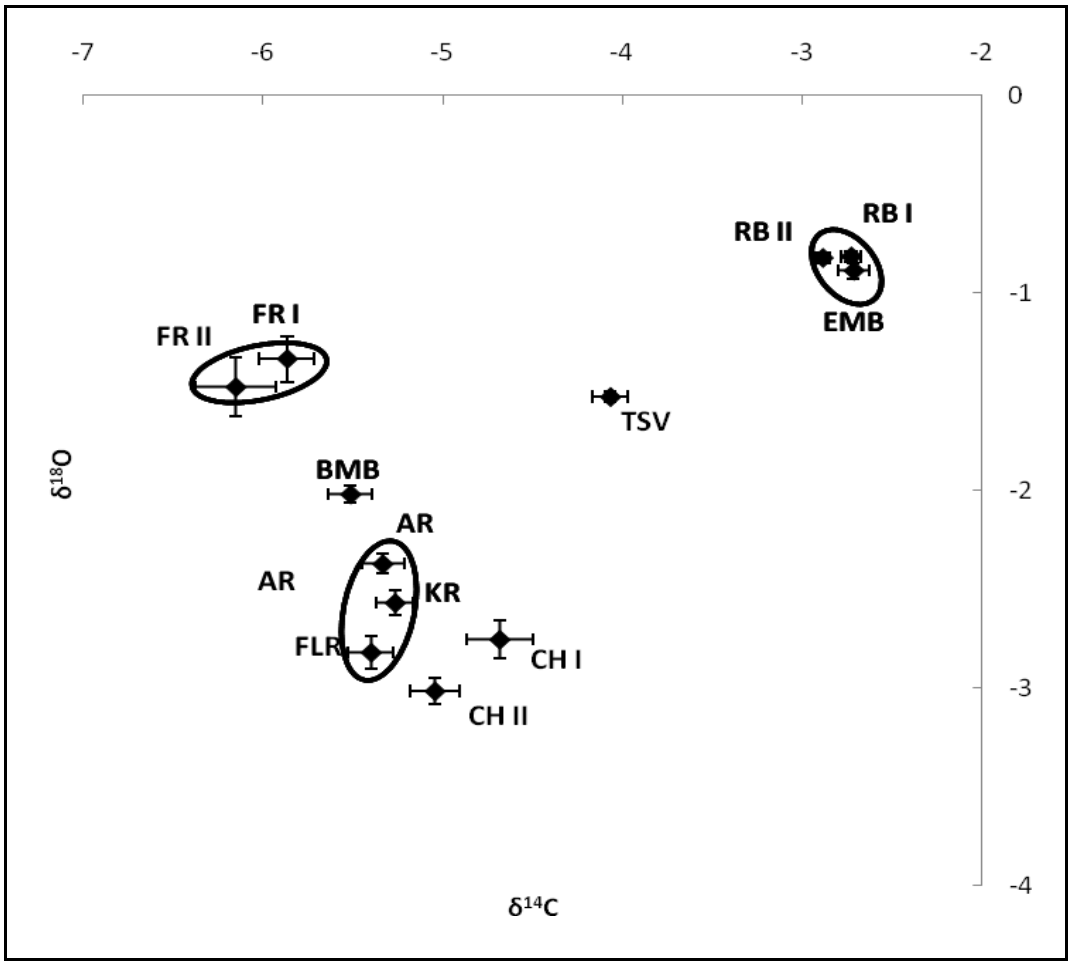


**Figure 8.4:** Mean $\delta^{18}$O values (± standard error) versus mean $\delta^{13}$C values (± standard error) of *P. macrochir* sagittal otolith carbonate for each location. Ellipses include those sites not statistically different from each other from the PERMANOVA. Locations defined in Figure 9.3.

This study has provided further evidence that measurement of stable isotope ratios in teleost sagittal otolith carbonate is a valuable tool in discerning fishery management units of adult fish across a species distributional range that includes waters with differing environmental regimes. The differences in $\delta^{13}$C and $\delta^{18}$O reflect the surrounding environmental conditions that an individual fish encounters during its life history (e.g. Gao and Beamish, 1999). The results from this study are a reflection of the different physical and biological characteristics present in the nearshore coastal areas across northern Australia. The oxygen stable isotope ratios are influenced readily by both water temperature and salinity differences in each water body. In contrast, the primary productivity and food resources incorporated within each water body influence carbon stable isotope ratios.

The separation of these nearshore stocks of *P. macrochir* may be reflective of fine-scale habitat macrocosms and/or local environmental preferences such as rivers, creeks, estuaries, river drainages,

beach systems and embayments. An area of further study is the examination of what mechanisms in these nearshore environments allow for local retention mechanisms of adult fish and/or larvae.

### Fishery management implications

The identification of fish stocks or management units and thus understanding population structure is a critical element in sustainable fisheries management. The $\delta^{18}O$ and $\delta^{13}C$ stable isotope values in this study have revealed distinct location-specific signatures, strongly supporting the premise of a multi-stock complex of *P. macrochir* across northern Australia. This multi-stock complex is consistent through time and can be separated into a number of distinct stocks or management units. These stocks are: (1) Eighty Mile Beach and Roebuck Bay [WA]; (2) Chambers Bay [NT NC]; (3) Blue Mud Bay [NT GoC]; (4) Albert, Flinders and Kendall Rivers [QLD GoC]; (5) Townsville [QLD EC] and (6) Fitzroy River [QLD EC]. Further evidence of spatial subdivision may be revealed with additional sampling at supplementary locations. As with other stock structure studies, the bounds of fishable stocks or management units are confined by the spatial scale of sampling possible given the logistics of sampling in remote locations. Spatial heterogeneity in the population structure of *P. macrochir* also implies that this nearshore coastal species may also exhibit spatial variation in demographic parameters and harvest rates.

While the adult assemblages of *P. macrochir* are effectively isolated from each other, recruitment to each stock or adult assemblage across northern Australia may be derived from, (1) a common gene pool (if there is an absence of significant genetic variation among stocks), or (2) a distinct gene pool (if there is significant genetic variation among stocks). Under scenario (1), high levels of connectivity or intermixing during the egg and larval stages across a multi-stock complex of separate and distinct adult stocks imply that the size of the total adult spawning stock (i.e. the combined sum of each of the separate adult stocks) could impact recruitment. Thus, fishing on any one stock could impact fishing on any other stock, through subsequent recruitment (resulting from a reduced spawner biomass). However, direct impacts of fishing on one stock should not affect adjacent stocks (or any fishing impact should be negligible).

In contrast, under scenario (2), limited or negligible levels of connectivity or intermixing during the egg and larval stages across a multi-stock complex of separate and distinct adult stocks imply that the size of the individual adult spawning stock in each distinct adult assemblage could impact recruitment. Thus, localised depletion on any one stock could adversely impact that stock directly by reducing the spawner biomass and directly impacting subsequent recruitment.

The distinct separation of stocks and the delineation of stock structure allow management units to be defined. The presence of a multi-stock complex of *P. macrochir* indicates that management can be applied separately to each of the stocks at the regional or location level along the northern Australian

coast. Importantly, there are no cross-jurisdictional or shared stock management issues for this species (although further sampling of populations close to jurisdictional boundaries is required to assess the efficacy of the results from this study).

Spatial heterogeneity in population structure and life history traits implies that it will be difficult to apply optimal harvest strategies over state or regional scales. Furthermore, spatial variation in population structure limits traditional stock assessment approaches. Given the likely dynamic state of these tropical nearshore fishery resources, management arrangements will be challenging. The fine scale spatial separation of stocks signifies that these spatial scales will be required for sustainable management, monitoring and assessment.

Monitoring and assessment will also need to be robust and indicators will need to be developed that can adequately reflect the dynamic state of the resource through space and time. Fisheries in these nearshore tropical coastal areas will need an adaptive response to the social, biological and environmental conditions and therefore flexibility in management arrangements will be required to accommodate the spatial variability in population structure, demography and harvest of *P. macrochir*. However, given the spatial subdivision evident, a precautionary approach to the sustainable management and harvest of *P. macrochir* stocks would be for each state and territory fisheries management agency to aim to maintain an adequate total spawner biomass within each fishable stock identified in this study and avoid localised depletion events. Note that localised depletion events will be exacerbated if there are limited or negligible levels of gene flow among these stocks.

**Appendix 8.1:** Summary of the sampling data, and results from the stable isotope analyses of the sagittal otolith carbonate of *Polydactylus macrochir* from locations across northern Australia (WA = Western Australia; NT = Northern Territory; QLD = Queensland; GoC = Gulf of Carpentaria; East Coast = EC). More specific locations details are listed in the methods section.

| Location (code) | Position (latitude, longitude) | Date of sampling | N | Length Range (FL, mm) mean (range) | Otolith weight (mg) mean (range) | $\delta^{13}C$ (‰ PDB) mean (range) | $\delta^{18}O$ (‰ PDB) mean (range) |
|---|---|---|---|---|---|---|---|
| Eighty Mile Beach, WA | 19°34'S, 121°25'E | April 2009 | 41 | 624 (231-1021) | 333.8 (286.0-994.0) | -2.72 (-4.62 to -1.68) | -0.89 (-1.59 to -0.16) |
| Roebuck Bay, WA | 18°05'S, 122°17'E | March 2008 | 40 | 716 (544-892) | 324.1 (152.0-672.0) | -2.73 (-3.42 to -2.04) | -0.82 (-1.24 to -0.52) |
| | | August 2008 | 40 | 584 (463-881) | 199.7 (107.8-575.1) | -2.89 (-3.26 to -2.19) | -0.82 (-1.19 to -0.52) |
| Chambers Bay, NT NC | 12°12'S, 131°53'E | February 2008 | 40 | 906 (730-1030) | 716.1 (325.0-1035) | -4.69 (-8.25 to -3.20) | -2.75 (-4.23 to -1.75) |
| | | May 2009 | 40 | 707 (540-850) | 292.9 (171.9-455.7) | -5.04 (-7.31 to -3.97) | -3.01 (-4.28 to -2.44) |
| Blue Mud Bay, NT GoC | 13°26'S, 136°03'E | September 2008 | 40 | 643 (435-825) | 214.8 (95.6-405.6) | -5.51 (-7.78 to –4.20) | -2.02 (-3.02 to -1.43) |
| Albert River, QLD GoC | 17°34'S, 139°50'E | October 2008 | 35 | 601 (520-715) | 239.1 (167.4-340.1) | -5.33 (-6.54 to -3.80) | -2.37 (-2.98 to -1.71) |
| Flinders River, QLD GoC | 17°35'S, 140°34'E | July 2007 | 40 | 633 (495-915) | 249.3 (168.6-387.9) | -5.40 (-6.47 to -3.13) | -2.82 (-3.66 to -1.15) |
| Kendall River, QLD GoC | 14°10'S, 141°35'E | September 2008 | 40 | 554 (330-905) | 184.2 (61.9-376.7) | -5.27 (-7.36 to -4.19) | -2.57 (-3.88 to -1.82) |
| Townsville, QLD EC | 19°15'S, 146°55'E | June 2008 | 40 | 693 (582-810) | 257.8 (200.7-333.1) | -4.07 (-5.54 to -2.92) | -1.53 (-1.90 to -1.26) |
| Fitzroy River, QLD EC | 23°32'S, 150°52'E | September 2007 | 39 | 690 (430-1126) | 291.5 (110.6-1032) | -5.87 (-7.87 to -3.38) | -1.33 (-2.77 to -0.21) |
| | | October 2008 | 33 | 741 (515-1290) | 354.6 (142.9-1361) | -6.15 (-8.63 to -3.09) | -1.48 (-2.97 to -0.31) |

# Chapter 9: Stock structure of king threadfin, *Polydactylus macrochir*, as indicated by life history parameters

**Brad R. Moore, Jason M. Stapley, Quentin Allsop, Stephen J. Newman and David J. Welch**

## 9.1 Introduction

The king threadfin, *Polydactylus macrochir* Günther, 1867 is a large, protandrous Polynemid that inhabits turbid coastal waters, estuaries and mangrove creeks across northern Australia and southern Papua New Guinea (Motomura *et al.* 2000). In Australia, the species' distribution extends from the Ashburton River in Western Australia across northern Australia to Brisbane in southeast Queensland (Motomura *et al.* 2000).

King threadfin support valuable commercial, recreational and indigenous fisheries across northern Australia and form the second most important target species for northern Australia's inshore net fisheries after the iconic barramundi (*Lates calcarifer*) (Pember *et al.* 2005), with a reported 780 tonnes harvested commercially across Australia in 2005. The bulk of this catch was from the waters of the Northern Territory and Queensland Gulf of Carpentaria (323 and 279 tonnes, respectively), whereas smaller catches occurred in the waters of Western Australia (70 t) and Queensland's east coast (108 t) (Matthews and Grace 2006; DPI&F 2007; Newman *et al.* 2007). The species is also heavily targeted by recreational anglers throughout its distribution, and is highly regarded as both a table and sport fish (Welch *et al.* 2002). For management and assessment purposes, single stocks are recognised in the waters of Western Australia and the Northern Territory, whereas two stocks are recognised in Queensland waters; one in Queensland's Gulf of Carpentaria waters and one on Queensland's east coast.

The current management arrangements for *P. macrochir* across northern Australia make no allowance for movement of fish between management jurisdictions. As the distribution of king threadfin crosses state and territorial boundaries, a need for cross jurisdictional cooperative management may exist. Conversely, king threadfin may be resident on a small spatial scale, with a number of localised stocks occurring within each jurisdiction. In this case management may be more appropriate on a fine scale. Irrespective of the scenario, knowledge of the stock structure is seen as critical to ensuring sustainable management of the king threadfin across northern Australia.

Life history parameters, such as age and growth patterns, mortality rates, reproductive profiles, fecundity, distribution and abundance, have been widely used to provide information on the stock structure of commercially exploited fish species (Ihssen *et al.* 1981a; Jennings and Beverton 1991; Pawson and Jennings 1996; Begg *et al.* 1999; Abaunza *et al.* 2008; Silva *et al.* 2008). Life history parameters are regarded as phenotypic expressions of the interaction between genetic and environmental influences (Begg *et al.* 1999). Estimates of life history parameters, such as age and growth relationships, mortality rates, length-at-first maturity of an individual fish are believed to representative of a putative stock (Begg 2005). Differences in life history parameters between populations are taken as evidence that such populations are geographically and/or reproductively isolated, thereby forming discrete units for management purposes (Ihssen *et al.* 1981a; Begg *et al.* 1999). In addition to providing information on the stock structure and connectivity of fish populations, knowledge of vital life history parameters can provide an indication of the species' vulnerability to over-exploitation (Jennings *et al.* 1998; Marriott *et al.* 2007), and provides useful baseline biological data for monitoring and stock assessment purposes.

There is some data to suggest that king threadfin life histories may vary across northern Australia. Pember *et al.* (2005) used the von Bertalanffy growth model to demonstrate Western Australia populations of king threadfin attain total lengths of 322, 520 and 945 mm by the end of years 1, 2 and 5, respectively. In contrast, Bibby and McPherson (1997) used the Schnute Case 3 growth model to indicate Queensland Gulf of Carpentaria populations of king threadfin attain approximate total lengths of 345, 490 and 790 mm by the end of years 1, 2 and 5, respectively. Differences in spawning times have also been noted for king threadfin across northern Australia. *Polydactylus macrochir* populations along the Pilbara and Kimberly coasts of Western Australia have been demonstrated to spawn from September to January (Pember *et al.* 2005), whist in the Gulf of Carpentaria king threadfin are reported to spawn between late winter (~August) and early spring (~September) (McPherson 1997). Based on a small number of samples, Russell (1988) concluded that king threadfin spawn along the east coast of Queensland from October to March. Whether the observed differences in demography across northern Australia reflects the occurrence of different stocks, or are merely artefacts of temporal variability in demographic patterns, is unknown.

The objective of this chapter was to evaluate the stock structure of king threadfin across northern Australia through a comparison of their life history characteristics. Key parameters investigated include growth rates and length and age-at-sex change.

## 9.2 Methods

### Sample collection

King threadfin were collected between July 2007 and December 2009 from nine estuarine and coastal sites across northern Australia (Figure 9.1). Whole fish or fish frames (whole skeleton remaining after filleting) were obtained directly from commercial fishers, fish processors or by fisheries-independent sampling. At each site fish were collected with gillnets of mesh diameter up to approximately 165 mm. For each fish collected the total length (TL), length to caudal fork (FL), and upper jaw length (UJL) was measured to the nearest millimetre. Sex and maturity stage was determined from a macroscopic examination of the gonads. As some fish were gutted at sea before processing it was not possible to obtain sex or stage information from all individuals. All macroscopic staging was assessed using the staging scheme of Pember *et al.* (2005). Gonads from a subsample of fish from each site where stored in either neutral buffered formalin or frozen for transport to the laboratory for verification by a second reader. Sagittal otoliths (hereafter referred to as otoliths) were removed, cleaned, dried and stored in paper envelopes until processing in the laboratory.

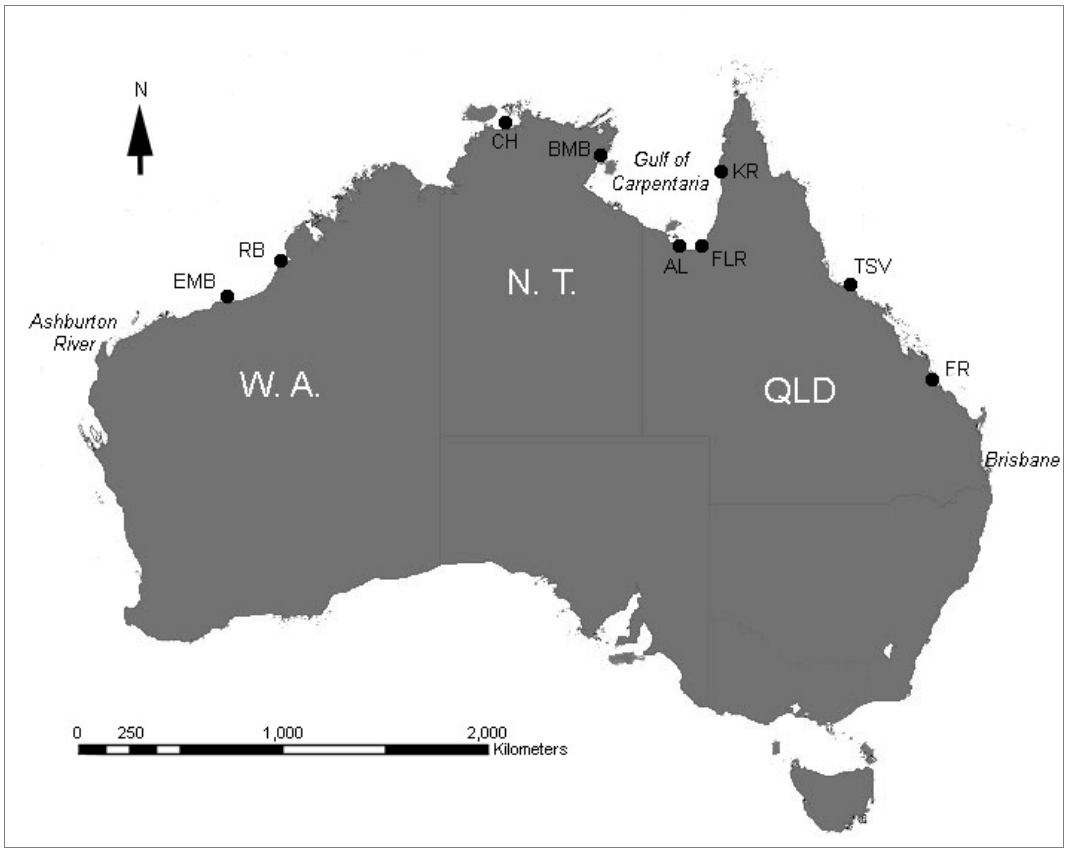


**Figure 9.1:** The nine sampling sites used in this study. EMB, Eighty Mile Beach; RB, Roebuck Bay; CH, Chambers Bay; BMB, Blue Mud Bay; AL, Albert River; FLR, Flinders River; KR, Kendall River; TSV, Townsville; FR, Fitzroy River.

### Age determination

A comparison of whole and sectioned otoliths was conducted to assess which structure would be used for ageing. Whole otoliths from a total of 460 individuals, taken from fish across a range of lengths and sites, were immersed in oil and examined microscopically under a reflected light against a black background and the number of opaque zones was counted. The same otoliths were then mounted in resin and up to four transverse sections measuring 300 μm were taken using a diamond-edged circular saw. Care was taken to ensure the primordium of the otolith was included in at least one section. All sections were cleaned and mounted on glass microscope slides with polyester resin. Otolith sections were examined under a stereo dissecting with reflected light against a black background.

An image of each whole and sectioned otolith was taken using a Leica DC 300 digital camera mounted to the dissecting microscope. The numbers of opaque bands, verified as annuli by Pember *et al.* (2005), were counted twice for both whole and sectioned otoliths. The precision of annuli estimates from whole and sectioned otoliths was calculated using the coefficient of variation (CV) (Chang 1982). Greater precision is achieved when the CV is minimised (Campana *et al.* 1995). Differences between the two reading methods was observed by age bias plots modified from Campana *et al.* (1995) and by plotting the CV of the whole otolith reads against the sectioned otolith age, based on the assumption that sectioned otoliths provided the best estimate of true annuli number (Beamish 1979).

The total CV of annuli estimates between whole and sectioned otoliths was 1.22%, indicating a high degree of overall precision between whole and sectioned otolith annuli estimates. However, when ages were analysed separately, the CV increased as the number of annuli increased (Figures 9.2 and 9.3), indicating an increasing divergence in annuli estimation between whole and sectioned otoliths, particularly beyond annuli counts of six. As a result, all otoliths were initially read whole. Otoliths in which six or less annuli were counted in the initial read were read whole again, whereas otoliths in which more than six annuli were counted in the initial read were sectioned. Once the method of reading was established each otolith was read twice. When annuli counts did not agree, a third reading was taken, and the two concurrent readings being accepted as the number of annuli. When all three counts differed, the otolith was rejected from further analysis.

For all whole and sectioned readings each otolith was assigned a marginal index category (Table 9.1). The advantage of including the marginal index category in the annuli counts is that this helps to minimise potential bias in age estimates due to variations in sampling date between sites. For fish with a margin category of 0 or 1, the number of annuli was taken as the age of the fish. Fish with a margin category of 2 were assigned an age equal to the number of annuli and had half a year added to their age, and a full year was added to fish with a margin category of 3. This method was used to age all fish, regardless of whether the spawning season or timing of otolith opaque increment completion was known, to allow for consistency in the derived ages between sites.

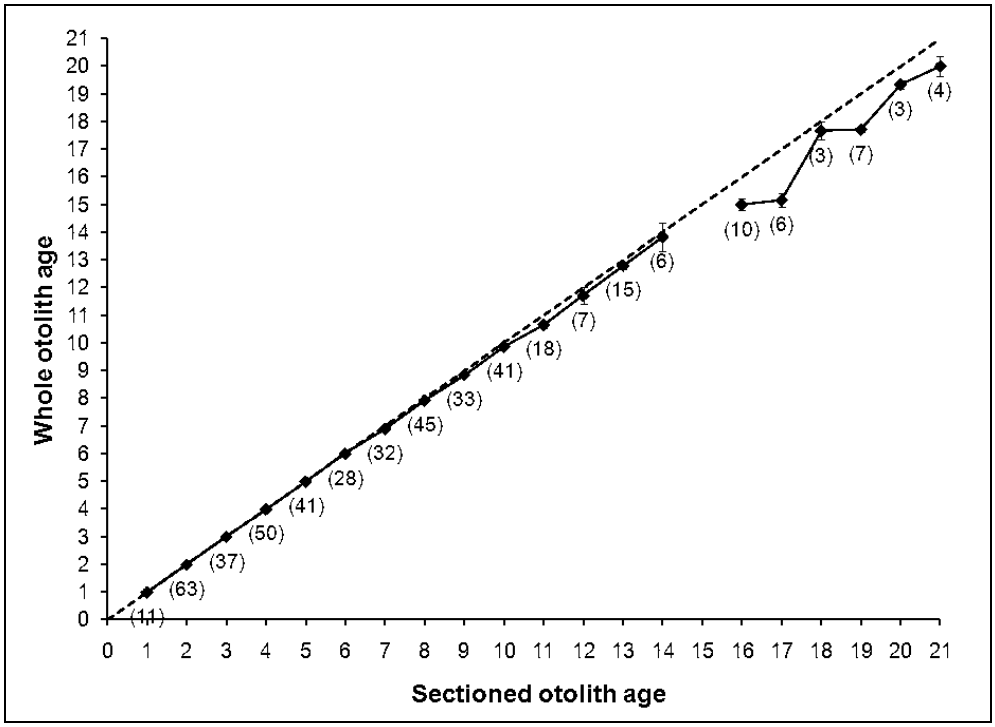


**Figure 9.2:** Comparison of ages estimated from whole and sectioned otoliths of king threadfin.

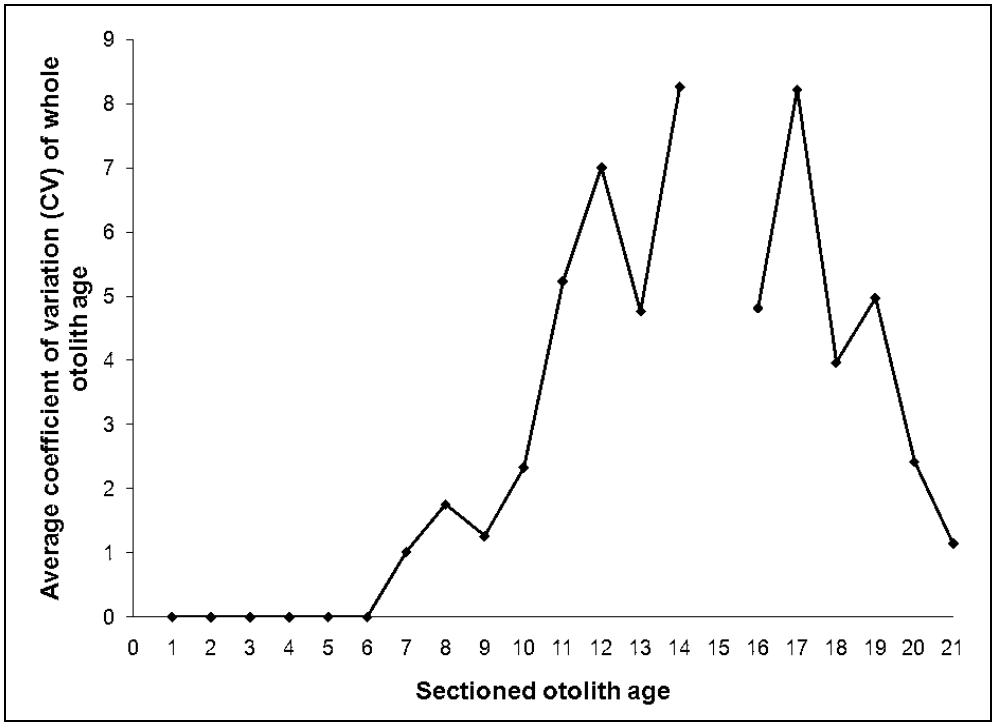


**Figure 9.3:** Average coefficient of variation (CV) of whole otolith reads compared to sectioned otolith reads of *Polydactylus macrochir* otoliths.

**Table 9.1:** Description of the otolith margin categories used to establish absolute ages of *Polydactylus macrochir* (Tobin and Mapleston 2004).

| Margin category | Description |
|---|---|
| 0 | Complete and continuous opaque band formed around edge of otolith, with no translucent material beyond the last opaque band. |
| 1 | Translucent band laid onto the outer edge comprising less than half the width of the previous translucent band. |
| 2 | Translucent band laid onto outer edge comprising roughly half the width of the previous translucent band. |
| 3 | Opaque band on edge of otolith, however band is not complete or continuous. |

## Age and growth

Growth was modelled using the von Bertalanffy growth function (VBGF), fitted by nonlinear least-squares regression of TL on age of *P. macrochir*. As king threadfin are protandrous, changing from males to females with increasing age, a single curve VB growth curve was fitted to TL at age. The form of the VBGF used to model length-at-age data was:

$$L_t = L_\infty(1\text{-}e^{-K(t-t0)}),$$

where $L_t$ is the length-at-age, $L_\infty$ is the asymptotic length, $K$ is the growth coefficient, and $t_0$ is the hypothetical age at which fish would have a length equal to zero.

Differences in the parameters derived from the VBGF ($L_\infty$, $K$ and $t_0$) were compared across sites using likelihood ratio tests. A common range of age classes (2-10½ years) was used in each analysis to assure validity of the comparisons (Haddon 2001). As not all sites could be compared using this method (see results), spatial patterns in growth were further examined through an analysis of length-at-age class. A one-way analysis of variance (ANOVA) was used to determine if there were differences in mean TL for the 2+ years and 3+ years age classes. Length and age were first $\log_e$ transformed to meet assumptions of normality and homogeneity of variance. In the comparison of length-at-age only age classes that had at least eight individuals per site were considered. This number is similar to the samples sizes used by Erzini (1994) and Abaunza *et al.* (2008) in their respective studies of variability of length-at-age of marine fishes. Significant results were examined using Tukey-Kramer post-hoc pairwise comparisons.

### Length and age at sex change

Logistic regression analysis was used to estimate the proportion of females (relative to males) in each age and fifty millimetre length class to estimate the age and length that *P. macrochir* changes sex at the various sites. The age and length of sex change was determined using the equation:

$$Ps = 1/\{1+\exp[-\ln(19)(s-s_{50})/(s_{95}-s_{50})]\},$$

where *Ps* = the proportion of females in each age or fifty millimetre length class *s*, *s50* and s95 is the age or length at which 50% and 95% of the population have changed to females, respectively, and ln = the natural logarithm. Transitional individuals were not included in the analysis.

## 9.3 Results

### Age and growth

A summary of the sample sizes, age and TL ranges, and number of male, female and transitional fish collected from each site is presented in Table 9.2. It was not possible to compare the VBGF among all sites due to the lack of older fish at some sites (particularly in the Gulf of Carpentaria and Townsville samples) (Figure 9.4). The Eighty Mile Beach, Roebuck Bay, Chambers Bay and Fitzroy River sites were considered to have adequate age ranges in the samples for robust comparisons among sites. Table 9.3 gives the estimated growth parameters derived for these sites. Likelihood ratio tests indicated that patterns of growth differed significantly between the four sites (Table 9.4). Fish from Eighty Mile Beach grew slower, but attained a greater asymptotic length to those from Roebuck Bay. Fish from the Fitzroy River attained a greater maximum size and lived longer and than those from the three other sites, reaching a maximum age of 22 years old.

**Table 9.2:** King threadfin sampled for growth and length and age of sex change.

| Site | *n* | Age range (years) | TL range (mm) | *n* males | *n* females | *n* transitionals |
|---|---|---|---|---|---|---|
| Eighty Mile Beach | 150 | 0.5 – 11.5 | 273 - 1242 | 41 | 99 | 10 |
| Roebuck Bay | 314 | 1.5 – 10.5 | 545 - 1068 | 260 | 55 | 4 |
| Chambers Bay | 126 | 1.5 – 11.5 | 625 - 1200 | 73 | 52 | 1 |
| Blue Mud Bay | 88 | 1.0 – 4.0 | 497 - 1010 | 72 | 2 | 0 |
| Albert River | 36 | 3.0 – 5.0 | 560 - 865 | 31 | 5 | 0 |
| Flinders River | 138 | 1.5 – 7.5 | 390 - 1120 | 83 | 49 | 5 |
| Kendall River | 63 | 1.0 – 3.0 | 375 - 940 | 57 | 6 | 0 |
| Townsville | 84 | 1.5 – 4.0 | 538 - 1060 | 67 | 2 | 1 |
| Fitzroy River | 592 | 2.0 – 22.0 | 520 - 1612 | 241 | 29 | 6 |
| **Total** | **1591** | **0.5 – 22.0** | **273 - 1612** | **925** | **299** | **27** |

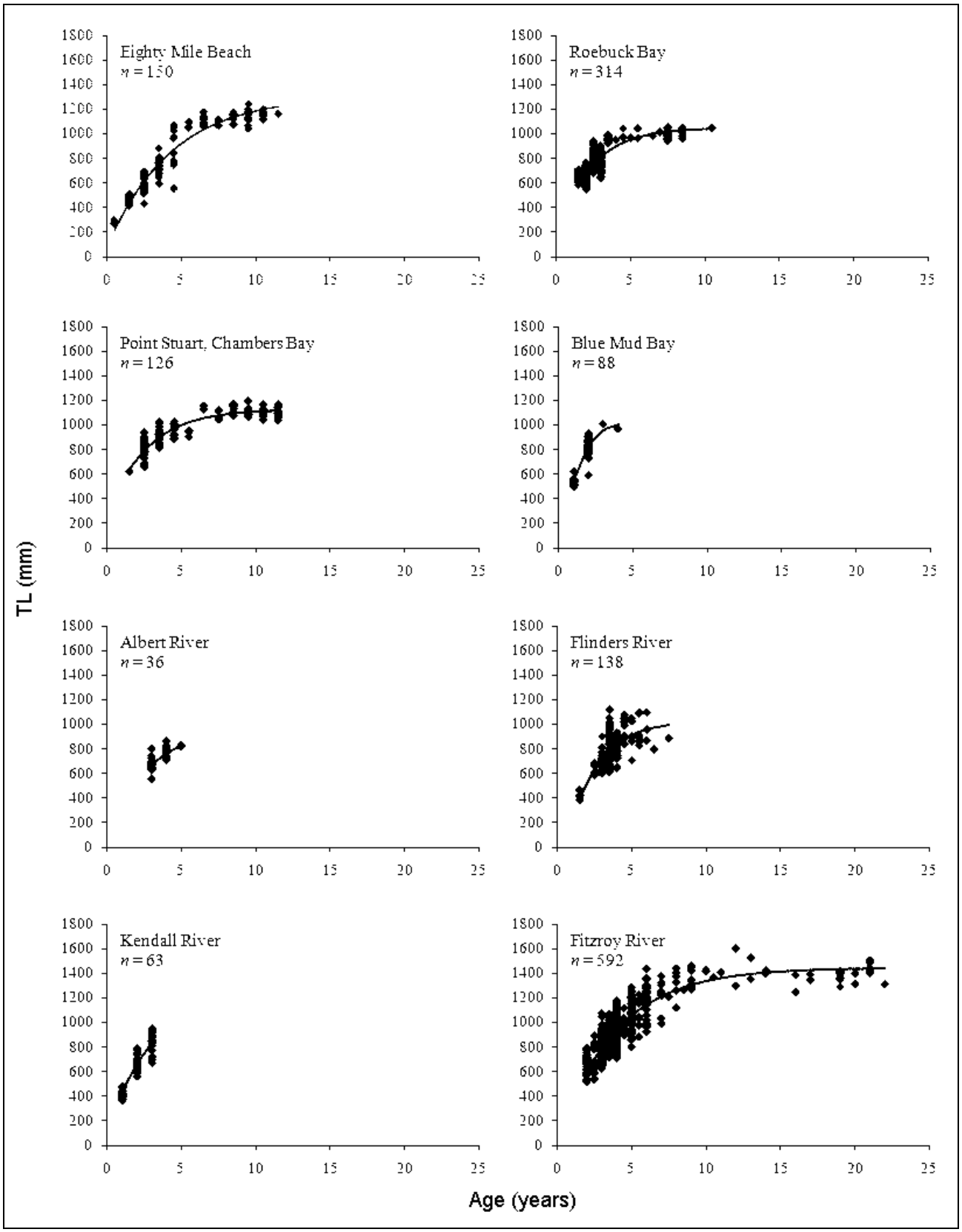


**Figure 9.4:** Total length (TL) at-age data and estimated von Bertalanffy growth curves from eight sites sampled in the present study. Note Townsville samples are not shown as we were unable to fit the VBGF to the data.

**Table 9.3:** Parameter estimates for the von Bertalanffy growth function for king threadfin collected from four sites across northern Australia.

| Site | VBGF growth function parameter estimates | | |
|---|---|---|---|
| | $L_\infty$(mm) | $K$ | $T_0$ |
| Eighty Mile Beach | 1303.35 | 0.2313 | -0.3059 |
| Roebuck Bay | 1048.46 | 0.4508 | -0.3188 |
| Chambers Bay | 1127.21 | 0.3829 | -0.7332 |
| Fitzroy River | 1448.43 | 0.2566 | -0.1346 |

**Table 9.4:** Results of maximum likelihood tests for differences in growth of king threadfin collected from four sites across northern Australia for fish ranging from 2 to 10½ years old. Listed in the last column are the growth parameters that differed significantly between sites.

| Comparison | $\chi^2$ | d.f. | $P$ | Parameters |
|---|---|---|---|---|
| EMB vs. RB | 244.74 | 3 | $P < 0.0001$ | $L_\infty$, $K$ |
| EMB vs. CH | 132.75 | 3 | $P < 0.0001$ | $L_\infty$, $t_0$ |
| EMB vs. FR | 118.54 | 3 | $P < 0.0001$ | $L_\infty$, $K$, $t_0$ |
| RB vs. CH | 40.82 | 3 | $P < 0.0001$ | $L_\infty$, $K$, $t_0$ |
| RB vs. FR | 217.44 | 3 | $P < 0.0001$ | $L_\infty$, $K$, $t_0$ |
| CH vs. FR | 141.73 | 3 | $P < 0.0001$ | $L_\infty$, $K$ |

Comparisons of mean length at age data provided a measure of the patterns in growth between neighbouring sites (Table 9.5). Fish from Roebuck Bay were significantly larger than those from Eighty Mile Beach in both the 2+ years and 3+ years age classes. Chambers Bay fish were significantly larger than fish from Eighty Mile Beach and Roebuck Bay for both the 2+ years and 3+ years age classes, but did not differ from those from Blue Mud Bay in length in the 2+ years age class. In the Gulf of Carpentaria, Blue Mud Bay samples appeared different to those from the Flinders and Kendall rivers. Although no 2+ year old fish were available from the Albert River, fish in the 3+ years age class from the Albert River were significantly shorter than those from other Gulf of Carpentaria sites. No significant differences in length were observed between the Flinders and Kendall rivers in the 2+ years or 3+ years age classes. On Queensland's east coast, fish from Townsville and the Fitzroy River were not significantly different at the 3+ years age class, suggesting that these fish either have a common history or have grown in a similar environment.

**Table 9.5:** Comparison of length-at-age class data of king threadfin across northern Australia. The number in the table corresponds to the age class for which mean length is significantly different at the 95% level of confidence based on Tukey-Kramer pair-wise comparison. Ns = Not significant, Nt = Not tested (insufficient samples sizes).

| Location | Eighty Mile Beach | Roebuck Bay | Chambers Bay | Blue Mud Bay | Albert River | Flinders River | Kendall River | Townsville | Fitzroy River |
|---|---|---|---|---|---|---|---|---|---|
| **Eighty Mile Beach** | | 2+ yrs, 3+ yrs | 2+ yrs, 3+ yrs | 2+ yrs, Nt | Nt, Ns | Ns, Ns | 2+ yrs, 3+ yrs | Nt. 3+ yrs | 2+ yrs, Ns |
| **Roebuck Bay** | | | 2+ yrs, 3+ yrs | 2+ yrs, Nt | Nt, 3+ yrs | Ns, 3+ yrs | Ns, Ns | Nt, Ns | Ns, Ns |
| **Chambers Bay** | | | | Ns, Nt | Nt, 3+ yrs | 2+ yrs, 3+ yrs | 2+ yrs, Ns | Nt, Ns | 2+ yrs, 3+ yrs |
| **Blue Mud Bay** | | | | | Nt. Nt | 2+ yrs, Nt | 2+ yrs, Nt | Nt, Nt | 2+ yrs, Nt |
| **Albert River** | | | | | | Nt. 3+ yrs | Nt, 3+ yrs | Nt, 3+ | Nt, 3+ yrs |
| **Flinders River** | | | | | | | Ns, Ns | Nt, 3+ | Ns, Ns |
| **Kendall River** | | | | | | | | Nt, Ns | Ns, Ns |
| **Townsville** | | | | | | | | | Nt, Ns |
| **Fitzroy River** | | | | | | | | | |

### Length and age at sex change

It was not possible to determine the length and age at sex change across all sites due to a lack of males or females at some of the sites (particularly Blue Mud Bay and Townsville). There were considerable numbers of small, young females from three sites in the eastern Gulf of Carpentaria. The lack of increase in the proportion of females at these sites, even when data for these sites was pooled, resulted in the logistic function providing a poor fit to the data (Figure 9.5).

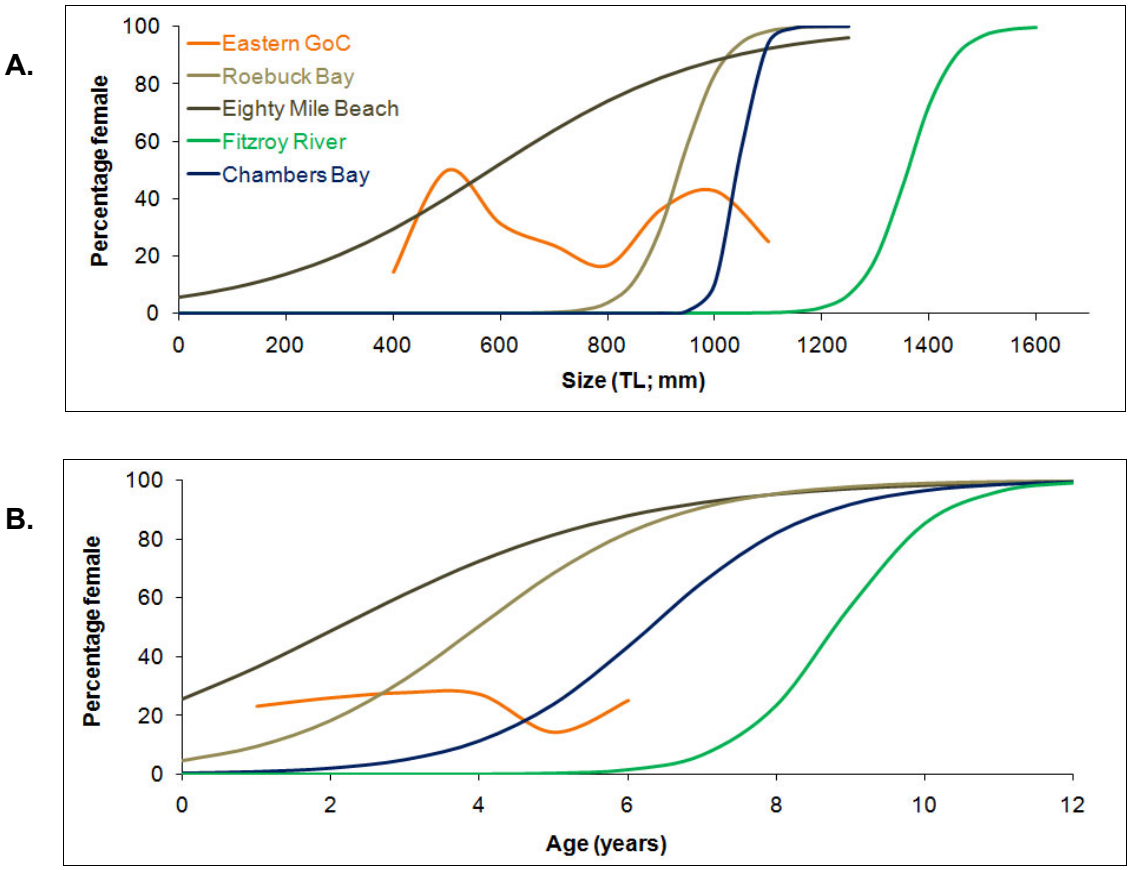


**Figure 9.5:** Logistic regression curves fitted to the percentage of female *Polydactylus macrochir* in each 50 mm length (A) and age class for sites across northern Australia (B). Note data for eastern Gulf of Carpentaria sites are pooled.

The estimated length at which 50% and 95% of individuals changed sex varied considerably between the sites where we had sufficient numbers of both sexes (Figure 9.5; Table 9.6). The length at which 50% of individuals changed sex was lowest for Eighty Mile Beach fish (581 mm TL), and highest for Fitzroy River fish (1,360 mm TL). The pattern of age at sex change was similar to the pattern of length at sex change. The age at which 50% of individuals changed sex was lowest at the Western Australia sites (2.1 and 4.0 years at Eighty Mile Beach and Roebuck Bay, respectively) and highest at the Fitzroy River (8.8 years).

**Table 9.6:** Length and age of sex change of *Polydactylus macrochir* across northern Australia.

| Site | Length (TL, mm) | | Age (years) | |
|---|---|---|---|---|
| | $L_{50}$ | $L_{95}$ | $A_{50}$ | $A_{95}$ |
| Eighty Mile Beach | 581 | 1194 | 2.1 | 7.9 |
| Roebuck Bay | 935 | 1056 | 4.0 | 7.9 |
| Chambers Bay | 1044 | 1103 | 6.3 | 9.6 |
| Blue Mud Bay | - | - | - | - |
| Albert River | - | - | - | - |
| Flinders River | - | - | - | - |
| Kendall River | - | - | - | - |
| Townsville | - | - | - | - |
| Fitzroy River | 1360 | 1482 | 8.8 | 10.8 |

## 9.4 Discussion

### Spatial patterns

The results of this study suggest that king threadfin form a number of isolated, non-mixing stocks across northern Australia. Patterns in growth rates and length and age at sex change were found to be significantly different between fish across northern Australia at various spatial scales, including between sites separated by as little as tens of kilometres (e.g. Eighty Mile Beach and Roebuck Bay). Such differences suggest that king post-recruitment populations of king threadfin are highly resident and form a number of isolated, non-mixing stocks for the purposes of fisheries management.

Due to the general absence of larger, older fish from some site it was not possible to compare growth across all sites using growth models such as the VBGF. To provide an indication of patterns of growth between all sites we used a comparison of length-at age of fish in the 2+ years and 3+ years age classes. In the mean length-at-age analyses, we found similarities between fish from the Flinders and Kendall Rivers in the eastern Gulf of Carpentaria, Chambers Bay and Blue Mud Bay in the Northern Territory, and the Fitzroy River and Townsville on Queensland's east coast, suggesting that they form single stocks in each region. Although we cannot rule this out from the growth data, it is considered that the observed similarities in growth are a result of similar conditions between the sites, rather than a mixing of fish. Given the fine scale spatial structure observed elsewhere in the present study (e.g. Eighty Mile Beach and Roebuck Bay), it is likely that fish from each of these sites form distinct stocks. Further techniques are warranted to accurately delineate the stock structure of king threadfin in these waters.

Although the mean length-at-age analyses provide evidence of fine scale spatial structure in the 2+ years and 3+ years age classes, they cannot be used to unequivocally discern the stock structure of the species across its entire life history. For example, although the mean length of fish from two sites may be significantly different at age 2 or 3, these fish may mix later in life, and could therefore be considered a single stock. As such, caution is advised when interpreting the stock structure solely from the mean length-at-age comparisons. However, when combined with the comparisons of VBGF parameters and length and age-at sex change analyses, they provide an additional line of evidence that helps to provide a robust, reliable approach to discerning the stock structure of *P. macrochir*.

Sampling different sites at different times may introduce error into the comparisons of mean length-at-age, as it can result in comparisons of fish of different fractional yearly ages. Although we perceive this to have a negligible effect on the analyses, as neighbouring sites were generally collected at the same time, further analyses are warranted to accurately define the differences in growth of king threadfin populations across northern Australia. Furthermore, the small number of age classes available to compare growth between the sites may also significantly reduce the ability to detect differences in growth. Analysis of additional age classes is warranted to investigate the patterns of growth in these waters.

Several possible factors may be driving the observed geographical differences in life histories of king threadfin across northern Australia. In the present study, fish from lower latitude sites were generally found to grow significantly faster and reach greater lengths at comparable ages than those from higher latitude sites for comparable coastlines. For example, along the Western Australian coastline, fish from Roebuck Bay generally grew faster and reach a greater length-at-age than those from Eighty Mile Beach. Fish from the two northernmost sites, Chambers Bay and Blue Mud Bay, grew faster than fish from all other sites. Fish from the Albert River, in the southern Gulf of Carpentaria, were significantly smaller at a given age than those from the Flinders or Kendall rivers to the north. Although fish from the Fitzroy River reached the greatest length and age, they generally grew at a slower rate compared to those from the northern sites. Such observations suggest that growth rates of king threadfin may be temperature related. Indeed, the relationship between water temperature and fish growth is well documented (Conover 1992; Durieux *et al.* 2009; Tolan and Fisher 2009).

Despite the general association between latitude and length-at age, a number of other factors may contribute to the observed spatial patterns in growth. Geographic variation in growth has been demonstrated to result from spatial variation in recruitment, coupled with density dependant processes (Doherty 1983; Doherty and Fowler 1994), or by factors that influence larval survival and settlement. Alternatively, other processes, such as inter and intra-specific competition (Jones 1987), or habitat and food availability (Hart and Russ 1996), may be responsible for the observed spatial differences in growth. Pember (2006) hypothesised that the faster growth rates observed in fish from Roebuck Bay

fish compared to those from Eighty Mile Beach could be due to greater productivity of the benthic invertebrate fauna in the region as well as increased growth of early age classes as compensatory response to the areas long history of exploitation. The Roebuck Bay area has a long history of commercial, recreational and indigenous fishing pressure compared to other areas of the Kimberley coast (Pember 2006). It should be pointed out that knowledge of the mechanisms responsible for differences in patterns of growth is not necessary when delineating stock structure; in lieu of knowledge of the mechanisms responsible, comparisons of growth may at least show which fish have a common life history, indicative of stock discreteness.

### Temporal patterns

The maximum age of 7½ years observed from fish from Queensland's southern Gulf of Carpentaria waters represents a considerable decrease to the observations of fish up to fourteen years of age reported by Bibby and McPherson (1997) for the same waters. Additionally, there has also been an apparent increase in the mean length-at age of fish observed in the present study when compared to the data of Bibby and McPherson (1997) (Figure 9.6). The differences in the growth model fitted to the data (i.e. VBGF for the present study, Schnute case 3 for that of Bibby and McPherson) is unlikely to account for these differences, as these observations are based on the actual data points, rather than inferred from the growth curve. Although we cannot rule out environmental effects on growth, the observed increase in size-at-age coupled with a reduction in larger, older fish in the present study, is consistent with growth being, at least partially, under density dependent control. A reduction in density can result in lower levels of competition and increased availability of food, resulting in faster growth and an increase in length-at-age. Indeed, truncation of age classes and reductions in numbers of large, old fish are common features of exploited populations (e.g. Silberschneider *et al.* 2009; Stewart and Hughes 2009) and have been implicated in driving increases in length-at-age in a number of studies (Millner and Whiting 1996; Sinclair *et al.* 2002; Hidalgo *et al.* 2009).

The large number of small (and young) females and the general lack of any pattern of sex change with length or age for southern Gulf of Carpentaria populations of *P. macrochir* observed in the present study is in contrast to that reported by Garrett (1988), who observed the classical sigmoid-shaped pattern of increasing number of females with length expected for protandrous species (Figure 9.7).

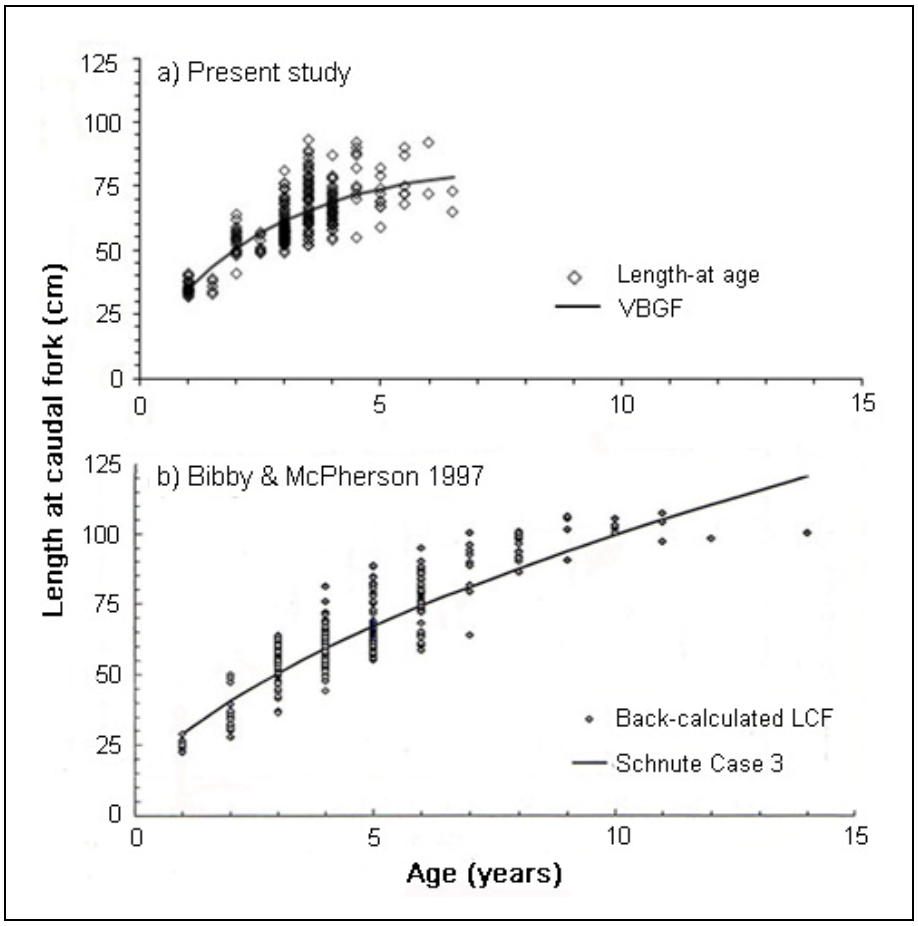


**Figure 9.6:** Comparisons of growth of *Polydactylus macrochir* between present study (top) and Bibby and McPherson (1997).

In many hermaphroditic species, the pattern of sex change is a social and phenotypically plastic in response to local conditions (Robertson 1972; Munday *et al.* 2006; Rodgers *et al.* 2007). For species that change sex, the size-advantage hypothesis (Ghiselin 1969; Warner 1975) predicts that sex change will occur when individuals experience higher reproductive success as one sex when small or young and a greater reproductive success as the opposite sex when large or old. By assessing the size-advantage hypothesis with respect to reproductive value, an individual is predicted to change sex based on its reproductive value as a male or female, relative to the size and sex composition of other individuals in a mating population (Warner 1988; Munday *et al.* 2006). Although individuals generally refrain from changing sex when small or young in populations with many large individuals of the secondary sex, sex change can occur at smaller sizes and younger ages when a population contains few individuals of the secondary sex (Munday *et al.* 2006; Hamilton *et al.* 2007). The targeted removal of the larger and older individuals of a protandrous species may result in male fish changing to females at a much smaller size and younger age in order to increase their reproductive value. Assuming sex-change is under social control in king threadfin, the removal of larger, older fish by size selected fishing

methods such as gillnets from the waters of Queensland's southern Gulf of Carpentaria, as hypothesised from the comparisons of age class structure and length-at-age, may have triggered males to change to females at smaller lengths (and younger ages) than historically documented, and thus may explain the large number of small, young female *P. macrochir* in the southern Gulf of Carpentaria.

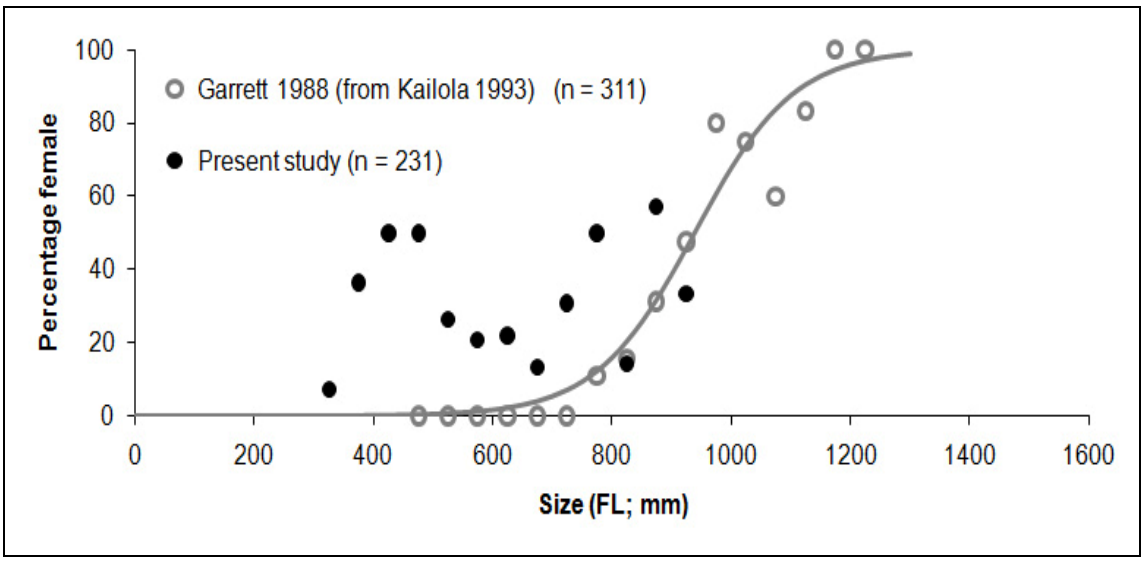


**Figure 9.7:** Percentage of female *Polydactylus macrochir* from Queensland's southern Gulf of Carpentaria waters for present (closed circles) and historical (open circles; (Garrett 1988, in Kailola *et al.* 1993)) data. The logistic curve has been fitted to the historical data.

Although the samples of Garrett 1988 (in Kailola 1993), Bibby and McPherson (1997), and McPherson (1997) were obtained using gillnets of up to 200 mm stretched mesh, it is unlikely that the observed differences in length at sex change, age class structure and length-at-age between these studies and the present study is a artifact of differences in sampling gears. Although the present study used nets smaller than the maximum size used by Bibby and McPherson (1997), a number of large, old fish were collected at sites outside of the Gulf of Carpentaria, including fish up to 22 years of age and 161 cm TL from the Fitzroy River. The observation of these fish in the samples suggest that they would have been collected by the sampling gear in the southern Gulf of Carpentaria during the present study had they occurred in these waters.

### Implications for fisheries management and future directions

The life history parameters examined in the study have shown distinct site-specific signatures which strongly support the hypothesis of fine spatial scale stock structure of *P. macrochir* across northern Australia. Our data limited robust comparisons of life history parameters to four sites across northern Australia and we found strong evidence that each site represents an isolated stock of king threadfin. Based on the spatial scale at which we found strong differences (tens of kilometres), it is likely that

each of the sites sampled in this study represent separate stocks. It is also likely that many more stocks of king threadfin exist across northern Australia than what we have been able to sample during this study, given the highly resident nature of the fish observed here.

King threadfin are currently managed separately by state-based management agencies in Western Australia, the Northern Territory, and Queensland (separated into Gulf of Carpentaria and east coast management arrangements). Little spatial structure is currently assumed in these jurisdictions. However, the findings of the current study suggest that post-recruitment populations of *P. macrochir* are highly sedentary, with little mixing between sites. As such, the long-term effects of fishing are likely to be highly localised within the current jurisdictional boundaries, implying little need for interstate co-operative management. The site-specific nature of adult *P. macrochir* observed in the current study renders the species vulnerable to serial depletion. As such, the development of harvest strategies and establishment of suitable fishery regulations should be conducted in a way that recognises the highly resident nature of adult *P. macrochir* in Australian waters.

The historical lack of recognition of the fine scale population structure of *P. macrochir* may have already resulted in localised depletions in the populations in the southern Gulf of Carpentaria. The observed changes in biology between the present study and those of Bibby and McPherson (1997) and the small proportion of fish less than five years old in the Queensland Gulf of Carpentaria samples is indicative of a fishery that is heavy exploited. More specific research and monitoring is urgently required to evaluate the status of *P. macrochir* populations in Queensland's Gulf of Carpentaria waters, and to test whether fishing is a contributing factor responsible for the observed differences in life history parameters of this species between the present study and the data of Garrett 1988 (in Kailola *et al.* 1993) and Bibby and McPherson (1997). In the meantime a cautious management approach to king threadfin fishing in the southern Gulf of Carpentaria should be adopted and, with confirmation of the indicators of overfishing observed here, urgent and decisive management intervention is warranted.

# Section 4
# Integration

# Chapter 10: Management framework for king and blue threadfins across northern Australia: integrated analysis

**David J. Welch, Stephen J. Newman, Lynne van Herwerden, Robert J. G. Lester, Neil A. Gribble, Quentin Allsop, Thor Saunders, Aaron C. Ballagh, John B. Horne, Brad R. Moore and Jason M. Stapley**

## 10.1 Introduction

The identification of the stock structure of exploited fish populations provides the necessary spatial basis for informed fisheries management, monitoring and assessment. It also provides the basis in formulating studies of fish populations (Secor 2005). To discriminate stocks there are many different and useful techniques available (Ihssen *et al.* 1981a; Cadrin *et al.* 2005), and these range from simple and qualitative methods such as the analyses of fisheries catch data, to more technical and quantitative methods that include mark-recapture experiments, molecular approaches (see Cadrin *et al.* 2005), parasite incidence (Mackenzie and Abaunza 2005), characteristics of scales and otoliths (e.g. microchemistry, shape, microstructure: Friedland and Cadrin 2005; Campana 2005), and life history characteristics (Begg 2005). Each of these techniques can be informative about fish stock structure at different scales spatially and temporally. For example, genetic analyses are typically used in identifying differences over large spatial and temporal scales, where gene flow is minimal. In contrast, otolith microchemistry and parasite incidence reflect the residence and movements of fish through different environments during its lifetime but are influenced by different factors, and may be used to resolve a genetically homogeneous population into discrete units of adult fish that may be more appropriate for management (Buckworth *et al.* 2007). Life history characteristics can be a function of both environmental conditions that the fish experiences and of its genetic history. Because each method addresses different aspects of the population, the choice of method depends on the specific research and/or management questions posed (Begg and Waldman 1999).

The concurrent use of different stock identification techniques or holistic approach provides a very powerful approach in determining the existence of different stocks (Begg and Waldman 1999). The use of this holistic approach for identifying fish stocks has been applied several times in the past using very early integrated techniques (Ihssen *et al.* 1981b; Claytor and MacCrimmon 1988; Safford and Booke 1991), as well as some unplanned comparisons of different techniques to identify stocks (Todd 1981; Graves *et al.* 1992). It is only relatively recently that multi-technique holistic approaches have been

recommended as the preferred approach for stock identification studies (Hancock 1998). The use of different complementary techniques, particularly when used on the same samples concurrently, provides greater power in the detection of different stocks, and by 'weight of evidence', provides greater certainty where stock separation is detected (or not). One of the limitations of single-technique studies is that in comparing fish from different locations the lack of evidence for stock differentiation cannot with any certainty conclude that they are not different stocks. The result may simply be a reflection of the discriminating power of the particular method or may reflect similar environmental conditions at the locations from which fish were sampled. With the use of a holistic approach a consistent result among techniques of no difference among different regions provides greater confidence (yet not proof) that the fish from the regions in question are actually part of a single stock. The use of life history characteristics within the holistic approach to stock identification also provides the added benefit of informing about the productivity of individual stocks detected and therefore helps determine the appropriateness, or otherwise, of common management strategies for each stock.

In this study we used a holistic approach to determine the stock structure for two fish species from the Family Polynemidae. These species are the blue threadfin, *Eleutheronema tetradactylum*, and king threadfin, *Polydactylus macrochir* and both are important for inshore fisheries in northern Australia. The null hypothesis being tested was that for each species there existed a single stock across northern Australia. The techniques used were genetics, parasite incidence, otolith microchemistry, and life history characteristics. For both species: the genetic analyses used mitochondrial DNA, however nuclear DNA (microsatellites) was also used for some of the blue threadfin samples; the otolith microchemistry analyses used stable isotope ratios (OIR); the parasite analyses used abundances of selected indicator parasite species and; the life history analyses used growth characteristics and size at sex change parameters. These techniques were applied to the same blue and king threadfin specimens thereby facilitating a holistic and integrated interpretation of the results (Abaunza *et al.* 2008). This chapter integrates the results from the respective techniques thereby facilitating the interpretation of the stock structure for each species, and therefore, the determination of an appropriate management framework for blue and king threadfin fisheries in Australia.

## 10.2 Methods

The details of the methods used for the respective techniques, including sample treatment and data analyses, are provided in the respective data chapters. The details of the results of each of the individual techniques are also presented and discussed in the respective chapters. The analyses of the data from the different techniques are specific to the types of data generated for each technique. For example parasite analyses compare mean values using uni- and multi-variate methods while life history parameters are compared using non-linear methods. The heterogeneous nature of data types across techniques therefore makes combining them into a single quantitative analysis problematic. Differences in the spatial and temporal scales at which each of the techniques are informative also make

interpretation of combined data challenging. This is a persistent issue now facing stock identification studies such as this one that utilise multiple techniques that require integration of results.

In this study, the basis for data analyses for each technique relied on pair-wise comparisons of each of the many locations sampled to determine stock structure. To integrate the results of the respective analyses and assist in their interpretation we developed a matrix based on the results of these pair-wise comparisons. This matrix gave an overview of the respective results, thereby clearly demonstrating where different stocks were detected and which methods discriminated these stocks. This also provided a basis for interpreting the mechanisms upon which stock structure was influenced. For example, it identified whether stock isolation was influenced by larval movement, adult movement, or a combination of the two mechanisms. The ability to readily assess the likely mechanisms behind stock structure patterns is important in identifying appropriate management responses. Similarly, the results of all temporal analyses of comparing locations sampled at different times are also combined in a matrix of comparison results.

Conventional tag-recapture data were also analysed for *P. macrochir*. Fish were tagged by recreational fishers using line fishing methods from estuaries and foreshore areas along the Queensland east coast and along the eastern coastline of the Gulf of Carpentaria. Fish were tagged using dart or anchor tags following methods outlined by Infofish services in the Austag Manual (www.info-fish.net). Data recorded for individual fish included the date of capture, total length, location and fisher name, and where fish were recaptured similar information was recorded. To further assess movements of king threadfin we extrapolated distance moved for each recapture as the shortest water distance between tagging and recapture locations.

## 10.3 Results

### Blue threadfin

Despite some of the results of the genetic analyses showing anomalies within the Gulf of Carpentaria (eg. Walker River (W GoC) was similar to the Love River (E GoC) whilst Arthur's Creek (halfway between the two) was different to Walker River), the strong message was that there are many genetically isolated blue threadfin stocks across northern Australia (Table 10.1). Results from the parasite analyses supported the genetics results and indicated that there is very little movement of adult fish among adjacent stocks. In fact all pair-wise regional comparisons had significantly different parasite assemblages, except for two regions in which the locations sampled were very close to one another (Keppel Bay – Port Alma, Archer River – Love River; both ~ 20 km apart). Analyses of otolith chemistry also revealed fine-scale sub-division of stocks even between the adjacent Keppel Bay and Port Alma samples, and the Eighty Mile Beach and Roebuck Bay samples. The apparent fine-scale isolation of

blue threadfin stocks was also evident from analyses of growth and size at sex change parameters (Table 10.1).

**Table 10.1:** Results matrix for **blue threadfin** of the pairwise comparisons among the four techniques. Significant results for each pairwise comparison are indicated by the capital letters: G – mtDNA genetics, P – parasites, O – otolith stable isotopes, L – life history characteristics. Non-significant results are indicated by "n", and where the analysis was not carried out is indicated with a dash '-'.

| Region | EMB | RB | BMB | WR | RR | AC | LR | AR | CB | KB |
|---|---|---|---|---|---|---|---|---|---|---|
| **EMB** | | | | | | | | | | |
| **RB** | G P O L | | | | | | | | | |
| **BMB** | G - O L | G - O L | | | | | | | | |
| **WR** | G P O L | G P O L | n - n L | | | | | | | |
| **RR** | G P O L | G P O L | G - O L | n P O L | | | | | | |
| **AC** | G P O L | G P O L | G - O L | G P O L | n P O n | | | | | |
| **LR** | G P O L | G P O L | G - O L | n P n L | n P O L | n P O L | | | | |
| **AR** | G P O L | G P O L | G - O L | G P n L | G P O L | G P O L | G n n L | | | |
| **CB** | G P n L | G P O L | G - O L | G P O L | G P O L | G P O n | G P O L | G P O L | | |
| **KB** | G P O L | G P O L | G - O L | G P O L | G P O L | G P O L | G P O L | G P O L | G P O L | |
| **PA** | G P O L | G P O L | G - O L | G P O L | G P O L | G P O L | G P O L | G P O L | G P O L | G n O n |

The temporal stability of populations sampled at least six months apart was assessed for three regions (Table 10.2). The Roebuck Bay temporal samples were true temporal replicates as they were sampled from very similar location. The re-sampled populations in the eastern GoC (LR and AR) and on the east coast (KB and PA) were not sampled from exactly the same location however were treated as temporal replicates due to their proximity to each other (~20 km respectively). Despite a lack of consistency across techniques, all temporal comparisons also indicate fine spatial scale stock sub-division for blue threadfin (Table 10.2). Of particular note are the genetic differences detected in the samples collected ~ 20 km apart indicating larval and adult separation at localised scales. Within Roebuck Bay a difference in stable isotope histories also indicated fine scale separation, even within small embayments.

**Table 10.2:** Results of the temporal comparisons for **blue threadfin** for each of the techniques using mitochondrial DNA (mt-DNA), parasites, and otolith stable isotope ratios (OIR). Significant = 'sig', non-significant = 'ns'.

| Region | Technique | | | |
|---|---|---|---|---|
| | Mt-DNA | Parasites | OIR | Life history |
| RB1 v RB2 | ns | ns | sig | ns |
| LR v AR | sig | ns | ns | sig |
| KB v PA | sig | ns | sig | ns |

## King threadfin

The genetic results demonstrated that king threadfin are also characterised by many stocks across northern Australia within small localised regions (Table 10.3). The clear and consistent genetic signal of separation indicates a lack of mixing between adjacent stocks of both larval and adult phases, and this is also corroborated by results from the parasites, otolith chemistry and, where robust analyses were able to be carried out, life history characteristics. All samples from the eastern GoC were similar genetically and in otolith chemistry but were different in their parasite assemblages. This would indicate that over this spatial scale (AL – KR = ~480 km coastline distance), at least in the GoC, there is larval and/or limited adult mixing.

**Table 10.3:** Results matrix for **king threadfin** of the pairwise comparisons among the four techniques. Significant results for each pairwise comparison are indicated by the capital letters: G – genetics, P – parasites, O – otolith stable isotopes, L – life history characteristics. Non-significant results are indicated by "n", and where the analysis was not carried out is indicated with a dash '-'.

| Region | EMB | RB | CH | BMB | AL | FLR | KR | TSV | FR |
|---|---|---|---|---|---|---|---|---|---|
| **EMB** | | | | | | | | | |
| **RB** | G n n L | | | | | | | | |
| **CH** | G P O L | G P O L | | | | | | | |
| **BMB** | G P O - | G P O - | G P O - | | | | | | |
| **AL** | G P O - | G P O - | G P O - | G P O - | | | | | |
| **FLR** | G P O - | G P O - | G P O - | G P O - | n P n - | | | | |
| **KR** | G P O - | G P O - | G P O - | G P O - | n P n - | n P n - | | | |
| **TSV** | G P O - | G P O - | G P O - | G n O - | G P O - | G P O - | G P O - | | |
| **FR** | G P O L | G P O L | G P O L | G P O - | G P O - | G P O - | G P O - | G P O - | |
| **BR** | G - - - | G - - - | G - - - | G - - - | G - - - | G - - - | G - - - | G - - - | G - - - |

The temporal stability of the populations sampled was assessed for the Roebuck Bay, Chambers Bay and Fitzroy River regions. All techniques indicated that stock structure is stable through time for both the Roebuck Bay and Fitzroy River regions (Table 10.4). The Chambers Bay replicate samples, although genetically similar, were different in their parasites and otolith chemistry. Newman *et al.* (Chapter 9) concluded that this difference was due to very different sizes and ages of fish collected between sampling periods (see Table 7.1 and Appendix 9.1) and this may also account for the differences in parasites.

**Table 10.4:** Results of the temporal comparisons for **king threadfin** for each of the techniques using mitochondrial DNA (mt-DNA), parasites, and otolith stable isotope ratios (OIR). Significant = "sig", non-significant = "ns".

| Region | Technique | | |
|---|---|---|---|
| | **Mt-DNA** | **Parasites** | **OIR** |
| RB1 v RB2 | ns | ns | ns |
| CH1 v CH2 | ns | sig | sig |
| FR1 v FR2 | ns | ns | ns |

From a total of 235 recaptures of king threadfin, 190 were from the east coast and 45 were from the Gulf of Carpentaria. Recaptures occurred between October 1986 and May 2010 on the east coast, and between September 1989 and March 2007 in the Gulf of Carpentaria. Summary statistics are provided in Table 10.5.

**Table 10.5:** Summary of tag-recapture data for the east coast and the Gulf of Carpentaria indicating the number of fish (*n*), minimum, maximum and mean ± standard error (s.e.) for length at the time of tagging ($L_t$), length at recapture ($L_r$), distance moved and time at liberty.

| | East coast | | | | Gulf of Carpentaria | | | |
|---|---|---|---|---|---|---|---|---|
| | ***N*** | **Min** | **Max** | **Mean ± s.e.** | ***n*** | **Min** | **Max** | **Mean ± s.e.** |
| $L_t$ (TL; mm) | 170 | 250 | 1180 | 534 ± 11 | 42 | 330 | 1010 | 674 ± 25 |
| $L_r$ (TL; mm) | 148 | 340 | 1425 | 663 ± 15 | 36 | 480 | 1080 | 774 ± 24 |
| Distance moved (km) | 177 | 0 | 70 | 9.3 ± 1.2 | 40 | 0 | 600 | 71.6 ± 11 |
| Time at liberty (days) | 190 | 0 | 2599 | 329 ± 27 | 45 | 2 | 1158 | 284 ± 41 |

For all fish the time at liberty did not appear to influence the distance travelled. On the east coast recaptured fish had moved on average only 9.3 km. Regression analysis indicated that larger fish were

more likely to move further ($r^2$ = 0.24, p<0.001; Figure 10.1). Despite this, the longest movement on the east coast was only 70 km. This was in contrast to the Gulf of Carpentaria where the average distance moved was 71.6 km and several fish moved distances greater than 100 km, with one fish travelling 600 km (Figure 10.1). Regression analysis also indicated that larger fish were more likely to move further in the Gulf ($r^2$ = 0.42, p<0.001). However, the pattern of long-distance movements was "knife-edge". That is, all fish showed limited movement regardless of size and it was only the largest fish (> 950 mm TL) that moved large distances. To illustrate this point, the average movement with the four largest fish removed was only 16 km.

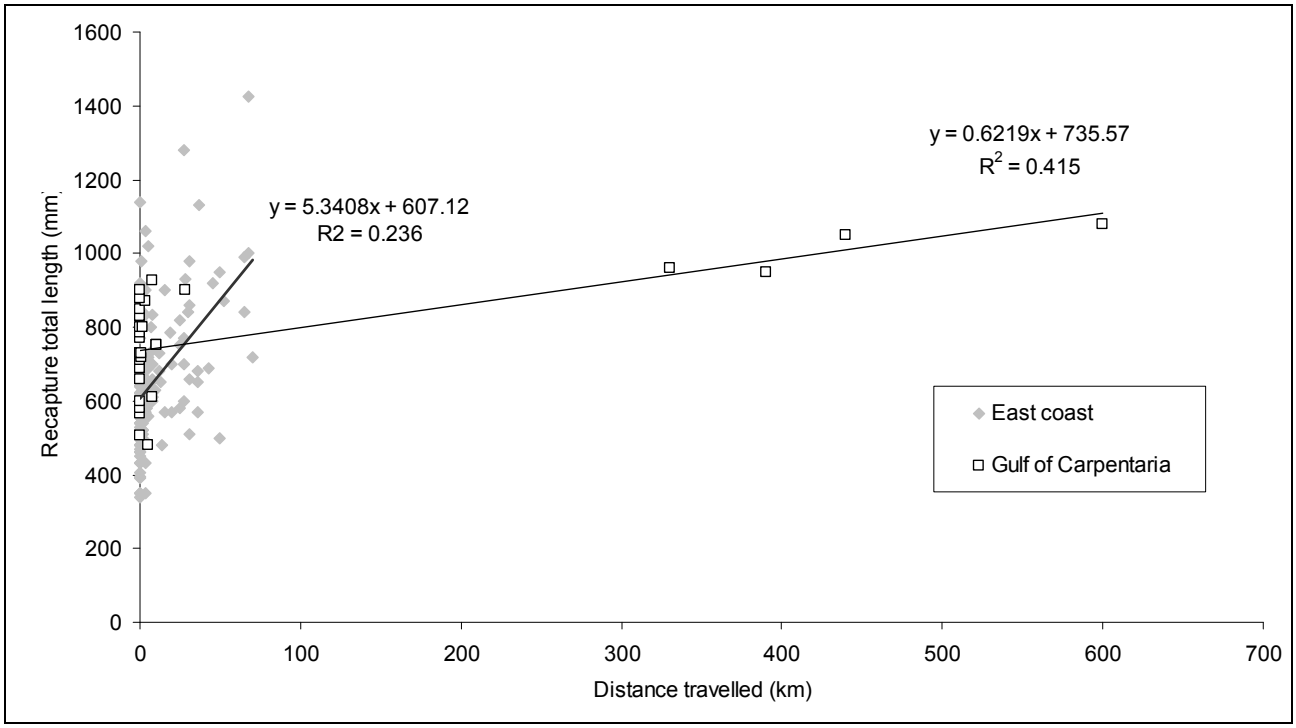


**Figure 10.1:** Length of recaptured fish and the distance travelled for fish tagged on the east coast and fish tagged in the Gulf of Carpentaria.

## 10.4 Discussion

In this holistic stock identification study we have been able to clearly demonstrate the existence of separate stocks of blue and king threadfin throughout the major inshore fishery regions across northern Australia. This information provides important guidance as to the appropriate spatial scale for monitoring, assessment and management of threadfin fisheries. The fine spatial scale at which the separation of stocks is evident for both species, particularly for the blue threadfin, is surprising given apparent lack of isolating barriers in areas such as the Gulf of Carpentaria. However, the use of multiple techniques simultaneously in determining stock structure, greatly increases the certainty of the results

of this study for each species, particularly given that there was a very high level of corroboration in the results across all techniques.

The benefit of using multiple techniques allows the use of complementary methods that on their own may be un-informative. Different techniques are potentially informative at varying scales in both space and time (Table 10.6). The use of genetic methods in determining stock structure very often will determine a single or few stocks due to the resolving power of the technique. This is because the detection of genetic change usually requires many generations of stock isolation, and mixing of only a few individuals between stocks may mask any difference. Other techniques, such as otolith chemistry and parasite incidence, are a reflection of the fish's history over the duration of their lifetime; in the case of blue threadfins and king threadfins, approximately 7 and 20 years respectively. While genetic techniques can be informative about the extent that stocks are indeed separate, it is the use of other techniques informative over smaller temporal scales (and spatial scales) that can resolve genetically homogenous stocks into discrete adult metapopulations (Buckworth 1998). It is these adult stock units that are more appropriate for management. Although both species exhibited fine spatial scale separation, the stock boundaries identified in this study were generally restricted by the sample sites used in this study (although boundaries did differ slightly among the two species). Given the high level of subdivision observed it is highly likely that further sampling would reveal even greater structuring. Despite this limitation, the stock boundaries observed will greatly aid sustainable management of these species

**Table 10.6:** Intrinsic time scales of the different stock identification techniques used in this study (Welch *et al.* 2009).

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

### Blue threadfins

The major feature of the stock structure of *E. tetradactylum* is the strong fidelity to local areas which was consistently indicated across all techniques. This is particularly apparent in the genetic results which show a strong pattern of isolation by distance indicating both limited larval dispersal and limited adult movement. This pattern is strongly corroborated by the results of otolith chemistry, parasite incidence, and life history parameter analyses. Despite this overall pattern there are some interesting

results from the Gulf of Carpentaria where the pattern of stock structure was less clear. Genetic differences between adjacent regions only ~ 20 km apart (Love River and Archer River) show that fine scale separation is still strongly evident, however, fish collected from western, southern and eastern locations in the GoC were genetically similar indicating that there is likely to be low levels of mixing of either larval or adult stages (or both). Recent research on swimming abilities and behaviour of *E. tetradactylum* larvae indicated that they show a "loop" swimming behaviour without directional tendencies (Leis *et al.* 2007). This is consistent in explaining the fine scale genetic stock structure in evidence as larval behaviour would tend to retain individuals in their local area of spawning. Despite the parasite, otolith chemistry and life history parameter results identifying unit stocks in the GoC where they were genetically similar, it is more likely that the similarities in genetics of fish in the GoC is due to very low levels of adult mixing.

This localised and fine spatial scale stock structure pattern is consistent with previous work on blue threadfin on Australia's east coast (Zischke *et al.* 2009). In that study the authors used parasite incidence and conventional tag-recapture data to infer that adult movement is limited with the majority of fish moving less than 10 km and only *ca.* 5 % moving distances greater than 100 km (max. = 180 km). They hypothesised that the species habitat preferences of shallow, turbid, soft bottom areas and geographic boundaries that separated these habitats, may limit movement. The results of the study here concur with the limited movement of blue threadfin found by Zischke *et al.* (2009) however it is possible that some individuals are capable of excursions of several hundred kilometres. In the GoC, where we suggest limited long-distance adult movement may be occurring, there is a continuous and relatively homogeneous habitat and coastline with no natural barriers. In contrast, the Eighty Mile Beach and Roebuck Bay locations in Western Australia are separated by *ca.* 100 km and yet were shown to be distinctly isolated by all techniques. These two embayments are separated by a series of rocky headlands that provide a disconnect in the fish's preferred habitat. However, overall the strong stock structure observed for this species, in this study, suggests that if these limited movements of adults are occurring they are not common.

The use of life history parameters as a complementary technique for identifying stocks was also utilised in this study and some surprisingly strong differences were found. These results not only demonstrate the plasticity in the fish's life history traits but also provides strong corroborating evidence of separate stocks at fine spatial scales. Further, the estimates of growth and size at sex change parameters for many of the different stocks of blue threadfin identified in this study provide an important basis for determining appropriate management strategies at the scale of each stock. Fortunately parameters of sex change suggest that all stocks of blue threadfin not only have matured well before the current minimum legal size limit of 400 mm TL, but also have changed sex and had the opportunity to spawn as females. The exception is for the east coast stocks which change sex at significantly larger sizes than any other location. This may be a concern for managers of east coast stocks given the disproportionate

contribution of large females to recruitment through more, larger and better quality eggs (Berkeley *et al.* 2004; Carr and Kaufman, 2009). Further, based on differences between results of mitochondrial and nuclear DNA analyses in this study, Chapter 3 hypothesised that there may be unequal female contribution from year to year in spawning due to fishing having reduced the number of females in those populations and a social hierarchy behaviour with dominant females changing from year to year.

### King threadfins

Similar to blue threadfins the results give a very strong and consistent signal of fine scale stock structure for king threadfin that was persistent in time for the years sampled. The genetic results in particular indicated significantly different stocks from all comparisons except in the eastern Gulf of Carpentaria where all three locations sampled were genetically indistinct. Results from the parasites and otolith chemistry analyses corroborated all regional comparisons except in the eastern Gulf of Carpentaria where the parasite data indicated that post-recruitment stages of the king threadfin life cycle form separate stocks. It is therefore likely that larval mixing is occurring in the eastern Gulf area and that movement of adults is limited.

The operational nature of the fisheries for king threadfin, the management questions under consideration and also the opportunistic nature of sampling meant that the locations sampled were generally separated by large distances (100's of km's apart). The three locations in the Gulf were, from west to east (AL – FLR – KR), approximately 80 and 400 km apart respectively. The Eighty Mile Beach and Roebuck Bay samples in Western Australia are separated by approximately 100 km and were shown to have limited gene flow as indicated by genetic differences, although parasites and otolith chemistry was similar. This was probably due to similar environmental conditions in these adjacent areas, and the differences in life history characteristics also suggest that fish from each region are isolated from one another. As postulated for blue threadfins it appears that bio-geographical barriers to preferred habitats of king threadfins, such as rocky headlands, may act as barriers to population exchange or mixing of stocks. Therefore, population connectivity is low and the probability of migrant exchange between populations decreases with distance. This hypothesis is supported by the analysis of tag-recapture data made available to the project. Movement of king threadfins was limited with the majority of recaptures coming from within 10 km of the tag location (Figure 10.2), with larger individuals more likely to move greater distances. However, on the east coast all movements were less than 70 km while in the Gulf of Carpentaria there were movements up to 600 km. The east coast has many sandy bays separated by rocky headlands that may well act as biophysical barriers that deter movement outside of this species preferred habitats. In the eastern Gulf of Carpentaria the coastline is more characterised by a continuous coastline of suitable habitat with no natural barriers to movement. Despite this, Gulf fish were still more likely to show limited movement. The largest distances moved by king threadfin in the Gulf of Carpentaria was only by the largest individual fish. These large fish are

more likely to be female and it is possible that only female king threadfins move around while males are more likely to remain in localised areas. These hypotheses would explain the limited gene flow and localised population structure observed in king threadfin.

Local recruitment of king threadfin has been shown to be strongly correlated to freshwater flows from a study in the Fitzroy River (Halliday *et al.* 2008). This study assumed low rates of movement between estuaries and their results suggest that king threadfin have a strong association with major river systems, and are consistent with the results in the current study. A similar study on barramundi, *Lates calcarifer*, in the same river system also found a strong correlation between recruitment and freshwater flows (Staunton-Smith *et al.* 2004). Studies on barramundi in northern Australia have demonstrated the localised genetic population structure of barramundi that is linked to major river systems (Shaklee *et al.* 1993). King threadfin and barramundi share many ecological and biological characteristics such as protandry, food preferences, and habitat preferences, and it may be that they also exhibit similar spatial dynamics. This strong association of king threadfin with major river systems also means that they are vulnerable to altered freshwater outputs, whether they be due to local water management strategies or climate change, which may have serious consequences on the demographics and genetic diversity of this species.

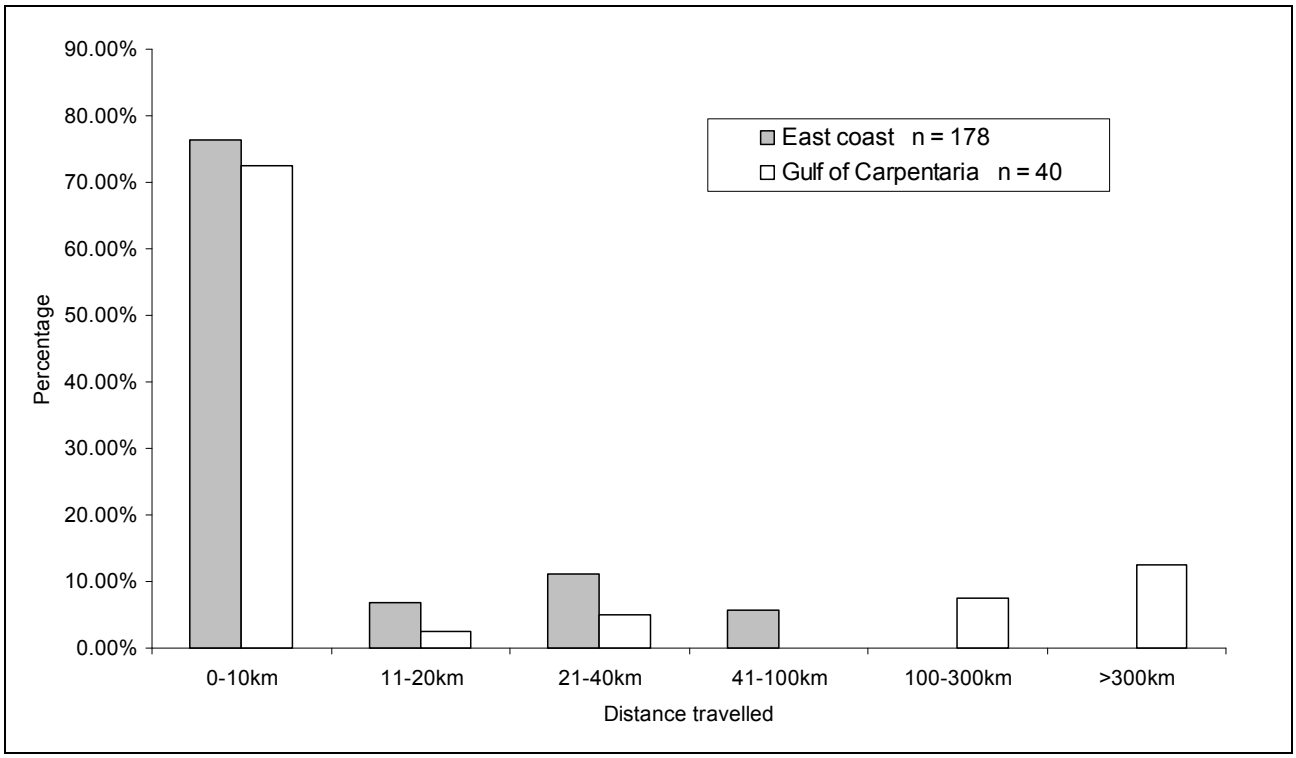


**Figure 10.2:** Distances travelled by king threadfin tag-recaptures for both the east coast and the Gulf of Carpentaria.

The additional use of life history parameters, although limited to only four locations due to sufficient sample numbers, provided corroboration of fine scale stock structure with significant differences in growth and size at sex change between adjacent samples. Estimation of these life history parameters for some of the stocks identified provides guidance to fisheries managers as to what management strategies may be appropriate for those stocks. Further, this information also gives an indication of the variability in these traits that may be observed for the remaining stocks where these parameters could not be estimated. The large size at which king threadfin change sex in all regions does not afford effective protection of the female spawning stock given the minimum legal size limit of 600 mm TL (in Queensland and WA only), as well as the tendency for fishing to selectively remove larger, older individuals for the respective stocks. Given these traits and the fine spatial scale stock structure shown here, king threadfin are a prime candidate for localised depletion of stocks. Consequently, the findings of Chapter 9 are of serious concern. They identified that current samples collected in the eastern Gulf of Carpentaria show truncated size and age structures as well as the presence of very small females. They also show that mean size at age has decreased over time. These are classic indicators of over-harvesting having occurred whereby large mature females have been removed from stocks and in response fish are changing sex at smaller sizes and younger ages to compensate, and in doing so less energy is being used for growth. Many studies have demonstrated this for size and age at maturity and other life history traits in exploited populations and warn that these indicators may be irreversible and should be seen as early warning signals for management intervention (Reznick *et al.* 1990; de Roos *et al.* 2006; Darimont *et al.* 2009).

### Management implications

Current management of both blue threadfin and king threadfin fisheries in northern Australia are based primarily on jurisdictional boundaries and include Western Australia, the Northern Territory and Queensland. In Queensland for convenience the fisheries are managed separately for the east coast and the Gulf of Carpentaria. For each of these major areas management of these species has historically assumed a single unit stock. This study identified that both blue and king threadfin form many discrete stocks throughout their fished range at spatial scales of less than 100 km. This spatial complexity can present challenges to management however, if ignored, can lead to overfishing and the likely collapse of less resilient stocks (see Hutchinson 2008).

Life history traits estimated for both species among the different regions demonstrate not only that each species are capable of phenotypic plasticity, but also the variation shows that different stocks are likely to exhibit different responses to exploitation, with some more resilient than others. This alone should alert fisheries managers as to the importance of considering the stock complexity shown. Further, in this study it was demonstrated that there is already the likelihood that exploitation may be impacting on king threadfin stocks in the eastern Gulf of Carpentaria. Coincidently this is the region where

commercial harvest of king threadfin has historically been highest across northern Australia. We recommend that fisheries management in all jurisdictions across northern Australia incorporate the known and likely stock structure of king and blue threadfin, based on observed spatial scales of separation observed here, into future monitoring and assessment of these fisheries. We also recommend that management consider the variation in life history characteristics observed among different regions in formulating strategies, and ensure the maintenance of stock size and age structure as well as female biomass. Finally, we recommend that the status of king threadfin stocks in the eastern Gulf of Carpentaria are assessed as a matter of urgency.

# Chapter 11: Conclusions

## 11.1 Benefits and Adoption

The results of this project fill crucial knowledge gaps for king and blue threadfins and facilitate improved management of threadfin resources. This will benefit the commercial, recreational and indigenous fishing sectors that target these species, as well as the seafood industry who run businesses selling product derived from threadfins. The identification of the appropriate spatial scale of management units for threadfins will assist managers in Western Australia, Northern Territory and Queensland in ensuring their fisheries sustainability. The determination of crucial life history parameters for separate stocks will also inform managers of the appropriateness of management strategies. Results of the project have recently been considered and incorporated into a review of the Queensland Gulf of Carpentaria Inshore Fishery Management Plan as well as an assessment of the stock status of blue and king threadfins in Queensland's fisheries. Fisheries managers in Queensland are also considering the indicators of overfishing on Gulf of Carpentaria king threadfin stocks indicated in this study. Future adoption and uptake of results from this study into management actions has been facilitated through the direct involvement in the project of fisheries managers from Western Australia (Fisheries WA), Northern Territory (DoR – Fisheries) and Queensland (GBRMPA, DEEDI). Project co-investigators from each jurisdiction (Welch – Queensland; Saunders – NT; Newman – WA) provide advice to key stakeholder advisory groups within their respective states/territory and have briefed their respective counterparts on project results. This will further facilitate adoption in the future where necessary.

## 11.2 Further Development

Recommendations for further research and development activities on blue and king threadfins include:

**HIGH PERFORMANCE**

- A well-planned monitoring and assessment program needs to be developed for king threadfin in the Gulf of Carpentaria as a matter of urgency with clear objectives that will assess the age structure of local stocks, determine maturity/sex change schedules, and assess the status of these stocks.
- Fisheries management in all jurisdictions across northern Australia should implement monitoring and assessment strategies of king and blue threadfins for their major fishery regions for these species. These activities should incorporate the known and likely stock structure of king and blue threadfin, based on observed spatial scales of separation observed here.
- Where pragmatic fisheries agencies should adopt spatially discrete management of threadfin resources.

- Management needs to consider the variation in life history characteristics observed among different regions in formulating appropriate strategies, and in doing so ensure the maintenance of stock size and age structure as well as female biomass.
- Maximum size limits should be investigated as a management strategy for maintaining (and restoring) female biomass of king threadfin.
- Research into developing simple but reliable indicators of the status of threadfin stocks would assist future resource assessments given their complex stock structure.

**MEDIUM IMPORTANCE**

- Management strategy evaluation should be carried out for threadfin that examines the implications of alternative management strategy responses on the resource given the highly localised stock structure.
- Research into the potential for sex-biased dispersal hypothesised for king threadfin whereby females may be moving far greater distances than males.
- Research into sex-biased reproductive success in blue threadfins whereby a female hierarchy behaviour may be influencing reproductive output of individual females from year to year.

**LOW IMPORTANCE**

- Future stock structure studies should adopt fisheries-independent sampling as much as possible to accurately determine the location of capture and to ensure the sample collected is representative of the catch as possible.
- Research into what mechanisms in nearshore environments allow for local retention mechanisms of adult fish and/or larvae of king and blue threadfin.

## 11.3 Planned outcomes

The planned outcomes this project has achieved include:

The project has determined that the appropriate spatial scale for management of blue and king threadfin fisheries is likely to be by state/territory and at local scales within these jurisdictions. The project identified that both king and blue threadfins show limited adult and larval movement between localised stocks separated by as little as several tends of kilometres. This means that the likelihood of straddling stocks of either species in Australia is unlikely however there is evidence for the exchange of genetic material among adjacent stocks within the Gulf of Carpentaria. This will greatly assist with compliance with the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* for

net fisheries in northern Australia by providing the necessary basis for robust assessment of the status of threadfin stocks, thereby helping to deliver sustainable their harvest.

The project provides the spatial framework for monitoring and stock assessment of threadfins in northern Australia. Fine spatial scale of stock separation will dictate the scale at which future monitoring and stock assessments for threadfins need to be carried out. Also, although not central to the project, the estimation of life history parameters for many of the stocks identified provide critical input parameters that will improve the certainty in future stock assessments. These parameters include growth and size at sex change. This has delivered considerable value to the project and further enhances management outcomes.

The project has delivered on threadfin research identified as a high priority. This study had been identified as a high priority for some time by the Fisheries Research Advisory Boards (FRAB) to the FRDC in both Queensland and the Northern Territory. It was also deemed a high priority by commercial, recreational, and fisheries management agencies in Western Australia, NT and Queensland.

The project provided further evidence for the utility of using a holistic approach in stock structure studies. The approach taken in this study followed that of recent FRDC studies in using multiple techniques simultaneously to identify fish stocks, and confirmed the value in adopting such an approach in providing greater certainty in the scale of stock separation and in interpreting the possible mechanisms that influence this separation.

The project provided significant human capital development opportunities. The samples collected during the project provided material for two PhD student projects to be carried out on the life history characteristics of both blue threadfin (Aaron Ballagh, James Cook University) and king threadfin (Brad Moore, James Cook University/University of Queensland). These significantly value-added to the stock structure study here (Chapters 5 and 9) and to the outcomes of the project. The analyses of parasites have also contributed to the PhD carried out by Brad Moore (Chapters 2 and 6).

The project further enhanced links between research, industry and management. Due to the inter-jurisdictional nature of this project fisheries managers from Western Australia, the Northern Territory and Queensland were formally consulted and included on the project team to facilitate the timely management uptake of research outcomes. This meant that contact with each of these managers was regular and they were kept reliably informed of project results. Sample collections were also largely reliant on the willing assistance of commercial fishers and required the establishment and maintenance of good relationships between the project team and industry.

Information provided by the project mean that management decisions about allocation of fished resources to meet the needs of the various sectors can be taken against a background of resource sustainability.

## 11.4 Conclusions

The conclusions from the project are:

- Both blue and king threadfin fisheries across northern Australia are comprised of many separate stocks that are highly localised.
- The highly localised nature of threadfin stocks throughout their Australian range pose a complex management issue since each stock represents a single management unit and management at such fine spatial scales are not likely to be pragmatic.
- Sustainability of threadfin fisheries is likely to require monitoring and assessment at small spatial scales given the risk of localised stock depletions and the protandric life history characteristic. This is particularly pertinent to king threadfins since this study found evidence of overfishing occurring in Gulf of Carpentaria stocks.
- Stock separation of both species is determined by both genetic and environmental factors, possibly influenced by each species ecological and behavioural characters, such as sex-biased adult movement, as well as by bio-geographical boundaries limiting mixing. Both species appear to be highly resident even when longshore movement is possible via continuous habitat.
- The use of the different techniques in identifying stocks proved extremely useful in delineating adults stocks from genetic stocks and in identifying possible mechanisms influencing the observed stock structure in each species.

# Appendices

# Appendix 1: Intellectual Property

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

# Appendix 2: Project Personnel

### Fishing & Fisheries Research Centre, James Cook University

David J. Welch (QPIF, DEEDI)..... Principal Investigator
Aaron Ballagh..... Student & Research Assistant
Bradley Moore (UQ) ..... Student & Research Assistant
Ann Penny..... Liaison Officer
Katia Bazaka ..... Administrative Officer
Leanne Currey..... Research Assistant

### Department of Employment, Economic Development and Innovation (DEEDI)

Neil Gribble..... Co-Investigator
Jason Stapley..... Fisheries Biologist
Mark Doohan..... Fisheries manager
Mark Lightowler ..... Fisheries manager

### Northern Territory Department of Resources – Fisheries

Blair Grace ..... Co-Investigator
Quentin Allsop ..... Fisheries Technician
Thor Saunders..... Co-investigator
Steve Matthews..... Fisheries manager

### The University of Queensland

Robert Lester..... Co-Investigator

### Department of Fisheries, Western Australia

Stephen Newman..... Co-Investigator

### Great Barrier Reef Marine Park Authority

Randall Owens..... Manager

### Contributing fishers and vessels

Andrew Tobin ..... Townsville, Queensland
Gary Ward ..... Karumba, Queensland

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# Appendix 4: Extension activities

- "Science to the rescue", The Courier Mail, Brisbane, QLD, 20/09/2007.
- "Push to save salmon stocks", The Northern Star, Lismore, NSW, 21/09/2007.
- "Threadfin salmon under the eye of scientists", The Advocate, Ayr, QLD, 21/09/2007.
- "Scientists to track threadfins", The West Australian, Perth, WA, 22/09/2007.
- "Scientists to track threadfins", Pilbara News, Pilbara, WA, 26/09/2007.
- "Threadfin salmon under the microscope", www.sail-world.com/Australia/Threadfin-Salmon-under-the-microscope/37641, September 2007.
- "Threadfin under the microscope", Fishing and Fisheries News, Edition 35, November 2007.
- Radio interview, ABC Far North (Cairns), 28/11/2007
- Radio interview, ABC Far North (Townsville), 28/11/2007
- Radio interview, ABC Western Queensland (Longreach), 29/11/2007
- Radio interview, ABC North West Queensland (Mt Isa), 29/11/2007
- "Spatial patterns of threadfin salmon", 'Hooked on Fish' eNewsletter, Issue 7, Queensland Government, DEEDI, December 2007.
- "Unravelling threadfin populations", Western Fisheries, WA, January 2008.
- "Spatial variation in parasites of king and blue threadfin in Australian waters: implications for fisheries", poster presentation, Australian Society for Fish Biology Conference, Sydney, NSW, September 2008.
- "Wanted – king threadfin", public poster for sample collections, Brisbane, QLD, October 2008.
- "Research project – king salmon in the Brisbane River", Brisbane Sportfishing Club newsletter, December 2008.
- "Threadfins – what are they, what are the main species and how do you tell them apart?", Fish and Boat, QLD, January 2009.
- "Threadfins – telling them apart", Western Fisheries, WA, March 2009.
- "Do you know your threadfins", Fishing and Fisheries News, Edition 40, March 2009.
- "Phlogeography of two Australian polynemids with drastically different species histories: implications for fisheries management", Oral presentation, 8$^{th}$ Indo-Pacific Fish conference and 2009 ASFB conference, Fremantle, WA, June 2009.

- "Threadfin fisheries across the north", Western Fisheries, WA, September 2009.
- "Life history of king threadfins", spoken presentation, Australian National Network in Marine Science conference, Hobart, TAS, December 2009.
- "Life history of blue threadfins", spoken presentation, Australian National Network in Marine Science conference, Hobart, TAS, December 2009.
- "Weird whiskers", Department of Fisheries Fact Sheet No. 15, WA, January 2010.
- Final project workshop, presentation project outcomes to fisheries managers and stakeholders, 10 March 2010, Townsville, QLD.
- "Variability in life-history characteristics of blue threadfin (*Eleutheronema tetradactylum*) across northern Australia: implications for adaptability to environmental change", Spoken presentation, ASFB conference, Melbourne, July 2010.
- "Stock structure of king threadfin, *Polydactylus macrochir*, in Australian waters; insight from life history parameters and parasite assemblages", Spoken presentation, ASFB conference, Melbourne, July 2010.