Adult migration, population replenishment and geographic structure for snapper in SA

A.J. Fowler, B.M. Gillanders and K.C. Hall

THE UNIVERSITY OF ADELAIDE
AUSTRALIA

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SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

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Project No. 2002/001

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Table of contents

Non-technical summary

2002/001 Adult migration, population replenishment and geographic structure for snapper in SA

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Objectives

(1) to determine whether adult fish from Spencer Gulf, Gulf St. Vincent and other regions migrate to the continental shelf, and where such fish then migrate in order to spawn - do they return to their regions of origin or is subsequent movement determined by other exogenous factors?

(2) to determine stock structure - does the South Australian snapper population constitute a single, large, inter-mixed population or is it divisible into numerous sub-populations?

(3) to determine whether adult fish collected from particular regions originate as juveniles from those regions or whether they constitute a mixture from different regions.

(4) to produce a final report that synthesises all information on snapper movement and stock structure in South Australia, including that from this study on otolith microchemistry with that from tagging studies and work on the analysis of genetics.

Outcomes achieved

This study has made new and significant contributions to what is now a substantial knowledge base on the population biology of snapper in South Australia. In particular, our understanding has been extended to the nature of limited nursery areas and the subsequent movement of young adult fish from these areas and their dispersion over significant distances to the other regions of State waters. These findings have profound significance for the temporal dynamics of the regional sub-populations, as well as for understanding the stock structure of the population. Furthermore, the study has helped explain the lack of genetic differentiation throughout South Australian waters, as documented by a previous study.

It now can be considered whether this new understanding of the life history of snapper can assist in explaining the differences in catch histories from the different regions that have been recorded over the past 20 years or so. Furthermore, the management strategy for this species should now be revisited in the light of the revised life history model and understanding of population biology.

Output from the project will be of interest to fishery biologists and managers who work with snapper in other states of Australia as well as in New Zealand. Furthermore, there will likely be considerable national and international interest in some of the methodologies used, particularly in relating the chemical profiles across otoliths to fish age, and the results of these analyses in terms of age-related fish movement. These results were presented at the Third International Symposium on Fish Otolith Research & Application in July 2004 and the annual conference of the Australian Society of Fish Biology in September 2004.

Non-technical summary

Through the early 2000s there was considerable concern about the South Australian snapper fishery, particularly about the continuation of poor catches from Gulf St. Vincent since they had crashed through the 1980s. It was suggested that the State-wide fishery would benefit from a regional management approach involving stock enhancement in Gulf St. Vincent. This suggestion clearly called into question the current management approach that treated the whole South Australian population as a single, large stock. Yet, in fact, the stock structure in South Australia was not sufficiently well understood to predict the likely success of such a regional management approach.

The stock structure of a population depends on the extent of mixing and exchange of individuals between different geographic locations. Such dispersal can occur at two stages of the life history, i.e. by transport of eggs and larvae or through active movement of fish as juveniles or adults. For snapper in South Australia the latter process has the most potential for dispersal of individuals, as suggested by results from earlier tagging studies. The need in this study was to provide a clearer understanding of adult movement patterns of snapper and the extent to which they affected the stock structure.

The main analytical approach was the analysis of the chemistry of otoliths of fish collected from the six geographic regions that constitute the main snapper fishing grounds in South Australia. As otoliths grow in the heads of fish some trace elements are incorporated into the otolith matrix at rates that are determined by the physical and chemical characteristics of the environment in which the fish is living at that time. The otoliths thus record and retain a natural chronological record of the environments experienced by that fish throughout its life. To address the objectives of the study the otoliths were sampled using a sophisticated analytical technique that allowed simultaneous sampling of numerous elements at extremely fine levels of resolution.

For otoliths to provide information on the movement of fish and the occupancy by those fish of different water masses, the otoliths must grow sufficiently throughout the year to record such

information. Thus, to some extent, interpreting data on otolith chemistry depends on having a fundamental understanding of how these structures grow. Such a model was developed for snapper otoliths based on the analytical technique of marginal increment analysis. The study determined that otolith growth was highly seasonal as approximately 75% of the annual deposition to the proximal surface was achieved in summer and autumn. For the otolith axis that was sampled for chemical analysis, i.e. between the core and the dorsal side of the sulcus, between 15-30 μm were added to the otoliths in each of winter and spring for 5-6 year old fish and only 3-15 μm were added in each of these seasons for 11-12 year old fish. Such small incremental growth to the otolith in each season must limit the potential for recording useful information that relates to fish movement.

The chemical profiles across otoliths were determined for up to 20 fish from each of the six regions of northern and southern Spencer Gulf (NSG, SSG), northern and southern Gulf St. Vincent (NGSV, SGSV), the west coast (WC) and the south east (SE) of the State. All fish were sampled in the same year and from the same age class, to minimise the influence of temporal variation on spatial comparisons. The fish were 9 years of age and were sampled from the 1991 year class. The measurements of Sr:Ca and Ba:Ca ratios along the transects across otoliths were related to fish age, and thus represented chronological profiles. These profiles showed considerable temporal variation manifested as within-year variation, differences between years and general ontogenetic trends. The highly individualistic nature of these profiles meant that they were difficult to interpret in terms of fish movement. Nevertheless, there were significant regional differences in the concentrations across otoliths. These regional profiles were similar for the first three years, but then diverged and became quite different between the 4^{th}-9^{th} annual increments. This suggests that fish from different regions had occupied different water masses between the ages of 4 – 9 years, thus indicating some sub-structure to the South Australian snapper population.

Other information was also assessed for evidence of regional differences, including: trends in catch statistics from the commercial fishery; population size and age structures of fish sampled from commercial fishery catches; and the optical and morphological characteristics of otoliths from these fish. Consistent regional differences in the temporal trends in fishery catches and in population structure suggested that different population processes were occurring in the different regions, whilst the optical characteristics of the otoliths also differed between regions. Such differences indicate that the fish from different regions must have spent considerable parts of each year in different water masses, and that this must have occurred consistently across years. Only the analysis of otolith morphometrics did not display regional variation, which may relate to the substantial within-sample variation that was evident in otolith shape.

Possible sub-structure in the snapper population was also assessed by comparing the chemistry of whole otoliths of 11 year old fish captured in 2002 from the five regions of NSG, SSG, NGSV, SGSV and the WC. Again the results demonstrated regional variation, indicating that the fish from different regions must have remained separate for sufficiently long periods of time to allow environmental differences to become manifested as chemical differences in their otoliths. This again is indicative of some population sub-structure.

It was considered important to determine whether the fish captured in the different regions as adults actually originated in those regions so as to determine whether there was the possibility of reproductively separate stocks. The alternative scenario to this is that the fish originated elsewhere and then moved to the various regions where they were captured. Here the cores of otoliths from fish captured in different regions were sampled and their chemical composition compared. The study determined no consistent difference in the multi-elemental chemical composition of these central cores. These data are consistent with the inner parts of the otoliths obtained from the transect data across the otoliths, which strongly suggested that the adult fish from different regions did in fact originate from only one or two places. The most likely such nursery area is NSG, whilst it is also possible that NGSV was a nursery area.

This study presented an overwhelming body of evidence indicating some regional sub-structure to the South Australian population of snapper. Yet these regional differences were only apparent for adult fish of 4 years of age and older. Data relating to the first three years of the fishs' lives showed little variation, indicating a lack of spatial differentiation with regards where fish originated. This suggests that most fish originated in only one or two places, most likely NSG and NGSV. The data suggest that the fish remained in these nursery areas for several years, but that significant movement to other regions occurred throughout their fourth year of life. Further evidence in support of this model comes from independent observations of recruitment and the history of fishery catches in the different regions.

The model presented above implies several types of movement behaviour by snapper. Firstly, it suggests that there was significant movement of young fish of 3-4 years of age away from the nursery areas, culminating in their being dispersed throughout State waters. However, once this movement took place it appears that subsequent movement of older snapper was relatively restricted. Tagging work has demonstrated that relatively few adults undertake movements over distances of several hundreds of kilometres, suggesting that the majority of fish of >4 years of age can be considered 'residents', whilst relatively few fish are 'migrants' that travel the longer distances recorded in earlier tagging studies.

This study suggests that most fish considered had a common origin, which clearly accounts for why there is no genetic differentiation amongst regional populations throughout South Australia. Nevertheless, the regional differences in population characteristics suggest that once the 3-4 year old fish moved and joined the regional sub-populations there was minimal subsequent movement between these ecological units. The sub-populations nevertheless were supplemented by immigration of 3-4 year old fish from the nursery areas.

Keywords

Snapper, *Pagrus auratus*, otoliths, otolith chemistry, movement, migration, population structure, fisheries, sparids

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We gratefully acknowledge the Fisheries Research and Development Corporation for providing the base funds to carry out this project (2002/001), and for their support during the project. We also thank SARDI and the University of Adelaide for the logistic and administrative support through the course of the project, including the use of laboratories and access to facilities.

There are numerous people who provided assistance at various stages through this project, ultimately contributing to its completion and success. Paul Jennings and Bruce Jackson assisted with the 5:00 am starts at the SAFCOL fish markets to collect the otoliths. Bruce Jackson maintained the biological snapper database as well as the otolith collection that were both used to select the otoliths from the appropriate fish for the various chemical and morphological analyses. Paul Jennings prepared the TS sections that were sampled using LA ICP-MS, and Travis Elsdon provided advice on laboratory procedures associated with the chemical analyses. John Tsiros, the manager of the ICP-MS facility at Monash University, provided advice and assistance to Dr Hall with regards operation of the equipment, and also ran the solution-based ICP-MS analyses.

1 General introduction

A.J. Fowler, K.C. Hall and B.M. Gillanders

1.1 Background

Species of marine organisms that have a wide distribution are generally divisible into a number of local populations that are effectively self-replenishing. In the fisheries context these are called 'stocks' and their distribution with respect to each other is called the 'stock structure'. From a fishery management perspective it is important to understand this stock structure as fisheries on different stocks should be managed independently, as each can potentially be overfished independent of the status of the populations and fisheries in adjacent areas (Pawson and Jennings 1996, Bailey 1997). Furthermore, nearby stocks can experience different rates of demographic processes, and so their population dynamics may function differently and vary independently.

In the past it has been common to base the determination of stock structure on genetic differences (Johnson et al. 1986, Utter 1991, Carr et al. 1995). However, it is now recognised that such genetic stock structure may not be that useful for the determination of the appropriate spatial scale for fishery management decisions (Carvalho and Hauser 1994, Pawson and Jennings 1996). The genetic stock structure identifies the separation of sub-populations that has developed over evolutionary time periods, whereas fishery management decisions are concerned with population processes over ecological time frames. The scale of the level of separation between sub-populations is important to this issue. For example, there may be an extremely low level of exchange of individuals between two sub-populations that is insignificant in an ecological sense but which is sufficient over long time periods to maintain sufficient gene flow to swamp the potential for genetic divergence. As such, these sub-populations may be genetically homogeneous despite being effectively ecologically isolated. These have been called 'harvest stocks' in contrast to genetically separated 'genetic stocks' (Carvalho and Hauser 1994).

The snapper (*Pagrus auratus*) is a member of the family Sparidae and is a highly significant fishery species throughout its distribution through the warm, temperate and sub-tropical waters of the Indo-Pacific region (Kailola et al. 1993). In Australia its distribution is effectively continuous around the coastline of the mainland below 18ºS (Fig. 1.1), where they are found in shallow, coastal, demersal habitats and offshore to the edge of the continental shelf across a depth range of 1-200 m. This broad distribution is divisible into a number of separate 'genetic' stocks. One significant division occurs at Wilson's Promontory in Victoria from where the eastern stock extends 2000 km up the coast of New South Wales (Sanders 1974) (Fig. 1.1). From Wilson's Promontory the

Victorian stock extends westward to the vicinity of the mouth of the River Murray, where there is evident a division between the Victorian and South Australian populations (Donnellan and McGlennon 1996) (Fig. 1.1).

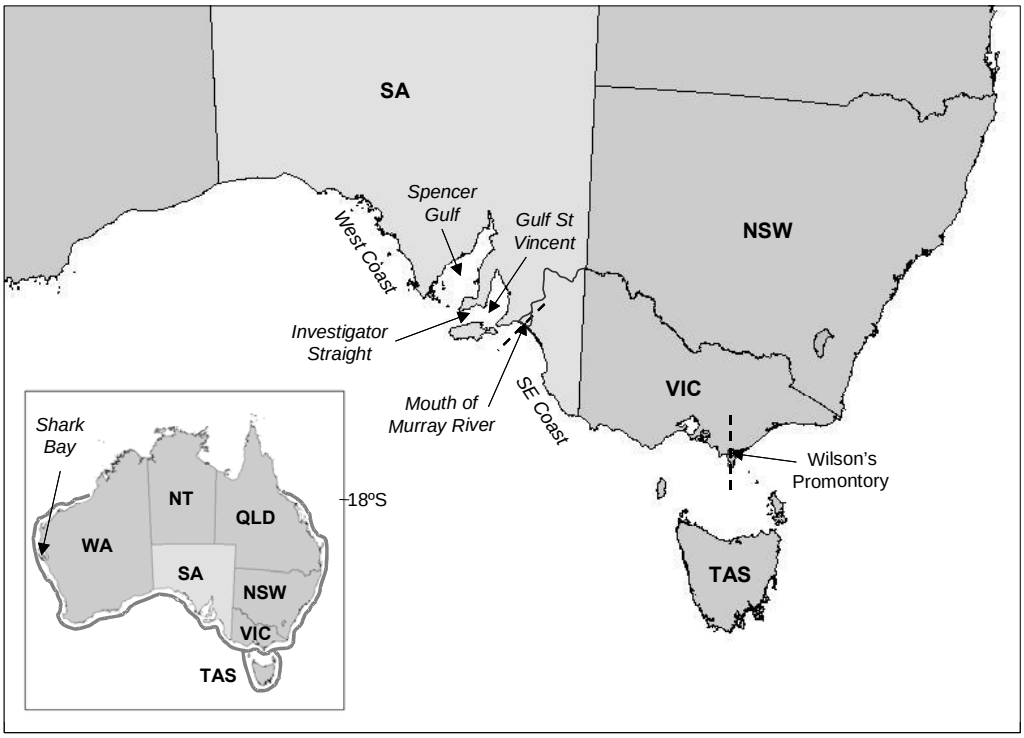


Figure 1.1 Map of south eastern Australia indicating the two genetic stock divisions (dashed lines) and the main components of the Gulf system of South Australian. *Inset:* Map of Australia with the snapper distribution indicated (thick grey line) and location of Shark Bay in Western Australia.

These stock divisions on the eastern side of Australia are quite coarse when compared with the much finer level of stock differentiation that is apparent for the Shark Bay area in the central region of Western Australia. A diverse complex of studies involving genetics (Moran et al. 2000), microchemical analysis of otoliths involving trace elements (Edmonds et al. 1989), and stable isotopes and Sr/Ca ratios (Edmonds et al. 1999, Bastow et al. 2002), fish tagging (Moran et al. 2003) and adult fish morphometrics (Moran et al. 1998) has revealed an unusually complex stock structure. The weight of evidence from these studies indicates that there is minimal exchange of individuals even over relatively short distances of 40 – 50 nautical miles, and that the populations in the Eastern and Western Gulfs of Shark Bay and the adjacent oceanic region are effectively separated from each other. In fact there is evidence for even finer scale structuring within the gulfs and oceanic sub-populations (Edmonds et al. 1989). Such unusually fine-scale stock structure in Shark Bay may relate to the particularly strong salinity gradients that exist in the Eastern and Western Gulfs (Bastow et al. 2002). It warns of the potential for a similar, small-scale level of stock differentiation for other regions where snapper occur.

In South Australia a recent comprehensive study on snapper genetics involving the analysis of mitochondrial DNA and allozymes (Donnellan and McGlennon 1994), could find no evidence for any finer-scale genetic structure outside of the known broad stock division between the Victorian and South Australian populations in the vicinity of the mouth of the River Murray. On the basis of this apparent panmixia the State-wide fishery has generally been managed as a single stock (Donnellan and McGlennon 1996). This single stock management approach is quite consistent with the currently accepted model of the life history of this species in South Australia, which says that: within the first few years after recruitment of 0+ individuals into the northern parts of Spencer Gulf and Gulf St. Vincent the fish move southwards, leave the gulfs and migrate to the continental shelf; it is suggested that from there they make annual spawning migrations over a number of years back into the gulfs during which time they are highly vulnerable to the fishery; and by approximately 12 years of age become permanent residents in the northern gulfs. Thus, the current life history model suggests that on the continental shelf there is a significant, mixed-age population of snapper that originates from age-related migration from a number of different regions. This would clearly provide an opportunity for the mixing of fish from different origins such as Spencer Gulf and Gulf St. Vincent.

Through the late 1990s and early 2000s there were some significant management issues with the South Australian snapper fishery that resulted in a renewed research focus and reconsideration of the single-stock management strategy. As part of this process it was suggested that the State-wide fishery could benefit from a regional management approach that involved a stock rebuilding program in one particular region, i.e. Gulf St. Vincent (McGlennon and Jones 1999). This, therefore, called into question the appropriateness of the 'single stock hypothesis' to describe the South Australian snapper fishery. Furthermore, reassessment of our understanding of the population biology indicated that in reality the current understanding of the life history of snapper in South Australia was too poor to clearly predict the likely consequences of such a regional management strategy. It was apparent that there was a need for a more comprehensive understanding of the movement patterns of adult snapper and their consequences for the stock structure.

It is traditional to describe adult migration from tagging studies and to determine stock structure by genetic analysis. Yet such studies for snapper in South Australia have been ambiguous about adult movement. Historic tagging work has been of limited value since there is minimal fishing effort on the continental shelf, thus restricting the opportunity for recaptures (Jones 1981). Furthermore, the general lack of genetic differentiation for South Australian snapper (Donnellan and McGlennon 1996), suggests a well-mixed population. However, that result could also reflect low rates of movement of individuals between regional populations over evolutionary time periods (Taylor and Dizon 1996). To overcome the ambiguity arising from these earlier studies, this project primarily

used an alternative methodology to elucidate both migratory behaviour of snapper and the degree of ecological separation amongst regional populations, i.e. the analysis of otolith chemistry. Furthermore, a number of other fishery and population characteristics were also compared amongst the different regional fisheries around the State. The null hypothesis tested was that the South Australian population of snapper constitutes one large, single, stock formed through the mixing of individuals that are initially derived from different regions. As such, there should be no differences in the chemistry of otoliths and other characteristics amongst fish collected from different regions.

1.2 Chemical analysis of otoliths

Otoliths are calcareous structures suspended within the inner ear of fish, which grow by the daily deposition of new material to an outer growing surface (Campana and Neilson 1985). Variation in the rate of deposition of calcareous, crystalline structure or protein matrix produces daily, seasonal or annual growth increments that can be seen in transverse sections of otoliths and are often used for age estimation (Campana and Thorrold 2001). As the otolith grows some trace elements and isotopes are incorporated into the new material at rates that are determined by the physical and chemical characteristics of the environment in which the fish is living at the time (Campana 1999). Because of environmental variation in the chemical composition of seawater (Bruland 1983), and since otolith material is metabolically inert once deposited, the otoliths retain a natural chronological record of the environments experienced by the fish throughout its life (Secor 1992, Campana 1999). Thus, it may be possible to reconstruct the sequence of environments used by a fish throughout its life based on the time series of trace element and isotopic concentrations in its otoliths (Secor et al. 1995, Thorrold et al. 1997, Clear and Kalish 2000, Milton et al. 2000).

In this study, the analysis of otolith chemistry was used to expose aspects of the life-history by addressing three questions: (1) do adult snapper in South Australia display a migratory life-history; (2) do the fish from across the state constitute a single, large population mixed from different regions or are they divisible into discrete, reproductively-separate regional populations; and (3) do the fish that are captured in particular regions originate as juveniles from the same region? There was a strong basis to expect that otolith chemistry could be used to answer these questions for snapper. First, similar studies for other fish species around the world have indicated that small environmental differences can result in measurable, multi-elemental differences in otoliths (Campana 1999). Furthermore, for snapper in particular there have been several successful studies where otolith chemistry has contributed to understanding some aspects of life history as well as the stock structure (Arai 1995, 1996; Edmonds et al. 1989, 1995, 1996; Gillanders 2002a,b; Gillanders and Kingsford 2003; Hamer et al. 2003).

Inductively coupled plasma - mass spectrometry (ICP-MS) is the preferred procedure for analysis of otoliths due to its capacity for rapid and accurate isotopic and elemental assays over a wide range of elements and concentrations, with resolution down to parts per billion (Campana 1999). Sample introduction by laser ablation, which vaporises microscopic amounts of material from precise points on the exposed surface of the otolith, can be used to analyse specific regions of the otolith such as the juvenile core or to sample continuously along a pre-determined transect line. When applied to the surface of a transverse section of the otolith, the resulting chemical pattern can be matched to the growth chronology, and signals that relate to specific events like migration can be matched to age and time of year (Campana 1999, Milton et al. 2000).

1.3 Water chemistry of South Australian waters

The success of studies using the chemical composition of calcified structures of marine organisms to infer population structure and movement patterns depends on the physico-chemical characteristics of regional waters being sufficiently different to produce distinctive signals in the otoliths. Otolith chemistry is particularly sensitive to variations in temperature and salinity (Fowler et al. 1995, Campana 1999, Elsdon and Gillanders 2002), which are two water properties that show large regional variation in South Australian waters. The shallow, semi-enclosed gulfs, i.e. Spencer Gulf and Gulf St. Vincent show greater seasonal variation in water temperature than adjacent continental shelf waters (Nunes and Lennon 1986), and much higher temperatures in their northern regions than elsewhere in the State (Fig. 1.2). The Gulfs are also hyper-saline in comparison with shelf waters due to limited freshwater inflow at the heads and high evaporation. Strong salinity stratification patterns are evident within the Gulfs from north to south, with extremely high salinities recorded at the heads of the gulfs during summer (42 to 46 ‰), particularly in northern Spencer Gulf (Fig. 1.3).

Other regional variations in water chemistry relate to the highly seasonal and localised distribution of the State's annual rainfall, and thus terrestrial run-off. Most rainfall occurs in winter and to a less extent spring, and primarily in the southeast and southern Gulf regions (Fig. 1.4). Terrestrial run-off generally contains higher levels of certain trace elements, in particular barium, in comparison with normal seawater. Another potential source of temperature variation and elevated barium is from localised areas of summer upwelling that occur along the west coast of the Eyre Peninsula and along the southeast coast of the State (Bye 1998) (Fig. 1.2*a*), which bring colder nutrient rich waters to the surface.

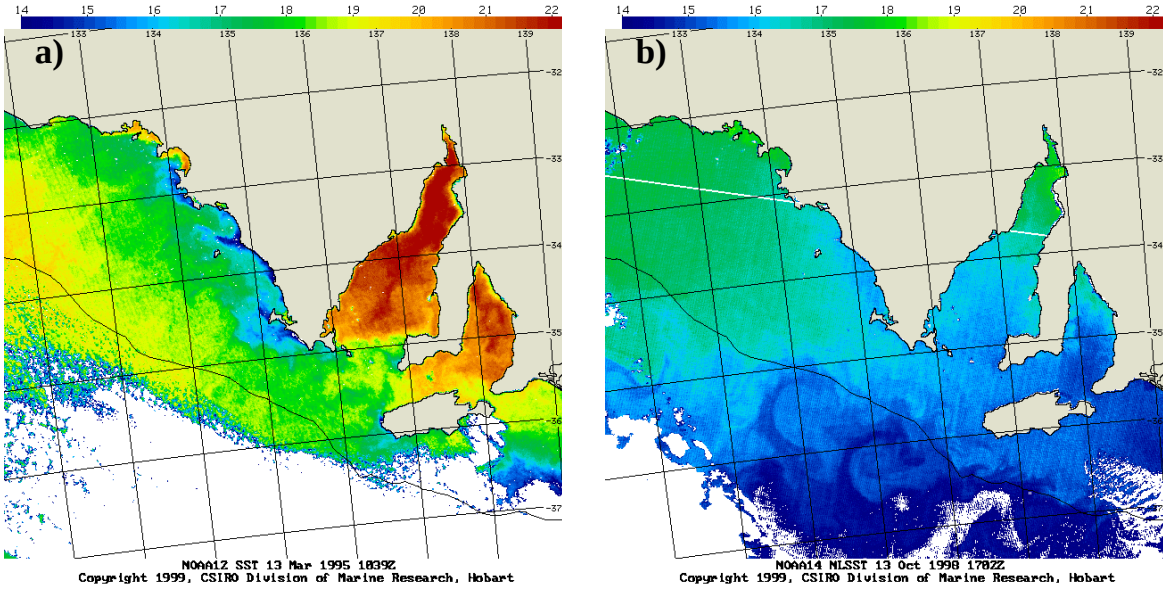


Figure 1.2 Sea surface temperatures in South Australian waters during summer *(a)* and winter *(b)*. The localised areas of summer upwelling along the western coast of the Eyre Peninsula are evident in bright blue *(a)*. Figures supplied by CSIRO.

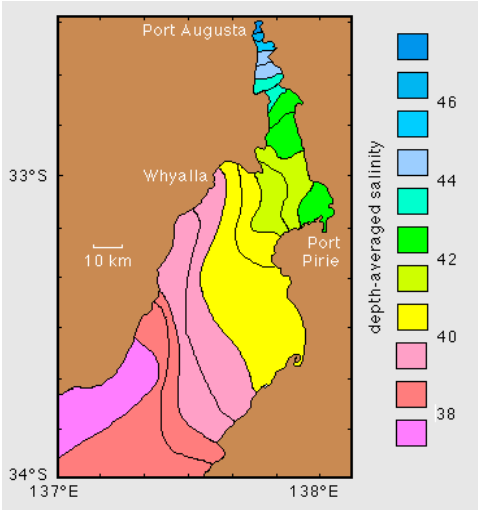
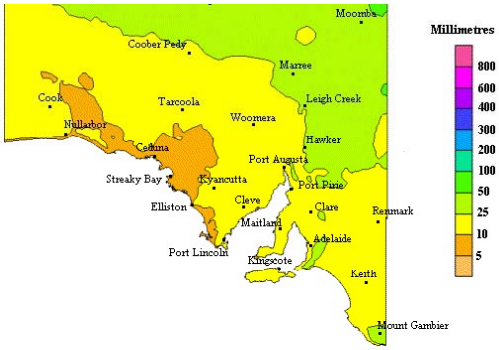
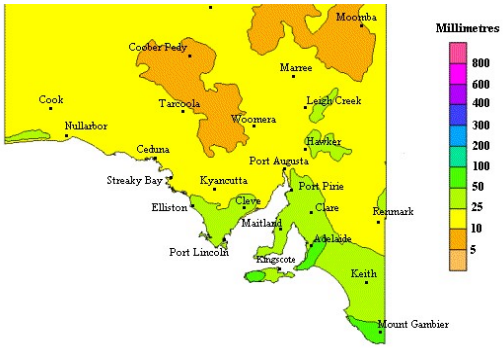


Figure 1.3 Depth-averaged salinity in northern Spencer Gulf, as observed in March 1984. Isohalines for salinities < 45 are shown in 0.5 unit spacing; for salinities > 45 isohalines are shown in 1 unit spacing. Figure reproduced with permission from M. Tomczak (1998).

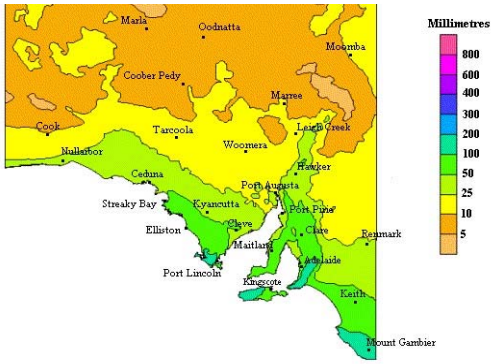
a) Summer (January)



b) Autumn (April)



c) Winter (July)



d) Spring (October)

Figure 1.4 Average monthly rainfall during summer *(a)*, autumn *(b)*, winter *(c)* and spring *(d)*, based on a standard 39 year climatology (1961 to 1990). Figures supplied by the Bureau of Meteorology, Commonwealth of Australia.

There are various sites along the coastlines of both gulfs that are likely to be significant point sources of trace elements that are derived from anthropogenic activities. The heavy industry of South Australia, including the largest lead smelter in the world, is centralized around the northern tip of Spencer Gulf and the Port River north of Adelaide (Edyvane and Boxall 1997). These have resulted in the significant elevation of concentrations of some trace metal in adjacent sediments (Ward and Young 1981, 1982; Edwards et al. 2001), which may influence the soluble concentrations in the ambient water available for incorporation in fish otoliths. Elevated levels of mercury and lead in the water have resulted in significant increases in their concentrations in otoliths (Geffen et al. 1998).

1.4 Need

The snapper fishery of South Australia has recently attained a level of heightened political sensitivity, reflecting the need to optimise the strategic approach to management. Nevertheless, it has become apparent that our understanding of the life history of this species is too poor to predict the likely outcomes of suggested regional management strategies.

For this snapper fishery there is a need to optimise management based on a better understanding of the life history and population biology, particularly with regard movement patterns of adult fish. The extent to which fish move between different geographic regions, and thus the extent to which such behaviour contributes to the natural processes of sustaining the different regional populations is currently unknown. Such adult movement will determine the extent to which regional sub-populations are independent and discrete. Adult movement and stock structure are fundamental to identifying the appropriate spatial scale at which population dynamics work, and thus the appropriate spatial scale at which fishery management should be applied.

1.5 Objectives

(1) to determine whether adult fish from Spencer Gulf, Gulf St. Vincent and other regions migrate to the continental shelf, and where these fish then migrate to in order to spawn - do they return to their regions of origin or is subsequent movement determined by other exogenous factors?

(2) to determine stock structure - does the South Australian snapper population constitute a single, large, inter-mixed population or is it divisible into numerous sub-populations?

(3) to determine whether adult fish collected from particular regions originate as juveniles from those regions or whether they constitute a mixture from different regions.

(4) to produce a final report that synthesises information on snapper movement and stock structure in South Australia, including that from this study on otolith microchemistry with that from tagging studies and work on the analysis of genetics.

1.6 Report Format

The study is reported in the following five chapters. The first provides a model of the seasonal growth of the otoliths of adult snapper, to help interpret the data presented in the following chapter. Chapter 3 relates the analysis of otoliths using laser ablation ICP-MS. Chapter 4 considers whether other fishery and population-based characteristics of snapper from South Australia show regional variation that would be indicative of regional stock structure and Chapter 5 presents the results of stock structure analysis based on solution-based ICP-MS. The final empirical chapter further explores the issue of stock structure by addressing whether adult fish collected from particular regions originate as juveniles from those regions or whether they represent a mixture of fish that come from different regions. The final chapter of the report is a General Discussion that integrates the empirical information into a life history model for snapper in South Australia and the stock

structure throughout the State. These findings are then discussed in the context of the historical understanding of the population biology of this species.

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2 Seasonal growth of otoliths from snapper in South Australia

A.J. Fowler and K. Schilling

2.1 Introduction

Otoliths are hard, calcified structures that constitute part of the inner ear of fish and which contribute to the fish's ability to hear and to orientate (Popper and Lu 2000). Their formation starts with the primordium, which is generally the first calcified tissue formed in the embryo (Morales-Nin 2000), after which they grow incrementally, adding a single increment to the growing surface on a daily basis (Campana and Neilson 1985). Each such daily growth unit is composed of an incremental zone (L-unit), which is the less dense part of the increment, and the discontinuous zone (D-unit), which is relatively narrow and dense. Otoliths initially grow in all directions, but as the fish becomes large the otolith growth along some axes stops and eventually becomes restricted to a dorsal opening on the floor of the skull (Morales-Nin 2000). Here the otoliths become thicker as the annual increments are stacked on top of each other towards the proximal surface (Mann-Lang and Buxton 1996).

As the otoliths grow some elements are incorporated at trace levels into the calcareous, crystalline structure and protein matrix at rates that are determined by the physical and chemical characteristics of the aquatic environment in which the fish is living at that time (Campana 1999). Since such environmental characteristics, including water temperature and salinity, vary spatially and temporally, the otoliths lay down a chronological record of the environments experienced by the fish throughout its life (Secor et al. 1995, Thorrold et al. 1997, Milton et al. 2000). Once deposited this material is not reworked because the otoliths are metabolically inert. Thus, it may be possible to access the life history information retained in the otolith across the entire lifetime of the fish. Such information can be useful for addressing numerous aspects of the life history and biology of the fish including determining the periods of residency and movement patterns between different water masses (Thorrold et al. 1997). Perhaps the best known example of this is for the striped bass *Morone saxatilis*, where the timing of movement and duration of residence of individual fish in water masses of different salinities have been determined for the Hudson River and Chesapeake Bay in north-eastern USA (Secor 1992, Secor et al. 1995, Secor and Piccoli 1996, Zlokovitz and Secor 1999, Secor and Rooker 2000).

To access the information on fish movement that is retained in the chronologically deposited structure of the otolith its chemistry must be related to the fish's age, with the latter determined

through interpretation of the otolith macrostructure (Halden et al. 2000, Kafemann et al. 2000). However, the interpretation of otolith structure and chemistry with respect to the time of the year can be difficult because otoliths do not grow constantly through time. Rather, otolith growth rate varies with respect to the season, slowing and potentially stopping during the colder months of the year (Victor and Brothers 1982). Thus, to relate information on fish movement the otolith must grow sufficiently through the period when the movement and occupation of the different water mass takes place so as to allow an interpretable signal to be retained in the otolith. Furthermore, the analytical methodology used for chemical analysis of the otolith must have sufficient spatial resolution to measure or detect the chemical signal that has been laid down.

The first objective of this project, as outlined in Chapter 1, is to determine whether the South Australian snapper display a complex, age-related migration phase during their life. If this is the case such migration should be evident in the chemical composition of the otoliths in relation to fish age. Chapter 3 of this report describes a study aimed at sampling the chemistry of otoliths of snapper between their core and proximal edge, using laser ablation inductively coupled plasma - mass spectrometry. The interpretation of results for the relative concentrations of different elements deposited at different times of the year requires some understanding of the growth of otoliths throughout the year. Current understanding of this for snapper in South Australia is poor. Consequently, the work described here was aimed at improving our understanding of the growth of adult snapper otoliths throughout the year to facilitate the interpretation of otolith chemistry in terms of possible migration behaviour of the fish. The specific objectives addressed were: to describe the optical characteristics of transverse sections (TS) of snapper otoliths; to identify the time of year of deposition of the opaque and translucent zones at several places on the surface of the otolith; and to determine the rate of growth of otoliths throughout the different seasons of the year.

This study focussed on the otoliths of fish from northern Spencer Gulf, as this is the most significant region in the fishery, is the one for which we have the most otoliths and which are the clearest for interpretation of optical characteristics. Otolith growth was determined through marginal increment analysis (MIA) of selected otoliths from fish of either the strong 1991 or 1997 year classes. MIA involved determining the proportion of each annual increment that was formed by each month of the year. This was described mathematically and the relationship was used to determine the percentage of annual growth accounted for by each season of the year.

2.2 Methods

TS sections of sagittae from the 1991 and 1997 year classes that had been prepared for age estimation in 2001 and 2002 were used for this study (Fowler et al. 2003). They were selected from those available on the basis of having a clear and interpretable macrostructure. All TS-sections had been cut with a diamond saw and were of a similar thickness of 0.5mm.

2.2.1 Measurement of optical density

The light intensity of the TS-sections of the otoliths was measured along two transects using the image analysis program Sigma Scan, whilst their digital images were also recorded along the same axes (Fig. 2.1). The light intensity measurements were converted to a percentage of blackness by the equation: 100 – [(original value/ 255) x 100]. The light intensity was related to linear distance along the transect by dividing the length of the transect by the total number of counts recorded along it. The relationship between light intensity and distance from the otolith core (µm) was graphed, with peaks representing blackness and troughs representing light transmittance. This graph was then aligned with the digital image of the otolith to confirm whether the peaks and troughs corresponded to the opaque and translucent zones, respectively.

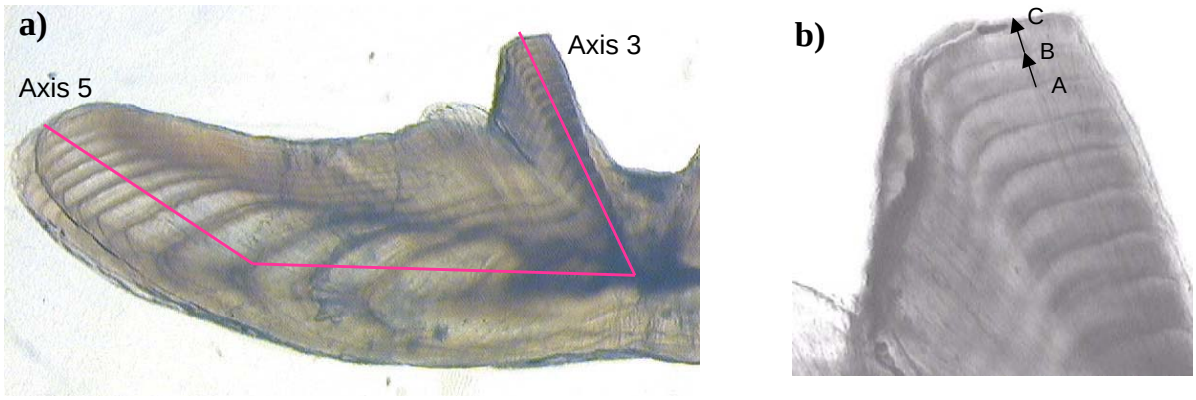

Figure 2.1 Photomicrographs taken under transmitted light of parts of a TS-section of a sagitta from a snapper collected from Northern Spencer Gulf. *(a)* Half of the section from the core region to the dorsal tip and proximal surface, with Axes 3 and 5 highlighted; *(b)* higher magnified view showing the increments towards the outer edge along Axis 3, with the measurements from the penultimate opaque zone (A) to the last opaque zone (B) and then to the otolith edge (C).

2.2.2 Marginal increment analysis

The growth of otoliths throughout the year was determined through MIA. Each TS section was smeared with immersion oil and then examined at x25 magnification using a binocular microscope and transmitted light. The image of the otolith was displayed on a video screen and examined

along Axes 3 & 5 (Fig. 2.1). For each axis the edge was identified as either opaque or translucent. Then the two most recently formed annuli were identified and measured using the image analysis software Videotrace. Two measurements were taken for each axis, i.e. from the initiation of the penultimate translucent zone to the outside edge of the last formed opaque zone, and from there to the edge of the otolith (Fig. 2.1b). Transverse sections with unclear annuli may have been interpretable along only one axis or were rejected. Outliers with particularly high or low values were checked by remeasuring.

The ratio of the marginal increment (I_m) to the penultimate increment (I_p) was calculated for each axis as, $R_m = I_m/I_p$. These ratios were plotted against the month and year of fish collection across the total period considered. Furthermore, the mean ratio ($\pm$SD) was calculated for each month and plotted against month and year. A mathematical relationship was derived to describe otolith growth throughout each year using the general equation: $R_m = R_{max}[1\text{-}e^{-k(m-a)}]$, where the parameters R_{max} = the estimated asymptote for the ratio, m = month number (i.e. January = 1, February = 2, March = 3 ….), and a = the estimate at which the relationship crosses the X-axis. The relationship was fitted using Solver in Excel, by minimising the least squares of the residuals. Each equation was then used to provide an estimate of the ratio at monthly time steps throughout the year. The estimated ratio for each month was then subtracted from that of the following month to provide an estimate of otolith growth for that month. To estimate the proportion of the total annual growth accounted for by each season the three monthly estimates of growth for that season were added. This methodology was applied independently for both Axes 3 and 5 for each of 2001 and 2002.

2.3 Results

2.3.1 Measurement of optical density

The high magnification view of Axis 3 in the TS section of a sagitta from a 13 year old snapper from Northern Spencer Gulf shows considerable variation in the transmittance of light across the section (Fig. 2.2). The core region represents the most opaque part of the TS section, with a general decreasing opacity from there to the edge. Within this is a series of alternating opaque and translucent zones, with the former represented by thin distinct lines, whilst the latter constituted the intervening, broader, lighter areas. Aligned with the picture is a graph (Fig. 2.2b) that shows the profile of light intensity as measured along the marked transect. The graph shows increasing blackness from the edge to the core, which is punctuated into a series of peaks and troughs, with the peaks indicating where the least amount of light was transmitted and the troughs indicating where most was transmitted.

The photomicrograph for Axis 5 also shows the thirteen annuli (Fig. 2.3a). As before, the core is the most opaque area whilst there is a general increase in translucency from there to the dorsal edge. The first few opaque zones are relatively broad compared to the thin opaque zones of increment numbers 6 to 13. The translucent zones are broad and contrast well with the opaque zones. The light intensity graph for the transect along this axis shows distinct peaks and troughs that align well with the various opaque and translucent zones.

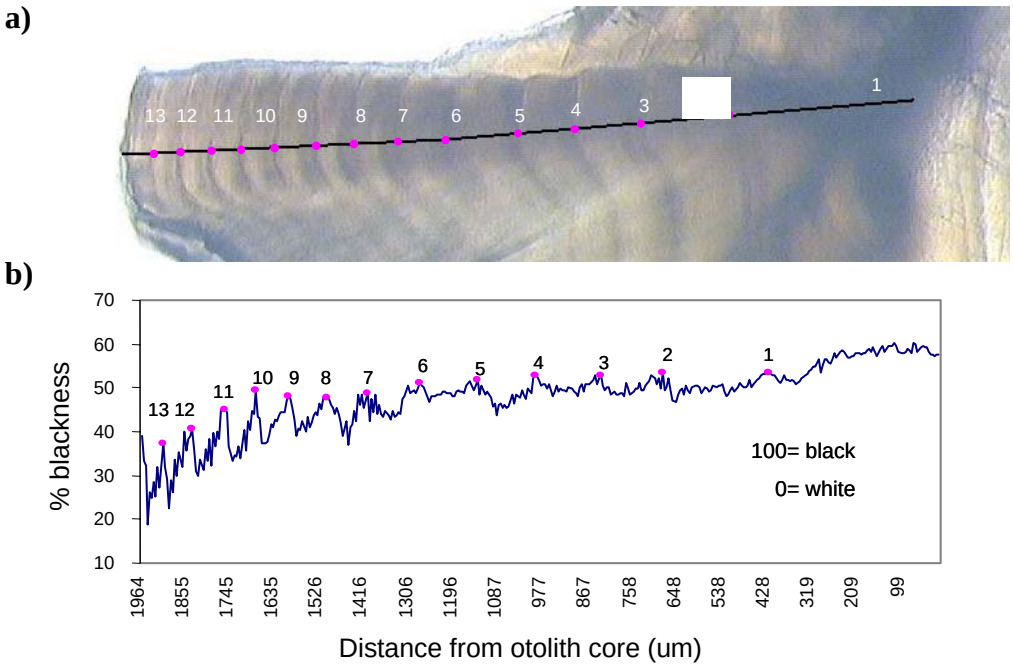

Figure 2.2 *(a)* Photomicrograph of Axis 3, i.e. from the core to the dorsal side of the sulcus of a TS-section of a sagitta from a 13 year old snapper from Northern Spencer Gulf, showing the line along which light intensity was measured using image analysis software; b) light intensity measurements recorded along the line indicated above. The dots and numbers in both figures show the places where they correspond.

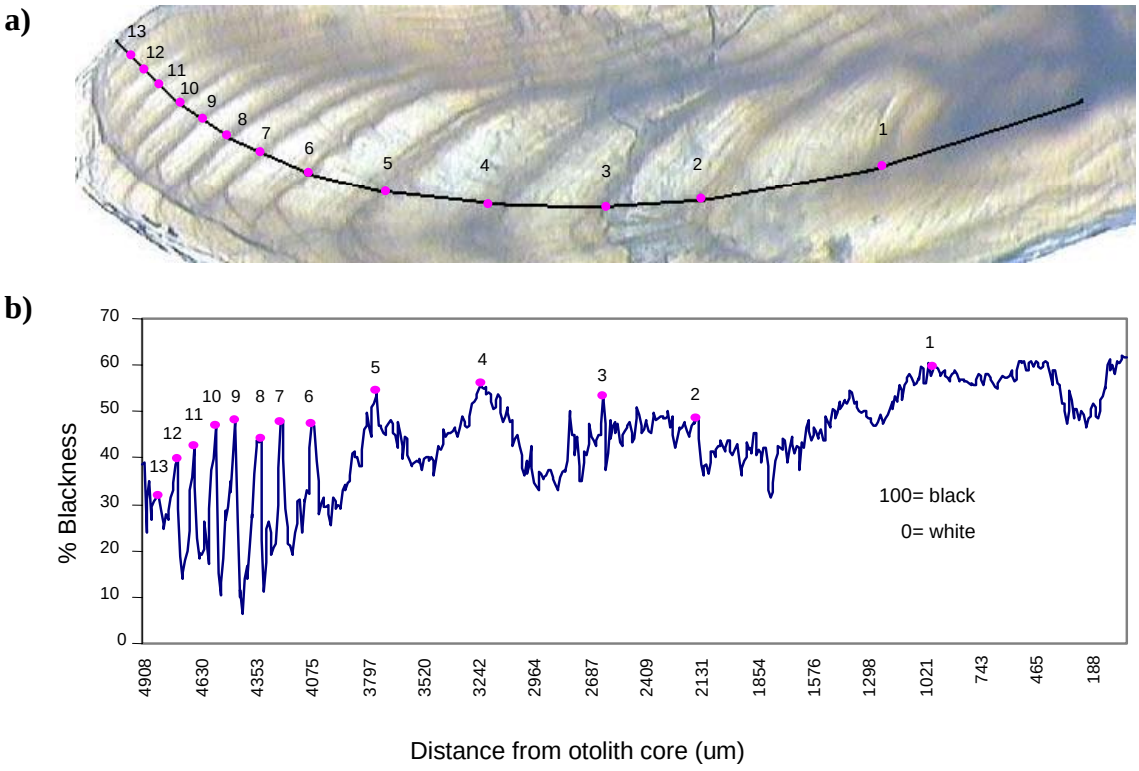

Figure 2.3 *(a)* Photomicrograph of Axis 5, i.e. from the core to the dorsal edge of a TS-section of a sagitta from a 13 year old snapper from Northern Spencer Gulf, showing the line along which light intensity was measured using image analysis software; *(b)* light intensity measurements recorded along the line indicated above. The dots and numbers in both figures show the places where they correspond.

2.3.2 Marginal Increment Analysis – 1997 year class

Edge Type

Of the 438 otoliths that were successfully interpreted from the 1997 year class only 22 had an opaque edge for Axis 3 (Table 2.1). The majority of these, regardless of whether they were the 4th, 5th or 6th opaque zone that formed in 2000, 2001 or 2002 respectively, were recorded in the months of November and December. Otoliths with completed opaque zones and newly initiated translucent material on the edges were recorded as early as November and December. These data indicate that for most fish in the age classes of 5 and 6 years, the opaque zone formed through the November/December period, whilst translucent material was laid down on the otolith edge throughout the rest of the year.

The data from the MIA for Axis 5 also suggest strongly that their formation peaked in November and December (Table 2.1). However, the opaque zones were recorded from as early as September in both 2000 and 2001, and in the latter year through until January 2002. Thus, they were apparent for more months of the year for Axis 5 than Axis 3.

Table 2.1 Results of 'edge type analysis' for Axes 3 and 5 for the sagittae from samples of the 1997 year class collected between September 2000 and December 2002. The results show the number of fish per sampling occasion with the indicated zone at the edge of the otolith (T = translucent zone, O = opaque zone).

		Axis 3							Axis 5					
Collection month/year	Sample size	4th T-	4th O-	5th T-	5th O-	6th T-	6th O-	Sample size	4th T-	4th O-	5th T-	5th O-	6th T-	6th O-
Sept 2000	15	15						16	10	6				
Nov 2000	30	10	6	14				32	3	9	20			
Dec 2000	18		1	17				19		1	18			
Feb 2001	7			7				8			8			
Mar 2001	14			14				13			13			
Apr 2001	26			26				28			28			
May 2001	36			36				38			38			
Jun 2001	33			33				30			30			
Jul 2001	17			17				16			16			
Aug 2001	8			8				6			6			
Sep 2001	19			18	1			18			13	5		
Oct 2001	2			2				2				2		
Nov 2001	46			40	6			38			9	27	2	
Dec 2001	18			11	4	3		22				6	16	
Jan 2002	21			3	3	15		23				7	16	
Feb 2002	36					36		36					36	
Mar 2002	30					30		30					30	
Apr 2002	15					15		12					12	
May 2002	7					7		6					6	
Jun 2002	2					2		2					2	
Jul 2002	11					11		12					12	
Sep 2002	10					10		9					9	
Oct 2002	6					6		6					6	
Dec 2002	10					9	1	7					5	2

Otolith Growth

The 438 sagittae were considered in the MIA to describe the formation of the 5th and 6th increments, mainly through 2001 and 2002 . For Axis 3, the marginal increment ratio (R_m) clearly changed throughout 2001, reflecting the growth of the otoliths through the year (Fig. 2.4). The raw data and monthly means indicate that growth was fastest through the months of summer and autumn, and then slowed considerably through winter and spring (Figs. 2.4). Despite this trend, the ranges in estimated ratios for all months were broad indicating considerable variation amongst otoliths. For example, in May 2001 the estimates of R_m ranged from 0.52 to 1.07, which indicates that at this time some otoliths had achieved half the growth of the entire previous year, whereas others had already grown more than that achieved throughout the entire previous year.

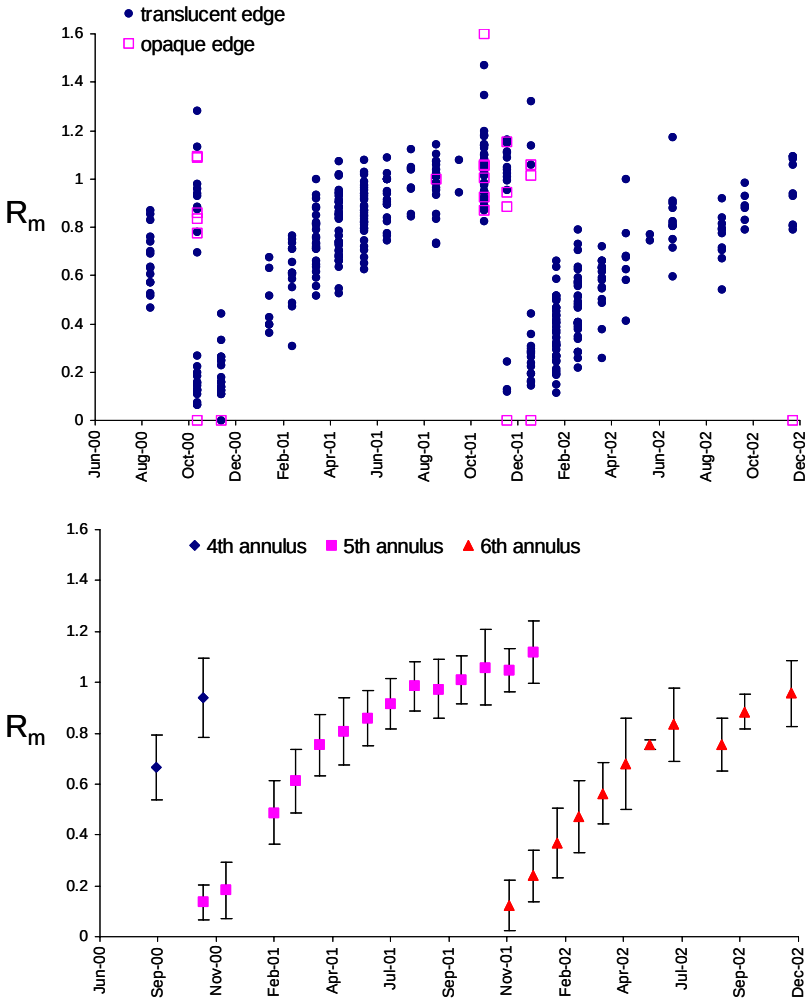

Figure 2.4 Results of MIA for otoliths from the 1997 year class sampled through the nominated months. Top graph shows estimates of R_m based on measurements along Axis 3, bottom graph shows the mean (±SD) of the estimated ratios in each month.

The results for formation of the 6th increment along Axis 3 through 2002 were similar to those described above, i.e. with growth fastest in summer and autumn and slower in winter and spring (Fig. 2.4). One difference was that the asymptotic size in 2002 was marginally smaller than for 2001. Furthermore, in 2002 the variation in monthly estimates was even greater, e.g. in February the estimates of R_m ranged from 0.11 to 0.66. In general, the trends described above for formation of the 5th and 6th annuli along Axis 3 also applied to Axis 5 (Fig. 2.5), except that variation in estimates of R_m was higher along Axis 5, e.g. in May 2001 estimates ranged from 0.42 to 1.13.

The average seasonal otolith growth relating to formation of the 5th and 6th increments through 2001 and 2002, were each described mathematically, and the parameters associated with these relationships are presented in Table 2.2. These growth equations were used to provide a monthly estimate of R_m over a 12 month period, and to describe seasonal growth independently for each of 2001 and 2002 (Table 2.2). Summer accounted for approximately 46% of the annual growth along Axis 3 in both years, whilst in autumn greater than 27% of the annual growth occurred. In winter and spring of both years, 16 and 10% of the annual growth of the otolith was achieved. Thus, the six months of summer and autumn accounted for approximately 75% of the formation of each of the fifth and sixth increments, leaving only 25% of annual growth that occurred in winter and spring.

The pattern for Axis 5 was similar, although summer accounted for a marginally lower proportion of the year's growth whilst winter and spring made up this difference (Table 2.2). Thus, for Axis 5 about 70% of the 5th and 6th annual increments were each formed in summer and autumn and the remaining 30% in winter and spring. A diagrammatic representation of these seasonal differences in growth patterns is presented in Fig. 2.6, which shows the 5th and 6th increments along Axis 5, partitioned into the proportion of annual growth achieved in each season between summer 2001 and spring 2002.

Table 2.2 Parameters of relationships describing growth of otoliths in each of 2001 and 2002 for fish of the 1997 year class. The general form of the equation was: $R_m = R_{max}[1-e^{-k(m-a)}]$. The table also shows the estimated percentage of the newly formed annual increment that grew in each season of that year.

Otolith axis	Year	Increment number	R_{max}	k	t_o	% of annual growth per season			
						Summer	Autumn	Winter	Spring
3	2001	5	1.2	0.1708	0.6247	46.0	27.6	16.5	9.9
	2002	6	1.05	0.1755	1.505	46.6	27.5	16.3	9.6
5	2001	5	1.2836	0.1423	0.2049	42.4	27.7	18.1	11.8
	2002	6	1.1232	0.1539	1.1488	43.9	27.7	17.4	11.0

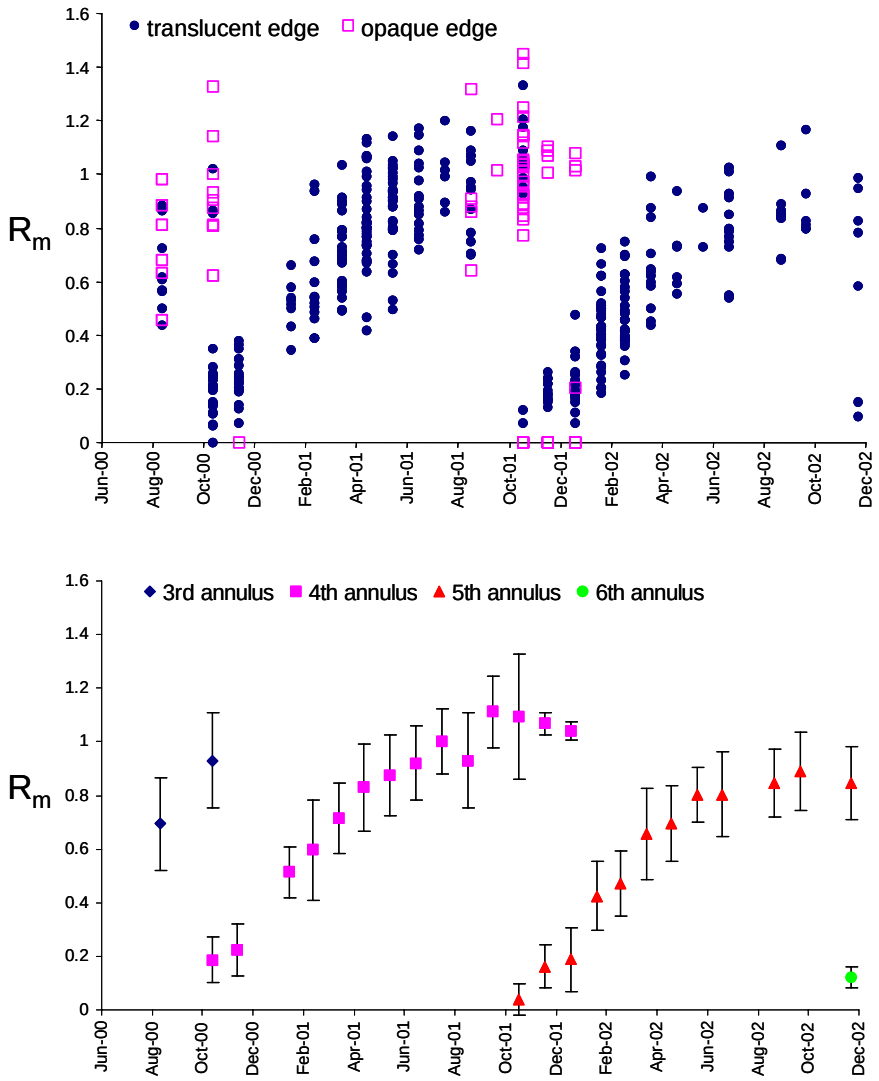

Figure 2.5 Results of MIA for otoliths from the 1997 year class for fish sampled through the nominated months. Top graph shows the estimates of R_m based on measurements along Axis 5, bottom graph shows the mean ($\pm$SD) of R_m in each month.

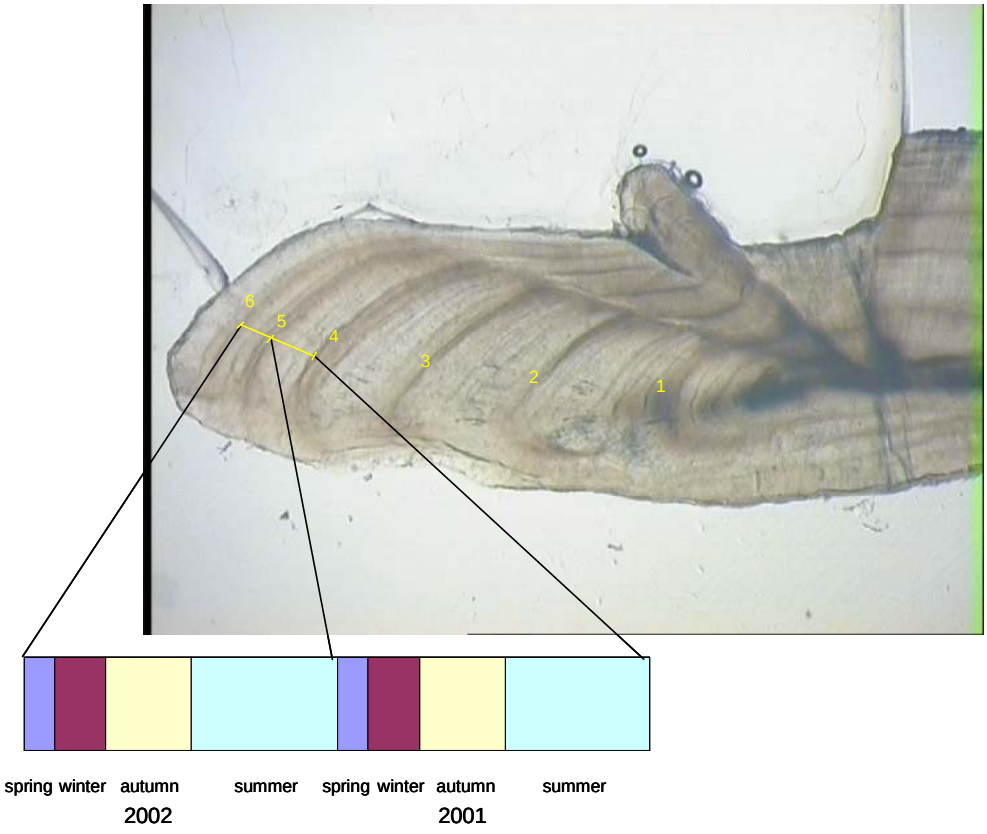

Figure 2.6 Picture shows the half of a TS section of a snapper otolith. The six completed opaque zones are enumerated along Axis 5, whilst the 5th and 6th increments are indicated with bars. The diagram shows the relative proportion of each of these increments that was deposited in consecutive seasons between summer 2001 and spring 2002, based on the estimate of growth for each season provided in Table 2.2

2.3.3 Marginal Increment Analysis – 1991 year class

Edge Type

Formation of the 11th and 12th translucent and opaque zones in otoliths of fish from the 1991 year class was documented by edge type and marginal increment analysis for 338 sagittae collected through 2001 and 2002. Otoliths with opaque edges along Axis 3 were recorded in 5 of 6 months between September and February, with the highest numbers in October and November (Table 2.3). For Axis 5 opaque margins were recorded in 6 of the 7 months from August to February 2002, whilst the highest proportions with opaque edges were recorded in October and November of both years.

Table 2.3 Results of 'edge type analysis' for Axes 3 and 5 for the sagittae from samples of the 1991 year class collected between September 2000 and December 2002. The results show the number of fish per sampling occasion with the indicated zone at the edge of the otolith (T = translucent zone, O = opaque zone).

		Axis 3							Axis 5					
Collection month/year	Sample size	10th T-	10th O-	11th T-	11th O-	12th T-	12th O-	Sample size	10th T-	10th O-	11th T-	11th O-	12th T-	12th O-
Sept 2000	14	14						14	13	1				
Dec 2000	13	5	5	3				14	3	8	3			
Mar 2001	20			20				22		1	21			
Apr 2001	1			1				4			4			
May 2001	9			9				8			8			
Jun 2001	13			13				16			16			
Jul 2001	5			5				8			8			
Aug 2001								2			1	1		
Sep 2001	12			11	1			12			11	1		
Oct 2001	23			14	9			25			8	17		
Nov 2001	25			16	9			29			7	18	4	
Dec 2001	6			6				6			5		1	
Jan 2002	18			14	2	2		21			6	5	10	
Feb 2002	12			4	1	7		19				11	8	
Apr 2002	10					10		10					10	
May 2002	17					17		15					15	
Jun 2002	14					14		13					12	1
Jul 2002	6					6		6					6	
Sep 2002	1					1		1					1	
Oct 2002	34					29	5	39					29	10
Nov 2002	41					33	8	36					18	18
Dec 2002	10					7	3	13					6	7

For formation of the 11th increment along Axis 3 in 2001 there was a substantial increase in R_m throughout the year, with the growth rate fastest through summer before slowing in winter and spring (Fig. 2.7). Nevertheless, the ranges in monthly estimates of R_m were broad. Opaque edges were detected for this axis over a six month period, i.e. from September 2001 until February 2002. Formation of the 12th increment along Axis 3 through 2002 also showed considerable variation (Fig. 2.7). Both the 11th and 12th increments along Axis 5 displayed the general seasonal pattern, but with an even higher within-sample variation than for Axis 3 (Fig. 2.8).

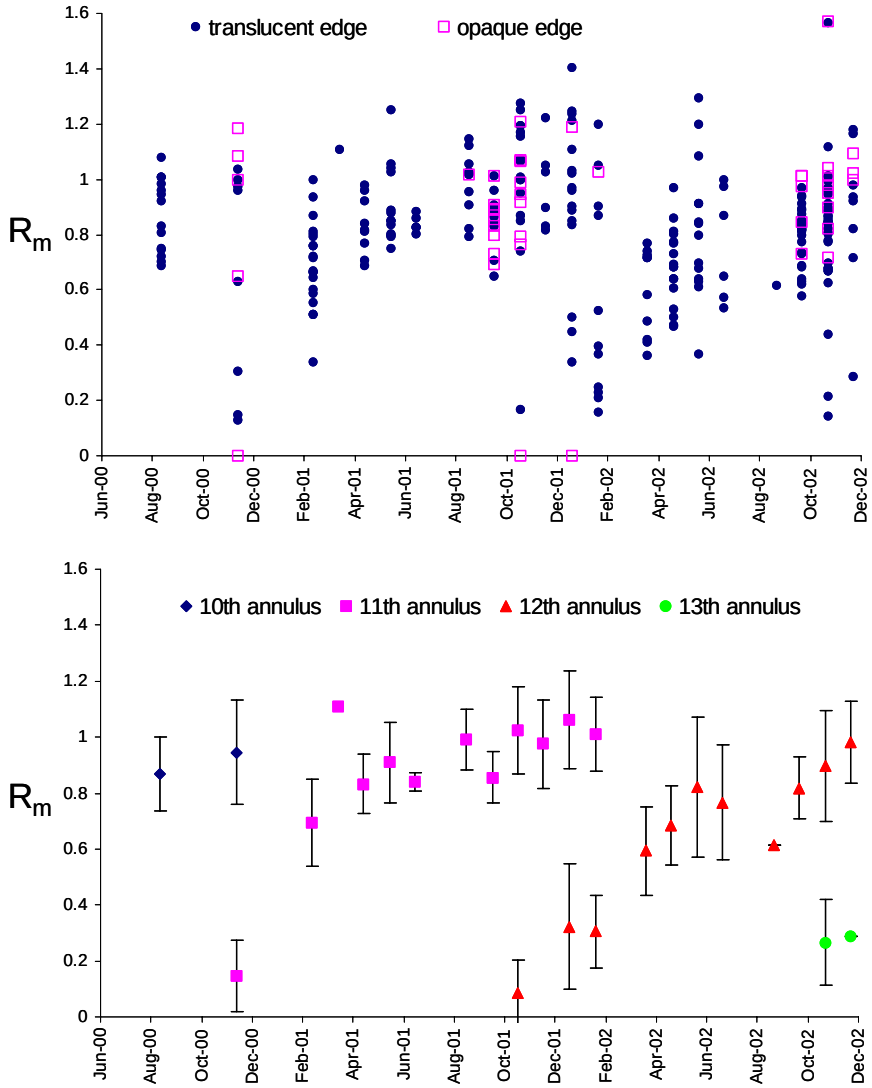

Figure 2.7 Results of MIA for otoliths from the 1991 year class of fish sampled through the nominated months. Top graph shows the estimates of R_m ratios based on the measurements along Axis 3, bottom graph shows the mean ($\pm$SD) of R_m in each month.

The seasonal variation in rate of development of the 11th and 12th increments (Figs. 2.7b, 2.8b), were each described mathematically, for which the parameters are presented in Table 2.4, along with the seasonal contributions to the annual formation of the 11th and 12th increments for both axes. Summer in 2001 accounted for >60% of the increment formation along both axes, whilst autumn was the second most significant season in this year. Thus, the two seasons together accounted for >85% of formation of the 11th increment in these otoliths. The bulk of the remaining annual growth occurred in winter, with only minor addition of new material in spring.

In 2002 the summer contribution was less than in the previous year along both axes, whilst the contributions in winter and spring were marginally higher (Table 2.4), indicating some inter-annual variation in seasonal growth characteristics of the otoliths.

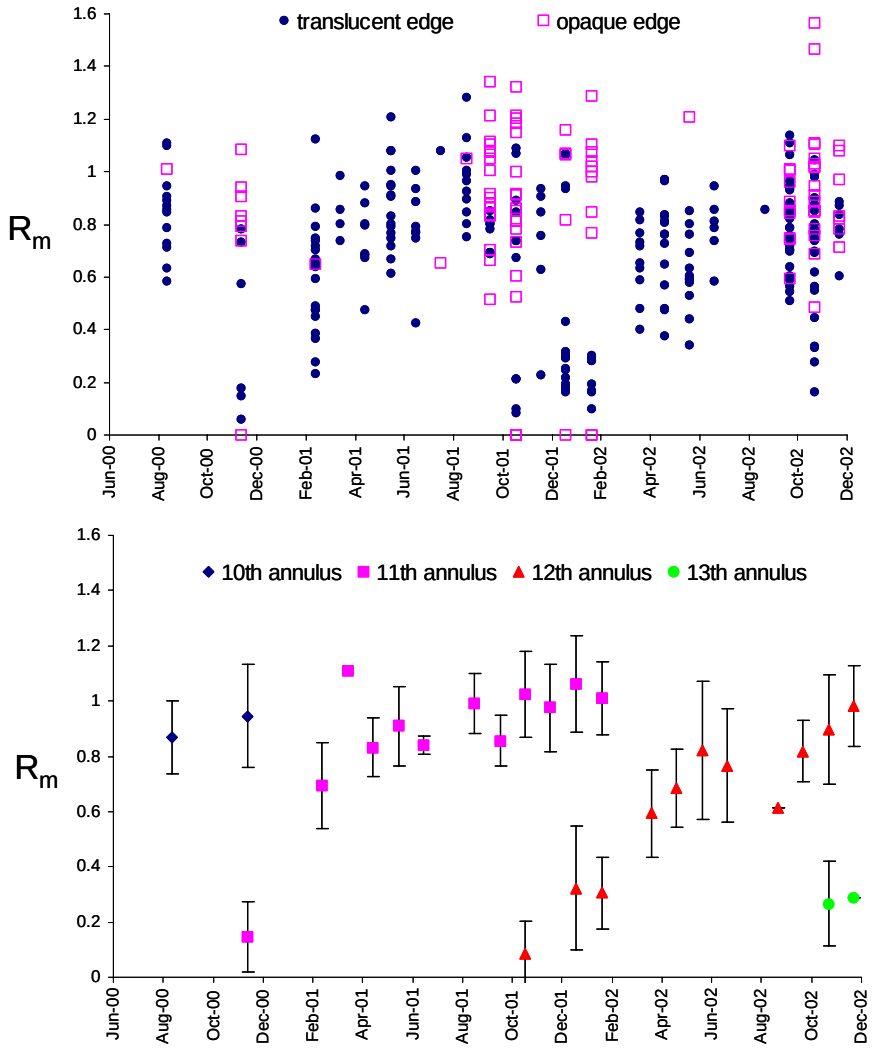

Figure 2.8 Results of MIA for otoliths from the 1991 year class of fish sampled through the nominated months. Top graph shows the estimates of R_m based on measurements along Axis 5 bottom graph shows the mean ($\pm$SD) of R_m in each month.

Table 2.4 Parameters of the relationships for growth of otoliths in each of 2001 and 2002 for fish of the 1991 year class. The general form of the equation was $R_m = R_{max}[1-e^{-k(m-a)}]$. The table also shows the estimated percentage of the newly formed annual increment that was accounted by each season.

Otolith axis	Year	Increment number	R_{max}	k	t_o	% of annual growth per season			
						Summer	Autumn	Winter	Spring
3	2001	11	0.9973	0.3385	1.4871	64.9	23.5	8.5	3.1
	2002	12	0.9553	0.2003	0.2107	49.7	27.2	14.9	8.2
5	2001	11	0.9641	0.3103	1.6676	62.1	24.5	9.64	3.8
	2002	12	0.9085	0.2603	2.0516	56.7	26.0	11.9	5.4

2.3.4 Seasonal otolith growth in absolute terms

So far in this chapter otolith growth has been described in terms of the proportion of the growth achieved in the previous 12 month period. It is also important to consider the seasonal growth in terms of the actual linear measurements. Here, the size of the penultimate increment of each otolith was used as an estimate of the annual growth of the otolith along the two axes. These sizes were then partitioned according to the proportions of annual growth that were accounted for by the different seasons, as presented in Tables 2.2 & 2.4. For the 1997 year class the penultimate increments measured in 2001 and 2002 along Axis 3 respectively, were each greater than150 μm (Table 2.5). Summer and autumn accounted for most of this growth, leaving approximately only 25 μm of growth that was achieved in winter and even less in spring. For Axis 5 the increment widths in both years were >350 μm and so the absolute growth in each season was relatively high. For the 1991 year class the average annual growth along Axis 3 was <100 μm (Table 2.5). When partitioned seasonally this meant that the estimated growth in both winter and spring was <15 μm per season. Despite the greater absolute growth along Axis 5 the estimated winter growth was still approximately only 20 μm, and spring growth was <10 μm.

Table 2.5 Results of the mean growth (μm) of otoliths along the nominated axes based on the size of the penultimate increment for each otolith sampled in that year. Also shown are the estimates of seasonal growth, based on apportioning the mean annual growth by the percentages specified in Tables 2.2 and 2.4.

							Otolith growth (μm)			
Yearclass	**Axis**	**Year**	**Mean**	**SD**	**Min**	**Max**	**Summer**	**Autumn**	**Winter**	**Spring**
1997	3	2001	172.3	28.6	108	286	79.3	47.6	28.4	17.0
	3	2002	154.6	27.4	72	235	72.0	42.5	25.2	14.8
	5	2001	399.1	89.0	341	700	169.2	110.6	72.2	47.1
	5	2002	353.6	74.7	164	549	155.2	97.9	61.5	38.9
1991	3	2001	96.9	17.5	63	152	62.9	22.8	8.2	3.0
	3	2002	97.1	21.2	49	187	48.3	26.4	14.5	8.0
	5	2001	187.4	48.6	93	325	116.4	45.9	18.0	7.1
	5	2002	166.2	44.0	77	356	94.2	43.2	19.8	9.0

2.4 Discussion

2.4.1 Measurement of optical density

There was considerable variation in the transmittance of light through the TS sections of snapper at two scales. Firstly, the section was most dense and opaque in the central region from where there was a gradual decrease in opacity towards both dorsal and ventral edges and the proximal surface. Secondly, there was a sequence of concentric opaque and translucent zones between the otolith centre and outside edge. The opaque zones were characteristically much thinner than the translucent ones, which meant that the majority of the surface area of the TS-section consisted of translucent material. Such otoliths are characteristic of fishes from the Order Perciformes that occupy relatively warm water environments (Fowler 2004). They contrast with otoliths from numerous high latitude, cold water species whose otoliths are generally opaque and for which the TS-section is dominated by broad opaque zones and thinner translucent ones (Campana 2000, Campana and Thorrold 2001).

2.4.2 Marginal increment analysis

We determined the timing of formation of the opaque and translucent zones in the otoliths of snapper through 'edge analysis' for large numbers of otoliths from the 1991 and 1997 year classes, sampled mainly throughout 2001 and 2002. Despite some obvious variation in timing, opaque zone formation peaked around November / December during formation of the 5^{th} and 6^{th} increments, and around October / November for the 11^{th} and 12^{th} increments. Thus, the highest frequencies of opaque zone formation were recorded in late spring and early summer, which concurs with results from an earlier study for snapper done in Northern Spencer Gulf (McGlennon et al. 2000). Nevertheless, the timing of opaque zone formation was quite variable, as otoliths with opaque edges were recorded for up to six months of the year. There was also some variation in timing of increment formation on different parts of the otolith. There were fewer months in which opaque edges were detected on Axis 3 than was the case for Axis 5 Also for the latter axis, opaque zones were detected earlier in the year. These results suggest that increment formation is not uniform across the growing surface of the otolith, i.e. the edge at the dorsal tip can be opaque whilst it is translucent on the dorsal side of the sulcus.

South Australian snapper conform to the large number of taxa of fish from different ecosystems around the world for which the opaque zone is formed in spring / early summer. This is the case for many temperate and tropical species of fish from the Order Perciformes (Beckman and Wilson 1995; Fowler 1995, 2004). The result differs from that obtained for juvenile snapper from New South Wales, where opaque zones were deposited during winter (Ferrell et al. 1992). Other species

also deposit the opaque zone during winter (Ferreira and Russ 1994, Beckman and Wilson 1995). At present there is no understanding of these seasonal differences for either within or between species. The data from this study lead to the overwhelming conclusion that a sequence of an opaque and a translucent zone is formed in the otoliths over the period of a year. This supports a similar finding that increment formation in otoliths of snapper from South Australia (McGlennon et al. 2000) and elsewhere (Ferrell et al. 1992, Francis et al. 1992), conform to an annual periodicity and can be used for age determination.

Consideration of growth of the snapper otoliths provided several obvious results. Clearly, the rate of deposition of the calcareous material varied seasonally. The growth rate was greatest through summer, decreased through autumn, and then was relatively low in winter and spring. As such, >75% of the annual deposition of material to the otolith surface was achieved in the six months of summer and autumn. Furthermore, there was considerable variation in the characteristics of otolith growth amongst different fish. This was manifested both as the within-sample variation in estimates of marginal increment, and also as the range of months for which the opaque zone was evident on the otolith edge. Such variability was evident despite that the fish from which the otoliths came were the same age and were sampled from the same region. This must relate partly to natural variation amongst fish. However, perhaps more importantly, the within-sample variation in estimates of R_m will likely depend on when the marginal zone was initiated in the different otoliths. Since some otoliths with opaque edges were detected for up to six months of the year, must mean that the timing of initiation of the following translucent zone is also highly variable, thus impacting on its width at any particular time. For example, for the wide range of R_m of 0.42 to 1.13 recorded in May 2001, the lower values may indicate initiation of the translucent zone within only a few months whereas the higher values may relate to initiation of the marginal translucent zone late in the previous year. This proposes that variation in the timing of zone formation may well influence the relative size of the marginal increment of an otolith.

The study also documented some variation between different otolith axes. Opaque edges were evident for more months of the year for Axis 5 than for Axis 3. For the reason presented above, the data for Axis 5 seem to be inherently more variable than do those for Axis 3. Finally, there were also differences in the characteristics of growth between 2001 and 2002. For all the growth functions derived, those for 2002 tended to have lower asymptotic sizes than those for 2001, which may be an environmental effect or a reflection of the slowing of growth with increasing age.

2.4.3 Significance of otolith growth to chemical analysis

The primary motivation for this chapter was to provide a comprehensive understanding of otolith growth to help interpret the profiles of elemental concentrations across the chronologies of the

otoliths (Chapter 3.0). Since otoliths do not grow constantly through the year it is necessary to relate the different parts of an annual increment to the time of formation against which the chemical profile can be aligned. This may assist in determining life history characteristics, such as whether the fish demonstrate regular migratory behaviour.

This study identified that otolith growth was seasonal. Average growth functions were derived to describe the development of the 5^{th} and 6^{th} annuli for the 1997 year class and the 11^{th} and 12^{th} annuli for the 1991 year class, and indicated that otolith growth rate declined in the colder months, but nevertheless remained positive. This means that, on average, otoliths should continue to record information about the physical and chemical environment throughout the year. Nevertheless, it is necessary to see whether there is sufficient addition of new calcareous material during the different seasons for any environmental variation in the chemistry to be detectable.

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3 Adult movement and stock structure – otolith chemistry analysis by laser-ablation ICPMS

A.J. Fowler, K.C. Hall and B.M. Gillanders

3.1 Introduction

A recent study for the snapper population of South Australia found no evidence for any genetic structure finer than the broad stock division between the Victorian and South Australian populations in the vicinity of the mouth of the River Murray (Donnellan and McGlennon 1996). This apparent panmixia supports the fact that the fishery has been managed as a single stock, and is consistent with the currently accepted life history model for snapper in South Australia (McGlennon and Jones 1997). This life history model hypothesizes that there are two components to the population, i.e. 'resident' fish and 'migrant' fish. The 'residents' are thought to originate from spawning activity and nursery areas in the two Gulfs, and then remain in the Gulfs throughout their lives. Whilst the 'migrants' also originate in the Gulfs, it is suggested that in the first few years of their lives they move to the continental shelf. The model suggests that as these fish grow and develop they join an annual spawning migration back to the Gulfs, as part of the reproductive cycle. After the reproductive season in late summer / autumn the 'migrants' then return to the continental shelf. The model further suggests that the numbers of fish that undertake the spawning migration increases dramatically from the age of 9 years onwards, but that the rate of return of fish after spawning to the continental shelf decreases from the age of 12 years to the extent that by 15 years of age the fish have become permanent residents in the Gulfs.

The model clearly suggests that the 'resident' and 'migrant' components of the population interact through a complex, age-related migration that is related to the annual reproductive cycle. The model largely originates from the seasonal and inter-annual variation in the fishery catch and population structure (McGlennon and Jones 1997). The potential existence of a population of 'migrants' on the continental shelf that are derived originally from the Gulfs has important implications for the population structure, as it would provide a clear opportunity for the mixing of fish that move out of regions such as Spencer Gulf, Gulf St. Vincent and Investigator Strait. The consequences of such mixing for the stock structure depend on the subsequent movement patterns of these fish. If they systematically return each year to the same area to spawn then regional population dynamics would remain independent and stocks would be separate. Alternatively, if fish move around in response to exogenous influences, suggesting that movement is far less deterministic, then there would be less opportunity for development of a regional stock structure.

This suggests that the appropriate spatial scale for management of the South Australian fishery is closely related to the movement behaviour of the adult fish.

Two broad questions were addressed in this chapter: 1) is there evidence to support the proposed complex age-related migration behaviour between the continental shelf and the Gulfs?; 2) and is there evidence of a regional stock structure? The analytical approach was based on analysis of chemical profiles across the chronological structure of otoliths using laser ablation inductively coupled plasma - mass spectrometry (LA-ICPMS). The specific objectives that were addressed were: 1) to sample the chemistry of otoliths and to determine whether chemical profiles in relation to fish age are consistent with a migratory life history; 2) if this is the case, to determine whether movement is systematic such that individuals return to the same area each year to spawn, or whether movement is less deterministic; 3) to compare the elemental profiles of otoliths amongst regions and to interpret these with respect to stock structure, i.e. whether there is evidence for one single, large population or for division into a number of smaller discrete sub-populations.

3.2 Methods

The snapper otoliths used for this study were collected from fish taken from the catches of commercial marine scalefish fishers in 2000, during routine sampling for stock assessment by SARDI Aquatic Sciences. The fish were caught at different times throughout the year in one of six fishery regions of South Australia, i.e. northern and southern Spencer Gulf (NSG, SSG), northern and southern Gulf St. Vincent (NGSV, SGSV), the west coast of Eyre Peninsula (WC) and the waters of the south-east corner of the State (SE) (Fig. 3.1). Four of these regions are associated with the extensive gulf system of South Australia. These include NSG, SSG and NGSV as semi-enclosed Gulf regions characterised by hypersaline waters and broad seasonal variation in water temperature. In this study SGSV included Investigator Strait and so had both an oceanic and a gulf influence. The WC and SE are both open, coastal regions that have a strong oceanic influence.

Where possible 20 individuals from the strong 1991 year class (i.e. 9 year old fish in 2000) were randomly selected from all fish collected from each region in that year. By selecting similar-aged individuals that were all collected in the same year, minimised the possibility of temporal confounding influences on otolith composition that might detract from spatial comparisons. A single sagitta was analysed from each individual, as the other sagitta had previously been sectioned and used for age estimation in the stock assessment work.

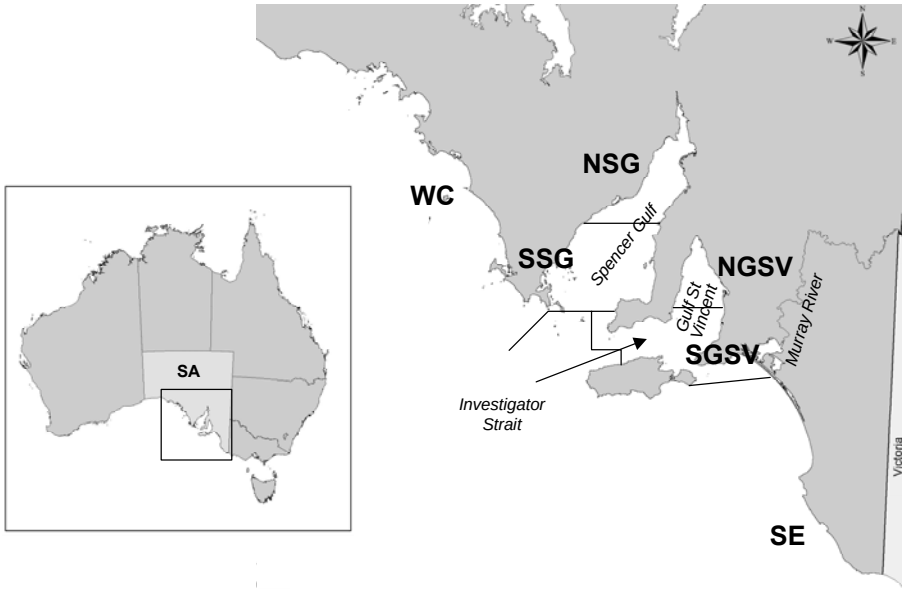

Figure 3.1 Map of the South Australian waters indicating the six geographic regions of the snapper fishery; west coast of Eyre Peninsular (WC), northern Spencer Gulf (NSG), southern Spencer Gulf (SSG), northern Gulf St Vincent (NGSV), southern Gulf St Vincent (SGSV) and south east coast (SE).

3.2.1 Otolith preparation

All plastic and glassware used for processing the otoliths was acid-washed in 10% nitric acid for 24 hours, triple rinsed in Milli-Q water and then dried in a laminar flow cabinet. Otoliths were always handled with acid-washed plastic forceps or powder-free plastic gloves and whenever possible were maintained under a dedicated class 100 laminar flow hood.

Before processing, each otolith was sonicated in ultra-pure (Milli-Q) water for 10 minutes and air-dried overnight. Each was then embedded in Dewars Epofix resin and a thin transverse section (TS-section) of approximately 350 µm thickness was removed from the core region using a slow speed gem saw lubricated with Milli-Q water. Each TS-section was polished with 9µm and 3µm aluminium oxide lapping film that was wet with Milli-Q water then triple rinsed, air-dried overnight and mounted on a geological microscope slide with resin. A digital image of each mounted TS-section was recorded using video capture software, with the image of the otolith displayed using a dissecting microscope fitted with a digital camera connected to a computer. After imaging, each TS-section was again sonicated for 5 minutes, triple rinsed with Milli-Q water, and air-dried under the laminar flow hood, before being stored in a separate plastic bag for transportation to the laser ablation facility.

3.2.2 Chemical analysis

Sections were analysed by LA-ICPMS at the School of Geosciences, Monash University, Melbourne. The system consisted of a Merchantek LUV266 petrographic ultraviolet laser (Nd:YAG) microprobe connected to a Thermo Finnigan MAT ELEMENT high resolution ICPMS. An otolith section was placed in the sealed perspex ablation chamber with helium atmosphere and viewed remotely via a microscope objective lens connected to a computer monitor. The laser was programmed to follow a transect path from the core to the outer edge of the otolith section. The laser was pulsed at a repetition rate of 6 Hz, a laser energy of between 70-100 mJ, and a scan speed of 1.3 $\mu m.s^{-1}$. These settings produced an ablation crater of approximately 40 μm diameter when held stationary, and concentrations were recorded for ablations every 2.67 ± 0.12 μm when in motion along transects. The ablated otolith material was entrained in an argon and helium gas stream (argon flow rate 14 $mL.min^{-1}$; helium flow rate 1.36 $mL.min^{-1}$) and carried to the plasma torch of the ICPMS for analysis. The elemental isotopes chosen for analysis were those thought to be metabolically inert, not under significant physiological control, and not subject to interference from other isotopes (Campana 1999). These included ^{25}Mg, ^{52}Cr, ^{55}Mn, ^{63}Cu, ^{59}Co, ^{64}Zn, ^{88}Sr, ^{114}Cd, ^{138}Ba and ^{208}Pb, as well as ^{44}Ca that was measured for use as the internal standard.

It was necessary to choose which axis of those available to sample in the main regional comparison. A pilot study was done where five otoliths were selected at random, and transects were analysed along four of the five different axes (Axes 2-5 of Fig. 3.2). Axis 1 was disregarded because it was often difficult to identify the opaque and translucent zones accurately, which would subsequently complicate the process of aligning the elemental profiles with age. For the main sampling protocol Axis 3 was chosen as the preferred axis for analysis (see Results Section), and a single transect was sampled along this axis for each TS-section.

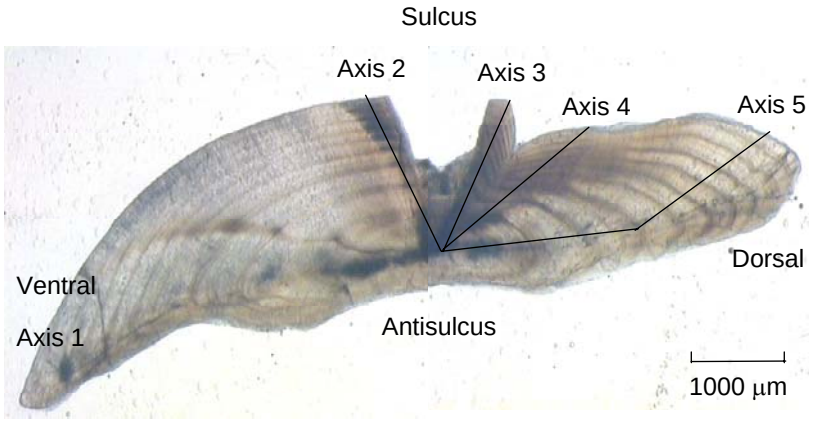

Figure 3.2 Transverse section of a sagitta of a 718 mm CFL, 9 year old snapper from the 1991 year class, illuminated with transmitted light.

The sequence for processing the TS-sections from the six regions was random, based on random numbers tables. It was only possible to sample between 10 to 12 TS-sections per day. At the beginning and end of each day, background counts were collected for 300 measurements, to estimate the limits of detection for each isotope, and the matrix-matched standard NIST 612 was analysed twice, to correct for instrument drift by linear interpolation and for use as an external standard to estimate isotope concentrations from sample counts. At the start of each transect, background counts were collected for 30 measurements, and the average was subtracted from sample counts to correct for background levels. The ablation chamber was purged for 20 s after each opening.

All data reduction was done off-line using spreadsheet programs. Calcium was used as an internal standard to correct for variation in ablation yield. Calcium concentration was assumed to be constant at 388,000 $\mu g.g^{-1}$, based on the published values for a certified reference material of otolith (Yoshinaga et al. 2000). The concentrations of other isotopes were estimated against this using the relative response factor of the instrument to the known concentration in an external, matrix-matched standard (NIST 612), and that recorded for the samples. Raw data were smoothed with a 9-point running median, followed by a 9-point running mean as described by Sinclair et al. (1998).

For an ablated crater size of diameter of 40 μm, the estimated detection limits (in $\mu g.g^{-1}$) during our analysis for the list of elements examined were estimated as the quantity of analyte required to produce a signal equivalent to three times the SD of the blank. These detection limits were estimated as: ^{25}Mg 0.21-1.61, ^{52}Cr 0.03-0.27, ^{55}Mn 0.20-1.76, ^{63}Cu 0.05-0.48, ^{59}Co 0.02-0.33, ^{64}Zn 0.02-0.28, ^{88}Sr 0.04-0.29, ^{114}Cd 0.05-0.70, ^{138}Ba 0.00-0.02 and ^{208}Pb 0.00-0.03.

3.2.3 Relating Chemical Profile to Fish Age

In order to compare the chemistry between otoliths of different sizes the elemental concentration profile across each otolith was matched to its optical macrostructure, using the annual increments as a temporal reference. A digital image of each ablated section was recorded using SigmaScan Pro Image Analysis software. From this, the width of each increment was measured to the nearest μm adjacent to the scar that resulted from the laser ablation process. Sections of the trace element concentration profile were then proportionally allocated to each increment. Only the chemical results from the core to the 9th opaque zone were considered in these analyses. The data for the marginal increment were not considered as the fish were collected at different times of the year throughout 2000.

3.2.4 Data analysis

The data were considered at two spatial scales, i.e. within and between regions. Firstly, the profiles for either ^{88}Sr or ^{138}Ba for each otolith were examined and each was categorised according to its characteristics, as described in Table 3.1. The first characteristic was the proportion of the length of the transect for which sinusoidal annual variation was evident. Those otoliths with a consistent pattern across the entire length of the transect were further sub-divided into those for which the peaks in elemental concentration aligned with the opaque zones, those that aligned with translucent zones and those for which there was no consistent alignment. For otoliths with only part of the transect showing systematic variation, the part of the otolith that this related to was recorded.

Table 3.1 Description of the main categories for the different patterns of variation in the concentrations of ^{88}Sr or ^{138}Ba across the otoliths.

Major category	**Sub-category**
Consistent pattern of annual variation in elemental concentration producing perceptible peaks and troughs along whole transect	▪ Peaks correspond with opaque zone for most increments ▪ Peaks generally antiphasic with opaque zones ▪ Peaks show no consistent alignment with opaque or translucent zones
Peaks and troughs in elemental concentrations apparent for only part of transect	▪ Variation only for first-formed increments ▪ Variation only for latter-formed increments ▪ Lack of annual variation in middle of otolith ▪ Annual variation only in middle
No annual variation	▪ Periodic variation exists but not annual ▪ Lack of obvious peaks and troughs

In the second approach for considering the within-region trends, the average concentration for each annual increment was calculated for each individual. Since there were 9 years of growth represented in each otolith, nine annual means were calculated for each otolith. This was done to facilitate the quantitative comparison of concentration profiles between fish from within and among different regions. An example of the alignment of the chemical and optical profiles, and the estimation of average elemental concentrations is provided in Fig. 3.3.

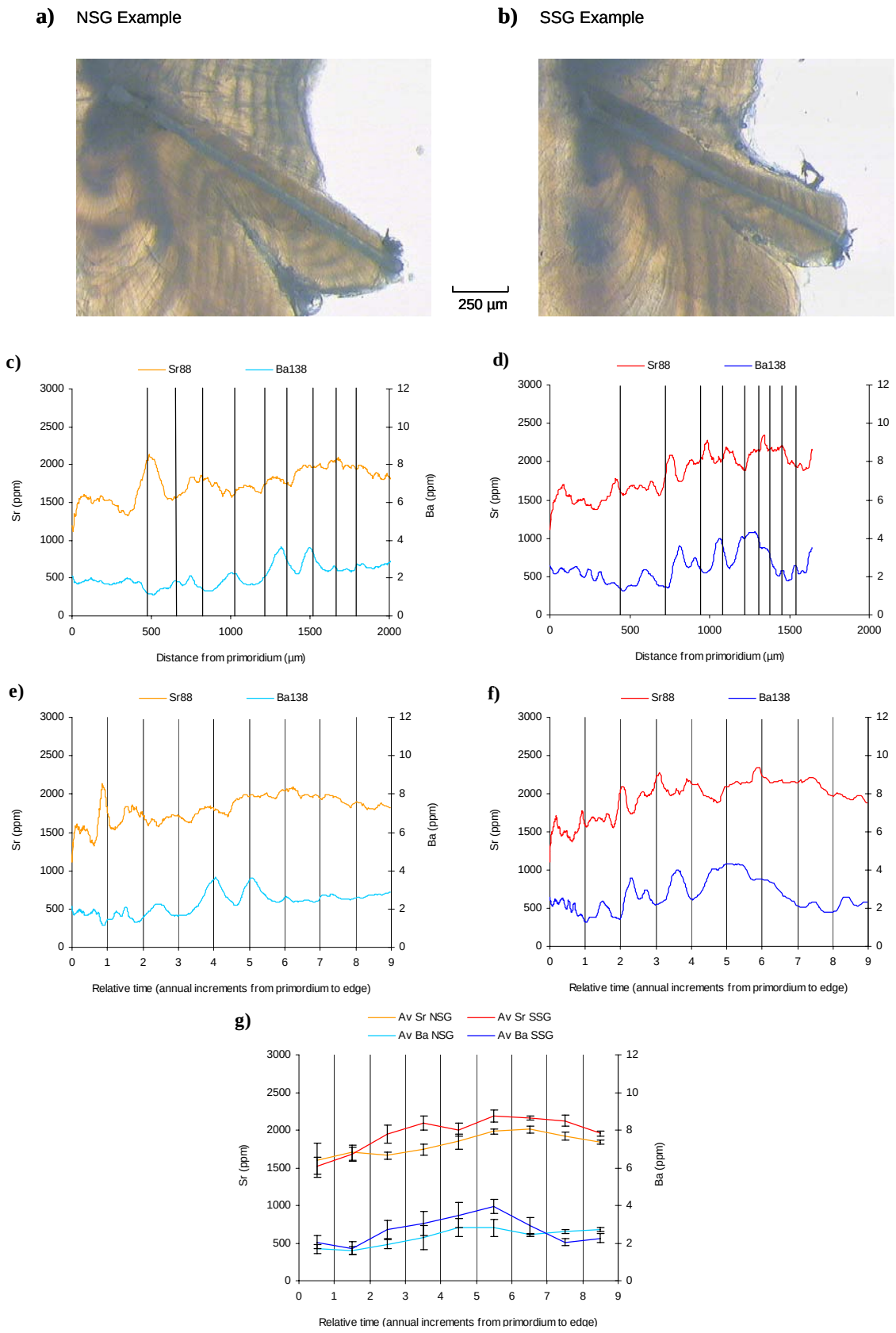

Figure 3.3 Examples of the results obtained from LA ICP-MS analysis of transverse sections of Axis 3 of *Pagrus auratus* otoliths: NSG *(a)* and SSG *(b)*. Barium and strontium concentration profiles for each individual *(c-d)* and with profiles proportionally adjusted to increment widths *(e-f)*. Average strontium and barium for each increment for the two individuals compared on the one graph *(g)*. The vertical lines indicate the location of opaque zones along the profiles.

The between-region comparisons involved repeated measures ANOVAs, MANOVAs, discriminant function analyses and maximum likelihood analyses of regional averages. The widths of successive growth increments are often auto-correlated, as the fastest growing fish in the earlier years tend also to be the fastest in subsequent years (Chambers and Miller 1995). In addition, multiple increment width and trace element concentration measurements from a single individual are likely to be longitudinal data and hence interdependent. This is unlikely to be the case for chemical data as the concentration of an element in one year is unlikely to be influenced by that in the preceding year, but will depend more on the location of the fish in the different years. Thus, repeated-measures ANOVA and MANOVA tests were used to compare individual growth increment profiles and average trace element concentration profiles over the nine growth increments between fish from the six geographic regions. The null hypothesis was that individual increment width and average concentration profiles do not vary between regions of capture. SPSS general linear models (GLM) was used for the main analysis. The three dependent response variables tested were increment width (IncW), average strontium concentration ($Av^{88}Sr$) and average barium concentration ($Av^{138}Ba$), with nine levels for each corresponding to the nine annual increments, as numbered from 1 to 9 from the otolith core to the outer edge. All three variables required transformation to satisfy the assumptions of normality and homogeneity of variance. IncW and $Av^{88}Sr$ were log transformed according to $\log_{10}(X+1)$ and $Av^{138}Ba$ was inverse transformed according to $1/(X+1)$.

Mauchly's test for sphericity was violated for all three variables so the Huynh and Feldt correction computed in SPSS GLM, based on a similar correction by Box and Cox (1964), was used to adjust F-critical values to protect against inflated Type I errors in repeated-measures ANOVA tests and the results of these were compared with those of repeated-measures MANOVA tests to ensure outcomes were reliable. In the latter case Wilk's Λ was used as the test statistic. As sample sizes varied between increments and regions, Tukey's honestly significant difference (HSD) tests were used for post hoc multiple comparisons of means between groups.

Linear discriminant function analysis (DFA) was used to assess whether the region of capture of individuals could be reliably predicted from the trajectories of increment width and average trace element concentrations for the nine increments. Due to the large number of predictors (3 variables by 9 levels each), which exceeded the number of samples in the smallest group, step-wise DFA was used to determine the most important predictors for the discrimination of group membership (Tabachnik and Fidell 2001). The entry of predictors to the analysis was determined by a statistical entry criterion of Wilks' Λ with a p(entry) = 0.05 and p(removal) = 0.25. The data were also analysed separately for each increment to assess changes in the pattern of group membership prediction with increasing age. For these analyses a standard DFA was used, such that all three variables were included for each increment. All DFA were completed using SPSS Version 10.

Maximum likelihood based analyses were also done to determine the ability of the increment widths and average trace element concentration data for the nine increments to estimate correctly the proportion of individuals from each region. A multipurpose simulation-bootstrap-analysis program was used for these analyses (Millar 1990), with each variable analysed separately. The program was run in simulation mode for 1000 simulations to estimate the variability of the estimator. The absolute error was estimated as the difference between the actual and known contributions of individuals to each region and the estimated contribution.

3.3 Results

3.3.1 The sampling and analysis protocol

The TS section of one sagitta from each of five fish were each sampled from the core to the otolith edge along 4 different axes. Of the eleven different isotopes measured along these axes the estimated concentrations for ^{52}Cr, ^{55}Mn, ^{59}Co, ^{63}Cu, ^{64}Zn, ^{114}Cd and ^{208}Pb were often below the detections limits of the analyses, and concentration profiles along the different axes were not similar (Fig. 3.4), suggesting that the data were not reliable. When the trajectories for ^{25}Mg were compared between axes some displayed numerous spurious and inconsistent peaks that were difficult to interpret, and so were also thought to be unreliable (Fig. 3.4). In contrast, the concentration profiles for each of ^{88}Sr and ^{138}Ba along the four different axes gave similar trends, suggesting that the peaks and troughs represented real variation in the chemical composition (Fig. 3.5). Consequently, these were the only two elements that were considered sufficiently reliable for further analysis.

The exercise of comparing between Axes 2-5 for the five TS sections also revealed that sampling with LA-ICPMS along otolith transects was time consuming, and therefore quite expensive. Consequently, since Axis 5 was the longest axis sampled, thus requiring the most sampling time to reveal the same information that appears to be evident along shorter axes, it was excluded from further consideration. Furthermore, earlier ageing work had shown that the optical structure along Axis 2 was ambiguous for ageing work and so it also was excluded. Finally, since the incremental structure along Axis 3 is oriented more perpendicular to the axis of sampling than is the case for Axis 4, making it easier to relate the chemical composition to time and fish age, then Axis 3 was chosen as the preferred one for chemical analysis using transect-based LA-ICPMS. This axis is relatively short, the increments are generally well-defined and are essentially oriented perpendicular to the axis of sampling. As such all results presented in the remainder of this chapter relate to sampling otoliths using LA-ICPMS along Axis 3.

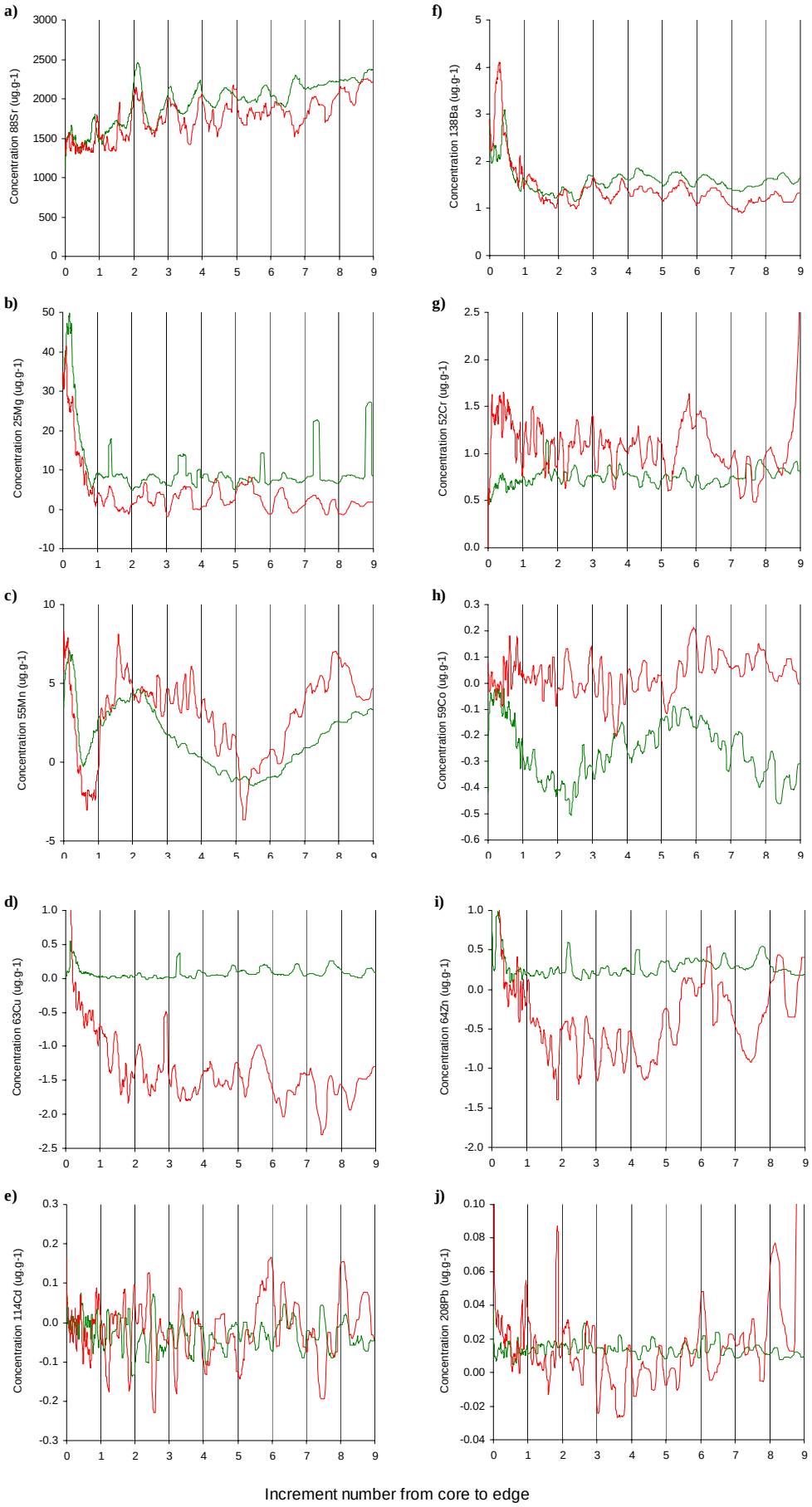

Figure 3.4 Isotopic concentration profiles for two transects along different growth axes of the same otolith from NSG plotted against relative time according to annual increments. Transects along axis 2 are indicated in red and along axis 3 in green.

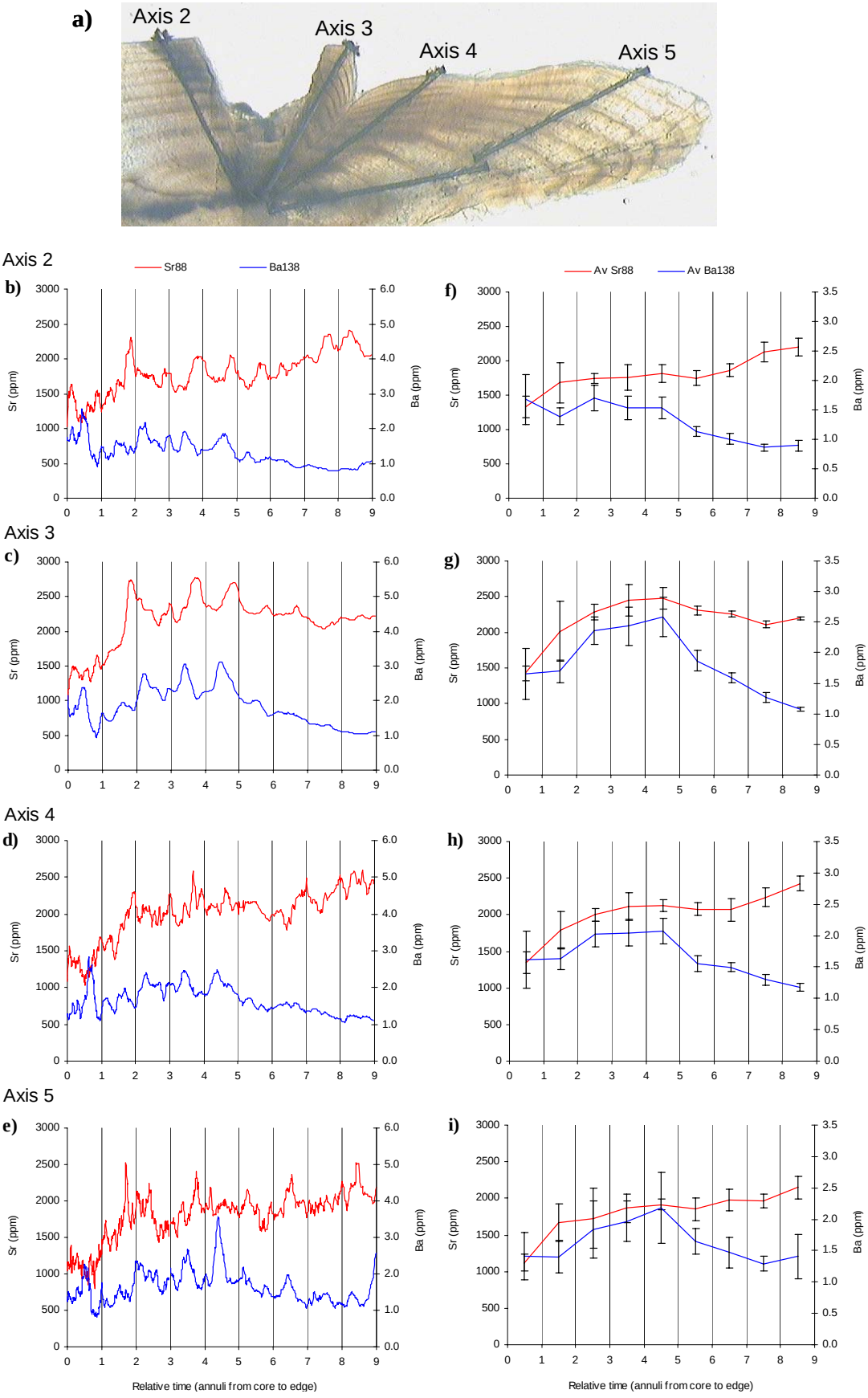

Figure 3.5 Transverse section of an otolith from the NSG region with 4 ablated transects along different axes *(a)*. For each axis, strontium and barium profiles plotted against relative time according to annual increments *(b-e)* and average strontium and barium for each increment *(f-i)*.

3.3.2 Trace Element Profiles – ^{88}Sr

The profiles for ^{88}Sr and ^{138}Ba concentration against age are presented for all otoliths in their different regions in the appendix (Chapter 9). The profiles for ^{88}Sr showed several different levels of variation. Firstly, most otoliths showed some level of systematic, sinusoidal variation with annual periodicity, indicating considerable within-year variation in the concentration of ^{88}Sr. There was also variation in the amplitude of the peaks and troughs across the transects, relating to between-year variation in the otolith concentration of ^{88}Sr. In some otoliths those peaks and troughs were discernible for all nine years considered along the length of the transect, but in others they were evident for only part of the length of the transect. There was also some variation with respect to alignment of peaks and troughs with the opaque and translucent zones.

The systematic, sinusoidal variation for ^{88}Sr was also superimposed on a larger scale trend. In some otoliths the trend was for an increasing concentration of ^{88}Sr with age, in others it was relatively flat, whilst in others the trend was for a decrease during the latter years of the life of the fish. This trend is evident in the age-related annual averages for ^{88}Sr when plotted against age (Fig. 3.6).

Within-region patterns

For NSG the largest peaks in ^{88}Sr tended to be associated with the younger age classes with some concentrations approaching 3000 $\mu g.g^{-1}$ (Chapter 9, Fig. A.1). The lowest troughs generally corresponded to the first one or two years, occasionally falling below 1500 $\mu g.g^{-1}$. NSG had the highest proportion of otoliths for which the peaks and troughs were discernible across the full transect length representing the nine years of growth (Table 3.2). For most such otoliths the peaks in ^{88}Sr aligned with the opaque zones, but there were others for which the peaks were associated with translucent zones. For several otoliths the annual variation flattened out after the first 3 – 6 annuli, and was not apparent along the whole transect length. However, for one otolith (Chapter 9, Fig. A.1, no. t), there was no apparent annual variation in the level of ^{88}Sr.

For seven otoliths there was a gradual trend of increasing concentration of ^{88}Sr with age, with most otoliths reaching the concentration of 2000 $\mu g.g^{-1}$ or greater by the 9^{th} year. The age-related annual averages for the different otoliths fanned out after a fairly uniform start (Fig. 3.6*a*). For most otoliths the concentration of ^{88}Sr either increased or was steady. However, two otoliths that showed little sinusoidal variation for the latter years demonstrated a decrease in concentration through these years.

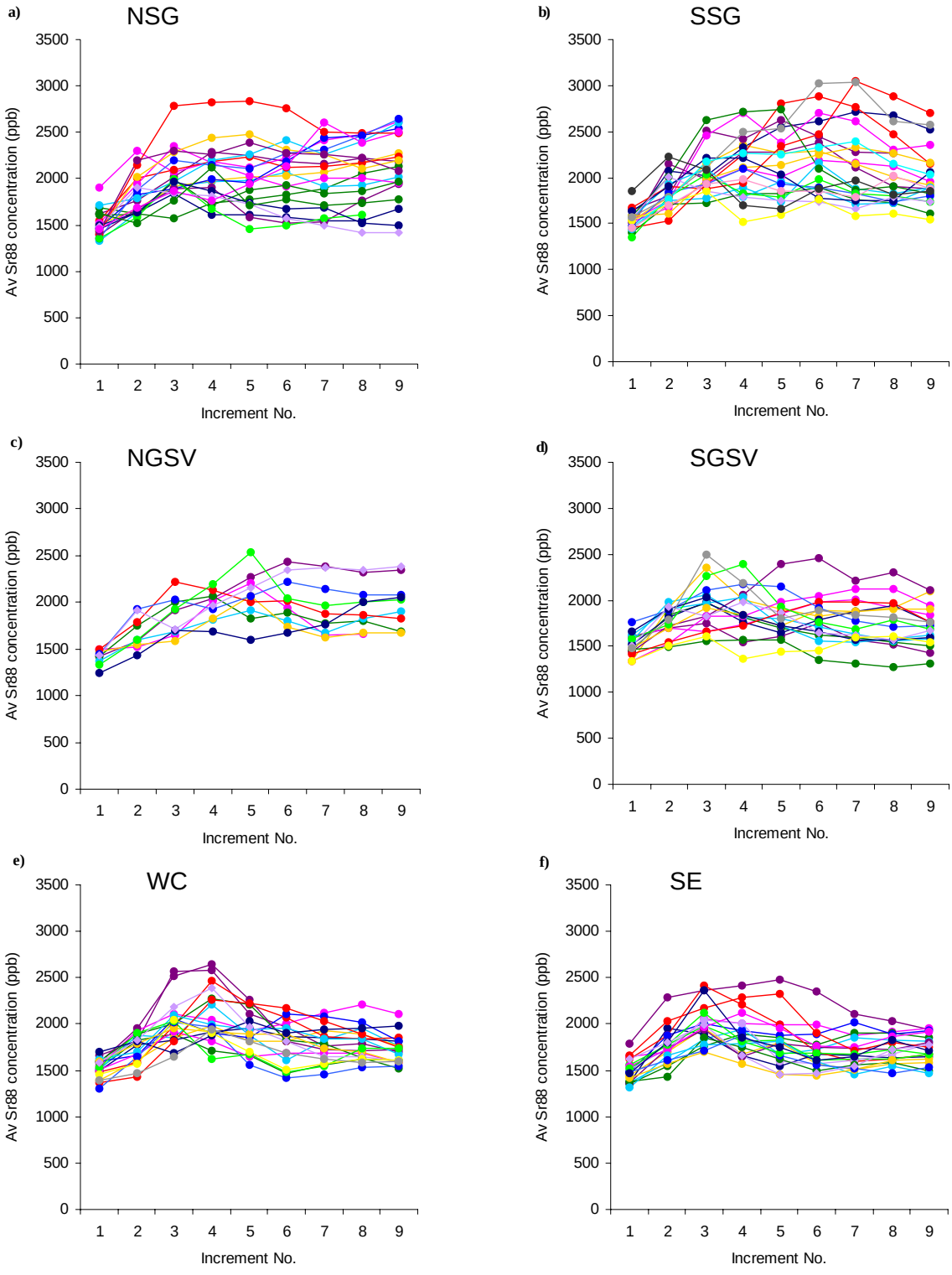

Figure 3.6 Profiles of average strontium concentration for each increment from the core to outer edge for each individual within each region.

Table 3.2 Results showing the number of otoliths from each region that were sampled for elemental concentrations, for which the pattern of variation in ^{88}Sr along Axis 3 was assigned to the various nominated categories.

Category	NSG	SSG	NGSV	SGSV	WC	SE
Annual variation across whole transect:	**10**	**5**	**4**	**3**	**2**	**2**
Peaks correspond with majority of annuli	5	2	4	-	-	-
Peaks generally antiphasic with annuli	2	1	-	1	2	-
Mixture of phasic and antiphasic	3	2	-	2		2
Annual variation across only part of transect:	**8**	**15**	**5**	**16**	**17**	**15**
In first part of transect	7	9	3	15	14	15
In latter part of transect	-	-	-	-	-	-
In first and latter part but not middle	1	6	2	1	1	
Only in middle					2	
No annual variation:	**2**	**2**	-	-	**1**	**3**
Periodicity is evident, but not annual	1	-	-	-	1	3
No obvious peaks and troughs	1	2	-	-	-	-
Total	**20**	**22**	**9**	**19**	**20**	**20**

For the otoliths from SSG the concentrations of ^{88}Sr increased through the first and second years, after which sinusoidal annual periodicity became apparent, and was evident for at least part of the remaining transect length. Compared with NSG there were less otoliths for which sinusoidal annual variation was consistent across the entire transect length, and more for which it broke down in the middle of the transect (Table 3.2). Two otoliths showed no obvious annual variation. There were two types of overall trends on which the annual periodicity was superimposed. For some otoliths the concentrations of ^{88}Sr levels remained relatively flat for the duration of the lifetime (Fig. 3.6*b*). For others the levels of ^{88}Sr showed a general rise and fall over a number of years.

For NGSV only 10 TS-sections were available for analysis. All concentration profiles for ^{88}Sr showed sinusoidal annual variation along at least part of the transect length. For four otoliths the peaks largely corresponded with the opaque zones across the length of the transect, whilst for the remainder the profile flattened out for either the middle or latter-formed increments (Table 3.2). For most otoliths the trend was quite flat from the fourth increment onwards (Fig. 3.6*c*).

For SGSV the highest variation in peaks and troughs was generally associated with the first few annuli. Systematic annual variation across whole transects was apparent only for three otoliths, whilst for the majority the annual variation was restricted to the first 2-5 increments (Table 3.2). The overall trends tended to be either flat or decreasing back to approximately 1500 $\mu g.g^{-1}$ (Fig. 3.6d).

For the WC, part or full annual periodicity was evident for most otoliths, with concentrations largely varying between 1500-2000 $\mu g.g^{-1}$. Maximum concentrations were generally attained around the time of formation of the second or third annuli, after which they declined considerably, with some trajectories becoming flat. Of 20 otoliths, only two showed consistent systematic annual variation across the whole transect (Table 3.2), with most of the remainder displaying annual variation for up to the 6th opaque zone, after which the trajectory either flattened out or did not vary systematically (Fig. 3.6e).

For otoliths from the SE the maximum peak for ^{88}Sr generally corresponded with the 2nd and 3rd opaque zones and then declined. Most otoliths only showed systematic variation for up to the 6th increment, after which the profiles flattened out. For two otoliths there was annual variation across the whole transect, whilst three others showed no annual periodicity (Table 3.2). The overall trend in this region was generally quite flat, particularly for the last five increments (Fig. 3.6f).

Inter-regional comparison for ^{88}Sr

As is evident from the descriptions above, there was considerable variation between otoliths in the profiles for ^{88}Sr. To determine whether there was evidence for regional differences the numbers of otoliths from each region that were classified to each of the three main categories described in Table 1 were compared using a Chi-squared contingency table test. The result ($X^2 = 19.46$, df = 10, p= 0.0348) indicates a significant regional difference in the frequencies between the categories. Both NSG and NGSV had the highest proportions of otoliths with systematic variation across the entire otolith with the remaining regions having higher proportions of otoliths with annual patterns discernible across only part of the transect lengths. Only few otoliths showed no annual variation in concentration of ^{88}Sr.

3.3.3 Trace Element Profiles – ^{138}Ba

Within-region patterns

The trajectories for ^{138}Ba demonstrated two levels of variation. Firstly, many otoliths displayed systematic, sinusoidal variation with an annual periodicity similar to that described for ^{88}Sr. This variation was often manifested as small within-year variation between peaks and troughs. In most cases the peaks in ^{138}Ba were aligned with the translucent zones of the annuli. There was also considerable variation across some otoliths in the relative sizes of peaks and troughs. In some cases this inter-annual variation involved substantial peaks that were several times the concentration of adjacent peaks, and an order of magnitude variation across a transect. Occasionally some peaks extended across several different years.

In NSG the barium concentrations were characteristically low, i.e. generally <3 $\mu g.g^{-1}$, only exceeding 5 $\mu g.g^{-1}$ in one otolith. Also, for another there was a pattern of annual periodicity consistent across the whole otolith. For nine otoliths there was some annual variation apparent for either the first-formed or the latter-formed annuli (Table 3.3). For half the otoliths there was no perceptible annual variation in concentration of ^{138}Ba. The plots of the trajectories of age-related annual averages for all otoliths were generally around 2 $\mu g.g^{-1}$ (Fig. 3.7a).

For SSG the barium concentrations were perceptibly higher and more variable than for NSG. Only for one otolith was annual periodicity discernible across the length of the whole transect (Table 3.3). For 12 otoliths, however, the periodicity in barium concentration did not conform to an annual time period. Occasionally there were substantial peaks (Chapter 9, Fig. A.2, nos. n,s,t) for which the ascending and descending arms of the peaks encompassed several years of otolith growth. The annual averages displayed far greater variation, particularly for the 6th increment, than was the case for NSG (Fig. 3.7b).

The general level of ^{138}Ba in most otoliths from NGSV was low, with several showing particularly low variation. For several otoliths there was evidence for annual variation along either part or the whole transect (Table 3.3), including two otoliths (Chapter 9, Fig. A.3, nos. g,h) for which there was a large peak in the translucent zone of the fourth increment. For three otoliths there were several substantial peaks in barium in the first annulus, after which there was only very minimal variation apparent. The annual means indicate that three otoliths display variable between-year patterns compared to the remainder which are largely invariant (Fig. 3.7c).

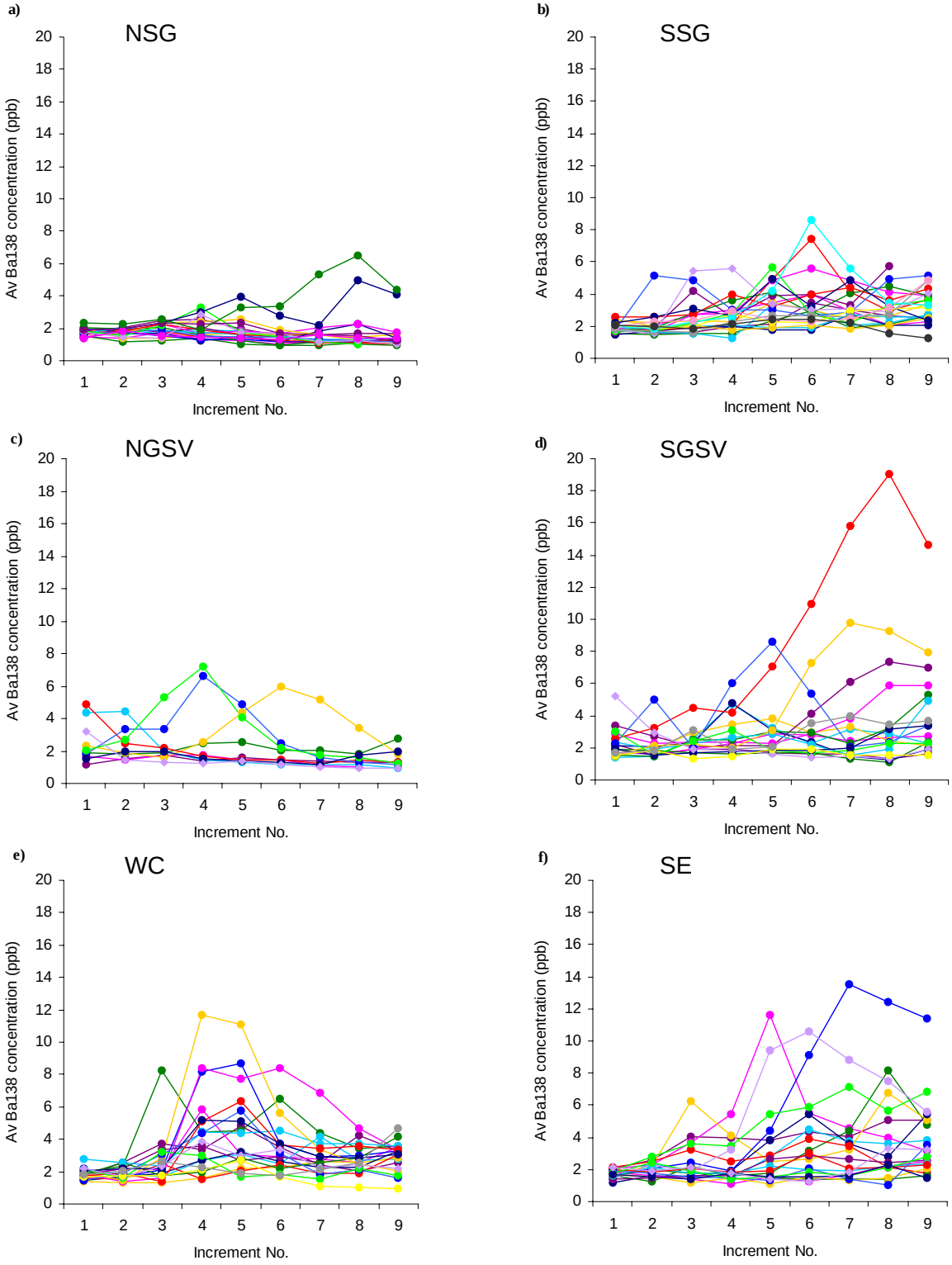

Figure 3.7 Profiles of average barium concentration for each increment from the core to outer edge for each individual within each region.

Table 3.3 Results showing the number of otoliths from each region that were sampled for elemental concentrations, for which the pattern of variation in ^{138}Ba along Axis 3 was assigned to the various nominated categories.

Category	NSG	SSG	NGSV	SGSV	WC	SE
Annual variation across whole transect:	**1**	**1**	**2**	**7**	**2**	**8**
Peaks correspond with majority of annuli	-	-	-	-		
Peaks generally antiphasic with annuli	1	-	-	5	2	7
Mixture of phasic and antiphasic		1	2	2		1
Annual variation across only part of transect:	**9**	**9**	**2**	**5**	**7**	**4**
In first part of transect	7	1	2	2	5	
In latter part of transect	2	3		1	2	2
In first and latter part but not middle		5		2		2
Only in middle						
No annual variation:	**10**	**12**	**5**	**7**	**11**	**8**
Periodicity is evident, but not annual	1	12	3	6	10	6
No obvious peaks and troughs	9		2	1	1	2
Total	**20**	**22**	**9**	**19**	**20**	**20**

Barium concentrations in SGSV were generally higher than those from NGSV, and were manifested in a range of different patterns across the transects (Table 3.3). Numerous otoliths showed annual variation along the entire transect length, three of which (Chapter 9, Fig. A.4, nos. a,h,k) shared a pattern of high barium that crossed the last four annuli. Five otoliths displayed annual periodicity along only part of the transect, whilst seven displayed variation that was not annual, including one otolith that had a peak in the first year that was similar to that described above for NGSV. The age-related annual averages demonstrated considerable variation compared to those from the two northern gulfs (Fig. 3.7d).

Barium concentration in the otoliths from the WC was relatively high. Seven otoliths displayed annual variation for at least part of the transect (Table 3.3). For numerous otoliths, however, the variation was less systematic showing no annual periodicity. Some had significant peaks in barium that were substantially above the background levels of 2-4 $\mu g.g^{-1}$. For example, each of five otoliths (Chapter 9, Fig. A.5, nos. c,j,k,o,q) displayed bimodal peaks that encompassed the 4^{th} and 5^{th} increments. These otoliths are clearly evident in the figure showing the annual averages (Fig. 3.7e).

For otoliths from the SE the concentrations of ^{138}Ba displayed considerable variation. For some there was an annual periodicity where the maxima corresponded to the translucent zones (Table 3.3). For the majority of the remainder the barium levels did not vary annually. This included some for which there were large variations in concentrations that encompassed several annuli. For four otoliths (Chapter 9, Fig. A.6, nos. e,g,q,s) there were substantial increases in concentration of ^{138}Ba for the last 2 annuli processed.

Inter-regional comparison for ^{138}Ba

The numbers of otoliths from each region that were classified to the three main categories in Table 3.3 were compared using a Chi-squared contingency table test. The result ($X^2 = 17.46$, df = 10, p= 0.0648) indicates no significant difference at the 0.05 level of significance..

3.3.4 Profiles of Increment Widths along Axis 3

To characterise otolith growth along Axis 3 the increment width profiles were measured for each fish and are presented by region in Figure 3.8. The first growth increment consisted of the opaque larval and juvenile core region and the first relatively narrow translucent zone. The subsequent increments were measured between the outer edges of the consecutive opaque zones, thereby encompassing a single, wide translucent zone. The width of the first increment varied considerably amongst individuals, particularly those from NSG and SSG, but less so in NGSV and SGSV (Fig. 3.8). After this there was an exponential decrease in increment width that occurred in every region. The difference in width between the first and second increments was less distinct for NGSV, but was most obvious for WC (Fig. 3.8). Overall, there was quite substantial variation in the width of increments between individual otoliths from each region for any given year.

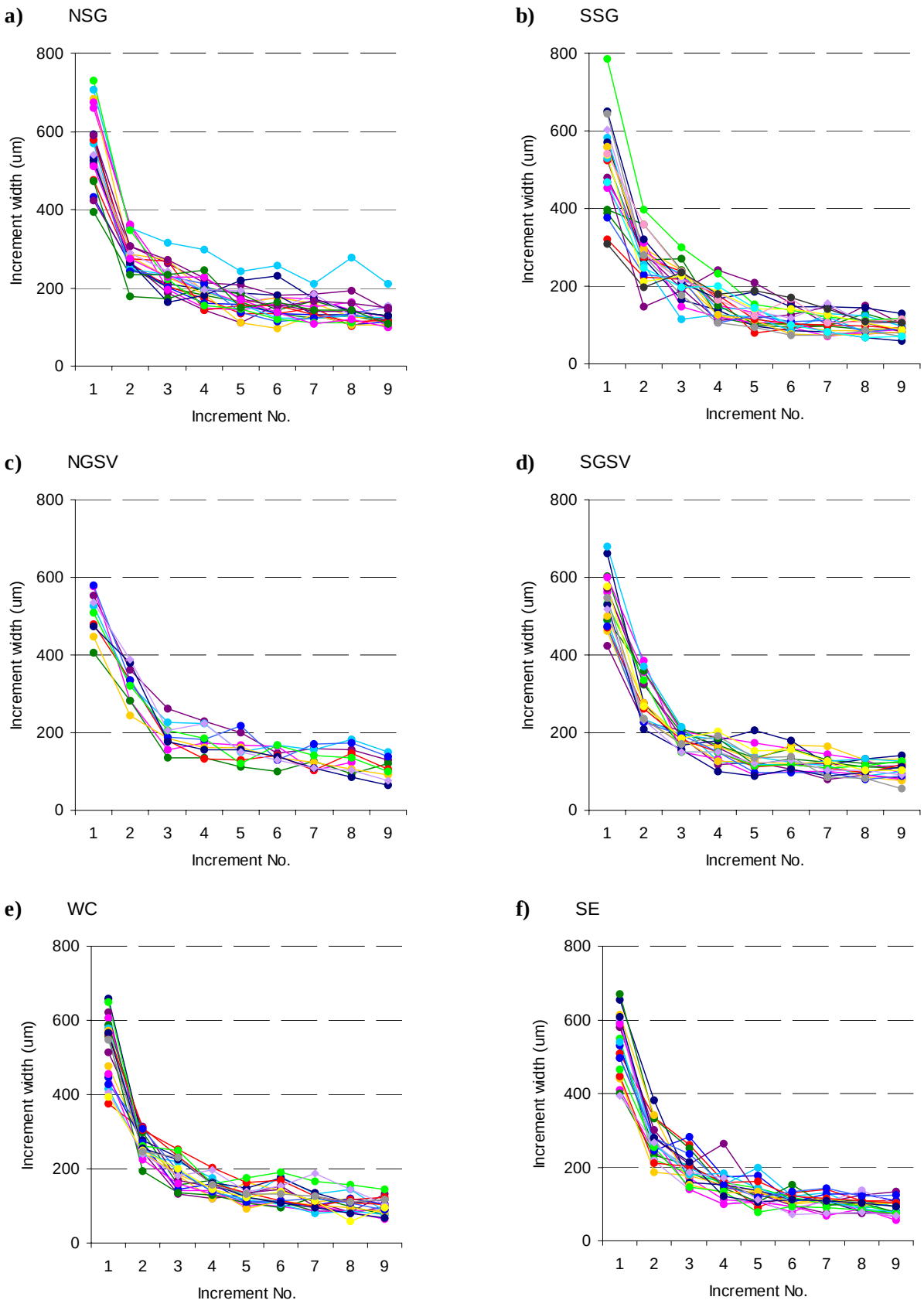

Figure 3.8 Increment width patterns across the nine increments from the core to outer edge for each individual within each region.

3.3.5 Regional variation in profiles

Single Variable Analyses

The age-related mean values for each of ^{88}Sr and ^{138}Ba for each fish (i.e. Figs 3.6, 3.7) and age-related increment widths constituted the basic data that were then considered in the statistical analyses. These results were compared amongst regions independently for each parameter, using a repeated measures ANOVA, a repeated measures MANOVA and a stepwise discriminant function analysis.

The average increment widths differed significantly amongst regions indicating spatial variation in the growth rates and development of the otoliths along Axis 3 (Table 3.4). The two northern gulf regions had higher mean increment widths for increment numbers 4 to 9, compared to those from the other four regions (Fig. 3.9a, Table 3.4). These differences were also apparent in the canonical variate plot, where the otoliths from NSG and NGSV separated from those of the other four regions according to Function 1, which was mainly influenced by the IncW of increment numbers 2, 5 and 8. There was also some separation amongst the latter four regions according to Function 2, mainly determined by increment numbers 5 and 6 (Fig. 3.9b).

The repeated measures ANOVA and MANOVA for annual averages for ^{88}Sr also indicated significant differences amongst regions (Table 3.5). The post hoc tests showed some overlap in the means between regions, but identified significant separation between SSG and both SGSV and the SE. The means were quite similar for increment numbers 1 – 3 after which they diverged, particularly for increment numbers 5 – 9 (Fig. 3.9c). The canonical variate plot shows separation between two groups of regions for Function 1 (Fig. 3.9d). The first group includes the three gulf regions of SSG, NSG and NGSV which have the higher levels of ^{88}Sr for increments 5 – 9, compared with the remaining regions of SGSV, WC and SE that have the lower strontium levels.

The repeated measures ANOVA and MANOVA for ^{138}Ba also indicated significant regional differences, from which the post hoc tests identified two distinct groups of regions (Table 3.6). The levels of barium were similar between regions for the first three increments, but were quite different for the remaining six increments (Fig. 3.9e). The canonical variate plot clearly shows the separation of NSG and NGSV from the other regions for Function 1, whilst there is also some separation amongst the remaining regions for Function 2 (Fig. 3.9f).

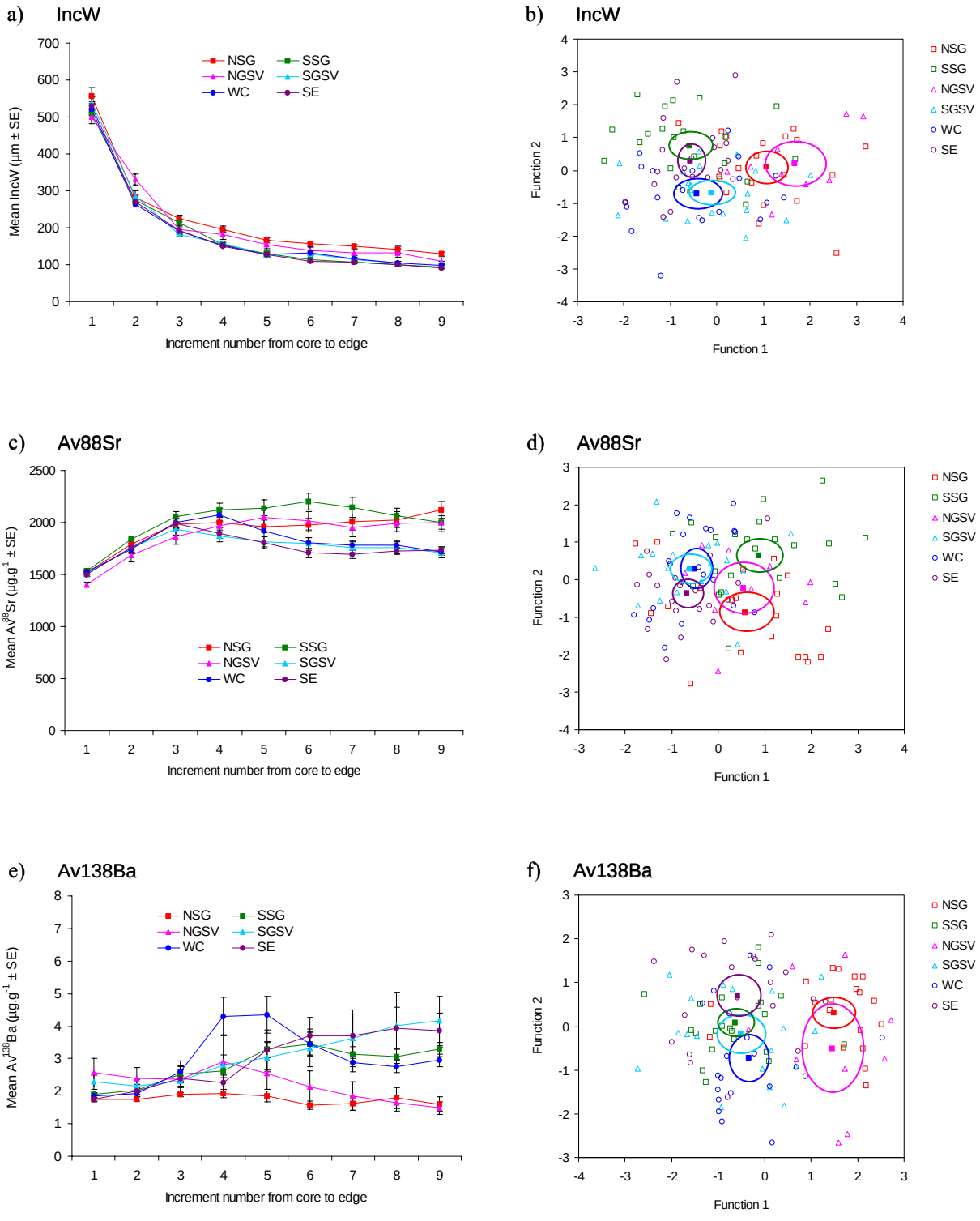

Figure 3.9 Profiles and step-wise discriminant function analyses results for the mean values IncW *(a)*, $Av^{88}Sr$ *(b)* and $Av^{138}Ba$ concentration *(c)* for the nine increments from the core to the outer edge of the otolith section, for each of the six geographic regions.

Table 3.4 *Top:* Results of repeated-measures ANOVA test (with Huynh-Feldt adjustment for non-sphericity) and repeated-measures MANOVA test for differences between the profiles of IncW across the nine increments and between the six geographic regions. *Bottom:* Results of Tukey's HSD post-hoc multiple comparison tests between regions for the profiles of IncW across the 9 increments.

Source of variance	F	df	P>F	Wilks' Λ	F	df	P>F
Between subjects effects:							
Region	10.87	5	0.000*				
Within subjects effects:							
Increment No	1073.6	6.5	0.000*	0.017	696.8	8,97	0.000*
Increment No * Region	2.92	32.5	0.000*	0.398	2.50	40,426	0.000*

Region	n	Subset A	Subset B
NSG	21	A	
SSG	22		
NGSV	10	A	B
SGSV	19		B
WC	20		B
SE	20		B

Table 3.5 *Top:* Results of repeated-measures ANOVA test (with Huynh-Feldt adjustment for non-sphericity) and repeated-measures MANOVA test for differences between the profiles of $Av^{88}Sr$ across the nine increments and between the six geographic regions. *Bottom:* Results of Tukey's HSD post-hoc multiple comparison tests between regions for the profiles of $Av^{88}Sr$ across the 9 increments.

Source of variance	F	df	P>F	Wilks' Λ	F	df	P>F
Between subjects effects:							
Region	6.04	5	0.000*				
Within subjects effects:							
Increment No	91.08	3.5	0.000*	0.142	73.49	8,97	0.000*
Increment No * Region	4.64	17.3	0.000*	0.364	2.78	40,426	0.000*

Region	n	Subset A	Subset B
NSG	21	A	B
SSG	22	A	
NGSV	10	A	B
SGSV	19		B
WC	20		B
SE	20		B

Table 3.6 *Top:* Results of repeated-measures ANOVA test (with Huynh-Feldt adjustment for non-sphericity) and repeated-measures MANOVA test for differences between the profiles of $Av^{138}Ba$ across the nine increments and between the six geographic regions. *Bottom:* Results of Tukey's HSD post-hoc multiple comparison tests between regions for the profiles of $Av^{138}Ba$ across the 9 increments.

Source of variance	F	df	P>F	Wilks' Λ	F	df	P>F
Between subjects effects:							
Region	10.37	5	0.000*				
Within subjects effects:							
Increment No	8.93	4.4	0.000*	0.675	5.83	8,97	0.000*
Increment No * Region	6.06	21.8	0.000*	0.361	2.80	40,426	0.000*

Region	n	Subset A	Subset B
NSG	21	A	
SSG	22		B
NGSV	10	A	
SGSV	19		B
WC	20		B
SE	20		B

Multiple Variable Analyses – Discriminant Function Analyses

The first discriminant analysis was done on the annual estimates of the concentrations of ^{88}Sr and ^{138}Ba after appropriate transformations (Table 3.7). Five discriminant functions were described with a combined χ^2 value of 152.2 ($df = 25$, $p < 0.001$) and five variables were included in the functions. The first two functions accounted for 54.9% and 18.9%, respectively, of the between-group variability.

The first discriminant function separated the two northern Gulfs from the other four regions, whilst the second function discriminated SSG from the remaining three regions (Fig. 3.10). The parameters that contributed most to the separation amongst regions for Function 1 were the $Av^{138}Ba$ concentration of the 9th increment and $Av^{88}Sr$ concentration of the 6th increment. The latter was also the variable that contributed most strongly towards separating SSG from the other three regions for Function 2 (Table 3.7).

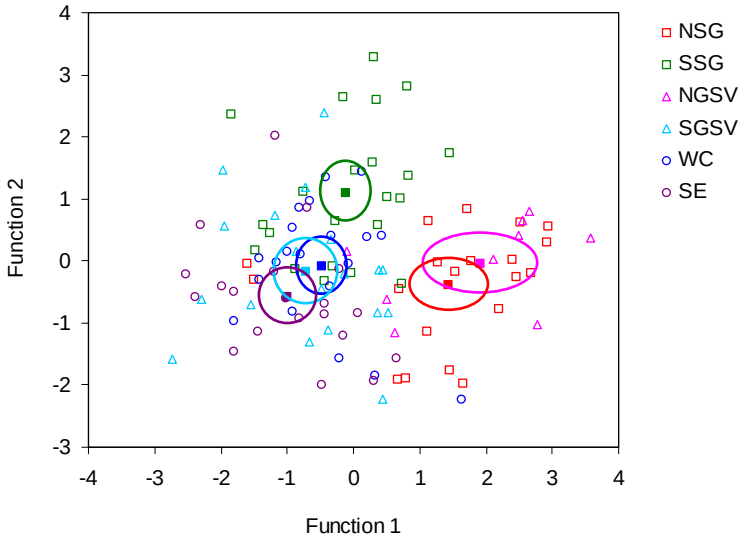

Figure 3.10 Scatterplot of the first two discriminant function scores for snapper otoliths resulting from a step-wise discriminant function analysis using the nine $Av^{88}Sr$ variables and nine $Av^{138}Ba$ variables. Group centroids with 95% CI are also indicated for each region.

Table 3.7 Pooled within-groups correlations between discriminating variables (in order of inclusion) and standardized canonical discriminant functions for the two-by-nine trace element profile variables.

Variable	Function 1	Function 2	Function 3	Function 4
InvBa9	0.929	-0.302	-0.125	0.323
LogSr6	0.534	0.862	0.158	-0.022
InvBa4	0.005	-0.192	0.694	-0.745
InvBa1	-0.091	-0.072	0.667	0.667
LogSr1	-0.287	-0.021	0.533	0.395
% of variance	54.9	18.9	15.4	9.4

Table 3.8 Classification success of step-wise discriminant function analysis with the two-by-nine trace element profile variables. The data presented are the percentage of otoliths from the region of origin (column) classified to each of the six regions (rows).

Region	NSG	SSG	NGSV	SGSV	WC	SE
NSG	65	4.5	11.1	5.3	5	5
SSG	0	63.6	0	10.5	5	5
NGSV	25	0	55.6	0	0	0
SGSV	10	18.2	22.2	26.3	10	10
WC	0	4.5	0	21.1	65	10
SE	0	9.1	11.1	36.8	15	70

The analysis resulted in the correct classification of only 58% of all otoliths to the region from which the fish were sampled. A total of 65% of otoliths from NSG were correctly classified, whilst the majority of the remainder were classified to NGSV (Table 3.8). Of the 36.4% of individuals from SSG that were misclassified most were allocated to other southern regions, with only 4.5% allocated to the northern gulfs. Half the otoliths from NGSV were correctly classified, whilst the remainder were allocated to the other northern gulf region, the neighbouring SGSV and the SE. SGSV had the lowest proportion of individuals correctly classified, with the remainder allocated broadly to other regions particular to NGSV and SSG, the two adjacent regions. The classification success for the WC and SE were each relatively high, and most of those misclassified were shared between the other southern oceanic regions.

The second discriminant function analysis involved the addition of the 9 IncW variables into the analysis. The step-wise discriminant analysis resulted in the description of four significant discriminant functions with a combined χ^2 value of 198.7 ($df = 40$; $p < 0.001$). Eight variables were included in the final functions (Table 3.9), the first two of which accounted for 56.4% and 19.4% respectively, of the between-group variability.

Compared to the previous analysis this one produced further separation between the two northern Gulfs as well as the SE from the other two southern regions, based primarily on Function 2 (Fig. 3.11). The main variables that influenced the second function were IncW for 3rd increment and $Av^{88}Sr$ for the 6th increment (Table 3.9).

The level of correct classification of individuals to the region from which they originated was increased to 65.5%. However, there remained considerable variation amongst regions in the classification success. Most individuals from NSG were correctly classified. For SSG, the individuals that were incorrectly classified were broadly distributed over the remaining regions. Most fish from NGSV were either correctly classified or were allocated to SGSV or the other northern gulf region. Fish from SGSV were broadly allocated amongst the three southern oceanic regions, with few to the gulf regions. For the WC and SE most fish were correctly classified, with the remainder allocated to the Spencer Gulf regions or the other shelf region.

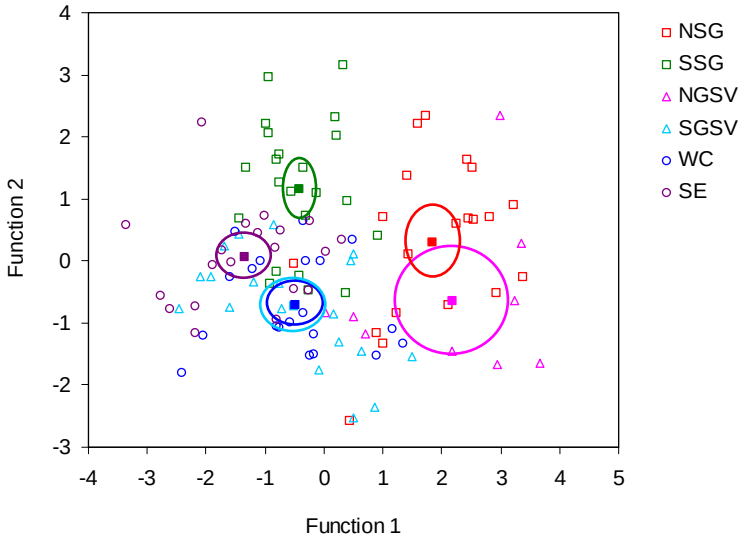

Figure 3.11 Scatterplot of the first two discriminant function scores for snapper otoliths resulting from a step-wise discriminant function analysis using all nine $Av^{88}Sr$, $Av^{138}Ba$ and IncW variables at the start. Group centroids with 95% CI are also indicated for each region.

Table 3.9 Pooled within-groups correlations between discriminating variables (in order of inclusion) and standardized canonical discriminant functions for all three-by-nine profile variables.

Variable	Function 1	Function 2	Function 3	Function 4
InvBa9	0.756	-0.120	0.078	0.158
LogSr6	0.580	0.513	-0.599	-0.075
LogInc6	0.260	-0.478	-0.285	0.502
InvBa4	0.040	0.346	0.681	-0.494
InvBa1	-0.181	0.330	0.332	0.730
LogSr1	-0.272	0.229	0.237	0.613
LogInc3	-0.094	0.595	0.042	0.220
LogInc7	0.474	0.025	0.252	-0.344
% of variance	56.4	19.4	12.8	8.6

Table 3.10 Classification success of step-wise descriminant function analysis with all three-by-nine profile variables. The data presented are the percentage of otoliths from the region of origin (column) classified to each of the six regions (rows).

Region	NSG	SSG	NGSV	SGSV	WC	SE
NSG	80	4.5	11.1	5.3	10	5
SSG	0	68.2	0	5.3	5	15
NGSV	5	0	55.6	5.3	0	0
SGSV	10	9.1	33.3	47.4	10	0
WC	5	9.1	0	15.8	60	5
SE	0	9.1	0	21.1	15	75

Multiple Variable Analyses – Maximum Likelihood Estimations

Overall, maximum likelihood estimators performed better at predicting the proportion of individuals from different regions than did the results from the discriminant function analyses. The maximum likelihood estimator derived from the nine IncW variables predicted the contribution of the different regions to the overall sample population to within 4.1% to 5.5% of actual contributions (Table 3.11). Actual contributions of regions were between 17.2 and 20%, except for the NGSV region, which only contributed 8.2% (Table 3.11). The highest errors in estimated contributions were associated with the classification of individuals from SGSV and WC. The variability in estimates of proportional contribution, ranged from 4.3% for NGSV to 11.5% for SE.

Table 3.11 Results of maximum likelihood analyses of the ability of the nine IncW variables to estimate correctly the proportion of individuals from different regions.

Region	Actual contribution	Estimated contribution	SD
NSG	18.2	18.0	6.7
SSG	20.0	19.6	10.0
NGSV	8.2	8.9	4.3
SGSV	17.3	22.8	11.4
WC	18.2	14.1	11.4
SE	18.2	16.7	11.5

The maximum likelihood estimator derived from the nine $Av^{88}Sr$ variables (Table 3.12) generally performed better than that resulting from the nine IncW variables. The predicted contributions of regions to the overall sample population were to within 1.6% to 4.8% of actual contributions (Table 3.12). Once again, the highest errors in estimated contributions were associated with the classification of individuals from SGSV followed by SSG. The variability in estimates of proportional contribution was similar, ranging from 4.4% for NGSV to 11.9% for SGSV.

Table 3.12 Results of maximum likelihood analyses of the ability of the nine $Av^{88}Sr$ variables to estimate correctly the proportion of individuals from different regions.

Region	Actual contribution	Estimated contribution	SD
NSG	18.2	17.0	7.0
SSG	20.0	18.5	7.3
NGSV	8.2	8.9	4.4
SGSV	17.3	22.1	11.9
WC	18.2	16.9	8.5
SE	18.2	16.6	10.6

The maximum likelihood estimator derived from the nine $Av^{138}Ba$ variables (Table 3.13) generally performed worse than the other two sets of variables. The predicted contributions of regions to the overall sample population were to within 2.6% to 4.0% of actual contributions (Table 3.13). In contrast to the other two sets of variables the highest errors in estimated contribution were associated with the classification of individuals from NSG and SSG. The variability in the estimates of proportional contribution ranged from 4.4% for NGSV to 11.5% for SSG.

Table 3.13 Results of maximum likelihood analyses of the ability of the nine $Av^{138}Ba$ variables to estimate correctly the proportion of individuals from different regions.

Region	Actual contribution	Estimated contribution	SD
NSG	18.2	22.1	6.1
SSG	20.0	24.0	11.5
NGSV	8.2	7.5	4.4
SGSV	17.3	14.7	10.4
WC	18.2	15.7	7.1
SE	18.2	15.9	8.0

When the three variables, IncW, $Av^{88}Sr$ and $Av^{138}Ba$, were analysed by discriminant function analyses for each of the nine increments separately (Fig. 3.12), similar trends as those derived from the profile analyses were obtained. For the first three increments, there was little regional variation in the three variables, the discriminant functions described were only marginally significant and poor classification resulted (Figs 3.12*a*-*c*; Table 3.14). Only NGSV separated from the other five regions, for which the centroids and 95% confidence limits were largely overlapping. However, for the fourth to ninth increments, significant regional variation was more apparent (Figs 3.12d-*h*; Table 3.14). The discriminant functions described were highly significant with better classification results. The separation patterns obtained for these latter increments were similar in appearance to that obtained for the trace element concentration data combined for all increments (Fig. 3.12).

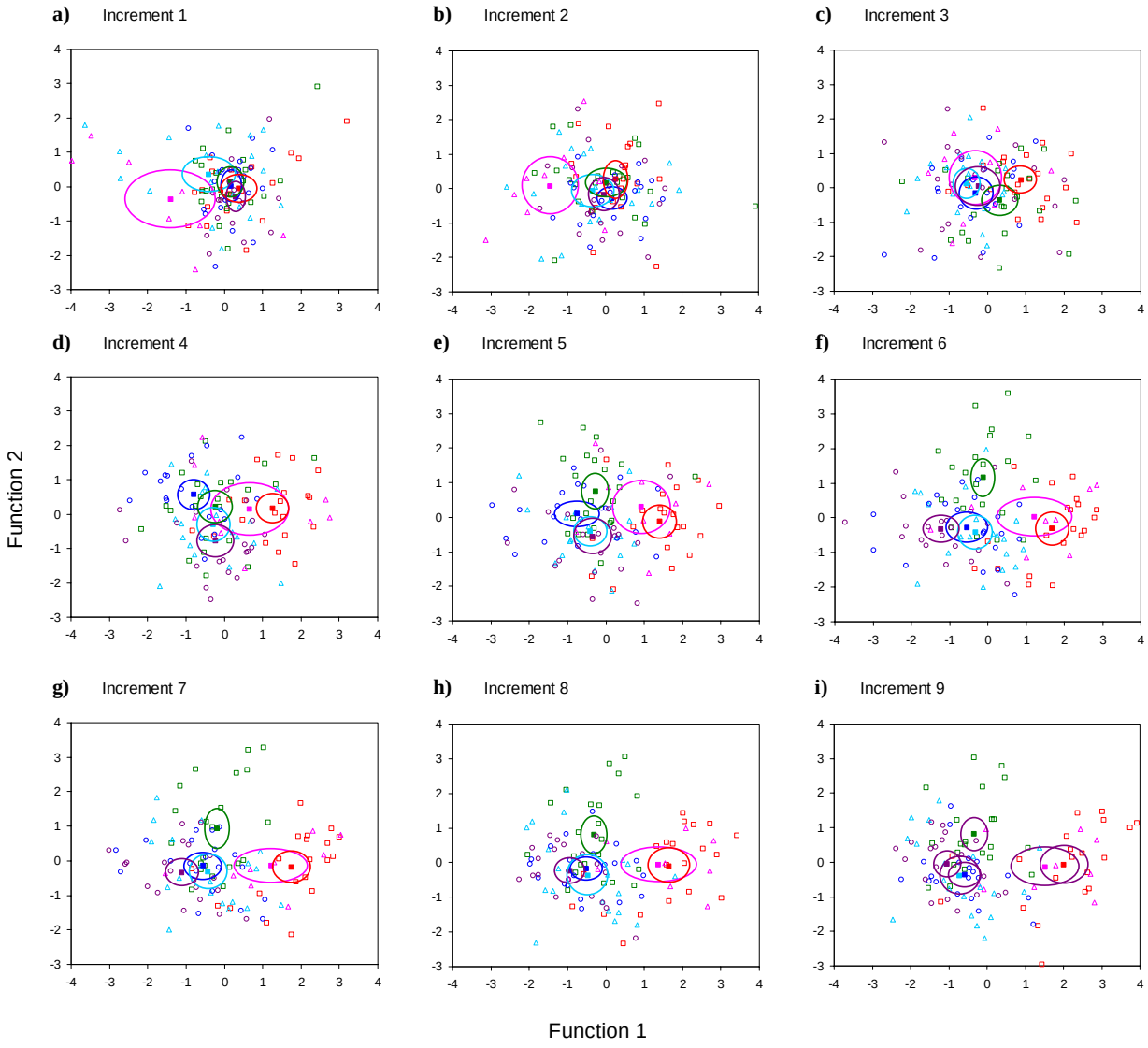

Figure 3.12 Canonical variate plots for each of the datasets for each of the nine increments analysed as separate datasets. □ = NSG, □ = SSG, △ = NGSV, △ = SGSV, ○ = WC and ○ = SE, solid squares indicate the centroid for each region, and ellipses represent the 95% confidence intervals about the centroid.

Table 3.14 Significance and classification results for ANOVA and DFA of the data for each of the nine increments analysed as separate datasets

Increment number	LogIncW Regions p	LogSr Regions p	InvBa Regions p	DFA χ^2 (1 to 3)	p	% correct classification
1	0.800	0.125	0.013	31.4	0.008	27.3%
2	0.031	0.227	0.273	26.7	0.031	23.6%
3	0.000	0.214	0.243	32.8	0.005	36.4%
4	0.000	0.023	0.000	69.1	0.000	40.9%
5	0.000	0.001	0.000	75.6	0.000	42.7%
6	0.000	0.000	0.000	116.7	0.000	49.1%
7	0.000	0.000	0.000	98.8	0.000	42.7%
8	0.000	0.000	0.000	88.1	0.000	39.1%
9	0.000	0.000	0.000	115.4	0.000	42.7%

3.4 Discussion

3.4.1 Patterns of Variation

This study was aimed at testing the migration life-history model for snapper in South Australia, and to determine the likely implications for stock structure. The fish considered in the study were all approximately the same age coming from the one strong year class that had recruited as 0+ individuals in early 1991. This year class was chosen to maximise the number of otoliths from which to select a random sub-sample for each region. The otoliths were selected from those that had been collected throughout the year of 2000, for which the 10^{th} translucent or opaque zone was being formed. The variation in chemical composition across the TS-sections of these otoliths was determined using LA-ICPMS, which was applied along transects from the otolith core to the edge along the short axis on the dorsal side of the sulcus. The results for the complete increments from the core to the 9^{th} opaque zone were considered in the statistical analyses. Thus, the same period of 9 years was considered for all otoliths, to ensure that any differences amongst otoliths related primarily to spatial differences amongst regions. Furthermore, the sampling protocol, which involved processing otoliths from all regions in random order, minimised the possibility of introducing artefacts that related to instrument drift.

Eleven different elements were sampled simultaneously, including ^{44}Ca as the internal standard. Of the remaining 10 elements, 8 were unreliable with concentrations either below the detection limits of the analysis or not reproducible along replicate transects. This left only ^{88}Sr and ^{138}Ba to be considered in the statistical analyses. The former was present at minor levels of approximately 1,500 – 2,500 $\mu g.g^{-1}$, whilst the concentration range for the latter was at trace levels of approximately 2 – 20 $\mu g.g^{-1}$. This difference of several orders of magnitude between the concentrations of the two elements is comparable to that recorded in numerous previous studies (Campana 1999).

Several levels of variability were apparent in cross-otolith profiles for both elements that relate to different temporal scales. The systematic sinusoidal variation along either complete or part transects between the core and the 9^{th} opaque zone of each otolith reflects variation within years. The peaks in strontium often coincided with the opaque zones that were generally thin discontinuities that separate broader translucent zones. The ascending and descending parts of these peaks in ^{88}Sr were associated with the translucent material on either side of the opaque zones. Thus, strontium concentrations often peaked in spring/early summer before decreasing again through summer and autumn. Conversely, the peaks in barium were generally associated with the broad translucent zones, for which the fastest growth occurred in summer and autumn (Chapter 2).

The second level of variation in concentration of both elements related to the considerable differences in amplitude of the peaks and troughs across transects, indicating considerable inter-annual variation in their rates of deposition relative to ^{44}Ca. Although the absolute concentrations of ^{88}Sr were far higher, the relative differences for ^{138}Ba between years were greater than for ^{88}Sr. There was greater variation in the timing and the width of peaks of barium, with some encompassing more than a single year of otolith growth. Other studies that involved sampling across otoliths have demonstrated considerable inter-annual variation in elemental concentrations, particularly for strontium (Sadovy and Severin 1992, 1994; Secor 1992; Fuiman and Hoff 1995).

Despite the variability in the ontogenetic elemental profiles displayed in otoliths from each region, when the age-related mean values for barium and strontium were compared between regions, significant differences were apparent. These regional differences were least evident for the first three years of the fish's lives. The regional patterns then diverged between the third and fifth increments, and then from the sixth increment onwards, consistent differences were apparent in the regional means for both ^{88}Sr and ^{138}Ba.

3.4.2 Factors Influencing Otolith Concentrations of ^{88}Sr and ^{138}Ba

The results summarised above suggest that the chemistry of otoliths from fish that were captured in different regions diverged from approximately the age of three years onwards. The question now is whether such regional differences reflect anything meaningful about the movement of individuals or the stock structure of the population. To date, numerous studies based on fish rearing experiments and field sampling have provided some insight into the processes of otolith formation and the factors that influence trace element incorporation. These studies indicate that although otolith formation is complex and is not yet fully understood, that at least some understanding of the influence of some environmental factors is emerging (Campana 1999, Bath et al. 2000, Secor and Rooker 2000, Milton and Chenery 2001, Kraus and Secor 2003).

Otoliths grow through crystallization of $CaCO_3$ in its aragonitic form from the endolymph onto the proximal surface of the otolith, i.e. the growing surface (Morales-Nin 2000). As this occurs, numerous elements are incorporated into the structure at trace and minor levels. Several experiments have identified that the majority of these ions are derived from the water that the fish occupy rather than from a dietary source (Farrell and Campana 1996, Milton and Chenery 2001). To eventually reach the otoliths from the water these ions must pass through three interfaces, i.e. through the gills to the fish's blood stream, then through a likely transcellular route into the endolymph and from there to the crystalline matrix (Campana 1999). This process provides ample opportunity for selection or rejection of ions of particular elements through physiological processes, and thus possible modification of the relative concentrations available in the

environment. As such, otoliths may not reflect the elemental or isotopic composition of the surrounding water.

For both strontium and barium, i.e. the elements of interest in this study, the method of incorporation and location of the ions in the crystalline matrix of the otoliths are likely to be through substitution for Ca or through co-precipitation of either $BaCO_3$ or $SrCO_3$ (Campana 1999). The rate that this occurs seems to be influenced by several factors. Firstly, it is now clear that the ambient concentration of these elements in the water has a major influence over their rate of incorporation into otoliths. This is based on several experiments where enhanced concentration of strontium or barium in the water occupied by fish led to their increased concentration in the otoliths (Bath et al. 2000, Milton and Chenery 2001, Kraus and Secor 2003). Presumably, an increase in ambient concentration of either element in the water modifies the element to Ca ratio in the endolymph, ultimately affecting the rate at which ions are incorporated into the crystalline matrix (Campana 1999).

Numerous studies have identified that other factors also affect Sr:Ca and Ba:Ca ratios in otoliths including water temperature, salinity and biological influences such as the growth rate and developmental or reproductive status of the fish (Secor and Rooker 2000). The effects of these factors may well be manifested through their influence over the growth rates of the otoliths (Campana 1999). The rate of crystallization of otoliths is likely to be controlled by the formation rate of the proteinaceous matrix on the growing surface of the otolith. Since the rate of such protein synthesis is likely to be strongly influenced by water temperature then this may account for experimental observations that temperature affects otolith chemistry (Fowler et al. 1995a, b; Elsdon and Gillanders 2002). Furthermore, protein synthesis is highly correlated with metabolic rate and somatic growth rate, which may also account for their presumed effects on otolith chemistry (Campana 1999).

There was once a presumed strong effect of salinity on otolith chemistry (Secor and Rooker 2000), but recent studies suggests that this may have been overstated. It now seems that effects that had been assigned to salinity *per se*, actually related to the relative Sr:Ca ratios in different water masses (Kraus and Secor 2003). Consequently, the variation in Sr:Ca ratios in the otoliths that has been related to movement of anadromous fish between fresh and saltwater, does not relate to the differences in environmental salinity, but to differences in the ambient Sr:Ca ratios. This finding further substantiates the significance of ambient Sr:Ca ratios for the elemental concentrations in otoliths. Similarly, since barium tends to occur in higher concentrations in freshwater than saltwater, Ba:Ca ratios in otoliths may also reflect the movement of fish between water masses of different salinities (Milton and Chenery 2001). Thus, recent studies have de-emphasized the immediate significance of salinity on otolith chemistry, which is consistent with the results from a

number of experimental studies where fish that were exposed to a range of salinities based on dilution of seawater, recorded minimal impact on otolith composition (Fowler et al. 1995a,b; Elsdon and Gillanders 2002).

Despite that strontium and calcium are considered conservative in seawater there can be considerable variation in Sr:Ca ratios between different water masses and depths (de Villiers et al. 1994). Surface depletions of 1- 3% for both elements can occur with respect to deeper waters, suggesting a 'labile nutrient-like' behaviour. The processes most likely to affect Sr and Ca cycling in the ocean include remineralization of organic matter and production and dissolution of $CaCO_3$ and celestite. Calcium depletion in surface waters is generally attributed to the biological cycling of $CaCO_3$, whereas Sr depletion most likely relates to production of celestite, i.e. $SrSO_4$ by the protozoan acantharia, the only marine organism known to use Sr as a skeletal component. Thus, relative rates of $CaCO_3$ and celestite production must influence the relative concentrations of Ca and Sr in the surrounding seawater. Increased concentrations of Ca and Sr at depth relate to the dissolution of $CaCO_3$ and celestite from dead organisms. Upwellings can then modify the Sr:Ca of surface waters by bringing these enriched deeper waters to the surface. Furthermore, river run-off generally leads to a modification of the Sr:Ca ratio of local waters because freshwater is generally depleted in Sr compared with Ca. This, however, is not always the case and the Sr:Ca ratios of freshwater input to the sea can occasionally exceed that of seawater (Kraus and Secor 2003). Wind-driven dust may be another continental influence that can affect the Sr:Ca of nearshore, coastal seawater (de Villiers 1999).

The concentrations of barium also vary naturally in the marine environment, with higher concentrations in riverine and near-coastal areas, suggesting a continental source to marine waters (Shen and Stanford 1990). However, there are also greater concentrations of dissolved barium in deep ocean environments where soluble Ba is thought to be regenerated as biological debris sinks and is oxidised or resolubilised (Bruland 1983). Such barium can then be returned to shallow water through up-welling events (Dove and Kingsford 1998).

3.4.3 Environmental variation in marine waters of South Australia

At present there is no information available for the relative concentrations of strontium and barium amongst the six fishery regions considered in this study. Nevertheless, there are substantial differences in the characteristics of the physical environmental regimes of the six regions that could very likely result in significant spatial differences in the environmental concentrations of ^{88}Sr and ^{138}Ba. These include differences in water temperature and salinity regimes, point sources of input and physical oceanographic processes (summarised in Chapter 1). Four of our regions were associated with the major gulf system of South Australia comprised of Spencer Gulf, Gulf St.

Vincent and Investigator Strait. Both gulfs experience warmer summer and cooler winter temperatures than nearby oceanic waters (Chapter 1, Fig. 1.1). Furthermore, they are also inverse estuaries for which the combination of high evaporation and low rainfall, in association with minimal run-off and groundwater supply, means that salinity increases from oceanic values at their entrances to hypersaline conditions (up to 48 ppt) in summer towards their heads (Chapter 1, Fig. 1.2; Nunes Vaz et al. 1990). Consequently, there are temperature and salinity gradients along the lengths of the gulfs that vary seasonally, contrasting with the less variable conditions of the three continental shelf regions of SGSV, WC and the SE. Another difference between regions is the location of point sources of anthropogenic inputs into the marine environment. The heavy industry of South Australia is largely centralised around the coastal margins of the northern part of Spencer Gulf, whilst metropolitan Adelaide, which supports approximately 80% of the State's human population, is located along the eastern shore of Gulf St. Vincent.

At several places along the southern oceanic coastline summer up-wellings occur. These are at the western tip of Kangaroo Island, the Coffin Bay Peninsula, Streaky Bay (Ward et al. 2000), and off the south-east coast between Cape Jaffa and Portland (Schalinger 1987). At each place cells of cool water of approximately 16˚C extend from the surface to the seafloor during late summer and early autumn that result from up-welling during periods of prolonged south-easterly winds that blow longshore (Ward et al. 2000). These sites seem to be determined by topographic and bathymetric features, suggesting that the locations of up-wellings will be consistent from year-to-year. Such cool, up-welled waters are relatively high in nutrients and may also be high in barium and/or strontium concentrations. Furthermore, there are also secondary oceanographic phenomena that may minimise the mixing of these enriched oceanic waters with gulf waters, at least through the summer/autumn period. Frontal zones establish at the southern end of Spencer Gulf and the mid-western end of Investigator Strait during summer, which are likely to reduce gulf/shelf exchange until the end of autumn. The two fronts result from the juxtaposition of warm waters from both gulfs and the cool water from the Great Australian Bight, and are thought to effectively separate the different water masses through this time. By mid-late autumn the oceanic water retreats and on cooling the saline waters of Spencer Gulf undergo convective overturning and form a density current that flows from the gulf to the shelf by early winter (Petrusevics 1993).

Clearly there are substantial differences in the characteristics of the marine environment that fish would experience in the six different South Australian regions, as well as physical oceanographic processes that minimise the mixing of different water masses during the summer/autumn period, when regional differences are most extreme. This period is also when the otoliths grow the fastest and achieve most of the year's growth (Chapter 2). There may also be regional differences in the ambient concentrations of ^{88}Sr and ^{138}Ba. The groups of 10 year old fish that were captured from these different regions had otoliths that differed in their elemental composition between the 4th and

9th annual increments. The inevitable conclusion from these observations is that between these ages these fish must have spent considerable periods of time in water masses that differed in chemical and/or physical characteristics. This observation is more consistent with the South Australian snapper being divided into regional sub-populations than constituting a single, large, intermixed population. The fact that the chemical signatures of the otoliths of fish sampled from most regions were similar up to the age of 3 years may be accounted for by several hypotheses. Firstly, these data may indicate that the majority of snapper originate in one region and then later radiate out to become established in other regions. Alternatively, the snapper may have originated in numerous regions that for the first three years had homogeneous chemistry, after which regional environmental differences developed that became manifested in the otoliths. The latter hypothesis is highly unlikely because of the temperature and salinity differences between regions, but nevertheless cannot be excluded completely at this stage.

Addressing the issue of movement of individual fish on the basis of elemental concentrations in otoliths is far more difficult than addressing the question of stock structure. The movement question depends on considering the variation in elemental profiles within and between years for individual fish. The expectation is that if fish do move between places that are characterised by different environmental characteristics and elemental concentrations then such differences will be manifested in the cross-otolith transects. It is tempting to suggest that the annual sinusoidal variation for both ^{88}Sr and ^{138}Ba did in fact relate to such annual migrations, as has been done for anadromous fishes that move between marine, estuarine and freshwater habitats (Secor 1992, Secor and Piccoli 1996, Secor and Rooker 2000). However, other species of strictly marine fishes have displayed similar patterns of systematic annual variation in strontium in the absence of movement between water masses of different salinities (Sadovy and Severin 1992, 1994; Fuiman and Hoff 1995). For two species, i.e. *Haemulon plumieri* and *Epinephelus guttatus,* there was correspondence between the maxima for Sr:Ca ratios and the opaque zones of the otoliths (Sadovy and Severin 1992, 1994), and in each case the Sr:Ca ratios were inversely related to somatic growth rates. In these cases the systematic variation in Sr:Ca ratios reflected the variation in physiology of the fish in response to environmental seasonality. Clearly, sinusoidal variation in elemental concentration alone is not sufficient to indicate deterministic movement patterns. Perhaps the best evidence for inter-regional movement, or the residency of fish in different regions would come from unusual patterns of variation in either ^{88}Sr or ^{138}Ba, or both elements. Thus, any unusually large peaks in strontium in the otoliths of fish captured in an oceanic region may represent the excursion of this individual into one of the northern gulfs, whilst any low flat levels of strontium in otoliths of northern fish may reflect periods of existence in one of the oceanic regions.

It was tempting to speculate about the possible movement and regional residence of several fish from the elemental profiles of their otoliths. Two fish from NSG (Chapter 9, Fig. A.1, nos. q,t)

displayed low concentrations of ^{88}Sr as well as low annual and inter-annual variation for at least the ages of 4 - 8 years, and in this respect were more similar to those collected from the continental shelf. This may indicate that these fish had only recently moved into NSG from such a region. This hypothesis may be even more convincing for another fish (Chapter 9, Fig. A.1, no. n), which showed similar characteristics for ^{88}Sr, but also displayed considerably higher levels of ^{138}Ba for the same age range than for other individuals from NSG.

The otoliths from three fish (Chapter 9, Fig. A.4, nos. a,h,k) that were each collected in March 2000 from SGSV displayed very high levels of ^{138}Ba for their 4 years prior to their capture. These fish may have moved into a barium-rich environment around the start of their 6th year, where they remained until capture. For at least two of these otoliths the strontium levels were particularly low and invariant, which is consistent with these fish occupying an oceanic environment. A number of fish from the WC (Chapter 9, Fig. A.5, nos. a,c,i,j,k,o,q) had high barium values through their fourth and fifth years, again suggesting that they may have occupied an area where barium was enriched through up-welling during this period.

3.4.4 Conclusions

Ten year old snapper were sampled from six different regions of South Australian marine waters, which differed considerably in their physical characteristics particularly with respect to annual temperature and salinity regimes, but possibly also with respect to ambient concentrations of strontium and barium. The otoliths from these fish were analysed by LA-ICPMS and the resulting elemental profiles were related to fish age. The profiles for individual fish were difficult to interpret in terms of fish movement. However, the regional age-related profiles based on averaging the data from all fish in each region differed significantly amongst regions with respect to the concentrations of ^{88}Sr and ^{138}Ba. However, these differences were not apparent for the first three increments, but related to the otolith region between the 4th and 9th annual increments. Such results are consistent with the fish from different regions having spent considerable periods of time throughout the ages of 4 – 9 years, in different water masses. These data are consistent with the division of the South Australian population of snapper into a number of regional sub-populations, but where division took a number of years to become established. One possible explanation for the uniform nature of the otoliths throughout the first three years is that most fish had a common regional origin.

The chemical structure of individual otoliths appears to not relate useful information on systematic migration behaviour. This is because the otoliths display a pattern of sinusoidal variation of both ^{88}Sr and ^{138}Ba that is most likely a reflection of the seasonal variation in physiology of the fish being manifested in the otoliths. However, the chemical profiles of some otoliths suggest that the

fish from which they were removed were resident for a number of years in water masses that had specific chemical characteristics.

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4 Analysis of stock structure I – regional variation in population characteristics

A.J. Fowler, K.C. Hall, W.B. Jackson and P.J. Jennings

4.1 Introduction

For the South Australian population of snapper there is no evidence for stock structure outside of the broad division from the Victorian population near the mouth of the River Murray (Donnellan and McGlennon 1996). As such, the South Australian fishery currently treats the population as a single stock, which is consistent with the currently accepted model of the life history in South Australia. This life history model proposes that through complex, age-related migration there exists on the continental shelf a significant, mixed-age population of snapper that derives originally from the gulfs (Chapter 3, McGlennon and Jones 1997). The model states that within the first few years after recruitment into the northern gulfs some young fish move southwards, leave the gulfs and migrate to the continental shelf. It is proposed that from there they undertake annual spawning migrations over a number of years back into the gulfs, at which time they are highly vulnerable to the fishery. Finally, it is thought that these fish become permanent residents in the gulfs from the age of about 12 years onwards.

The existence of a population on the continental shelf that results from movement of fish from the gulfs must provide a clear opportunity for the mixing of fish that originate from different regions, such as Spencer Gulf and Gulf St. Vincent. The consequences of this for the stock structure depend on the subsequent behaviour of the fish. If they return from the continental shelf to areas from which they originated, as is the case for the populations of Atlantic cod (*Gadus morhua*) in the North Atlantic (Campana et al. 2000), then the population dynamics of snapper in the two gulfs would be independent and their regional populations would be isolated. As such the regional fisheries would need to be managed independently and there would be hope for success of a regional management strategy. Furthermore, it would be expected that such a stock structure would be apparent as regional differences in population and fishery characteristics. Alternatively, however, if fish did not show such systematic movement patterns but responded more to exogenous factors, then fish from different regions would become mixed with respect to their place of origin. In this situation there would not be apparent any stock structure or any regional differences in population and fishery characteristics, and snapper would conform to the 'single stock hypothesis'. Under these circumstances the fishery should be managed as a single, large, inter-related population, and a regional management strategy would not be useful.

The second model described above is the currently accepted one for the stock structure of snapper in South Australia. It states that the State-wide population is not differentiated into a number of local sub-populations, but rather constitutes a single, large stock that is derived originally and mixed from different regions throughout the State. In this chapter this hypothesis was tested, by comparing the fishery and population characteristics from different regions from throughout the State. The characteristics considered were: fishery catch and effort trends; population size and age structures; growth characteristics; the optical characteristics of otoliths; and the morphometrics of the otoliths. A lack of regional differences in these characteristics would support the 'single stock' hypothesis. Alternatively, systematic differences in some or all of these characteristics would indicate that fish from different regions spend considerable parts of their lives in different water masses, which is more consistent with a regional stock structure.

4.2 Methods

4.2.1 Commercial catch statistics

Since 1983-'84 the commercial marine scalefish fishers of South Australia have been obliged to provide information on their catch and effort at the spatial scale of prescribed marine fishing areas (Fig. 1). Fishers are required to report the weight of their catch by species per fishing area and month by each gear type. Here, this information was used to calculate annual totals of catch and effort by region and gear type as well as total regional catch. The six different regions considered in the South Australian snapper fishery are Northern Spencer Gulf (NSG), Southern Spencer Gulf (SSG), Northern Gulf St. Vincent (NGSV), Southern Gulf St. Vincent (SGSV), the West Coast of Eyre Peninsula (WC), and the South East region, i.e. east of the mouth of the Murray River (SE) (Fig. 4.1). The temporal trends in annual catches were compared between the four most significant regions, i.e. NSG, SSG, NGSV and SGSV for the period of 1983/84 to 2001/02 using correlation analysis.

4.2.2 Measuring Program

Since January 2000, SARDI has maintained a biological sampling program to provide regional and temporal information on population structure and other biological information. This has involved two related sampling programs:

1. from January 2000 to December 2002, SARDI researchers carried out a market measuring program at the Adelaide SAFCOL fish market. On each Friday morning, a research team visited the market and recorded the number of landings of snapper, the number of fish bins in each landing and measured the fish from as many landings as possible. The landings for

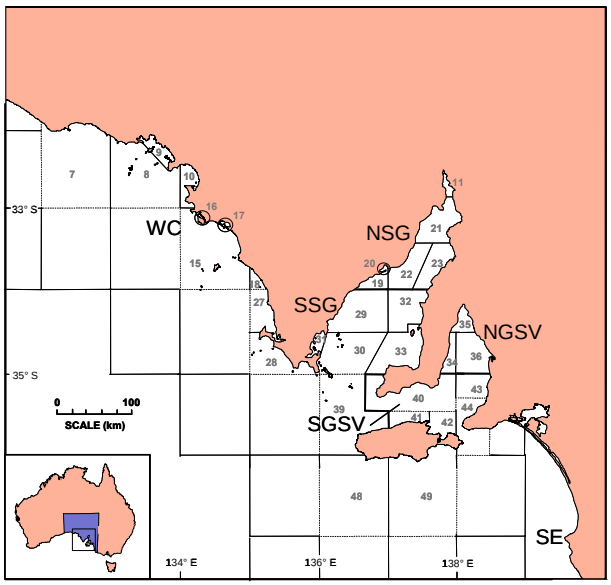

Figure 4.1 Map showing the numbered Marine Fishing Areas of South Australia, which represent the smallest spatial scale to which the commercial catch data are reported. The map shows the six regions for which data were compared in this study.

which fish were measured were chosen to represent as broad a geographic range as possible. Furthermore, the name of the fisher was recorded so as to relate the biological information to the fisher's catch record, which provided the required information on fishing method and marine fishing area. Throughout 2001 and 2002 the otoliths from some fish were also collected. This could only be done for fish that were already gutted as it involved removing several vertebrae from behind the head through the empty body cavity to provide access to the otoliths;

2. in the second sampling protocol throughout the period of January 2000 to December 2002 the research team worked directly with some fishers from Spencer Gulf and Gulf St. Vincent who provided access to their fishery catches. Where possible these whole fish were measured, weighed, gutted and had their otoliths removed to provide information on size, weight, age, sex and stage of reproductive development and maturity.

One otolith from each fish was processed to provide an estimate of fish age. The otolith was sectioned in the transverse plane through the middle using a diamond saw to produce a section that was approximately 500 µm thick. This TS-section was polished lightly on both sides using lapping film and then mounted on a microscope slide with a drop of resin. The otolith was examined using transmitted light and the opaque zones were counted. This count was interpreted to provide an estimate of age in years based on the time of year of capture, the birth date, and the time of year when the opaque zones are formed (McGlennon et al. 2000).

For each region, the data from the two sampling programs were combined to produce an annual estimate of the size frequency distribution of the catch taken by the handline and longline geartypes, which provide 84% and 14.5% of the total catch of snapper, respectively (Fowler et al. 2003). These were based on data combined across all those landings for which it was possible to assign a fishing method and a marine fishing area from the catch returns of the fishers. The estimates of age from the otoliths were used to develop a specific age/length key for each of the four main regions. Each age/length key was then used to produce an annual estimate of the age structure for the landings by the two major geartypes for each region, based on the annual size frequency distribution.

4.2.3 Optical characteristics and morphometrics of otoliths

The optical characteristics of the otoliths were compared among the regions. Each otolith was examined and assigned a score on the basis of the clarity and interpretability of the structure of the increments. The relative proportions of otoliths that were assigned each score were compared amongst regions using a Chi-square contingency analysis. The scores are defined in Table 4.1.

Table 4.1 Scores used for the classification of otoliths according to their clarity and interpretability.

Score	Description
1	Otolith is unreadable and is rejected as uninterpretable
2	Incremental structure is poorly defined resulting in low confidence in the count
3	Incremental structure is clear and confidence in the count is high
4	Incremental structure is very clear resulting in no ambiguity in the count

The otoliths were also considered in a morphometric study to determine whether otolith shape varied between regions. Approximately 50 otoliths were selected from those available from 12 year old fish that had been sampled through 2003 in the four regions of NSG, SSG, NGSV and SGSV. For each otolith several digital images were recorded using Video Capture software. The images were then processed using Sigma Scan to provide estimates of four linear measurements, as well as a measure of the perimeter and area of the otolith. The linear measurements were: the length and breadth through the core; the length between the anterior and posterior tips with the otolith oriented side-on; and the depth of the otolith from the proximal surface to the line connecting the lowest points of the anterior and posterior tips. Since the digital images provide two-dimensional images of a complex three-dimensional structure that has a significant concave shape, the estimates of perimeter and area relate more to the shadow of the structure on a flat plane than to the real measurements.

The various measurements of otolith size were graphed against fish length, revealing significant linear relationships with fish size. Therefore, prior to analysis by multivariate techniques, the data had to be transformed to remove the effect of fish size to ensure that only otolith shape and not otolith size was influential in the multivariate analyses. The common-within group residual method was used to achieve this (Reist 1985, 1986). These transformed data were then considered in two multivariate analyses. Principal Components Analysis was used to reduce the dimensionality of the data, the results of which were plotted on new sets of axes. Then a discriminant function analysis was done to test the hypothesis that the group means for the different variables differed between the four regions. This analysis was done using SYSTAT Version 10.

4.3 Results

4.3.1 Commercial catch statistics

The total State-wide catches of snapper in each of 1999/00, 2000/01 and 2001/02 were 576, 578 and 648 tonnes, respectively (Fig. 4.2). There was an uneven contribution to these totals by the six different regions around the State that was consistent through time. In each year NSG and SSG provided the highest catches, which together comprised 85-90% of the total catch. In comparison NGSV and SGSV provided only 6.2 to 7.9% of the catch. These substantial and consistent differences in catch between regions are strongly suggestive of a persistent large difference in the density of snapper supported in each. The WC and SE contributed minimally to the total catch.

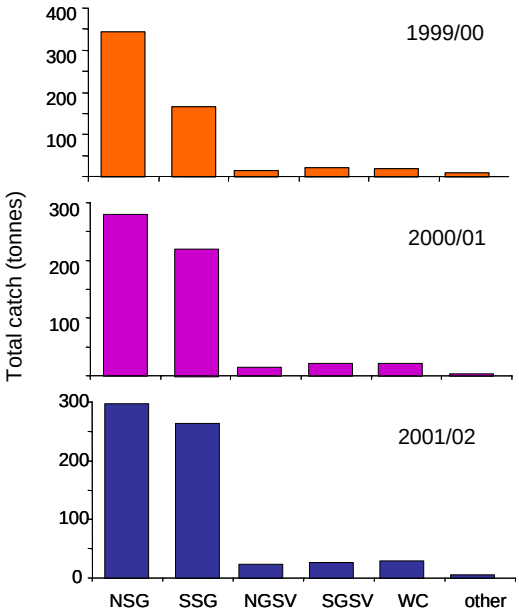

Figure 4.2 Regional contribution to the total catch of snapper in each financial year of 1999/00, 2000/01 and 2001/02.

The annual trends in catches in NSG and SSG decreased through the late 1980's, then attained a small maximum in 1990/91, decreased again to 1994/95, before increasing to record high catches in the early 2000's (Fig. 4.3). In contrast, the catches in NGSV and SGSV decreased significantly through the late 1980s to minima in 1993/94 and 1994/95 respectively, but have never subsequently recovered to the levels of the early to mid 1980s. Not surprisingly, the temporal trends were significantly correlated between NSG and SSG, and also between NGSV and SGSV, but were not correlated between the two gulfs (Table 4.2). These differences in catches between the two gulfs are thought to reflect trends in biomass because the latent effort in this fishery would likely take advantage of any increase in biomass available to fishers. The different temporal trends suggest that different influences have affected the gulf populations over the twenty years.

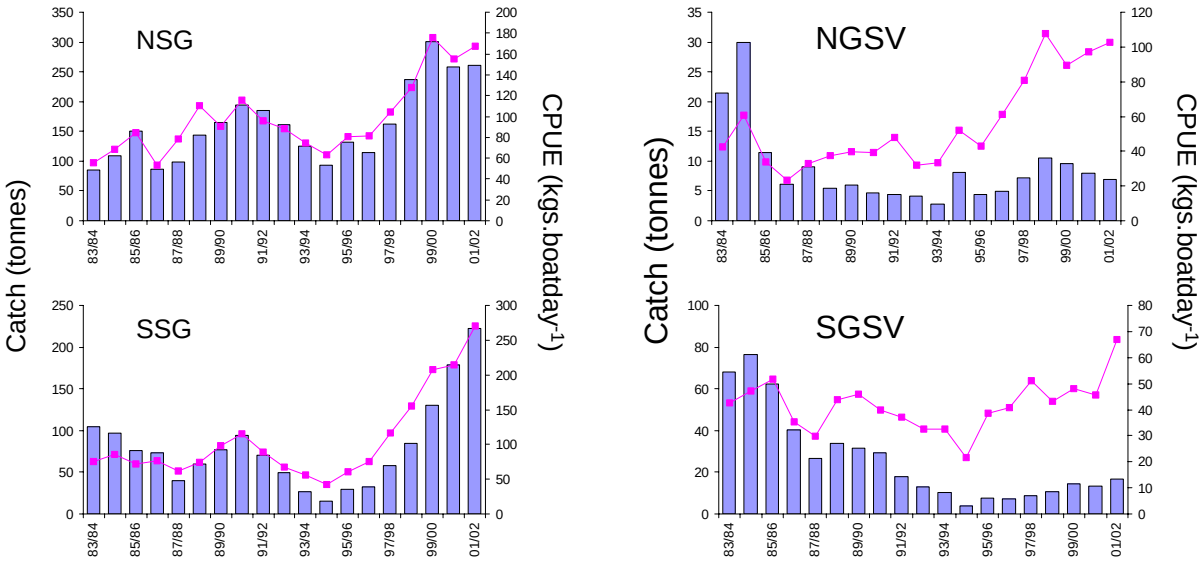

Figure 4.3 Historical record of the targeted catch (bar charts) and CPUE (line graphs) for snapper by the commercial handline sector for each of the four main fishery regions.

Table 4.2 Results of correlation analyses for the temporal patterns in the variation in handline snapper catches between the nominated regions for the period of 1983/84 to 2001/02. Data presented are the correlation coefficients with 17 degrees of freedom (* = significant at p = 0.05, ns = not significant at p = 0.05).

Region	NSG	SSG	NGSV	SGSV
NSG	-	0.6990^{*}	-0.2045^{ns}	-0.3594^{ns}
SSG	-	-	0.2225^{ns}	0.1586^{ns}
NGSV	-	-	-	0.7683^{*}
SGSV				

4.3.2 Population structure – Comparison between NSG and SSG

Size and age structures

If the regional differences in snapper catches described above are the consequence of different demographic processes, it is likely that this would also be evident as differences in population structure at this spatial scale. Consequently, the size and age structures from fishery catches were compared. For the two Spencer Gulf regions there were sufficient data to consider the catches from both handlines and longlines. In NSG the size distribution of fish taken by longlines compared with handlines clearly showed a much higher number of large fish of >80 cms CFL (Fig. 4.4), which is consistent with previous observations that these two geartypes have different selectivity for size of fish (McGlennon and Jones 1997). Thus, to establish a realistic impression of population structure it is necessary to consider the catches from both geartypes. By so doing for NSG in 2002, it is clear that there were several modes in the population, particularly in the size classes of 35 cms, 44-50 cms, 70-80 cms, and 85-93 cms CFL (Fig. 4.4). In contrast, in SSG the size structures of catches from both gear types were very similar and displayed only two distinct modes, i.e. 30-40 cms CFL and around 70cms CFL. Thus, compared with NSG the relative abundance of fish in the 44-50 cm size range was far lower, whilst the 85-93 cm fish were completely absent.

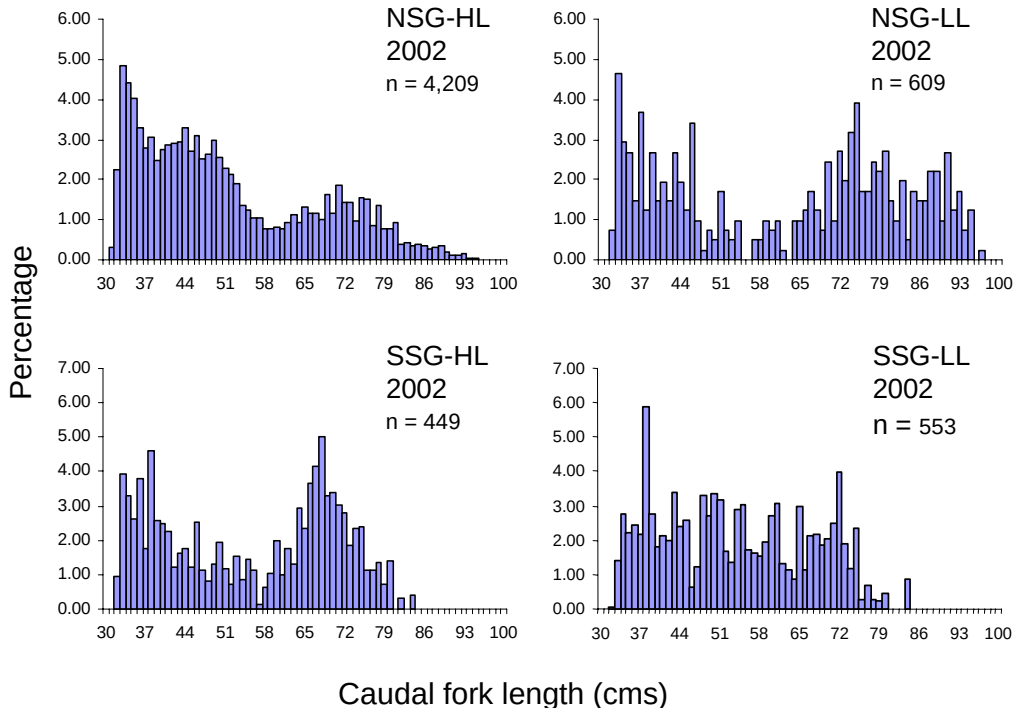

Figure 4.4 Annual size frequency distributions for commercial snapper landings by the handline and longline sectors in NSG and SSG in 2002.

The comparison of age structures from the handline sector of the fishery, which provides the majority of the catch, also shows differences between NSG and SSG (Fig. 4.5). The handline catches of NSG in 2000, 2001, and 2002 were each dominated by the 1991 and 1997 year classes. In the first of these years, the 1997 year class was not fully recruited to the fishery and so contributed less fish than the 1991 year class (Fig. 4.5), but in the following two years the 1997 year class was numerically dominant. However, in SSG the 1991 year class was numerically dominant in each year contributing >60% of all fish taken, whilst the 1997 year class contributed only 10-20% of the numbers taken. Thus, the 1991 and 1997 year classes were the most significant in both regions, but the relative contribution of each year class to the total catch differed between the two regions. Such a difference is not consistent with the 'single stock' hypothesis.

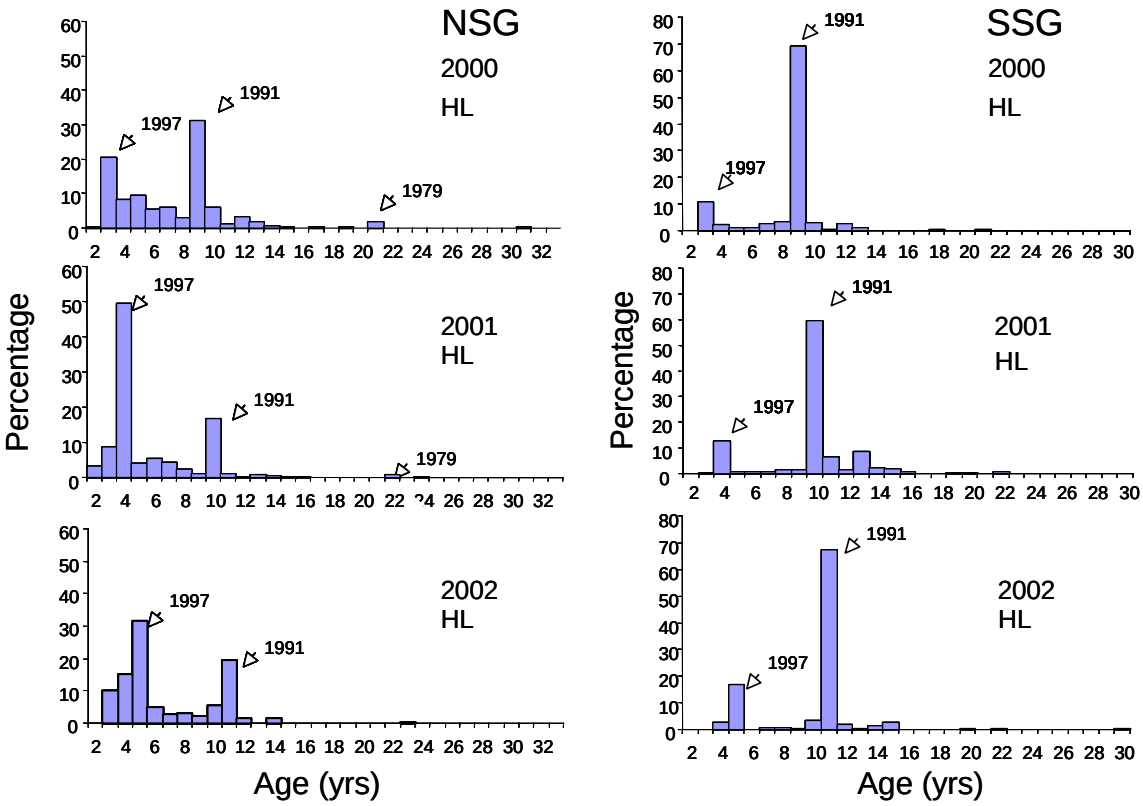

Figure 4.5 Age structures of the handline catches for snapper in NSG and SSG in each of the nominated calendar years.

Size-at-age information

The relationships between size and age for NSG and SSG also demonstrated significant regional differences that were consistent through time. For NSG those fish older than the 1991 year class (i.e. 9 yrs in 2000, 10 yrs in 2001 and 11 yrs in 2002) show a tight, asymptotic relationship between size and age (Fig. 4.6). Alternatively, the younger age classes showed far greater variation in size-at-age. This may be a density dependent effect resulting from the high abundance of the 1991 year class. In comparison, in SSG there were far fewer older fish, the size range for each age class was broader, whilst the estimate of the asymptotic size was greater than 30 cms less than that for NSG (Table 4.3). In fact, the mean size-at-age for each age class from the age of about 6 years onwards for fish in SSG was substantially less than for NSG (Fig. 4.6).

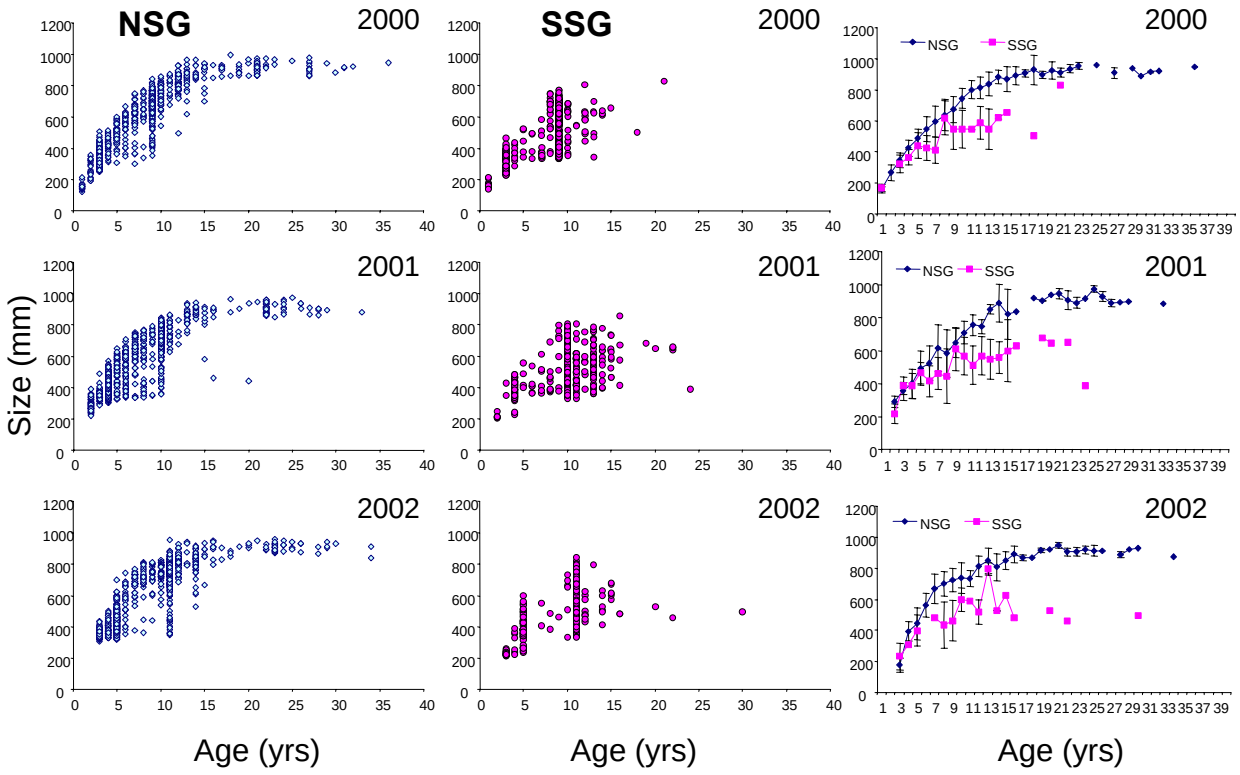

Figure 4.6 Relationships between size and age for NSG and SSG in each of 2000, 2001 and 2002. The right hand graphs compare the mean sizes per age class between regions for the three years.

Table 4.3 Estimates of von Bertalanffy growth parameters based on samples of fish collected from the four main fishery regions.

Region	L_∞ (mm)	K	t_0
NSG	984	0.1238	-0.3470
SSG	632	0.2101	-0.1077
NGSV	907	0.1585	-0.0818
SGSV	895	0.1311	-0.0619

To further explore this issue of variation in size-at-age, the size structure of the catch of the dominant 1991 year class in each of 2000, 2001 and 2002 were compared between NSG and SSG. NSG had consistent unimodal distributions, although with a low number of individuals that were small for their age (Fig. 4.7). In contrast, each size distribution from SSG was clearly multimodal with there being an obvious mode of small fish and another of large fish. It is apparent that for this region there is a significant component of the population that is 'stunted' in size that is virtually absent from NSG. Alternatively, the mode of larger fish from SSG does overlap with some component of the population in NSG.

The multiple modes of fish from the 1991 year class that were taken in 2000, 2001 and 2002 in SSG may relate to different sized fish moving into the region at different times of the year. This hypothesis was assessed by examining the size structures of fish taken in different seasons of the year (Fig. 4.8). The data indicate that individuals from 30 to 80 cm CFL were taken in each season, with no seasonal differences in size structures that were consistent across years (Fig. 4.8). Thus, it appears that the multimodal size distribution represented fish that were present in the region throughout the entire year.

4.3.3 Population structure – Comparison between NGSV and SGSV

There were considerably less data available for comparing population structure between these regions by virtue of the low number of landings and few fish that pass through the SAFCOL market from this Gulf. The size distributions were quite similar between regions, displaying approximately similar modes (Fig. 4.9). All age structures were dominated by the 1991 year class but the 1997 year class was not apparently significant until 2002 (Fig. 4.9). Nevertheless, the growth relationships for the two regions were subtly different. For SGSV the range of sizes for all age classes showed greater variation than for NGSV, whilst the mean size of fish for each age class was lower (Fig. 4.10). These differences are quite apparent when the size distributions of the abundant 1991 year class are compared. In Fig. 4.11 the size distributions for both NGSV and

SGSV are presented in combination for the 9, 10 and 11 year old fish that were captured in 2000, 2001 and 2002, respectively. Although both size distributions are unimodal and normally distributed, the sizes of fish from SGSV were more variable and had a lower mean compared with those from NGSV.

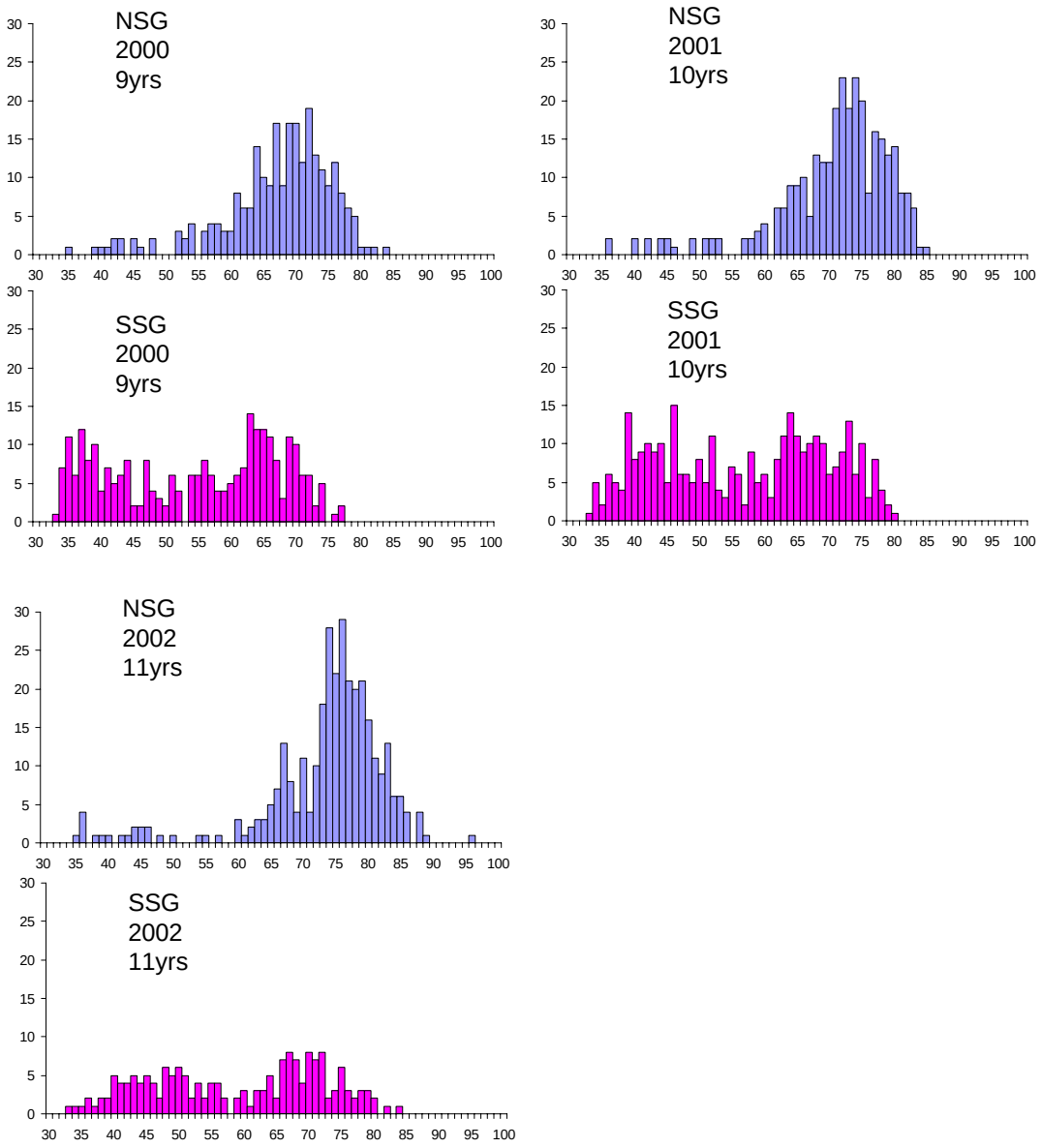

Figure 4.7 Comparison of the size frequency distributions of the 9, 10 and 11 year old fish captured in NSG and SSG in 2000, 2001 and 2002, respectively.

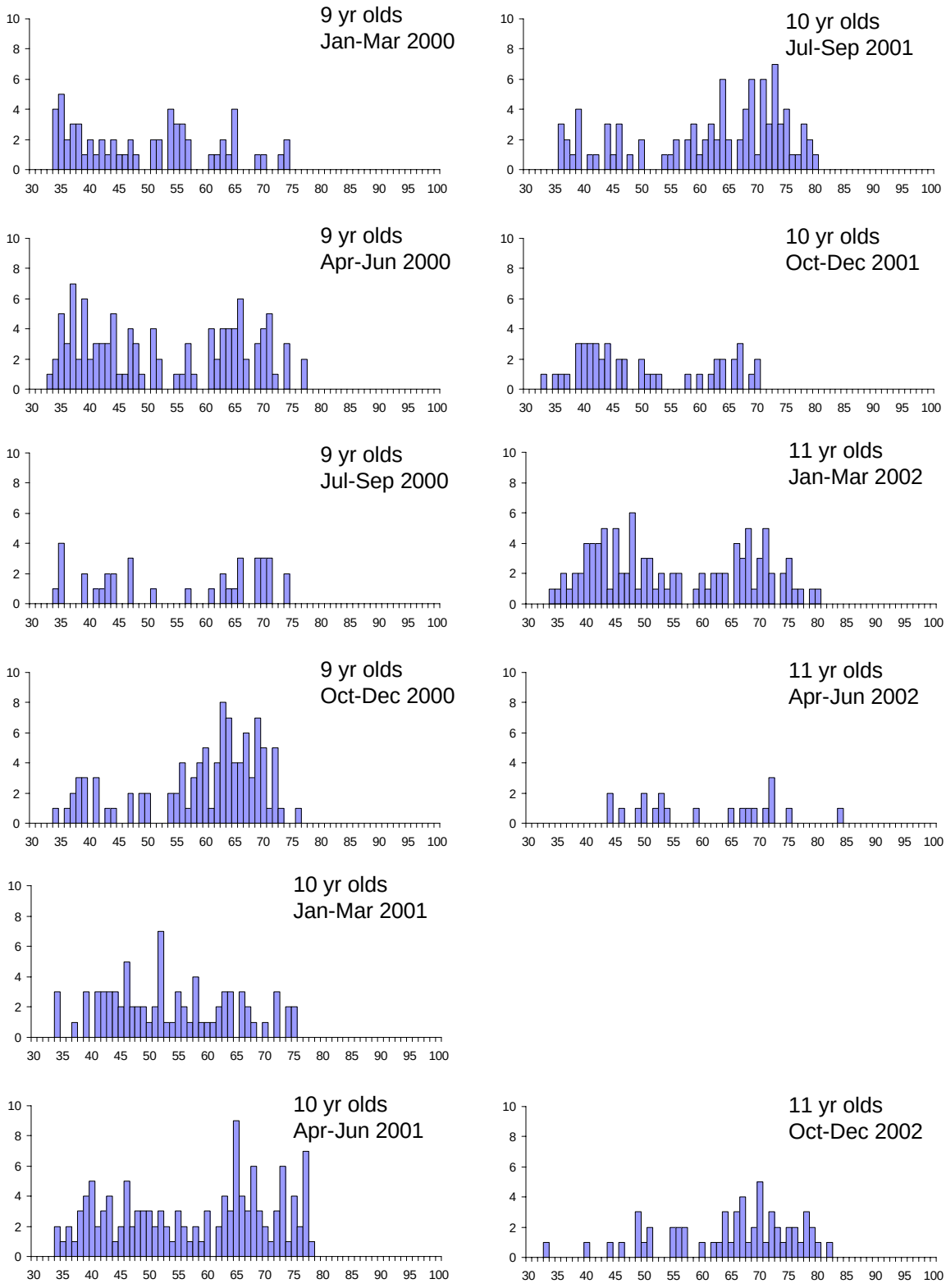

Figure 4.8 Seasonal size frequency distributions for the 1991 year class of snapper sampled from SSG during 2000, 2001 and 2002.

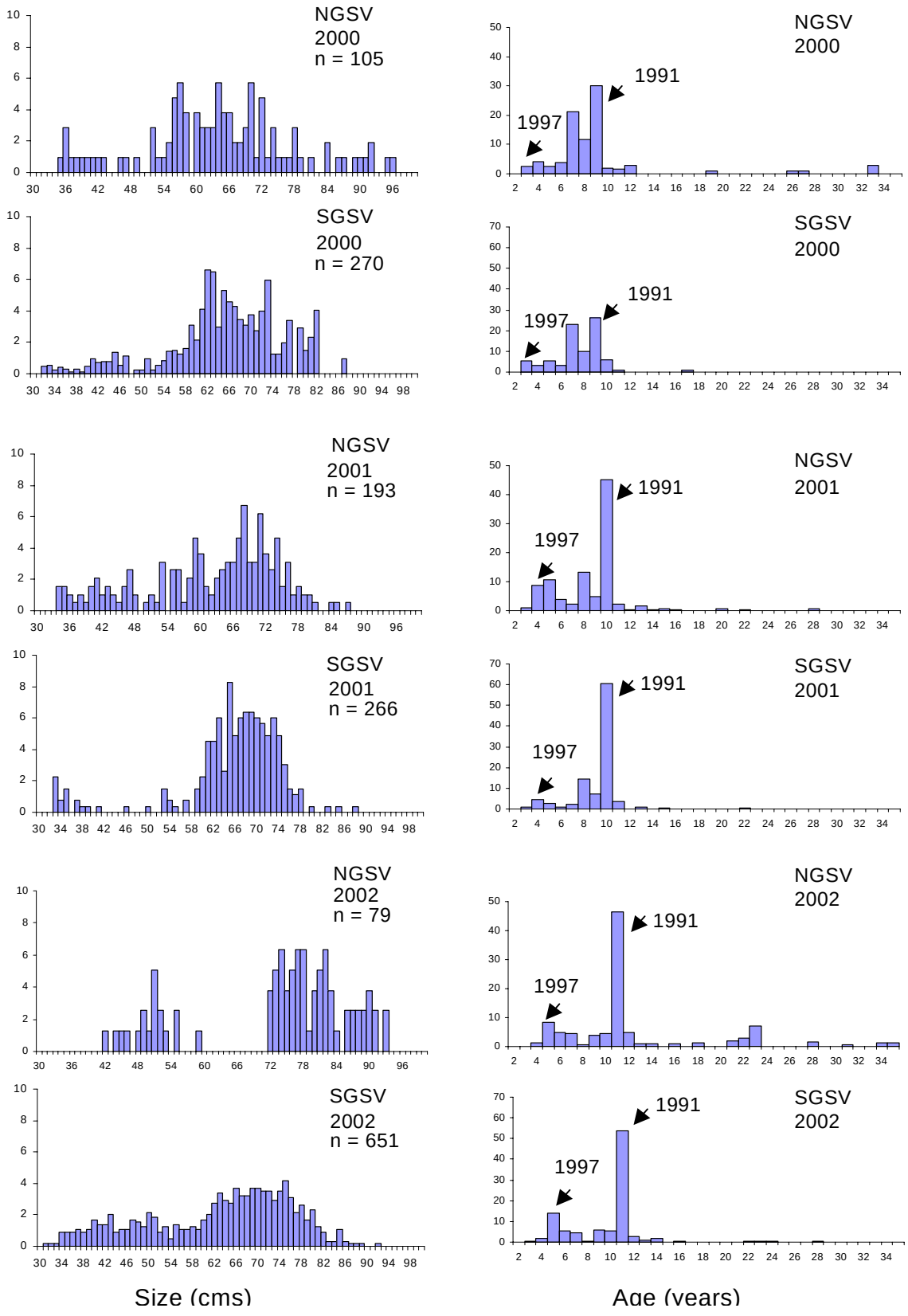

Figure 4.9 Left hand graphs compare the annual size frequency distributions between NGSV and SGSV for each of 2000, 2001 and 2002. Right hand graphs show the age structures that the size distributions relate to.

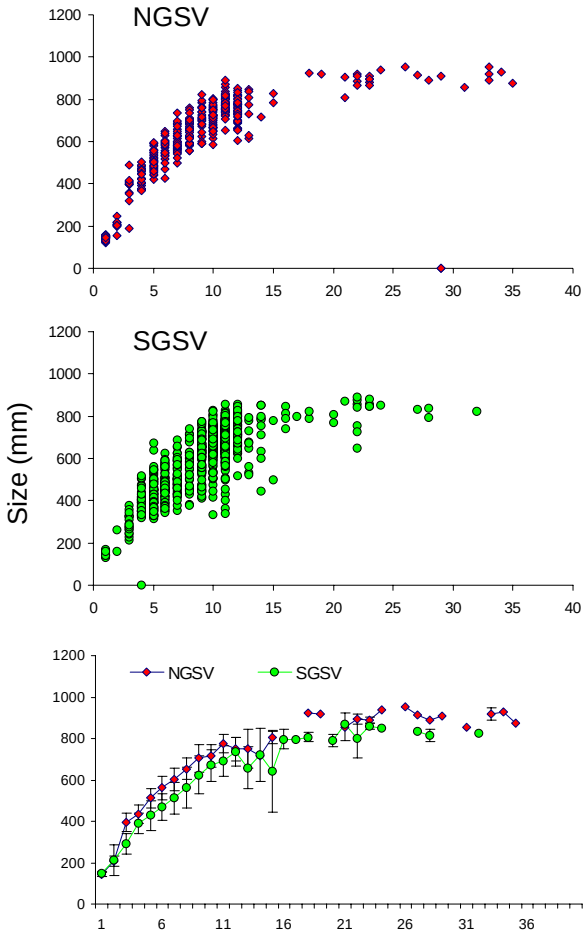

Figure 4.10 Relationships between size and age for NGSV and SGSV based on data collected in 2000, 2001 and 2002. The third graph compares the estimates of mean (±SD) size per age between regions.

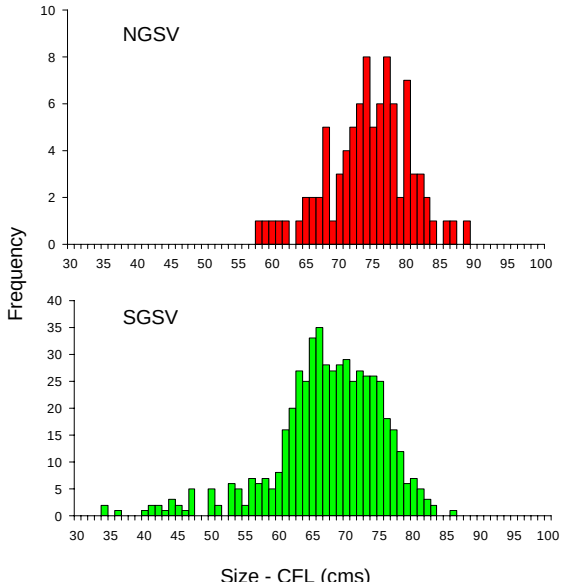

Figure 4.11 Comparison of size frequency distributions for NGSV and SGSV compiled from the 9+, 10+ and 11+ age classes that were collected in 2000, 2001 and 2002, respectively.

4.3.4 Optical characteristics and morphometrics of otoliths

A total of 6,356 otoliths were examined for snapper between 2000 and 2002, for which a score was assigned based on the clarity and interpretability of the structure of the transverse section. The relative frequencies of the different scores assigned to the otoliths from the 6 regions are presented in Fig. 4.12. There was a highly significant difference between regions in the relative frequencies of the scores (Chi-square value = 424.54, df = 2, 15, $p < 0.001$*), and some geographic structure to this variation. NSG and NGSV had the highest number of high quality otoliths. The WC and SE had the lowest number of high quality otoliths and the highest number of poor grade ones. The two regions with intermediate scores were SSG and SGSV, which are geographically located between the other two groups.

For the PCA done on the otolith morphometric data, the first two axes accounted for 98.7% of the variance in the data. However, the plot resulting from the PCA shows considerable overlap amongst the datapoints from the four regions (Fig. 4.13). This suggests the lack of any significant difference in otolith shape amongst regions. To further consider this result the discriminant function analysis (DFA) was used to determine whether otoliths from particular regions could be accurately classified to their region of origin based on the otolith measurements. The resulting classification matrix (Table 4.4) was most strongly influenced by the linear measurements along axes 1, 2 and 3. The classification matrix was particularly mixed and does not suggest strong differentiation amongst the regions. Only for NSG, where 62% of otoliths were correctly classified was there some indication for regional structure. For the remaining regions, particularly SSG, the

classification success was poor. Thus, the results of the PCA and DFA were in agreement in indicating that there was poor differentiation amongst regions on the basis of otolith shape.

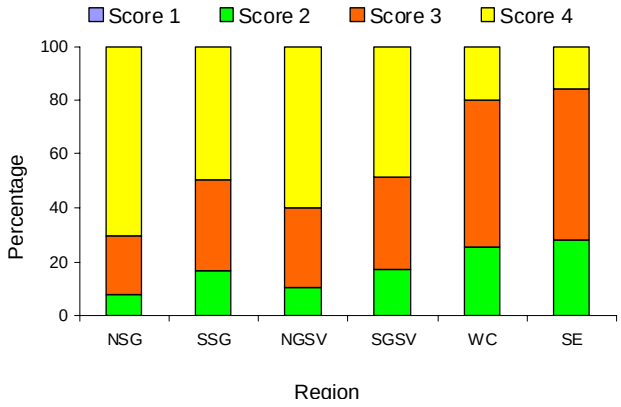

Figure 4.12 Relative frequencies of the different scores assigned to otoliths examined from the six regions (sample sizes for number of otoliths considered per region are: NSG = 3,414; SSG = 1,489; NGSV = 368; SGSV = 892; WC = 86; SE = 107). The scores 1-4 are defined in Table 4.1.

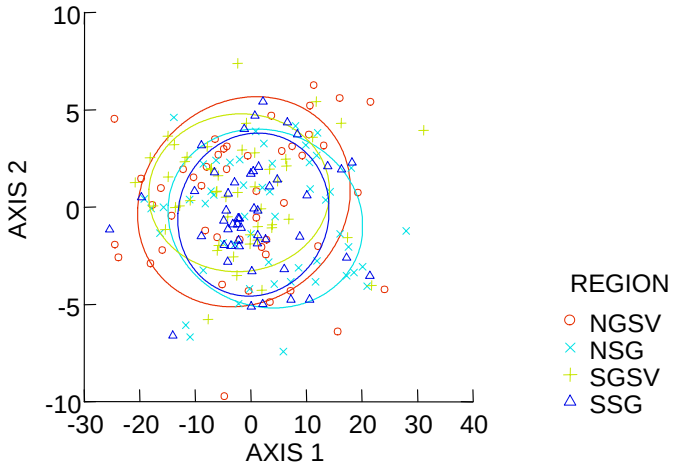

Figure 4.13 Result of the PCA on otolith morphometric data comparing among the four regions of NSG, SSG, NGSV and SGSV.

Table 4.4 Classification matrix from discriminant function analysis on the basis of linear measurements, perimeter and area of otoliths.

Region	NGSV	NSG	SGSV	SSG	% correct
NGSV	16	12	14	5	34
NSG	6	32	9	5	62
SGSV	10	13	20	7	40
SSG	13	21	7	9	18
Total	45	78	50	26	39

4.4 Discussion

This chapter was aimed at discriminating between the alternative hypotheses that the South Australian population of snapper constituted a 'single stock' or was divisible into a number of 'regional stocks'. Rather than using a direct test, as is the case in Chapter 5.0, the population and fishery characteristics were compared amongst regions, particularly NSG, SSG, NGSV and SGSV. The logic here was that the lack of any differences at the regional scale would provide evidence that the population constituted a single, large, intermixed population. Alternatively, consistent differences would indicate that fish caught in different regions did in fact occupy different water masses for significant parts of their lives, which is consistent with a regional stock structure.

4.4.1 Fishery Catches

The substantial and consistent differences in catch between the two gulfs suggest that there are large and consistent differences in snapper abundance between gulfs. Such a difference in biomass could eventuate from several causes. There may be habitat differences, where Spencer Gulf provides more resources and can support a relatively larger population per unit area. Alternatively, however, the differences in catch between gulfs may also be indicative of different demographic processes. The temporal trends in fishery catches also varied between the two gulfs, but not within each gulf, i.e. Spencer Gulf enjoyed record catches in each of 1999/2000, 2000/01 and 2001/02 whilst the catches in Gulf St. Vincent have never returned to the high levels that were recorded in the early 1980s. Such differences between gulfs strongly suggest different population processes occurring at this spatial scale. This provides a significant inconsistency with the 'single stock' hypothesis for snapper in South Australia.

4.4.2 Population structure

The population size and age structures provided evidence of even finer scale differences between regions. The size structures of catches in SSG were quite different to those in NSG, which was a consequence of subtle differences in age distributions and growth functions between regions. In general, the fish have slower growth, do not attain the same size-at-age, or the same asymptotic size compared with NSG. Furthermore, a substantial number of fish in SSG are stunted compared with elsewhere. Such 'stunted' fish appear to represent approximately half of the abundant 1991 year class, whereas very few such fish are recorded outside this region. The persistence in SSG of a group of fish that are small for their age indicates that these fish do not eventually diffuse out into other regions over time. This result is again contrary to there being one large population derived from different regions that become mixed through an annual process of fish migration. Clearly, some fish are resident in particular regions for periods of years.

There were two subtle differences in population structure between NGSV and SGSV. Firstly, the age structure was broader in NGSV, i.e. more old fish were caught. Secondly, the average size per age class was larger in the north, indicating that a different growth function applied in this region. Such significant regional differences are consistent with the fish from the two adjacent regions having spent considerable parts of their lives experiencing different habitats, and thus occupying different water masses. This provides a further observation that is not consistent with the 'single stock' hypothesis.

4.4.3 Optical characteristics and morphometrics of otoliths

The growth patterns of otoliths can vary amongst fish that live in different places, resulting in subtle variation to their morphology (Begg and Brown 2000). This, however, was not the case in this study where otoliths from a single age class were compared amongst the regions from which the fish were captured. There was no significant variation in shape evident that was consistent with regional structure. Alternatively, however, there was significant variation in the clarity and interpretability of otoliths from fish collected from the different regions. Similar findings for tropical species have been related to the seasonal variation in water temperature (Fowler 1991, 1995). Here, the northern gulfs had the highest number of clear and interpretable otoliths, and are the regions that experience the greatest range in seasonal water temperature regimes. Alternatively, the continental shelf regions that experience the lowest seasonal variation in water temperature and have a strong oceanic influence, had otoliths that were less clear and were harder to interpret.

The significance of this finding in the context of this study is that for the different water temperature regimes to influence the optical characteristics of the otoliths requires that the fish be

resident within the various regional waters for considerable parts of the year. That the fish have consistent clarity across their otoliths also suggests that they must be resident in these regions over periods of years. This observation is inconsistent with the concept of there being one large, inter-mixed population of snapper, but rather is strongly suggestive that fish occupy particular water masses over considerable periods of time, i.e. the 'regional stock structure'.

4.4.4 Summary and conclusions

In this chapter fishery and population characteristics were compared amongst six regions of the South Australian snapper fishery. Some regions were part of South Australia's extensive gulf system, whereas others were on the continental shelf and experienced an oceanic environment. The catch information from the commercial sector indicated different temporal trends in catch history between the two gulfs. Population size and age structures, as well as patterns of growth, also differed between regions. Although the shape of otoliths did not vary amongst regions, the clarity of the incremental structure did vary in a way that was consistent with the geographic structure. Such spatial differences could only arise if most fish from the different regions had spent considerable parts of their lives living under different environmental conditions, i.e. had occupied different water masses. Therefore, these systematic differences are not consistent with the hypothesis of a single, large, mixed population but are more consistent with the population being divisible into numerous sub-populations of adult fish. The hypothesis that the snapper population of South Australia is divisible into numerous regional sub-populations is further explored in the following chapter.

4.5 References

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5 Analysis of stock structure II – otolith chemistry analysis by solution-based ICPMS

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5.1 Introduction

Management of a fishery should be based on a sound understanding of the stocks (i.e. arbitrary groups of fish which have similar life history characteristics and are large enough to be self-reproducing), such that differences in population parameters and dynamics for each stock can be incorporated into population models and stock-specific strategies for management can be developed (Begg & Waldman 1999). Stock structure also requires some knowledge of the degree of mixing and exchange between stocks from different geographic locations. Failure to recognize stock structure can lead to changes in biological attributes and productivity rates of a species, including overfishing and depletion of some stocks. Indeed, worldwide there are many examples of depleted fish stocks (e.g. anchovy, capelin, Atlantic cod, haddock, herring, orange roughy, Atlantic salmon, sardine, see references in Begg & Waldman 1999).

Stock identification can be based on a variety of approaches including mark-recapture data, catch data, life history characteristics, parasites, otolith chemistry, morphology (e.g. meristics, morphometrics, scale and otolith analyses), and genetics (e.g. protein variation, mitochondrial DNA, nuclear DNA) (Begg & Waldman 1999). Although many studies utilise genetic techniques, these techniques will not detect differences where there are low levels of mixing between stocks. More definitive methods are required under such circumstances. One alternative that has received considerable attention is the analysis of the chemical composition of calcified structures (Campana 1999, Thresher 1999). This approach is based on the water bodies that are inhabited by different stocks differing in the concentration of trace elements. Because the otoliths are composed predominantly of $CaCO_3$ and trace elements that are derived from the water, different stocks would be expected to show differences in the chemical signatures of their otoliths (Campana 1999). Either whole otoliths representing a life-time integrated signature (e.g. Edmonds et al. 1989, Edmonds et al. 1991, Edmonds et al. 1992, Campana & Gagne 1995, Campana et al. 1995, Edmonds et al. 1995, Begg et al. 1998) or portions of the otolith corresponding with particular growth stages (e.g. Kalish 1990, Sie & Thresher 1992, Campana et al. 1994, Thresher et al. 1994, Proctor et al. 1995) can be analysed.

Many studies have found differences in otolith chemistry for fish collected from different areas and have assumed that such differences represent different stocks (see Table II in Gillanders et al.

2001). Most studies have used fish representing a number of age classes, despite ontogenetic effects and age-related differences in exposure history known to contribute to differences in elemental signatures of different size classes collected from the same area (e.g. Papadopoulou et al. 1980, Edmonds et al. 1989, Campana et al. 1995, Campana et al. 2000). We are not aware of any study focusing on adult fish, which have targeted a common year class to determine stock structure.

Otolith chemistry has been used to determine stock structure of snapper (*Pagrus auratus*; Sparidae) in Western Australia (Edmonds et al. 1989, Edmonds et al. 1995, Edmonds et al. 1999, Bastow et al. 2002) and to determine whether juvenile snapper show differences in elemental signatures among estuaries in New South Wales (Gillanders 2002b, Gillanders & Kingsford 2003) and Victoria (Hamer et al. 2003). In addition, connectivity between juvenile and adult populations of snapper has also been investigated (Gillanders 2002a). To date, otolith chemistry has not been applied to the potential stock structure of snapper in South Australia.

Snapper in South Australia is currently managed as a single stock, which is consistent with the genetic analysis of the population that suggests a well-mixed population (Donnellan & McGlennon 1996). Genetic analyses, however focus on evolutionary time scales and even low levels of movement may result in a genetically homogeneous population, despite substantial population structuring occurring. Mark-recapture studies have been of limited value since there is minimal fishing effort on the continental shelf outside of the gulf regions, which limits the opportunity for recaptures (Jones 1984). Thus, the lack of representative tagging and recapture effort is likely to prevent the successful use of mark-recapture methods for determining stock structure of snapper in South Australia (see Begg & Waldman 1999). Catch data for the two gulf regions (Spencer Gulf and Gulf St. Vincent) suggest that these stocks may be discrete (Chapter 4). In addition, life history characteristics (e.g. age and size structure, growth rates) of the four main regions of the fishery (Northern Spencer Gulf, Southern Spencer Gulf, Northern Gulf St. Vincent and Southern Gulf St. Vincent) also suggest population structuring (Chapter 4). Otolith chemical profiles obtained from LA-ICPMS analysis across adult snapper otoliths found significant regional differences (Chapter 3). Thus, the current study was undertaken to further clarify the stock structure of snapper collected from the six fishery regions. Specifically, we were interested in determining whether there were significant differences in the whole otolith signatures, as determined by solution-based ICP-MS, that might suggest that fish from the different regions have been separated for most of their life time.

5.2 Methods

The snapper otoliths used for this study were collected from fish taken from the catches of commercial marine scalefish fishers in 2002, during routine sampling for stock assessment monitoring by SARDI Aquatic Sciences.

Where possible, 30 individuals from the strong 1991 year class (i.e. the 11-year-old fish in 2002) were randomly selected from all fish collected for each of the six snapper fishery regions of South Australia; Northern Spencer Gulf (NSG), Southern Spencer Gulf (SSG), Northern Gulf St. Vincent (NGSV), Southern Gulf St. Vincent including Investigator Strait (SGSV), the west coast of the Eyre Peninsula (WC) and the South East region (SE), i.e. east of the Murray River (Fig. 3.1). The selection of snapper from a single year class that were collected in the same year minimised the possible effects of inter-annual variation in otolith composition that might detract from spatial comparisons. A single sagitta was analysed for each individual. The other sagitta had previously been sectioned for age estimation (Chapter 2 and 5).

5.2.1 Otolith preparation

Ultra-pure (Milli-Q) water and nitric acid (HNO_3; AristaR, BDH chemicals) were used throughout for otolith cleaning and digestion and the preparation of standard solutions. All plastic and glassware was acid-washed in 10% HNO_3 for 24 h, triple rinsed in Milli-Q water and dried in a laminar flow cabinet before use. A dedicated class 100 laminar flow cabinet was used for all drying and the preparation of otolith and standard solutions to minimise contamination. Otoliths were always handled with acid-washed plastic forceps or powder-free plastic gloves.

Before processing, each otolith was ultrasonically cleaned in Milli-Q water for 5 min, rinsed, dried overnight and then weighed to within 0.0001g on an electronic balance. After weighing, otoliths were decontaminated in 1% (by volume) HNO_3 for 5-10 s, rinsed three times in Milli-Q water and dried in acid-washed Teflon caps overnight. Ten randomly selected otoliths were reweighed to assess the effect of cleaning in 1% HNO_3 on otolith weight. Weight changes were found to be negligible.

Each otolith was placed in a 5 ml Teflon tube and digested in 60% (by weight) concentrated HNO_3 overnight. The amount of acid used for digestion was determined according to the weight of the otolith, and ranged between 1 mL for an otolith weight of 0.2-0.3 g and 2.5 mL for an otolith weight of 0.6-0.7 g. After digestion, additional Milli-Q water was added to each tube to bring the volume of the concentrated otolith solution up to 5 mL. Blank solutions were prepared according to

the same procedure, using an initial volume of 2.5 mL of concentrated acid and with no otolith material present.

Final sample solutions were prepared in polypropylene tubes from an appropriate quantity of concentrated otolith solution (370-1,150 μL) diluted with Milli-Q water to produce a 50 mL sample with a final otolith concentration of 10 $g.L^{-1}$ and acidity of 5% (by volume) HNO_3. Acidity was adjusted with concentrated HNO_3 before the final volume was obtained. Thus a similar mass of otolith material was analysed for each individual, irrespective of original otolith size. Blank samples were prepared in a similar manner, with a standard aliquot of 1,000 μL of the initial concentrated acid solution used.

5.2.2 Chemical analysis

Sample solutions were analysed by a Thermo Finnigan MAT (Bremen, Germany) ELEMENT^{TM} high resolution ICPMS, that was located at the School of Geosciences, Monash University. Samples were introduced by a Cetac ASX 500 microautosampler into the water-jacket-cooled cyclonic spray chamber of a self-aspirating nebulizer (Glass Expansion, Victoria, Australia), operated at an aspiration rate of 0.1 $mL.min^{-1}$. The sample aerosol was carried by argon gas to the ICP torch (Finnigan guard electrode) and was ionised by an argon plasma. The ICP-MS was operated in low-resolution mode ($m/\Delta m = 300$) and "Both" detection mode, which automatically selected between analogue and counting modes depending on the concentration of metals detected.

Samples were analysed in randomly allocated blocks of ten, with matrix-matched multi-element standards and external calibration standards before and after each block to correct for instrument drift and for calibration calculations of the following isotopes: ^{25}Mg, ^{27}Al, ^{51}V, ^{52}Cr, ^{55}Mn, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{85}Rb, ^{88}Sr, ^{97}Mo, ^{114}Cd, ^{118}Sn, ^{133}Cs, ^{138}Ba and ^{208}Pb. Matrix-matched standards were prepared from a laboratory standard *Pagrus auratus* otolith powder, ground to micron-sized particles, dissolved in concentrated HNO_3 and made up to 5% (by volume) HNO_3 solution. Known concentrations of primary standards (Merck) were also included to cover the range of elemental concentrations found in otolith samples, i.e. 0 ppb, 1 ppb, 10 ppb and 100 ppb. Standards for external calibration of ^{88}Sr concentrations were also prepared with a 5% HNO_3 background and no otolith matrix, at concentrations of 500 ppb, 1,500 ppb and 3,000 ppb. Blank solutions were run at the start and end of each day for blank corrections and to calculate the limits of detection for each element. A wash solution consisting of 5% nitric acid was used in between samples.

5.2.3 Data analysis

The detection limits of each element examined were estimated from the concentration of analyte required to produce a signal equivalent to three times the SD of the blank. Only elements with all estimates of sample concentrations above the detection limits were used in the statistical analyses. Mean estimates of precision (% relative standard deviation) were calculated from replicate measurements of the laboratory standard (n = 22).

Univariate and multivariate analyses of variance (ANOVA and MANOVA, respectively) were used to determine whether the concentration of single elements and multi-element "signatures", respectively, varied between the otoliths of fish taken from the six different regions. Unfortunately, no fish from the SE region were obtained and therefore this region was not included in the statistical analyses. SPSS Version 10.0.1 general linear models (GLM) was used for all analyses. The five dependent response variables tested were concentrations of ^{25}Mg, ^{59}Co, ^{60}Ni, ^{88}Sr and ^{138}Ba. All required transformation to satisfy the assumptions of normality and homogeneity of variance. ^{138}Ba was inversely transformed according to 1/(X+1), whilst the remaining variables were log transformed according to $\log_{10}(X+1)$. Analyses of covariance (ANCOVA) were used in the first instance to test the influence of otolith weight on the concentration of elements. Although, significant relationships were found for LogMg, LogNi and LogSr, the effect of otolith size was not removed from the variable. A single year class of fish was used for the study and as such variations in otolith size were considered a meaningful and inherent feature of the data. However, these effects should be considered during the interpretation of results.

Linear discriminant function analysis (DFA) was used to assess whether region of capture of individuals could be reliably predicted from the trace element concentration data. Only elements that significantly differed between regions were included in the multi-element analyses. The DFA was completed using SPSS Version 10.0.1 DFA.

Maximum likelihood based analyses were also used, to determine the ability of the trace element concentration data to estimate correctly the proportion of individuals from each region. A multipurpose simulation-bootstrap-analysis program was used for these analyses (Millar, 1990). The program was run in simulation mode for 1000 simulations to estimate the variability of the estimator. The absolute error was estimated as the difference between the actual and known contributions of individuals to each region and the estimated contribution. Once again, only those elements that significantly differed between regions were included in the analyses.

5.3 Results

Only five elements were consistently measured in the whole otolith samples of snapper at concentrations above the estimated detection limits (Table 5.1). These were ^{25}Mg, ^{59}Co, ^{60}Ni, ^{88}Sr and ^{138}Ba (Table 5.1). The concentrations of ^{59}Co and ^{60}Ni in both the otolith samples and laboratory standards were considerably higher than previously reported in other studies of fish otoliths and for the laboratory standard (Campana 1999; Gillanders unpub. data). Furthermore, the precision of the ^{59}Co measurements was questionable (16.52% RSD), especially in comparison with the other elements measured in concentrations above the detection limits (1.6-6.6% RSD; Table 5.1). The elevated concentrations were unlikely to have resulted as an artefact of contamination during preparation, as background counts and blank preparations both recorded relatively low concentrations of ^{59}Co and ^{60}Ni. In addition, the recovery of spiked matrix-matched standards of the two elements were relatively satisfactory. The most likely cause of these elevated levels was a matrix effect, with interference from ^{43}CaO or ^{43}CaOH, due to the high levels of Ca present in otolith samples (Scoog et al. 1998). Although these effects usually cause a reduction in the analyte signal, under certain circumstances the signal is enhanced. Therefore, as a precautionary measure, all statistical analyses were completed twice, once with and once without ^{59}Co and ^{60}Ni included, to assess the relative influence of these measurements on the outcomes.

Regardless of which combination of elements were included, MANOVA tests indicated significant differences in the trace element concentrations between regions, when the transformed concentration data were analysed in combination as elemental signatures (Table 5.2). ANOVA tests for each individual element showed significant differences between regions for all elements, except ^{25}Mg (Table 5.2). The patterns of variation among regions differed between elements, which was reflected in the different homogenous groupings obtained for each element from post hoc Tukey HSD comparisons (Fig. 5.1).

In the case of ^{59}Co, otoliths from SGSV and WC had higher concentrations than those from the two Spencer Gulf regions of NSG and SSG, whilst otoliths from NGSV had intermediate levels (Fig. 5.1*b*). Whereas, for ^{60}Ni, those from SGSV and SSG had the higher concentrations than those from NSG, NGSV and WC, which all had lower concentrations (Fig. 5.1*c*). Therefore, although both elements were likely to have been influenced by matrix effects, different patterns of regional variation were evident.

Table 5.1 The limits of detection (LOD), mean concentration for samples and the laboratory standard and precision (% relative standard deviation of laboratory standard measurements) for each of the isotopes analysed. Mean concentration for the laboratory standard, as measured previously by another independent laboratory is also indicated (Gillanders unpub. data).

Isotope	LOD ($\mu g.g^{-1}$)	Mean sample ($\mu g.g^{-1}$)	Mean lab standard ($\mu g.g^{-1}$)	Precision (% RSD lab standard)	Mean lab standard previous
^{25}Mg	1.59	8.0 ± 2.1	20.9	2.9%	
^{27}Al	0.88	*0.1 ± 1.3*	1.0	9.3%	
^{51}V	1.19	*NQ*	*NQ*	*NQ*	0.01
^{52}Cr	0.79	*0.2 ± 0.4*	0.4	24.2%	0.2
^{55}Mn	0.56	*0.4 ± 0.1*	1.1	2.2%	
^{59}Co	1.10	4.6 ± 1.4	5.7	16.5%	0.8
^{60}Ni	0.82	60.0 ± 4.7	53.0	5.5%	13
^{63}Cu	1.86	*NQ*	*NQ*	*NQ*	0.8
^{66}Zn	0.83	*NQ*	2.7	26.7%	10
^{85}Rb	0.58	*0.04 ± 0.01*	0.06	6.6%	0.05
^{88}Sr	0.94	2191 ± 234	2091	2.5%	1954
^{97}Mo	0.63	*NQ*	*NQ*	*NQ*	0.01
^{114}Cd	0.20	*NQ*	*NQ*	*NQ*	*NQ*
^{118}Sn	0.41	*NQ*	*NQ*	*NQ*	*NQ*
^{133}Cs	0.67	*NQ*	*NQ*	*NQ*	0.7
^{138}Ba	0.49	3.5 ± 2.6	7.3	1.6%	7.9
^{208}Pb	0.41	*NQ*	0.6	5.2%	0.5

Concentrations in italics indicate those that were below detection limits and *NQ* indicates that elemental signals were consistently at or below the level of the blank signal and hence "not quantifiable".

Table 5.2 Results of ANOVA tests for differences between the mean concentrations of each element between the five geographic regions and a MANOVA test with all elements combined as "elemental signatures" for each individual. Asterisks (*) indicate significant differences.

Variable	Source of variance		F	df	P>F
LogMg	Region		2.21	4	0.119
LogCo	Region		8.46	4	0.000*
LogNi	Region		6.65	4	0.004*
LogSr	Region		7.51	4	0.008*
InvBa	Region		5.75	4	0.000*
Mg,Sr,Ba	Region	Wilks' Λ = 0.731	3.71	12,355	0.000*
Mg,Co,Ni,Sr,Ba	Region	Wilks' Λ = 0.548	4.36	20,439	0.000*

For ^{88}Sr, the otoliths from NSG and SSG had the highest concentrations, whilst unexpectedly NGSV had the lowest concentrations (Fig. 5.1*d*). For ^{138}Ba, the otoliths from the two Spencer Gulf regions had lower concentrations and those from SGSV had higher concentrations. However, the otoliths from the WC had only marginally higher concentrations than those from the three Gulf regions, which did not differ significantly.

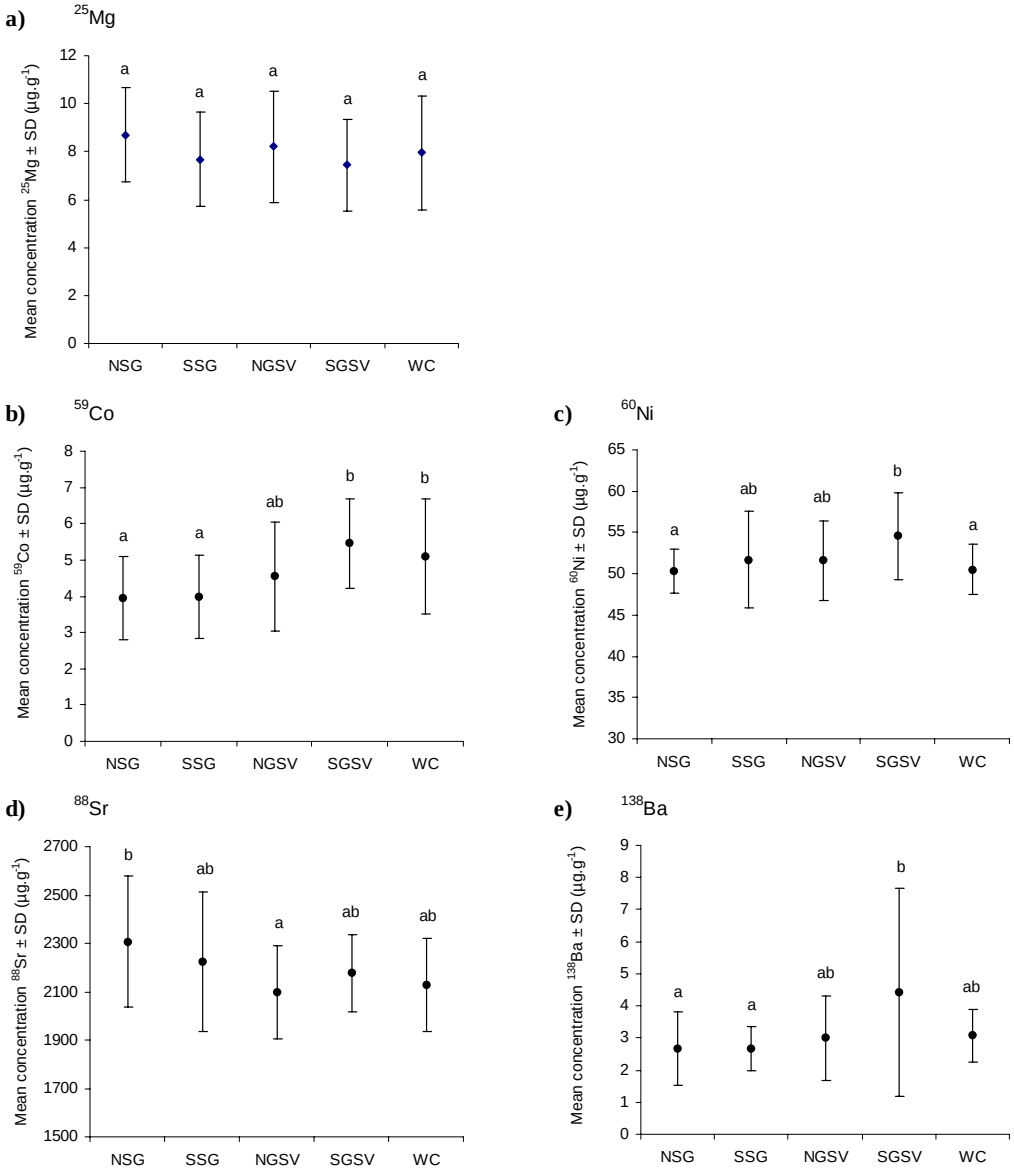

Figure 5.1 Regional variation in the mean concentrations of ^{25}Mg *(a),* ^{59}Co *(b),* ^{60}Ni *(c),* ^{88}Sr *(d)* and ^{138}Ba *(e)* for whole otoliths of snapper. SE region was not included, as the sample size was too small. Homogenous groups from post hoc means comparisons tests on the transformed data are also indicated by the letters a and b (Tukey HSD; $\alpha = 0.05$; harmonic mean sample size = 25.3).

The removal of the two potentially matrix-affected variables of LogCo and LogNi, and the non-significant variable of LogMg, left only two variables for consideration in the DFA, i.e. LogSr and InvBa,. The analysis resulted in the description of two discriminant functions with a combined χ^2 value of 35.9 (df = 8; p = 0.000). The two functions accounted for 76.7% and 23.3%, respectively, of the between-region variation.

The first discriminant function separated NSG and to a less extent SSG from the other three regions, while the second function separated SGSV from NGSV region (Fig. 5.2). The loading matrix of correlations between predictors and discriminant functions (Table 5.3) indicated that the predictor that contributed most to the separation of the Spencer Gulf regions from the others was InvBa according to Function 1, whilst the separations along Function 2 were mainly due to differences in LogSr (Table 5.3). The analysis resulted in very poor classification success, with only 33.3% of all individuals correctly classified to their region of capture. The worst classification results were for SSG and WC (Table 5.4). The misclassified individuals for all regions were broadly spread over the remaining regions.

With the addition of LogCo and LogNi, two significant discriminant functions were described with a combined χ^2 value of 73.9 (df = 16; p = 0.000). The two functions accounted for 77.6% and 14.48%, respectively, of the between-region variability.

A greater degree of separation between the regions resulted from the addition of the two extra elements (Fig. 5.3), with the pattern of separation similar to that described above when only two elements were included. The first discriminant function separated NSG and SSG from the other three regions and SGSV from NGSV (Fig. 5.3). The second function further separated SGSV from NGSV and WC. The loading matrix of correlations between predictors and discriminant functions (Table 5.5) indicated that the variables responsible for the separations along the two functions were primarily LogCo and LogNi respectively, rather than the other two more analytically reliable trace element variables. With the addition of the two extra elements, the level of correct classification of individuals according to region was increased from 33.3% to 43.3%, and the classification success for the WC region in particular was noticeably improved (Table 5.6). For all regions, most misclassified individuals were allocated to the region nearest to the region of capture.

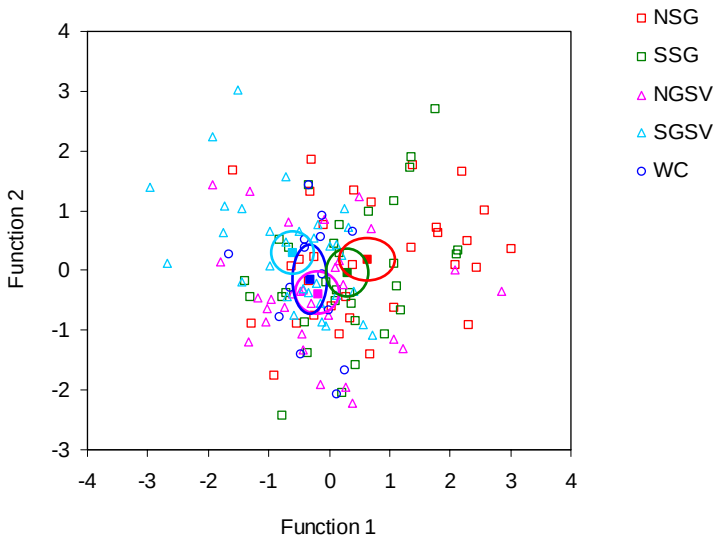

Figure 5.2 Scatterplot of the first two discriminant function scores for snapper otoliths resulting from a discriminant function analysis using the transformed concentration variables of LogSr and InvBa. Ellipses indicate the 95% CI around the group centroid for each region.

Table 5.3 Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions for the two concentration variables.

Variable	Function 1	Function 2
LogSr	0.58	0.82
InvBa	0.86	-0.51
% of variance	76.7%	23.3%

Table 5.4 Classification success (% of individuals captured in each region, as indicated by the header row, classified into each of the five regions) for discriminant function analysis using variables LogSr and InvBa. The shaded areas indicate the percentage of fish correctly classified to each region.

Region	NSG	SSG	NGSV	SGSV	WC
NSG	43.8	34.4	12.9	6.3	7.1
SSG	6.3	15.6	16.1	12.5	0
NGSV	25.0	28.1	35.5	25.0	35.7
SGSV	21.9	15.6	16.1	46.9	42.9
WC	3.1	6.3	19.4	9.4	14.3

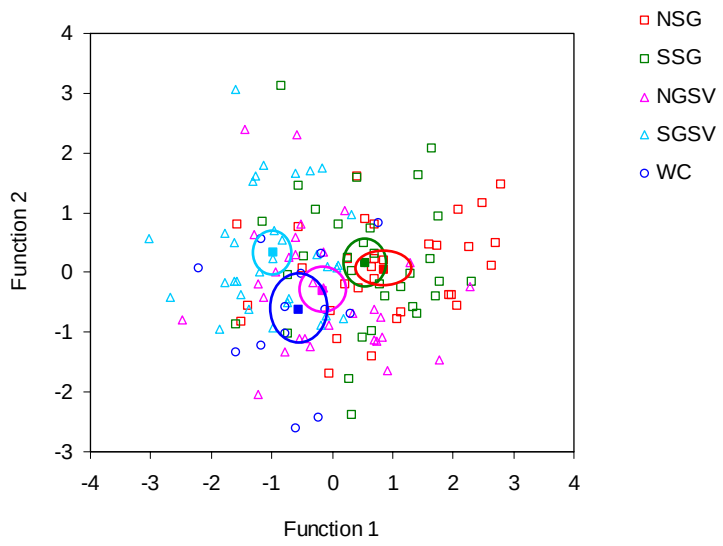

Figure 5.2 Scatterplot of the first two discriminant function scores for snapper otoliths resulting from a discriminant function analysis of the four concentration variables of LogCo, LogNi, LogSr and InvBa. Ellipses indicate the 95% CI around the group centroid for each region.

Table 5.5 Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions for the four concentration variables LogCo, LogNi, LogSr and InvBa.

Variable	Function 1	Function 2
LogCo	-0.74	-0.56
LogNi	-0.06	0.91
LogSr	0.52	0.54
InvBa	0.55	-0.37
% of variance	77.6%	14.4%

Table 5.6 Classification success (% of individuals captured in each region, as indicated by the header row, classified into each of the five regions) for descriminant function analysis of the four concentration variables LogCo, LogNi, LogSr and InvBa. The shaded areas indicate the percentage of fish correctly classified to each region.

Region	NSG	SSG	NGSV	SGSV	WC
NSG	50.0	43.8	12.9	3.1	7.1
SSG	18.8	25.0	9.7	6.3	7.1
NGSV	15.6	15.6	35.5	12.5	7.1
SGSV	6.3	9.4	25.8	56.3	21.4
WC	9.4	6.3	16.1	21.9	57.1

With just the two elements of LogSr and InvBa included, the maximum likelihood analysis vastly underestimated the contribution of SSG to the mixed population, and overestimated the contribution of the WC (Table 5.7). Overall, performance of the classification was poor relative to the results obtained for laser ablation classifications (Chapter 3) and with very high levels of variability in the estimator (12.8-19.6%; Table 5.7). With the addition of the two extra elements of LogCo and LogNi, the analysis performed worse in terms of error and marginally better in terms of the level of variability in the estimator (8.5-15.4%; Table 5.8). The contribution of SSG was again vastly underestimated whilst that of NGSV was overestimated (Table 5.8).

Table 5.7 Results of maximum likelihood analysis of the ability of LogSr and InvBa to estimate correctly the proportion of individuals from different regions.

Region	Actual contribution	Estimated contribution	SD
NSG	22.7%	26.7%	13.1%
SSG	22.7%	11.0%	17.9%
NGSV	22.0%	25.0%	18.8%
SGSV	22.7%	19.5%	12.8%
WC	9.9%	17.9%	19.6%

Table 5.8 Results of maximum likelihood analysis of the ability of the four element concentrations (LogCo, LogNi, LogSr and InvBa) to estimate correctly the proportion of individuals from different regions.

Region	Actual contribution	Estimated contribution	SD
NSG	22.7%	29.3%	11.4%
SSG	22.7%	8.7%	13.7%
NGSV	22.0%	32.0%	15.4%
SGSV	22.7%	24.1%	9.4%
WC	9.9%	5.8%	8.5%

5.4 Discussion

The elemental analysis of whole otoliths of snapper collected from different regions of South Australian waters showed significant variation. NSG and SSG showed some overlap in elemental signatures, while NGSV and SGSV showed significant variation. The WC overlapped with NGSV. Many other studies have found differences in elemental composition among locations which have been used to infer differences in stock structure (Edmonds et al. 1989, Edmonds et al. 1991, Edmonds et al. 1992, Campana & Gagne 1995, Campana et al. 1995, Begg et al. 1998), pollution regimes (Dove & Kingsford 1998), and natural tags of area of origin for juveniles (Gillanders & Kingsford 1996, Thorrold et al. 1998a, Thorrold et al. 1998b, Gillanders & Kingsford 2000, Gillanders 2002b, Hamer et al. 2003).

Despite differences in elemental composition of otoliths, the mechanisms generating these differences are not well understood. Water chemistry is likely to vary considerably among the different regions and may be influenced by freshwater input from rivers, oceanography (e.g. upwelling and currents) and the level of industry and urban development (Chapter 3). These factors are not only likely to influence the concentration of elements in the water, but the salinity and temperature of the water mass also vary along the length of the gulfs. Several studies have found a positive relationship between elements in the water and otolith chemistry for elements such as Sr and Ba (Gallahar & Kingsford 1996, Pollard et al. 1999, Bath et al. 2000, Elsdon & Gillanders 2003), whilst temperature and salinity have also been found to influence the chemistry of otoliths (Fowler et al. 1995b, Elsdon & Gillanders 2002, Kraus & Secor 2004).

Temperature and salinity show large regional variation in South Australian waters (see Chapter 1). Both gulf regions have greater seasonal variation in water temperature than adjacent continental shelf waters (Nunes & Lennon 1986) and much higher temperatures in the northern regions than elsewhere in the State (Fig. 1.1). Strong patterns of salinity stratification are also evident within the gulfs from north to south, and extremely high salinities at the heads of the gulfs are found during summer (de Silva Samarasinghe & Lennon 1987). Thus, the northern gulf regions are hypersaline with respect to shelf waters (e.g. West Coast, South East) due to limited freshwater inflow and high evaporation. Elsdon and Gillanders (2002) found a significant interactive effect of temperature and salinity on levels of strontium and barium in otoliths of black bream such that at low temperatures (e.g. 16°C) there was little effect of salinity on levels of Sr and Ba in otoliths, but at higher temperatures (e.g. 24°C) increased amounts of Sr and Ba in otoliths were found with increasing salinity. The maximum salinity in their experiments was only 30‰, whereas gulf waters may reach 42-46‰ during summer. No experiments have examined the influence of such high salinities on otolith chemistry. Less research has focused on elements other than Sr and Ba.

Freshwater input to the sea through rainfall is highly seasonal and localised in South Australia with most rainfall occurring in winter and to a lesser extent in spring. The high inputs of freshwater are likely to occur in the south east and southern Gulf regions (Fig. 1.3). Terrestrial and stream runoff may contain higher levels of trace elements (e.g. Ba) in comparison to seawater. Elevated levels of barium may also occur in localised areas due to summer upwelling along the west coast of Eyre Peninsula (West Coast region) and along the southeast coast of the State (South East region), which brings cold nutrient rich waters to the surface (Middleton & Platov 2003). Thus, a combination of natural processes are likely to influence otolith chemistry of fish in South Australia.

Information on levels of trace elements in the waters of South Australia is limited, but there are likely to be significant point sources of trace elements from anthropogenic activities. For example, heavy industry including a lead-zinc smelter is focused around northern Spencer Gulf where liquid effluent containing 250 t of zinc and 100 t of lead is discharged annually (Edwards et al. 2001). Previous studies in northern Spencer Gulf have found elevated levels of heavy metals (e.g. Pb, Cd, Cu, Zn) in sediments, seagrass, and flesh of marine fish (Ward & Young 1981, Ward et al. 1986, Ward 1987, Edwards et al. 2001). Barker Inlet, located just north of Adelaide on the eastern shore of Gulf St. Vincent, is also a major area for urban and industrial runoff including sewage effluent, saline discharges, thermal effluent and other contaminants from stormwater drains (Edwards et al. 2001). Thus, areas may exist where ambient levels of trace elements are elevated providing a more concentrated source for incorporation into otoliths. Elevated levels of mercury, copper and lead in the water have resulted in increases in otoliths (Geffen et al. 1998, Milton & Chenery 2001).

Previous studies have found that a range of other factors (e.g. growth rates, ontogenetic and physiological effects) may also influence otolith composition in some species (Kalish 1989, 1991, Sadovy & Severin 1992, Fowler et al. 1995a, Limburg 1995). Our analyses were limited to fish from the strong 1991 year class (i.e. 11-year-old fish in 2002) thus minimising possible ontogenetic effects. Although ontogenetic effects were minimised, substantial differences in size at age were found, especially for the two regions of Spencer Gulf (see Chapter 4). Despite differences in size at age for Southern and Northern Spencer Gulf fish, similarities were found in otolith chemistry of these two regions. Differences between the two regions of Gulf St. Vincent may, however, reflect differences in size at age, which were found for fish older than 3 years of age. We did not remove the effect of size from the data because we used a single age class and therefore differences in size of fish among regions represented a meaningful feature of the data.

Although significant differences in otolith chemistry were found among regions, the accuracy with which fish could be assigned to their region of collection was relatively poor (classification success ranged from 25-57% when using 4 elements) using a discriminant function approach. Spencer Gulf fish tended to be mis-assigned to the other Spencer Gulf region. Likewise, fish from Gulf St.

Vincent were generally mis-assigned to the other GSV region, although for SGSV a large number of fish were assigned to the West Coast. The maximum likelihood analysis showed less error in estimating the proportion of individuals from the different regions (error rates ranged from 1-14%). Different sample sizes, number of variables measured (e.g. elements) and number of stock groups (e.g. regions) all affect the relative performance of classification and maximum likelihood estimators (Millar 1990). Additional elements and the use of stable isotopes may increase the success of such methods. Although a wide range of elements was screened for use in the current study, many were below detection limits (e.g. Mn, Cd, Pb). In addition, the concentrations of two of the elements used (Ni, Co) are considered relative due to possible matrix-related interferences.

Some of the overlap in elemental signatures among regions may also relate to possible movement patterns of snapper. The life history model suggests that fish from the gulfs show a complex age-related migration where the young fish migrate to the continental shelf and then undertake annual spawning migrations over a number of years back into the gulfs. If this model is correct, it suggests that fish from all regions may incorporate a continental shelf signature at some part of their life cycle. Since the solution-based approach used in the current study integrates the entire lifetime signal, differences in otolith chemistry would not be as great as if the edge region (reflecting where the fish were caught) were analysed. Results using a laser based approach targeting sections of the otolith that represent each age increment can be compared to the solution based analyses. While the same year class of fish was used for both types of analyses, otoliths used for the solution-based analyses were from 11 year old fish, and those used in the laser-based analyses were from 9 year old fish. The laser results suggested that otoliths from the two Spencer Gulf regions differed from age 4, whereas the solution-based results suggested these regions were similar. Both methods suggested that the Gulf St. Vincent regions differed, with the laser approach suggesting that the fish from these regions had different signatures from age 4. Likewise, the West Coast overlapped with Northern Gulf St. Vincent for both methods and for most age increments. Although the South East was analysed using a laser-based approach, no samples were obtained from this region for the solution-based approach.

Other methods for determining stock structure of snapper in South Australia have shown mixed results. Variation in mtDNA and allozyme markers showed no sub-structure of snapper populations west of the River Murray mouth, but significant differences between fish east of the River Murray mouth (South East region) and the rest of South Australia (Donnellan & McGlennon 1996). These results contrasted with a previous allozyme study that suggested some structuring of populations within Spencer Gulf (MacDonald 1980, reported in Donnellan & McGlennon 1996). Molecular studies may show little population structuring if population subdivision has occurred relatively recently, and if low rates of female migration occur. Limited tagging data have shown movements of adult snapper from Spencer Gulf into Investigator Strait (Jones 1984), supporting the

limited population structure that was found using molecular data. Differences in the temporal histories of catches suggest that the populations in Spencer Gulf and Gulf St. Vincent may be discrete (Chapter 4). In addition, life history characteristics (e.g. age and size structure, growth rates) of the four main regions of the fishery (NSG, SSG, NGSV, SGSV) also suggest population structuring (Chapter 4). For example, fish from SSG have slower growth, and do not attain the same size-at-age or the same asymptotic size as fish from NSG (Chapter 4). Fish from NGSV have a more extensive age structure including more old fish and a larger size at age than fish from SGSV (Chapter 4). Chemical profiles from LA ICP-MS analysis across the otoliths of adult snapper suggested that significant regional differences can occur including differences between regions within each gulf (Chapter 3). In combination these various datasets suggest some sub-structure in the population of snapper in South Australia.

Studies of snapper in Western Australia also suggest that population structure is complex (e.g. Johnson et al. 1986, Edmonds et al. 1989, Edmonds et al. 1995, Edmonds et al. 1999, Bastow et al. 2002, Moran et al. 2003). In particular, fine-scale structuring of snapper populations in Shark Bay, where 70-80% of WA's snapper catch is taken, has been found. Shark Bay represents a smaller area than Spencer Gulf, but has a similar physical environment (e.g. hypersaline waters) to the South Australian gulfs (Nunes & Lennon 1986). Whole otolith analysis of minor and trace elements (e.g Na, K, Sr, Mg, S, P), as well as stable isotopes (^{18}O, ^{13}C), head morphology, parasite load, long-term movement patterns, and genetics all support at least three essentially non-mixing breeding populations occurring in Shark Bay (Johnson et al. 1986, Williams et al. 1993, Edmonds et al. 1995, Moran et al. 1998, Bastow et al. 2002, Moran et al. 2003). Tagging studies elsewhere suggest limited movement of snapper out of embayments (Sumpton et al. 2003).

While the snapper fishery in South Australia is currently managed as one stock, the fishery regions may not necessarily match the biological population structure. Current management and reporting likely reflect political (e.g. state) and administrative boundaries and as such the regional boundaries are relatively arbitrary. The boundary between the South East and Southern Gulf St. Vincent is however based on different genetic stocks. Despite the relative nature of the other boundaries the stock structure appears complex which suggests a risk averse strategy to management should be adopted (Stephenson 1999). Thus, it may be more prudent to manage the fishery as separate stocks as most available evidence supports substantial stock structuring. If such stock structuring is not recognised there is potential to lead to the demise of a stock from localised overfishing.

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6 Natal origins of adults – otolith cores chemical analysis by laser-ablation ICPMS

K.C. Hall, B.M. Gillanders and A.J. Fowler

6.1 Introduction

The geographic structure of marine populations is greatly influenced by the movement patterns of individuals of different life history stages. For most marine organisms, the larval stage is planktonic and has traditionally been considered the main dispersive stage (Swearer et al. 2002). Larvae were generally assumed to move long distances from natal populations and to recruit to distant populations. Hence local populations were considered to be demographically "open systems" with high levels of connectivity between them over large distances (Cowen 2002). As a result, marine fisheries tended to be managed as single large stocks.

Recently, however, increasing evidence suggests that for many marine fish species, population structure may be more complex than previously thought. In some instances, behavioural or physical mechanisms have been described that could limit larval dispersal, such that most larvae are retained and recruited to natal populations (Jones et al. 1999; Swearer et al. 1999; Cowen et al. 2000; Swearer et al. 2002). Furthermore, for a growing number of mobile species, 'homing' behaviour has been described where mature adults systematically return on an annual basis from mixed feeding grounds to spawn at natal spawning areas (e.g. Campana et al. 2000; Thorrold et al. 2001). Both scenarios could lead to high levels of self-recruitment, such that local populations were effectively "closed systems" that were reproductively isolated from adjacent populations. These populations should be considered separate stocks for fisheries management purposes. Therefore, knowledge of the extent of connectivity between local populations is vital to understanding stock structure and for the sustainable management of exploited populations (Cowen et al. 2002). Unfortunately, for many species such understanding is lacking due to the difficulties associated with tracking immense numbers of tiny larvae within vast marine systems (Jones et al. 1999; Thorrold et al. 2002).

For snapper *Pagrus auratus*, the first 3 to 4 weeks of life are spent as pelagic larvae, followed by settlement to benthic nursery areas (Francis et al. 1992; Fowler and Jennings 2003). Juveniles are generally found associated with soft bare substrate in sheltered estuaries or bays (Francis 1995; Gillanders 2002*b*; Fowler and Jennings 2003). In South Australia, juveniles have only been reported from the northern parts of both Spencer Gulf and Gulf St Vincent (Fig. 6.1*a*) (Fowler and

Jennings 2003). However, there has been little dedicated sampling for juveniles outside of these areas.

Ripe females with hydrated oocytes, taken to indicate imminent spawning, have been captured throughout all regions of the State (Fowler unpub. data), which suggests that spawning is not restricted to the localised areas where juveniles have been found. However, evidence from tagging studies and the analysis of commercial catch statistics also suggests that some adults migrate into the Gulfs from the continental shelf during the spring and summer to spawn (Jones 1984). Thus, a life history model involving complex age-specific migrations has been proposed for snapper in South Australia (McGlennon and Jones 1999). Juveniles are thought to recruit into the northern gulfs and then move southwards after a few years, leave the gulfs and migrate to the continental shelf. From there adults undergo annual spawning migrations over a number of years back into the gulfs, where they are highly vulnerable to the fishery. Eventually, at around 12 years of age, they are thought to become permanent residents in the northern gulfs.

The consequences of this life history for stock structure ultimately depends on the extent of larval dispersal between regions and the direction of subsequent spawning migrations. A number of hypothetical scenarios have been conceptualised (Fig. 6.1), each involving a different level of complexity of stock structure. The first (Model I, Fig. 6.1*b*) involves complete mixing of the population throughout the State. Spawning and nursery grounds occur in all waters, larval or adult dispersal is widespread, and adults do not return to natal areas to spawn. Hence, the populations are not reproductively isolated and should be considered "open systems" with recruitment occurring from a heterogenous source. The second scenario (Model II, Fig. 6.1*c*) consists of a single contracted spawning or nursery area, with widespread larval or adult dispersal and adults return to the common grounds for spawning. Hence, the populations are not reproductively isolated and should again be considered "open systems", but with recruitment from a single homogenous source.

The third scenario (Model III, Fig. 6.1*d*) involves localised spawning or nursery areas (for example the northern Gulf regions) and relatively widespread larval or adult dispersal. The determining factor for this model, is whether or not adults return to natal areas to spawn. If most adults return, the two gulfs would represent reproductively isolated populations and separate fishery stocks. Alternatively, if adults were guided by other endogenous factors and migrated randomly to either spawning area, the populations in the two gulfs would not be reproductively isolated, but rather one large inter-related stock. Finally, the last scenario (Model IV, Fig. 6.1*e*) involves spawning and nursery areas in all waters, but larval and adult dispersal is limited or adults return to natal areas to spawn. Hence, populations in different regions are essentially self-recruiting, "closed systems" that should be considered separate fisheries stocks. Obviously these models do not include all possible scenarios but they do provide a basis for the formulation of hypotheses to be tested.

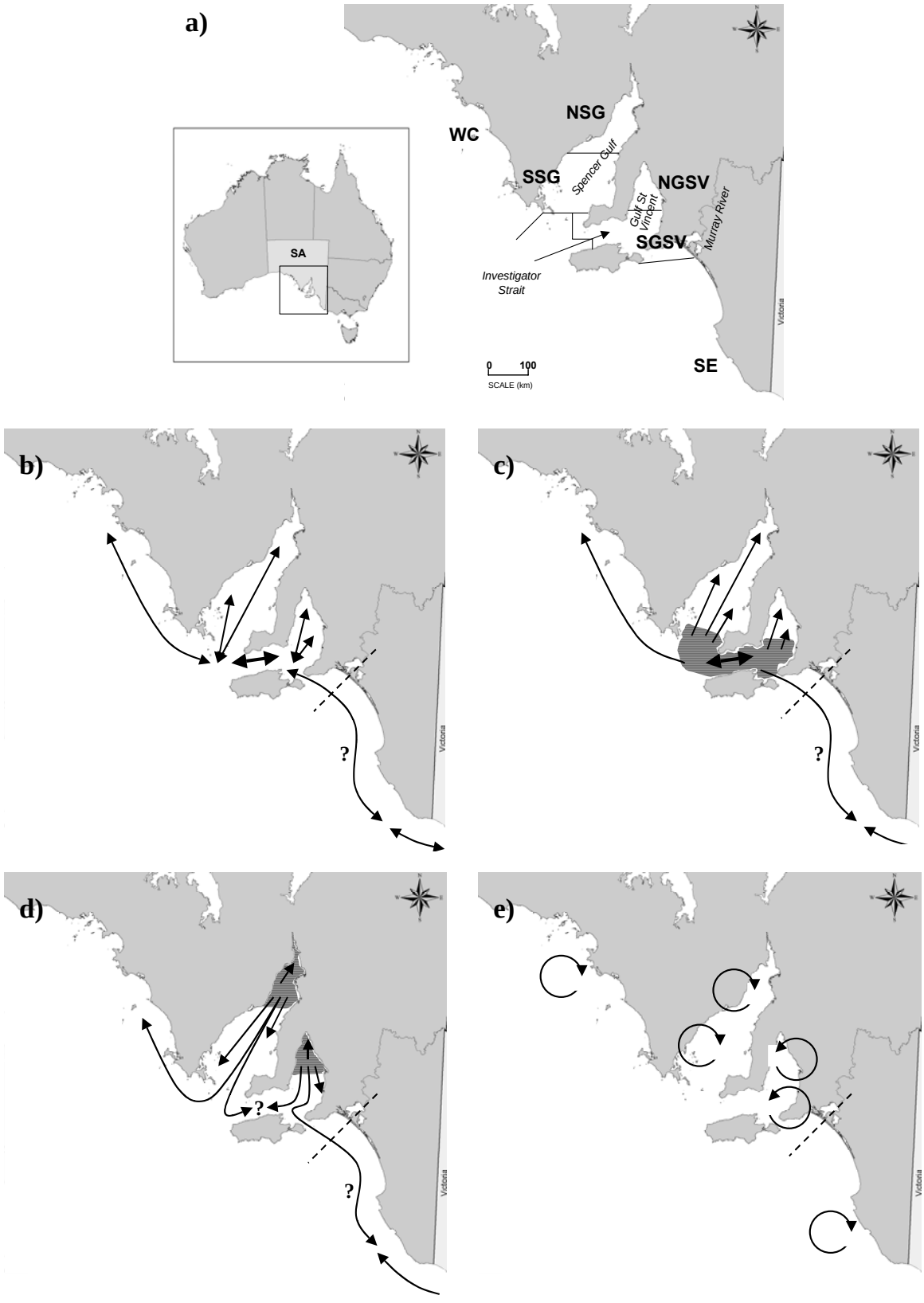

Figure 6.1 *(a)* Map of South Australia with regional breakdown of waters indicated. *(b-e)* Hypothetical scenarios for snapper dispersal in South Australian waters. *(b)* Spawning and nursery areas occur in all waters, larval or adult dispersal is widespread and adults do not return to natal areas to spawn; *(c)* Spawning or nursery areas are contracted but larval or adult dispersal is widespread and adults return to common grounds to spawn; *(d)* Spawning or nursery areas are localised, larval or adult dispersal is widespread and the extent of connectivity is determined by the direction of subsequent spawning migrations; *(e)* Spawning and nursery areas occur in all waters, but larval and adult dispersal is limited or adults return to natal areas to spawn. Hatched areas represent contracted spawning or nursery areas; solid lines represent dispersal patterns; thick double ended arrows suggest mixing between gulfs; dashed lines indicate the division of South Australian and Victorian stocks at the Murray River mouth as determined by genetics.

Given the limited information available, all of the above scenarios are plausible for snapper stocks in South Australia. However, the snapper fishery is currently managed as a single large inter-related stock (McGlennon and Jones 1999). This was primarily based on results of a genetic study, which found no indication of finer-scale stock structure in South Australian populations, other than the broad division between South Australian and Victorian populations near the mouth of the Murray River (Donnellan and McGlennon 1996). Trends in fisheries statistics and population age and size structure, however, suggest that regional population structure may exist, and that the populations in the two gulfs are separate stocks (Chapter 4). Furthermore, the chemical composition and clarity of otoliths of fish taken from different geographic regions show significant regional differences that imply the possibility of a finer-scale regional stock structure (Chapter 3 and 5).

The objective of the present study was to improve our understanding of the stock structure of snapper in South Australia by investigating the natal origins of adults collected from different geographic regions. Our approach was to analyse the chemical composition of the central part or core of the otoliths of adult fish. Since the core is laid down during the larval and immediate post-settlement life history stages it should have a chemical signal that is representative of the spawning and nursery areas for each individual (Thorrold et al. 2002). The chemical composition of each annual increment of adult snapper otoliths had previously been analysed with laser-ablation transects from the core to the outer edge (Chapter 3). The first two increments were found to be remarkably similar for fish collected from different regions. However, only two elements were considered in detail in that study, namely ^{88}Sr and ^{138}Ba and the transects did not always pass directly through the core due to the slight curvilinear nature of the growth axis. Hence, the aim of the work described here was to specifically target the central core of the transverse section of each adult otolith using laser-ablation ICP-MS analysis and to use the multi-elemental results for each core as a "larval signature" to compare the natal origins of fish collected from different geographic regions.

We tested the null hypothesis that there would be no significant difference in the chemical composition of otolith cores of adults collected from different regions in South Australia. We hypothesised that such a result would be expected if all larvae and juveniles originated from a common spawning or nursery area (e.g. Model I above), or if there were more than one spawning or nursery area but that these were subsequently mixed with respect to the region of adult capture (e.g. Model II above). This result is also consistent with there being multiple nursery areas with similar water chemistry. Alternatively, rejection of the null hypothesis would suggest that adults captured in different regions originated from different larval sources (e.g. Model III or IV above). Furthermore, if the multi-elemental "larval signatures" grouped according to the region of capture

of adults, it would suggest either that larvae were retained in the region of origin or that individuals returned to or became resident in natal regions as adults (e.g. Model IV).

6.2 Methods

The snapper otoliths used for this study were collected from the catches of commercial marine scalefish fishers in 2000, during routine sampling for stock assessment monitoring by SARDI Aquatic Sciences. The otoliths were randomly sub-sampled as detailed in Chapter 3, such that up to 22 individuals from the strong 1991 year class (i.e. the 9 year old fish in 2000) were analysed from each of the six snapper fishery regions of South Australia, i.e. northern and southern Spencer Gulf (NSG and SSG, respectively), northern and southern Gulf St. Vincent (NGSV and SGSV, respectively), the west coast (WC) and the south east (SE) (Fig. 6.1*a*; Table 6.1). The selection of snapper from only a single year class, that were all been collected in the same year, minimised the possible effects of inter-annual variation in otolith composition that might detract from spatial comparisons. Thus, the juvenile portion of all otoliths analysed should have been deposited at approximately the same time in 1991.

Table 6.1 Samples of 9 year old snapper *Pagrus auratus* randomly selected from each of the six geographic regions for analysis of otolith cores by LA-ICPMS.

Region	n	Size range (mm)
NSG	21	618 - 778
SSG	22	335 - 736
NGSV	10	588 - 785
SGSV	18	411 - 765
WC	20	493 - 761
SE	20	514 - 618

6.2.1 Otolith preparation

The transverse sections of otoliths considered in this study had been previously sampled with LA-ICPMS for the work described in Chapter 3 and the methods used for their preparation were detailed in that chapter. Since the mounted sections had been previously ablated, they were ultrasonically cleaned for 5-10 min in Milli-Q water, rinsed and dried overnight in a laminar flow cabinet. The slides were then placed in individual plastic bags for transportation to the LA ICP-MS facility.

6.2.2 Chemical analysis

Sections were analysed by LA-ICPMS at the School of Geosciences, Monash University. The system consisted of a Merchantek LUV266 petrographic ultraviolet laser (Nd:YAG) microprobe connected to a Thermo Finnigan MAT ELEMENT high resolution ICPMS. The sections were placed in a sealed perspex ablation chamber with helium atmosphere and viewed remotely via a microscope objective lens connected to a computer monitor. Although the sections had previously been analysed with laser-ablated ICPMS transects from the core to the outer edge along Axis 3 (Chapter 3), the slight curvilinear nature of the growth axis, meant that the transects rarely started directly in the core, but slightly to the side of it. Therefore, here the laser was focused directly onto the central core and fired continuously in the one location for 70 pulses, with a repetition rate of 6 Hz and laser energy of between 70 and 100 mJ. This produced an ablated crater size of approximately 40 μm diameter. Ablated material was entrained in an argon and helium gas stream (argon flow rate 14 $mL.min^{-1}$; helium flow rate 1.36 $mL.min^{-1}$) and carried to the plasma torch of the ICPMS for analysis. The elemental isotopes chosen for analysis were those thought to be metabolically inert, not under significant physiological control, and not subject to interference from other isotopes. These included ^{25}Mg, ^{52}Cr, ^{55}Mn, ^{59}Co, ^{63}Cu, ^{64}Zn, ^{88}Sr, ^{114}Cd, ^{138}Ba and ^{208}Pb, as well as ^{44}Ca, which was measured for use as an internal standard.

For most sections only one spot was analysed as close to the centre of the core as possible. To determine whether the precise location of the spot influenced the concentration of elements obtained, 18 sections were randomly chosen and three replicate spots were ablated in each core. The resulting concentrations were compared within and between fish.

Sections were analysed in randomly allocated blocks of 10 to 12. At the beginning and end of each block, background counts were collected for 70 measurements, to estimate the limits of detection for each isotope. A glass standard (NIST 612) was analysed twice to correct for instrument drift by linear interpolation and for use as an external standard to estimate isotope concentrations from sample counts. At the start of each sample, background counts were collected for 30 measurements, and the average was subtracted from sample counts to correct for background levels. The ablation chamber was purged for 20 s after each opening.

All data reduction was carried out off-line using spreadsheet programs. Calcium was used as an internal standard to correct for variation in ablation yield. Calcium concentration was assumed to be known and constant at 388,000 $\mu g.g^{-1}$, based on the published values for a certified reference material of fish otolith (Yoshinaga et al. 2000). The concentrations of other isotopes were estimated against this using the relative response factor of the instrument to the known concentration in an external standard (NIST 612), and that recorded for the samples.

6.2.3 Data analysis

The detection limits of each element examined were estimated from the concentration of analyte required to produce a signal equivalent to three times the SD of the blank. Only elements with more than 90% of sample concentrations above the estimated detection limits were included in statistical analyses. Values below the detection limits were left unchanged. Mean estimates of precision (% relative standard deviation) were estimated based on replicate measurements of the NIST 612 standard (n = 12).

Univariate and multivariate analyses of variance (ANOVA and MANOVA, respectively) were used to determine whether the concentration of single elements and multi-elemental "larval signatures", respectively, varied between the otolith cores of fish from different geographic regions. Analyses were completed using SPSS Version 10.0.1 general linear models (GLM). The five dependent response variables tested were concentrations of ^{25}Mg, ^{52}Cr, ^{66}Zn, ^{88}Sr and ^{138}Ba. All required log transformation according to $\log_{10}(X+1)$ to satisfy the assumptions of normality and homogeneity of variance in the first instance. A combination of linear regression analyses and analyses of covariance (ANCOVA) were used to test the influence of the width of the first increment, used as a proxy for growth rate, on the concentration of elements in the otolith core. Although, a significant linear relationship was found for LogMg ($p = 0.017$; $r^2 = 0.051$), the effect of growth rate was not removed from the variable. A single year class of fish was used for the study and as such variations in growth rate were considered a meaningful and inherent feature of the data. However, these effects are mentioned here as they should be considered during interpretation of the results.

6.3 Results

The crater that resulted from each ablation was circular and approximately 40 μm in diameter. The central core of a snapper otolith, however, is not circular but compressed along the proximal-distal axis and elongated dorso-ventrally (Fowler and Jennings 2003). The diameter of the pre-settlement core can be less than 40 μm along the compressed axis. Therefore, even if the spot was located directly over the core it would sample all the pre-settlement increments (larval growth period) and some of those formed immediately after settlement.

To determine whether the precise location of the spot influenced the concentration of elements obtained, three replicate spots were ablated in the central cores of 18 randomly chosen fish. One was located directly over the central core and the other two were adjacent to it and hence encompassed more of the post-settlement material. The resulting elemental concentrations were compared within and between fish. For ^{66}Zn, ^{88}Sr and ^{138}Ba, the level of variation in concentrations among replicate spots for each fish (34-42%) was less than that between fish (66-58%). However,

for ^{25}Mg and ^{52}Cr, the variation among replicate spots (75.7% and 55.3%, respectively) was greater than that between fish (24.3% and 44.7%, respectively). Nevertheless, for all elements measured above the detection limits, the overall difference between fish was sufficiently large to detect significant differences (p = 0.000-0.047).

Of the original 10 elements sampled in the main sampling program, only five were recorded in concentrations that were above the estimated detection limits. These were ^{25}Mg, ^{52}Cr, ^{66}Zn, ^{88}Sr and ^{138}Ba (Table 6.2). The ^{66}Zn concentrations were measured with much lower precision (27.3% RSD) than the other elements (Table 6.2). The variation in the mean concentration of each element in the central core was assessed between regions using ANOVA (Fig. 6.2; Table 6.3). No significant differences were detected among regions for the log-transformed concentrations of ^{25}Mg, ^{52}Cr, ^{88}Sr or ^{138}Ba, whereas a difference was found for ^{66}Zn. Post hoc analyses for ^{66}Zn concentrations indicated that the central cores of otoliths from SSG were significantly lower in ^{66}Zn than for the two Gulf St Vincent regions and the SE, but did not differ from the values of the NSG or WC regions (Fig. 6.2). This result was significant despite the poor precision of ^{66}Zn measurements (Table 6.2). However, when combined with the other elements as multi-elemental "larval signatures" for each individual, the results from a MANOVA indicated no difference among regions (Table 6.3; Fig. 6.3).

Table 6.2 The limits of detection (LOD), mean concentration for samples and the NIST 612 standard and precision (% relative standard deviation of NIST 612 standard measurements) for each of the isotopes analysed. Mean concentration for the NIST 612 standard is also indicated for comparison (Hollocher and Ruiz 1995).

Isotope	LOD ($\mu g.g^{-1}$)	Mean sample ($\mu g.g^{-1}$)	Mean NIST standard ($\mu g.g^{-1}$)	Precision (% RSD NIST standard)	NIST standard literature
^{25}Mg	10.3	37.6 ± 7.7	77.1	6.2%	77.4
^{52}Cr	0.7	1.4 ± 0.3	31.3	2.9%	31.2
^{55}Mn	5.8	*7.6 ± 3.7*	37.7	3.1%	37.7
^{59}Co	4.0	*NQ*	32.9	2.0%	32.8
^{63}Cu	0.7	*0.5 ± 0.4*	33.8	17.5%	33.5
^{66}Zn	0.3	2.4 ± 2.3	34.8	27.3%	34.1
^{88}Sr	0.9	1489 ± 162	76.6	2.9%	76.3
^{114}Cd	0.2	*NQ*	28.6	4.0%	28.4
^{138}Ba	0.1	1.8 ± 0.6	37.6	3.1%	37.5
^{208}Pb	0.04	*0.05 ± 0.1*	40.8	6.6%	40.6

Concentrations in italics indicate those that were below detection limits and *NQ* indicates that elemental signals were consistently at or below the level of the blank signal and hence “not quantifiable”.

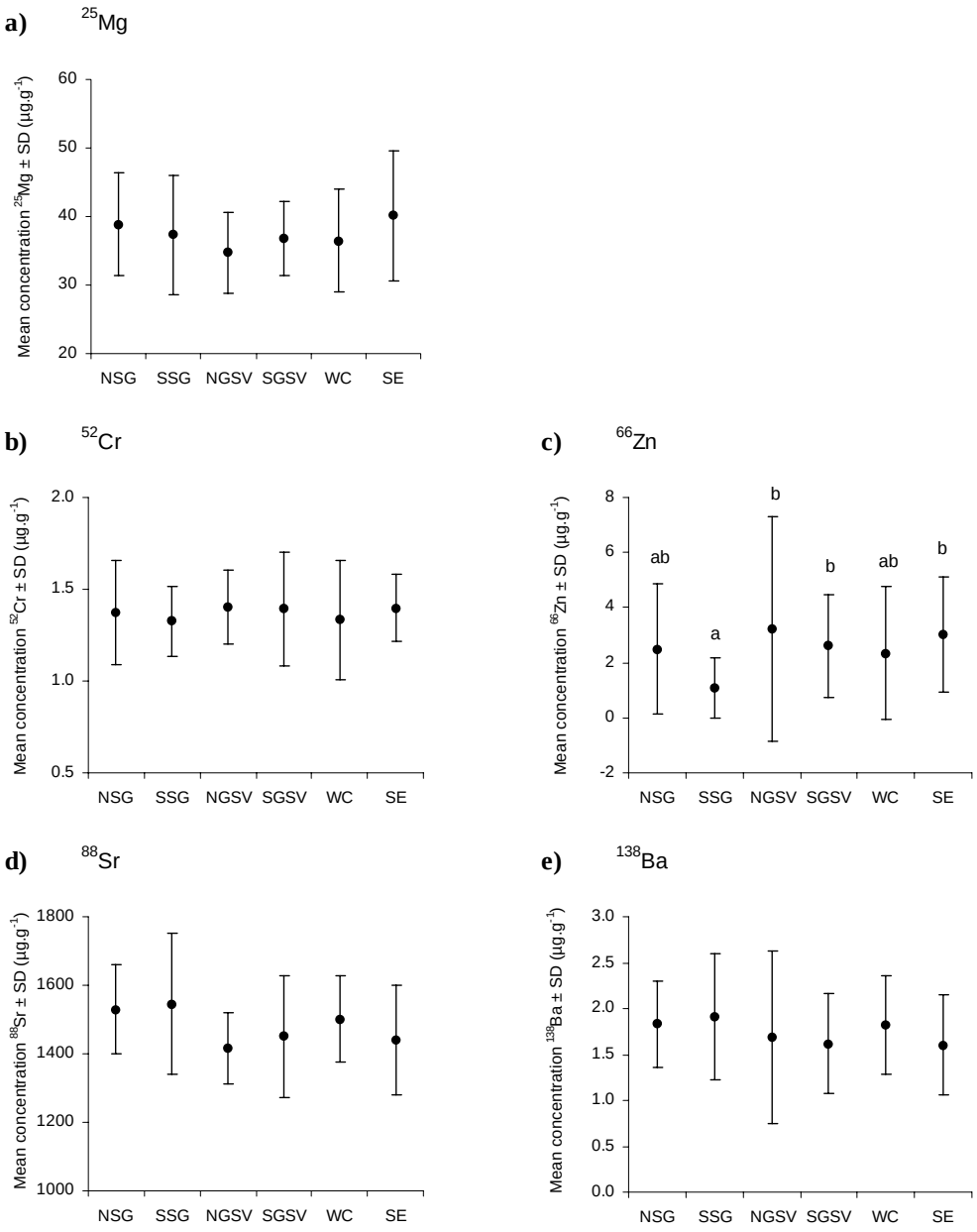

Figure 6.2 Mean concentrations of *(a)* ^{25}Mg, *(b)*^{52}Cr, *(c)* ^{66}Zn, *(d)* ^{88}Sr and *(e)* ^{138}Ba, in the central cores of snapper otoliths collected from six geographic regions. Homogenous groups from a post hoc test on the LogZn data are also indicated by the letters a and b (Tukey HSD; $\alpha = 0.05$; harmonic mean sample size = 17.2).

Table 6.2 Results of ANOVA tests for differences between the mean concentrations of each element between the six geographical regions and a MANOVA test with all elements combined as "larval signatures" for each individual. Asterisks (*) indicate significant differences.

Variable	Source of variance		F	df	P>F
LogMg	Region		0.82	5	0.540
LogCr	Region		0.36	5	0.872
LogZn	Region		3.71	5	0.004*
LogSr	Region		1.84	5	0.112
LogBa	Region		0.98	5	0.432
MANOVA	Region	Wilks' Λ = 0.771	1.09	25,377	0.348

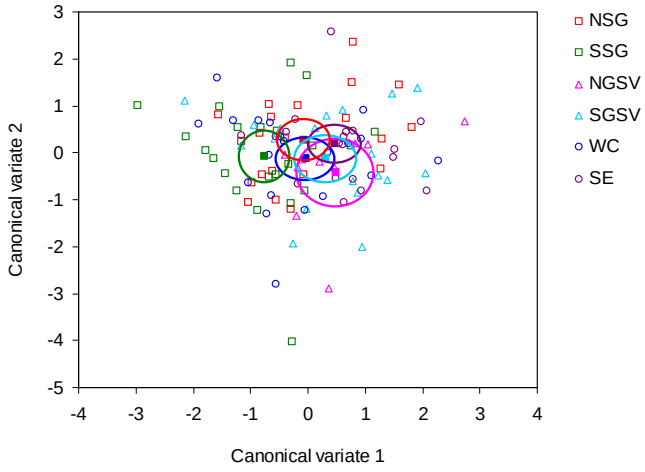

Figure 6.3 Scatterplot summarising the variation in "larval signatures" of adult snapper otoliths from each of the six geographic regions. Solid squares indicate the mean values for each region and ellipses represent 95% confidence intervals around the means.

6.4 Discussion

The aim of this study was to investigate the natal origins of adult snapper *Pagrus auratus* in South Australian waters. To do this, we analysed the trace element composition of the central cores of otoliths of 9-year-old adults collected from six different geographic regions, which were up to several hundred kilometres apart. A laser-based approach was used to specifically target the central core of each otolith, which sampled the daily increments that were deposited through the entire larval period as well as the first few days after settlement. A similar approach was successfully used to investigate the natal origins of adult weakfish *Cynoscion regalis* in northeast America, tropical shad *Tenualosa ilisha* in Bangladesh and India, and snapper *Pagrus auratus* in New South Wales (Thorrold et al. 2001; Milton and Chenery 2001; Gillanders 2002*a*).

The results of the present study indicated no consistent regional differences in multi-elemental chemical composition of the central cores of adult snapper otoliths. Only the concentrations of ^{66}Zn differed significantly amongst regions, with those from SSG having lower concentrations than those from NGSV, SGSV and the SE. The otoliths from NSG and WC were intermediate with regard the concentration ^{66}Zn. Despite this difference, the region of capture of adults could not be discriminated based on the multi-elemental "larval signatures".

To successfully discriminate stocks of fish, the among-stock variation must exceed the noise of within-stock variation (Waldman 1999). Since this was not achieved in this study, either: (1) there was little regional variation to detect, which is consistent with the null hypothesis; or (2) the level of within-region variation was too high to detect differences between regions. If the latter was the case, we risk committing a Type 2 error by accepting the null hypothesis. Thus, it is pertinent to assess possible factors that could have increased the level of within-region variation.

One factor that could artificially inflate within-region variation is deviation in the precise location of spots within each core. We assessed the potential of this by analysing replicate spots in cores of 18 randomly chosen otoliths. Concentrations were generally less variable among replicate spots than between individual fish. Only ^{25}Mg showed higher variation among replicate spots than between fish, and even then the difference between fish remained significant. Therefore, it is not likely that the lack of regional difference in chemical composition of the central cores resulted from variation in the precise location of spots in the core. Gillanders (2002*a*) also reported greater variation between otoliths of different fish than within otoliths of the same fish when the central cores of juvenile and adult *Pagrus auratus* from New South Wales estuaries were analysed with replicate LA-ICPMS spots. Similarly, replicate laser-ablation spots in different locations on sections of juvenile (0+) snapper otoliths from Victorian estuaries found less variation within than between otoliths for all elements analysed (Hamer et al. 2003).

Another factor that might contribute to high within-region variation in "larval signatures" is an extended spawning season. Larvae hatching at different times would be exposed to different water chemistry profiles during their first growth periods, which could translate into different chemical composition of their otoliths. *Pagrus auratus* in South Australia do have a considerable spawning and settlement period (Fowler and Jennings 2003). For the summer of 1999/2000, the estimated settlement dates of juveniles covered an 82-day period. However, in the three years of 2000, 2001 and 2002 most juveniles settled during a much shorter (3-week) period of peak settlement. Furthermore, other studies that have investigated variation in otolith chemistry among months within a year found minimal temporal effects that would confound spatial differences (Thorrold et al. 1998; Gillanders and Kingsford 2003; Hamer et al. 2003). Differences among years were generally more significant than within years.

Large within-region variation might also result from differences in growth rate between early and late spawned individuals. Large differences in growth rate between early and late hatched larvae have been demonstrated for snapper in NSG through the back calculation of hatch dates and daily somatic growth increments from juvenile snapper otoliths (Fowler and Jennings 2003). If growth rate did influence the rate of elemental incorporation, a significant relationship between elemental concentration and the width of the first increment, used as a proxy for growth rate in the first year, might be expected. For example, Sadovy and Severin (1994) found a consistent inverse relationship between strontium/calcium ratios and log body growth rate for red hind *Epinephelus guttatus* otoliths. In this study only the log-transformed concentrations of ^{25}Mg showed a significant linear relationship with the width of the first increment, which only accounted for 5% of the variation in the data. This suggests that the chemical composition of the central core of the snapper otoliths showed little variation in relation to otolith growth rate during the first year of life.

In this study the central cores of adult otoliths were analysed, as opposed to the otoliths of juveniles collected from known nursery areas. Thus, any "larval signatures" corresponding to specific nursery areas may have been mixed by the subsequent movement of adults and consequently be mixed with respect to region of capture (e.g. Model I above). This would result in large variation in "larval signatures" of adult otoliths collected from a single region, with no significant differences between regions. However, the analysis of chemical composition of all increments along laser-ablation transects across entire sections of adult snapper otoliths showed little divergence in chemical composition until after the third opaque zone (Chapter 3). If regional variation in "larval signatures" was masked by the subsequent movement of adults, greater variation in the earlier increments of the transects might have been expected.

Therefore, due to the absence of any compelling evidence that variation in chemical composition of the central cores within regions was unduly high, we accept the null hypothesis that there was no significant difference in the "larval signatures" of adult otoliths collected from the six different regions. This result, however, must be interpreted cautiously since it is not possible to positively demonstrate homogeneity of stocks (Waldman 1999). Rather one can only demonstrate differences between stocks. Failure to find evidence of multiple larval sources in the present study does not preclude another approach or level of sampling from revealing positive evidence. This quandary probably accounts in part for the lack of results in the literature that show little or no variation in otolith chemistry (Gillanders et. al. 2001).

To further complicate matters, a negative result for stock discrimination studies based on analysis of otolith chemistry may result if the physico-chemical properties of regional waters are not sufficiently heterogenous between locations to produce distinctive signals in the otolith material being deposited (Thresher 1999). Thus, the present results do not necessarily imply that all

juveniles originated from a common nursery area (such as that depicted for Model II above), but rather that the environmental conditions of the larval area/s appear to be similar for all adults sampled. Thorrold et al. (1997) also found no significant differences in the elemental concentrations of the central cores of otoliths of Atlantic croaker *Micropogonias undulatus*. This negative result was attributed to the probable homogenous environment of the oceanic larval areas.

For the geographic regions of South Australia sampled in this study water chemistry, is known to vary considerably, particularly during summer when spawning and settlement of juvenile snapper occurs. The shallow semi-enclosed estuaries of Spencer Gulf and Gulf St Vincent show greater seasonal variation in water temperature and salinity than adjacent continental shelf waters (Nunes and Lennon 1986), and much higher temperatures in their northern regions during summer than elsewhere in the State (Chapter 1). The Gulfs are also hyper-saline in comparison with shelf waters due to limited freshwater inflow and high evaporation (Tomczak 1998). Therefore, it is unlikely that the water chemistry was insufficiently heterogenous across all six regions.

Furthermore, the adult portions (> 3 y.o.) of laser-ablation transects across annual increments showed significant regional variation in chemical composition (Chapter 3), indicating that water chemistry differed sufficiently between regions to produce different chemical signatures in snapper otolith material. Admittedly, the adult portions were deposited during a full range of seasonal variation in water chemistry, whilst the central cores were deposited during only a single season (i.e. summer). There may also be ontogenetic differences in the way elements are incorporated into snapper otolith material. Nevertheless, the summer period when recruitment occurs is when the marine environment in the Gulfs is likely to differ most from continental shelf waters. In addition, significant differences have been detected in the chemical composition of otoliths of juvenile snapper collected from different estuaries in both Victoria and NSW (Hamer et al. 2003; Gillanders 2002*a*,*b*), suggesting that if snapper larvae had been subjected to different environmental regimes we should have detected different chemical signatures in the cores of the adult otoliths.

Overall, we suggest that the adult snapper collected in this study originated from a similar natal environment. This does not preclude spawning from occurring in all regions, rather that larvae and juveniles may only survive over a narrow range of conditions, resulting in all survivors showing a similar "larval signature". Either way the results appear to support a scenario similar to Model II above, which consists of a single contracted spawning and/or nursery area. However, Model II is not consistent with the reported limited distribution of juveniles to both northern Gulf regions. This observation is more in alignment with Model III above. However, for Model III to be correct, we would have expected to find more than one significant "larval signature" due to the multiple spawning or nursery areas. The one significant result from this study did provide support for Model III. The ^{66}Zn concentrations of central cores from the SSG were significantly different from those

from the two Gulf St Vincent regions and the SE, but not from NSG and WC. If the chemical environment of the two northern gulfs were similar, it might account for the fact that no other significant differences were detected in other elemental concentrations. Both gulfs are inverse estuaries with little freshwater inflow, and high summer salinities and temperatures in the northern reaches during the summer. Certainly, in the analysis of the adult portions of transects, individuals from the NGSV and NSG grouped out together, particularly when only the chemical data were considered in the statistical analyses (Chapter 3). Hence, it appears that the results of this study cannot distinguish between scenarios such as Model II and Model III above.

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7 General discussion

A.J. Fowler

Management of the snapper (*Pagrus auratus*) fishery of South Australia currently treats the population as a single, large stock (Fowler et al. 2003), an approach that is consistent with the lack of genetic differentiation (Donnellan and McGlennon 1996), and the current understanding of the life history of the species in this State (McGlennon and Jones 1997). However, there are some indications of regional population sub-structure that may indicate the existence of separate stocks. Resolving this issue is important because disregarding such sub-structure when determining an appropriate management protocol for a fishery can lead to localised depletion and over-fishing (Bailey 1997). Indeed, this may have already occurred for snapper in the Gulf St. Vincent fishery, where catches have never recovered after the high catches taken during the early – mid 1980s (Fowler et al. 2003). This reduction in catch in Gulf St. Vincent led to the recommendation that a regional approach be adopted for fishery management that involved stock enhancement in Gulf St. Vincent (McGlennon and Jones 1999). Clearly, however, such an approach could only be beneficial for this local fishery if the regional population was, to some extent, separate from those in other regions, otherwise there could be wholesale movement of fish out of this region.

The considerations above highlighted the lack of understanding of the extent to which the South Australian population of snapper is differentiated into sub-populations. Such determination of stock structure requires some understanding of the extent of mixing and rate of exchange of individuals between different geographic locations throughout the fishery. For marine organisms such movement can occur at two stages of the life history, i.e. through the early life history stage by transport of eggs and larvae involving physical oceanographic processes, or through the active movement of fish at some time throughout the juvenile and adult stages. To date the former has not been studied directly, but it is considered unlikely that eggs and larvae are transported over extensive distances. This is because of the relatively weak currents typical of the nearshore coastal areas of South Australia (Fowler et al. 2000), and because of seasonal oceanographic phenomena that serve to effectively separate regional water masses during summer (Petrusevics 1993), the reproductive season for snapper. Thus, it is unlikely that there would be wholesale transport of eggs and larvae in either direction between Gulf St. Vincent and Spencer Gulf, or from either Gulf to the West Coast or the South East. This suggests that if mixing does occur between regional populations it would most likely involve individuals of the post-settlement phase of the life history. Indeed, tagging work for snapper in this State has shown that some adult fish have successfully moved over distances of hundreds of kilometres across regional boundaries (Jones 1984). Whilst this demonstrates the potential for movement of adult snapper the question remains, however, as to

whether the numbers of fish that undertake such movements are high enough to result in sufficient mixing of fish from all regions that would prevent the development of any such population structure. The numbers of fish that carry out such movements may be relatively low, which means that the ecological consequences of such movement across regional boundaries would be minor.

This report was concerned with determining the stock structure of snapper in South Australia and identifying the significance of the mixing of fish as a result of adult movement. Three objectives were addressed in the five empirical chapters. Firstly, the report was concerned with determining whether there was evidence for sub-structuring of the whole State-wide population. The second focus was to provide a better understanding of the movement patterns of adult fish. Finally, it was concerned with determining the origins of fish collected from different regions. This is because even if adult fish were shown to have restricted patterns of movement this would not necessarily mean that the fish originated in the regions where they were captured. The issue here was whether any observed sub-structure in the characteristics of adult fish was also indicative of reproductive separation between such sub-populations. Primarily these issues were addressed through analysis of the chemistry of otoliths of fish collected from the six main geographic regions that constitute the main snapper fishing grounds in South Australia. These datasets were, however, augmented with several others that related to fishery and population characteristics for the regional populations. Here it is worth reviewing the major findings that were presented in each of these chapters.

7.1 Overview of results

7.1.1 Growth patterns of otoliths

The work described in this chapter was done to facilitate interpretation of the results from otolith chemistry presented in Chapters, 3, 5 and 6. The conceptual basis was that for otoliths to relate information on fish movements they must grow sufficiently through the period when fish move and occupy different water masses. This requires having some understanding of the pattern of growth of otoliths. This chapter focussed on developing a comprehensive description of otolith growth for two age classes of fish, using the methodology of Marginal Increment Analysis.

The most significant result of this work in the context of the whole study was the determination that otolith growth is highly seasonal. In fact >75% of the annual deposition of new material to the otolith surface was achieved in the six months of summer and autumn. The mean annual growth of the otolith along Axis 3 was approximately 150 – 170 μm for 5-6 year old fish and 100 μm for 11-12 year old fish. Of this growth, however, there was <30 μm per season for each of winter and spring for 5-6 year olds and <15 μm growth per season for the 11-12 year olds. Such restricted

seasonal growth may limit the potential for otoliths to record significant environmental information that relates to fish movement.

7.1.2 Age-related variation in otolith chemistry by LA ICP-MS

This chapter focussed on age-related movement patterns and regional stock structure. LA ICP-MS was used to describe the chemistry of otoliths for up to 20 fish that were from the 9-year age class from each region. Each otolith was sampled between the otolith core and the outside edge along the short axis to the dorsal side of the sulcus. Although 11 elements were sampled along the length of the transect for each otolith only the data for ^{88}Sr and ^{138}Ba relative to ^{44}Ca could be considered in the analyses. These chemical profiles were aligned with and related to fish age.

Although the chemical profiles were highly variable amongst individual fish, nevertheless significant regional differences were apparent. Whilst the regional profiles were similar for the first three years, they diverged and were quite different between the 4^{th} to 9^{th} annual increments. Although several hypotheses may account for such a result, the most likely is that from the age of 4 to 9 years the fish from different regions occupied different water masses whose physical and chemical properties resulted in different ^{88}Sr and ^{138}Ba levels being laid down in the otoliths. The lack of any regional differences in otolith chemistry for the first 1 – 3 years suggests the possibility that most fish from different regions occupied a common water mass through this period.

In contrast to the useful information provided about possible stock structure unfortunately the results of chemical profiles were impossible to interpret in terms of migratory behaviour. It was initially thought that the annual sinusoidal variation in ^{88}Sr and ^{138}Ba, reflecting seasonal variation in their rates of deposition, may relate to regular movement of fish between water masses with different characteristics. It seems, however, that physiological explanations provide more likely explanations for such data (Sadovy and Severin 1992, 1994). A further limitation to our ability to interpret these data is the lack of information available on the ambient concentrations of ^{88}Sr and ^{138}Ba for the different regional waters of South Australia. Given the complexity of factors that can influence otolith chemistry it now seems that to correctly interpret the chemical profiles across otoliths with any certainty in terms of fish movement, then data on ambient concentrations in different water masses are also required.

From Chapter 2 it was evident that for otolith chemistry to relate information on fish movement the otoliths must grow sufficiently to allow an interpretable signal to be recorded, and the analytical methodology for chemical analysis must have sufficient spatial resolution to measure or detect the chemical signal that has been laid down. Also in that chapter we demonstrated that otolith growth on the dorsal side of the sulcus was between 15-30 μm per season in winter and spring for 5-6 year

old fish and only 3-15 μm per season for 11 –12 year old fish. This indicates that the growth of otoliths along the axis that was sampled in winter and spring were generally low and decreased with age. Since the otolith sampling was done with a laser that was 40 μm in diameter the spatial resolution of the analysis was coarse relative to the small seasonal growth of the otoliths. Even for 5-6 year old fish it would have been impossible to sample either the winter or spring growth without contamination from adjacent material deposited in either the preceding or following seasons. This would certainly compromise the ability to demonstrate annual migrations that occurred at these times.

7.1.3 Population structure – regional variation in population characteristics

This chapter was primarily concerned with assessing the evidence other than that from otolith chemistry for the existence of regional population structure. The conceptual basis to this was - if snapper did constitute a single, large population where fish from different regions intermixed freely, then there should not be apparent any regional differences in fishery and population characteristics other than those that relate to regional differences in habitats. Consequently here a number of population and fishery characteristics were compared amongst regions including: fishery catch trends; population size and age structures; the optical and morphometric characteristics of otoliths.

The study identified consistent regional differences in the temporal trends in fishery catches as well as differences in population size and age structures, suggesting that different population processes occurred in different regions and that fish were not redistributed significantly. For example, between NSG and SSG there was a consistent difference in the relative strength of the 1991 and 1997 year classes. Such a difference could only be maintained if there was not significant redistribution of fish between these adjacent regions. Similarly, the persistence in SSG of a 'stunted' component of the population could happen only if the majority of fish were not continually reorganised across regional boundaries, but rather were resident in these regions. The optical characteristics of otoliths provided further support for this conclusion at two temporal scales. The observed regional differences in optical characteristics demonstrate that the fish from different regions must spend substantial parts of their year in different places and that this must occur consistently across years. The only characteristics that were compared amongst regions, which did not display a difference at this scale were the morphometric characteristics of the otoliths. This lack of a regional difference seems to relate to the extremely high variability in otolith shape amongst individual fish regardless of where they came from.

7.1.4 Population structure – regional variation in otolith chemistry by SB ICP-MS

This chapter was concerned with the same aim addressed in Chapter 4, i.e. whether there was evidence for sub-structuring to the South Australian snapper population. The work here, however, involved a more sophisticated analytical procedure and longer description and so was reported separately from that in the former chapter. This study involved the comparison of the chemistry of whole otoliths of 11 year old snapper that were captured in 2002 in each of NSG, SSG, NGSV, SGSV and the WC (no otoliths available from SE). The analytical methodology was solution-based ICP-MS. The results demonstrated significant regional variation in the chemistry of these otoliths, indicating that the fish that occupied these regions must have remained separate from fish in other regions for sufficiently long periods for environmental differences to become manifested as chemical differences in their otoliths. Despite this, however, there was considerable variation in the within-region results, which detracted from the accuracy with which fish were assigned to the region from where they were caught. This meant that either the environmental differences between regions were not sufficiently strong to result in a strong regional 'fingerprint' in the chemistry of the otoliths of all fish, or that not every fish was in fact a resident of the region from which it was collected. The data available cannot differentiate between these hypotheses.

7.1.5 Regions of origin by LA ICP-MS

The previous three chapters presented compelling evidence that for adult fish collected from different regional water masses, the within-sample variation for the various characteristics was generally less than the between-region differences, indicating real regional differences in the characteristics examined. If it could be demonstrated that the fish also originated in the regions where they were captured, this may indicate reproductively separated sub-populations. Alternatively, the fish may originate elsewhere and then at some point move into the various regions. The aim of work done in this chapter was to improve our understanding of the stock structure by comparing the regions of origin of adult fish collected in the different regions. This was done by chemical analysis of the cores of the otoliths of these fish using LA ICP-MS.

The study determined that there were no consistent regional differences in the multi-elemental chemical composition of the central cores of these adult fish otoliths. There was no compelling evidence of particularly high within-sample variation in the dataset and so we accepted the null hypothesis that there was no difference in the chemical composition of the cores of the otoliths collected from different regions. Given that similar analyses for snapper done in other states have demonstrated regional differences in the otolith cores (Gillanders 2002, Hamer et al. 2003), and that the differences that we documented for adult fish from South Australia, suggest that the reasonable interpretation of these data is that the adult fish collected from different regions did in

fact originate from only one or two common places. These places are likely to have been Northern Spencer Gulf and Northern Gulf St. Vincent, the only places where newly recruited and juvenile snapper have yet been caught.

7.2 Revision of life history model

There was an overwhelming body of evidence presented in Chapters 3, 4 and 5 that indicated that there was some regional population sub-structuring evident for the snapper population of South Australia. This evidence included data on the chemical composition of whole otoliths and across the age profile of otoliths, as well as augmentative datasets on fishery statistics, population size and age structures and growth functions. Such regional differences in characteristics could only develop if the fish living in different places experienced different environmental regimes for significant periods of their lives. Clearly, therefore, South Australian snapper do not represent a single, mixed population that is the consequence of redistribution of adult fish across regional boundaries on a regular basis.

The population structure evident in South Australia is, to some extent, analogous with the situation in Shark Bay Western Australia, where numerous studies involving genetics, otolith chemistry, adult tagging, and morphometrics have demonstrated a fine-scale level of stock structure (Edmonds et al. 1989, 1999; Moran et al. 1998, 2003). The difference, however, is that in South Australia there is no phylogeographic structure, despite the fish being distributed over a much bigger area than is the case for Shark Bay (Donnellan and McGlennon 1996). Nevertheless, this panmixia in South Australia is consistent with the results of tagging studies that indicated that some adult fish did move distances of hundreds of kilometres and crossed the arbitrary regional boundaries that we considered in this study (Jones 1981, 1984). It now appears that such movement occurs at a sufficiently high rate to prevent the development of regional genetic differentiation from developing over evolutionary time periods. Only relatively low rates of inter-regional movement are required to achieve such spatial homogeneity in genetic structure (Carvalho and Hauser 1994). At the same time, it appears that adult movement between regions did not occur at a sufficiently high rate to prevent regional structure in population characteristics from becoming apparent. This suggests that the movement patterns of most snapper in South Australia are relatively local, and so the fish can be considered ‘residents’, whilst only a few fish undertake more significant movement and can be considered ‘migrants’. This is consistent with the findings of most studies on snapper movement based on tagging adult fish that have been done in New Zealand (Paul 1967, Crossland 1982, Willis et al. 2001, Hartill et al. 2003), Western Australia (Moran et al. 2003), South Australia (Jones et al. 1981, 1984), Victoria (Sanders 1974, Coutin et al. 2003), and Queensland (Sumpton et al. 2003).

The age-related chemical composition provided a further insight as to why the panmictic population showed evidence of some sub-structure. The analyses of the cores and along the transects across otoliths using LA ICP-MS provided consistent results that related to the places from where the 9+ year old fish considered in these analyses originated. These data relating to the first three years of the fishes' lives showed very little variation, indicating a lack of spatial differentiation with regards where they originated. The most parsimonious explanation of this is that most fish, regardless of where they were eventually captured, had in fact originated in only one or two places that experienced similar environmental characteristics. The most likely regions where these nursery grounds were located are NSG and NGSV. Clearly, this hypothesis suggests that most fish, regardless of where they were captured, originated from localised spawning activity and accounts for the lack of genetic differentiation amongst regional populations.

The age-specific otolith chemistry data also suggested that the fish occupied the nursery areas for several years. Between the 3rd and 4th opaque zones in the otoliths the chemical signals began to become much more diverse and the regional patterns were quite different. This suggests that through the fourth year of the fishes' lives they moved from the nursery area(s), and redistributed themselves throughout the different regions of the State. This model is consistent with some other population characteristics and recruitment information. The otoliths that were considered in the chemical analyses of this study were all from fish of the 1991 year class that were captured in either 2000 or 2002. We know that the summer of 1990/91 produced an exceptionally strong year class of 0+ snapper in NSG, based on the high catches of baby snapper as by-catch in prawn trawl surveys around the Point Lowly area at that time (Carrick 1997, Carrick unpublished data; Jackson pers. obs). Since then the population of snapper in every region throughout South Australian waters has become dominated by the 1991 year class, whilst the overall regional catch rates of snapper have increased considerably, presumably as a consequence of the increase in biomass attributable to this exceptional year class (Fowler et al. 2003). These increases in fishery catches first became evident in Spencer Gulf and Gulf St. Vincent in 1995/96, when the 1991 year class were approximately 4 years of age, and one year later for the west coast of Eyre Peninsula in 1996/97. According to the interpretation of the otolith chemistry the timing of the increase in biomass in regional populations corresponds to the time when the fish were leaving NSG and redistributing themselves throughout the other regions of South Australia.

In conclusion, this study has presented a suite of information that is consistent with a model relating to the recruitment of snapper to the regional populations throughout South Australia. The model suggests that in this case the fish originated from the strong year class that recruited as 0+ individuals in the early months of 1991. The fish remained in the nursery area up to the age of approximately three years, when they began to disperse throughout the other regions of the State, indicating a significant age-related movement. By four years of age these fish were broadly

distributed throughout the gulf system and were beginning to contribute to the fishery catches in these different regions. Having established themselves in these places the majority of fish have remained resident and continued to contribute significantly to each regional fishery, whilst the regional populations have remained separated since they moved out of NSG.

This life history model suggests that most individuals that comprise the overall State population have a common origin, which appears to be in northern Spencer Gulf. This region offers the low energy waters and soft fine sediments that constitute the preferred settlement site for this species (Francis 1995). After several years some fish show age-related emigration from this region and join the populations in other regions that are located up to hundreds of kilometres away. These regional populations tend to be low in abundance relative to Spencer Gulf (Fowler et al. 2003). Thus, in this situation one major population is the source of numerous fringe populations. As such, the population represents a single, large mixed stock composed of numerous ecologically-related sub-populations. The management of the fishery should clearly acknowledge the significance of the source populations.

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8 Concluding sections

8.1 Conclusions

Objective 1 – to determine whether adult fish from Spencer Gulf, Gulf St. Vincent and other regions migrate to the continental shelf, and where these fish then migrate to in order to spawn – do they return to their regions of origin or is subsequent movement determined by other exogenous factors?

The sampling of transects across otoliths from the cores to the edges provided chronological profiles of the chemical composition relating to the environments experienced by each fish throughout its life. These profiles, however, did not prove useful for elucidating regular, annual migration of the fish. Thus, we remain unsure as to the significance of annual migration between inshore coastal areas and the continental shelf. This failure relates in part to the limited growth of the otoliths in some seasons of the year and also to a lack of data on ambient concentrations of different elements throughout the coastal waters of South Australia. Nevertheless, two types of movement were inferred from all the data collected in this study. Firstly, there was evidence of systematic movement of fish away from the nursery area(s), which occurred during the fourth year of the fishes' lives. This resulted in the redistribution of the year class of fish across the range of the species in South Australia. These fish then appeared to become residents of particular regions. From tagging work it is highly likely that some older fish did undertake movement of up to several hundred kilometres.

Objective 2 – to determine the stock structure, i.e. does the South Australian population constitute a single, large inter-mixed population or is it divisible into numerous sub-populations?

The snapper population constitutes a single, large, inter-mixed stock. The regional populations appear to be replenished by immigration of 3 – 4 year old fish that originate from specific nursery areas. Furthermore, previous tagging studies suggest some movement of older fish across regional boundaries that would lead to further mixing. Nevertheless, it appears that from approximately the age of 4 years and onwards, the movement was restricted and so the adult fish became restricted to their region of occupancy. As a consequence, numerous fishery and population characteristics as well as otolith chemistry and optical characteristics, varied amongst adult fish sampled from different regions. Such differences could only arise if these fish had experienced different environmental conditions, i.e. had occupied different water masses for considerable periods of time.

Objective 3 – to determine whether adult fish collected from particular regions originate as juveniles from those regions or whether they constitute a mixture from different regions.

The analysis of the cores and the first few annual increments in the otoliths using otolith chemistry suggests that, regardless of from where the fish were captured, they appear to have originated in only one or two places, most likely the northern parts of Spencer Gulf and Gulf St. Vincent. This confers onto these restricted nursery areas considerable significance for sustaining the entire State-wide population and its component regional sub-populations. That the different regional sub-populations had a common origin is consistent with the lack of genetic differentiation that has been previously described for South Australian snapper.

8.2 Benefits and beneficiaries

This project has significantly enhanced our understanding of the life history, population biology and demography of snapper in South Australia. This is particularly with respect to clarifying the inter-relationship between adult movement and population dynamics. It seems that inter-annual variation in recruitment determines year class strength, whilst adult movement patterns determine the distribution of these fish throughout the State's coastal waters.

This enhancement of our understanding and its demographic consequences will be relayed to the fishery managers of PIRSA, as well as the South Australian Marine Scalefish Fishery Management Committee. Such information will be invaluable in future discussions about the disparity in fishery catches and the possibility of stock enhancement in specific regions.

The findings of the study will also be used to improve the fisheries management model 'SnapEst' that was developed as part of FRDC project no. 99/145 by Dr Rick McGarvey from SARDI. Improved parameter estimates and better understanding of the spatial scale over which population processes occur should lead to more accurate estimates of output parameters and lead to improved advice about the status of the stock and its future management.

8.3 Planned outcomes

There were several planned outcomes for this project identified in the original project proposal. The specific ones with regard the population biology of snapper were: to develop a better understanding of the life history with respect to the timing and frequency of age-based migration between places; and to help determine whether the population of snapper is divisible into independent, self-recruiting sub-populations. The final planned outcome was to use this better understanding of the biology to identify the appropriate spatial scale for fishery management in this State and to help explain the differential in fishery catches between regions.

The project has proposed a significant refinement to the life history model for snapper, i.e. the movement of fish away from nursery area(s) when fish were 3- 4 years old, to become distributed broadly throughout the waters of the State. Furthermore, the study also suggested that the nursery areas for this species are restricted and that fish that are ultimately broadly distributed do originate from these areas. Clearly, therefore the adult movement patterns are highly significant for the replenishment of regional sub-populations. Because of this, it is inevitable that the whole State-wide population be described as one, single, large stock. However, this is complicated to some extent by the fact that fish do in general become residents from the age of about 4 years onwards, which means that there appears to be minimal interaction between regional sub-populations after this age. Therefore, this provides some impression of population sub-structure.

The future management of the fishery should take into consideration a number of findings from this study, including: the limited number of nursery areas; the dispersal of fish from these areas at 3- 4 years of age that then replenish regional sub-populations; and the residential nature of the fish after they have reached approximately 4 years of age.

8.4 Further development

This project has concluded with the development of a new life history model for snapper in South Australia, which largely accounts for the spatial dynamics in the distribution and abundance of the species throughout the State's waters. The further development of the project should be in two directions. Firstly, such information should be used in the short-term management and for

developing the long-term management strategy for this species. For example, the information can be used to refine the spatial scale of use of the fishery model SnapEst that is used in the annual stock assessment process for this fishery. The SnapEst model was developed as part of FRDC project no. 1999/145.

The second direction for further development from this project relates to the testing of the life history model that has been developed. Given the value of the commercial, recreational and charter boat sectors of the snapper fishery it is important to test the newly-developed model. This could be done again based on otolith chemistry analysis, but this time utilising stable isotope analysis. The ratio of $^{18}O/^{16}O$ in otoliths is known to reflect the water temperature in which fish have been living. Thus, the 'emigration' hypothesis proposed in this study, could be tested by determining the regime of water temperatures experienced by a fish throughout its life and comparing these between fish captured in different regions.

8.5 Intellectual Property

There are no Intellectual Property issues associated with this project.

8.6 Appendix: Staff

Dr Anthony Fowler	(SARDI)	Principal Investigator
Dr Bronwyn Gillanders	(University of Adelaide)	Co-Investigator
Dr Karina Hall	(SARDI)	Research Associate
Krystle Schilling	(SARDI)	Summer Scholarship Student

9 Appendix – elemental profiles from LA ICP-MS

The following figures show the elemental profiles for both ^{88}Sr and ^{138}Ba as related to fish age for each otolith sampled for analyses presented in Chapter 3. Each otolith was taken from a 9 year old fish that was collected in the year 2000, and so was from the strong 1991 year class. A transverse section was prepared from each otolith which was then sampled along a transect between the core and the outside edge on the dorsal side of the sulcus. The analytical methodology was LA ICP-MS. The figures are organised into six groups numbered A1-A6. Each figure contains multiple graphs - one for each fish considered from that region. Each graph shows the profile for both ^{88}Sr and ^{138}Ba. Fig. A.1 relates to the 20 otoliths sampled from NSG, Fig. A.2 to the 22 otoliths from SSG, Fig. A.3 to the 10 otoliths from NGSV, Fig. A.4 to the 19 otoliths from SGSV, Fig. A.5 to the 20 otoliths from the WC and Fig. A.6 to the 20 otoliths from the SE.

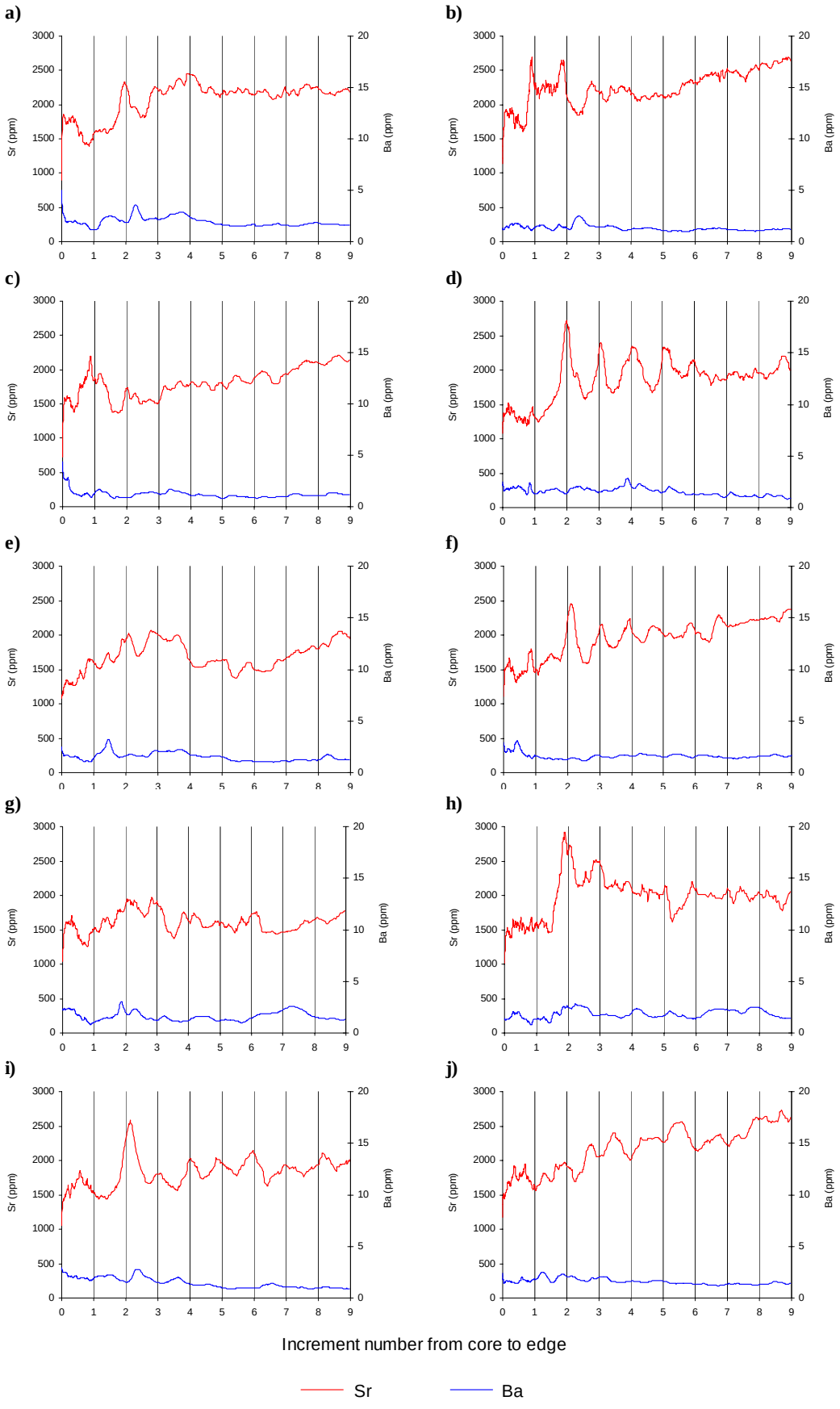

Figure A.1 Individual elemental profiles for Sr and Ba for otoliths from fish from NSG.

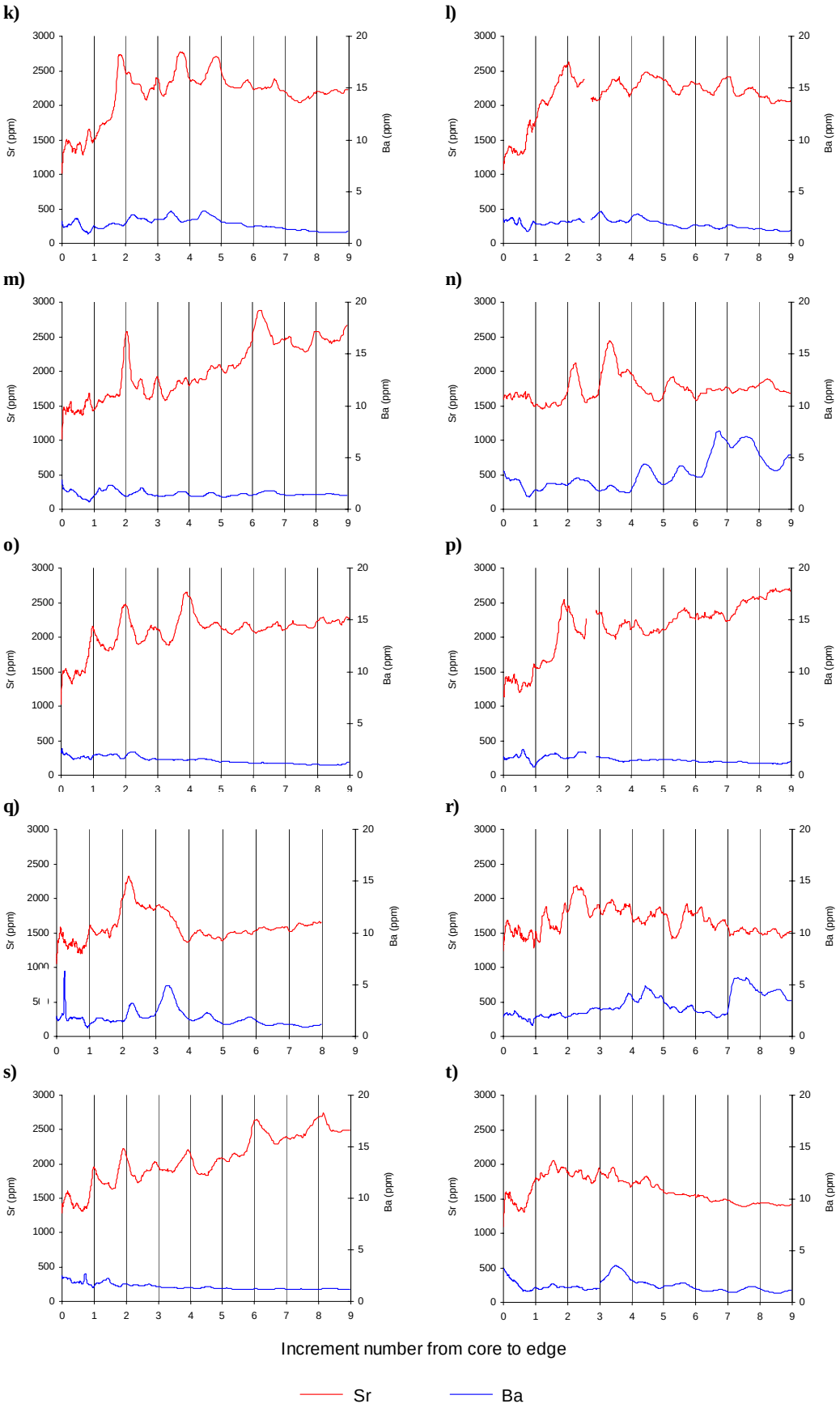

Figure A.1 cont…..

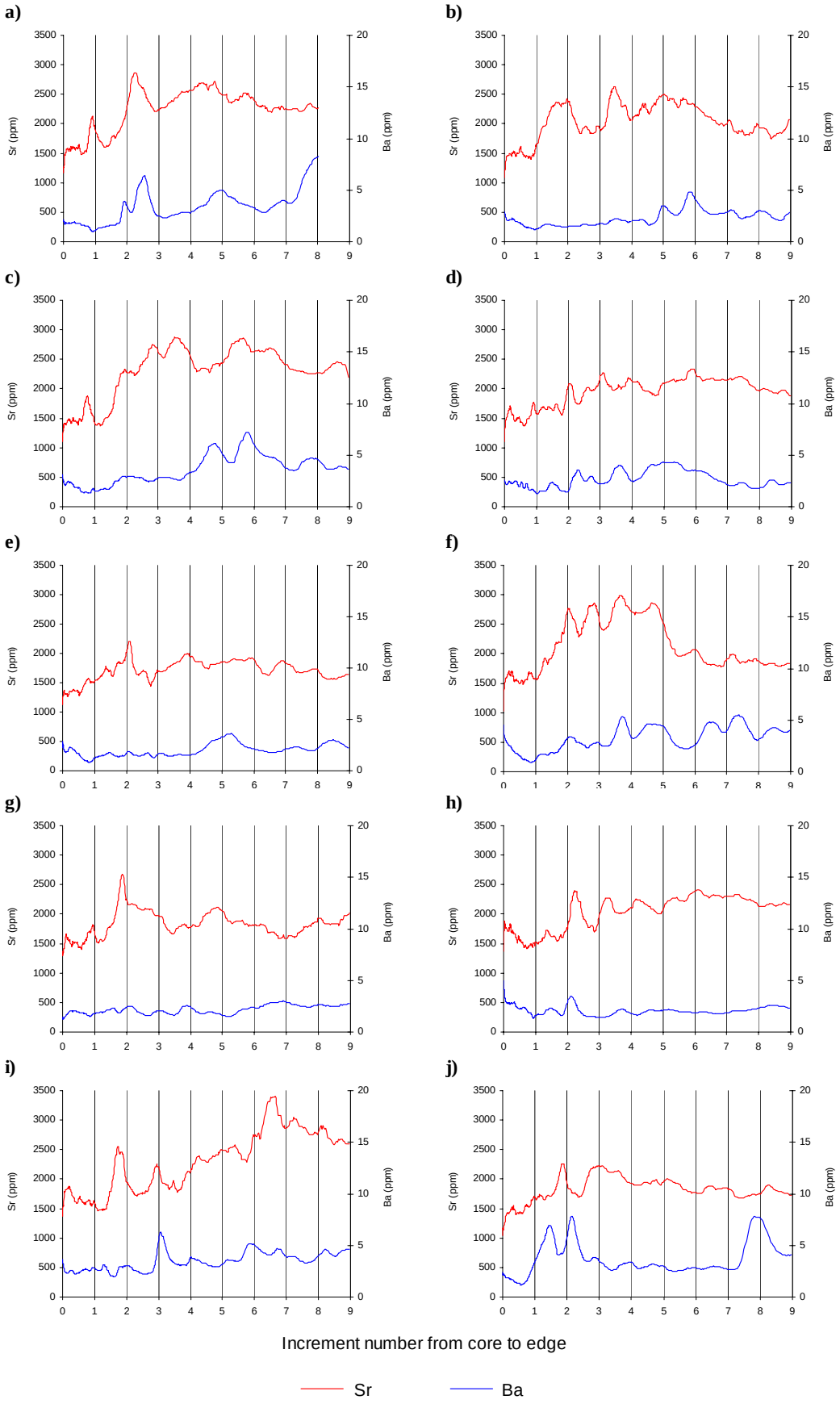

Figure A.2 Individual elemental profiles for Sr and Ba for otoliths from fish taken from SSG.

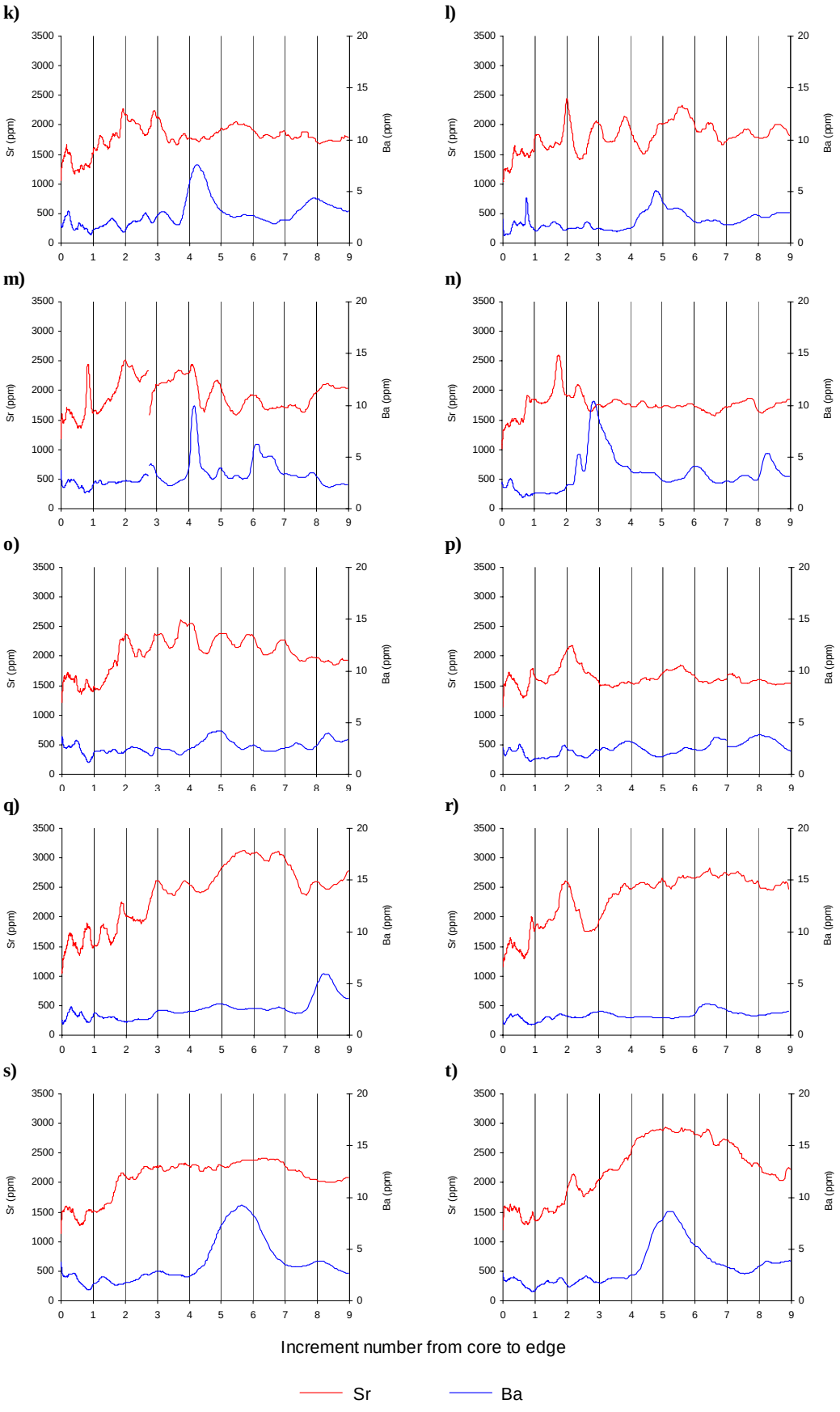

Figure A.2 cont…..

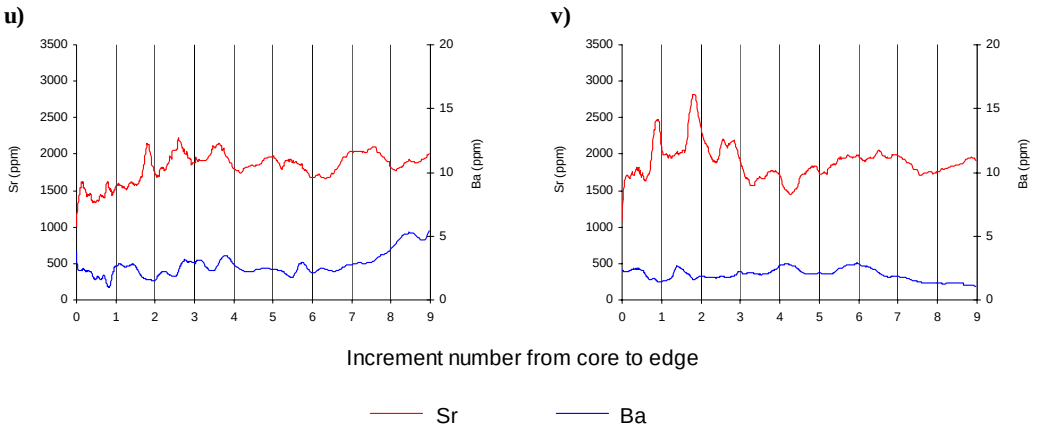

Figure A.2 cont…..

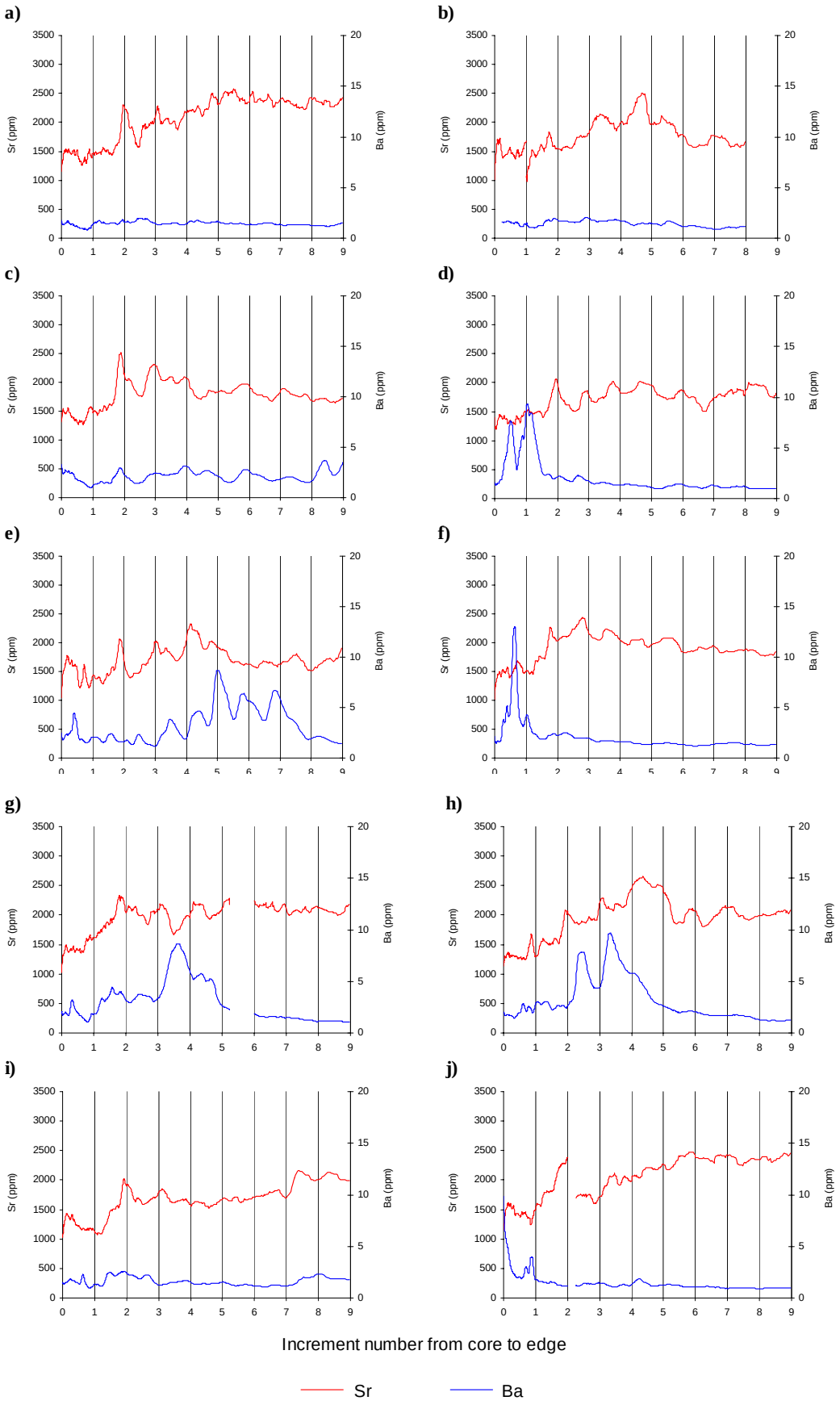

Figure A.3 Individual elemental profiles for Sr and Ba for otoliths from fish taken from NGSV.

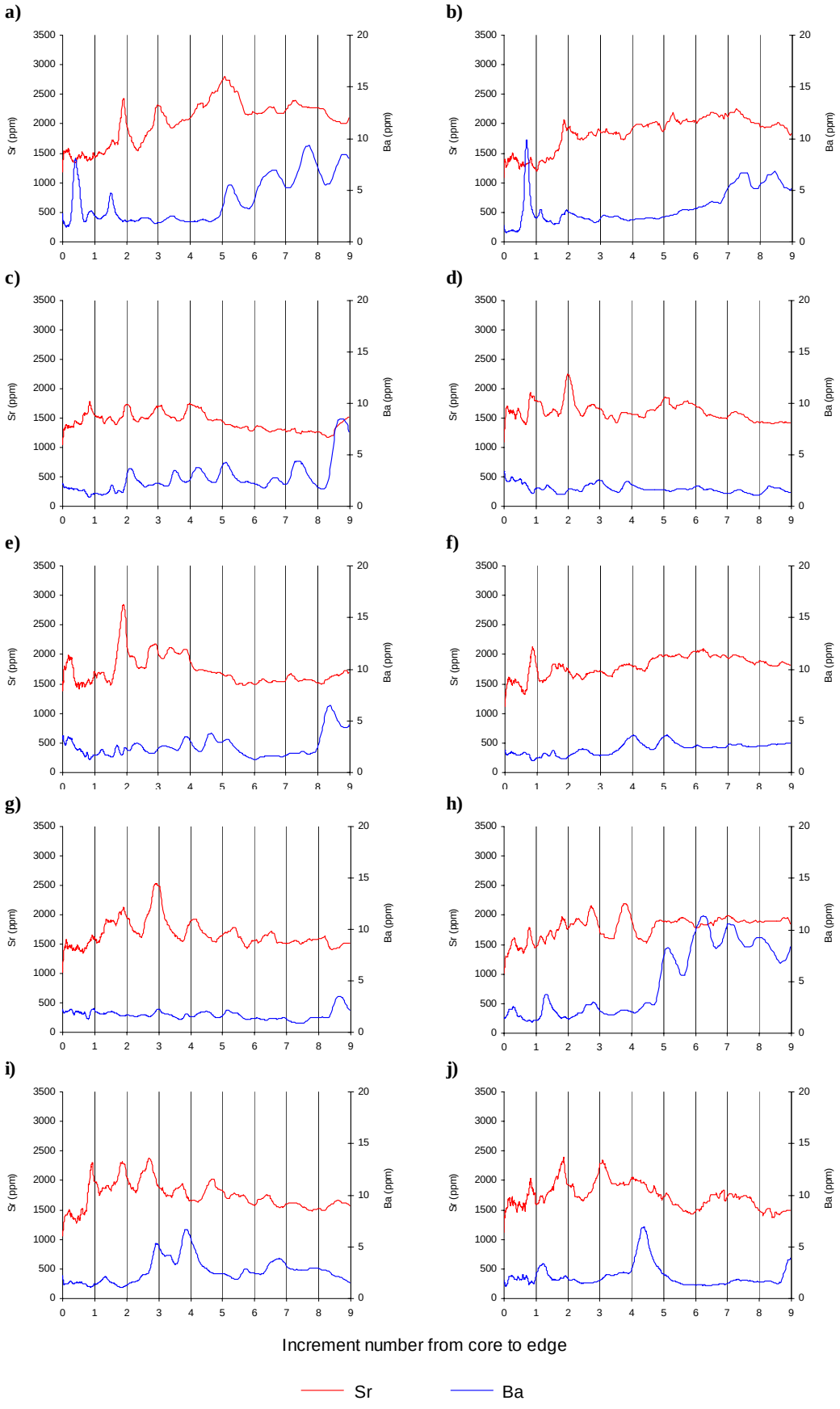

Figure A.4 Individual elemental profiles for Sr and Ba for otoliths from fish taken from SGSV.

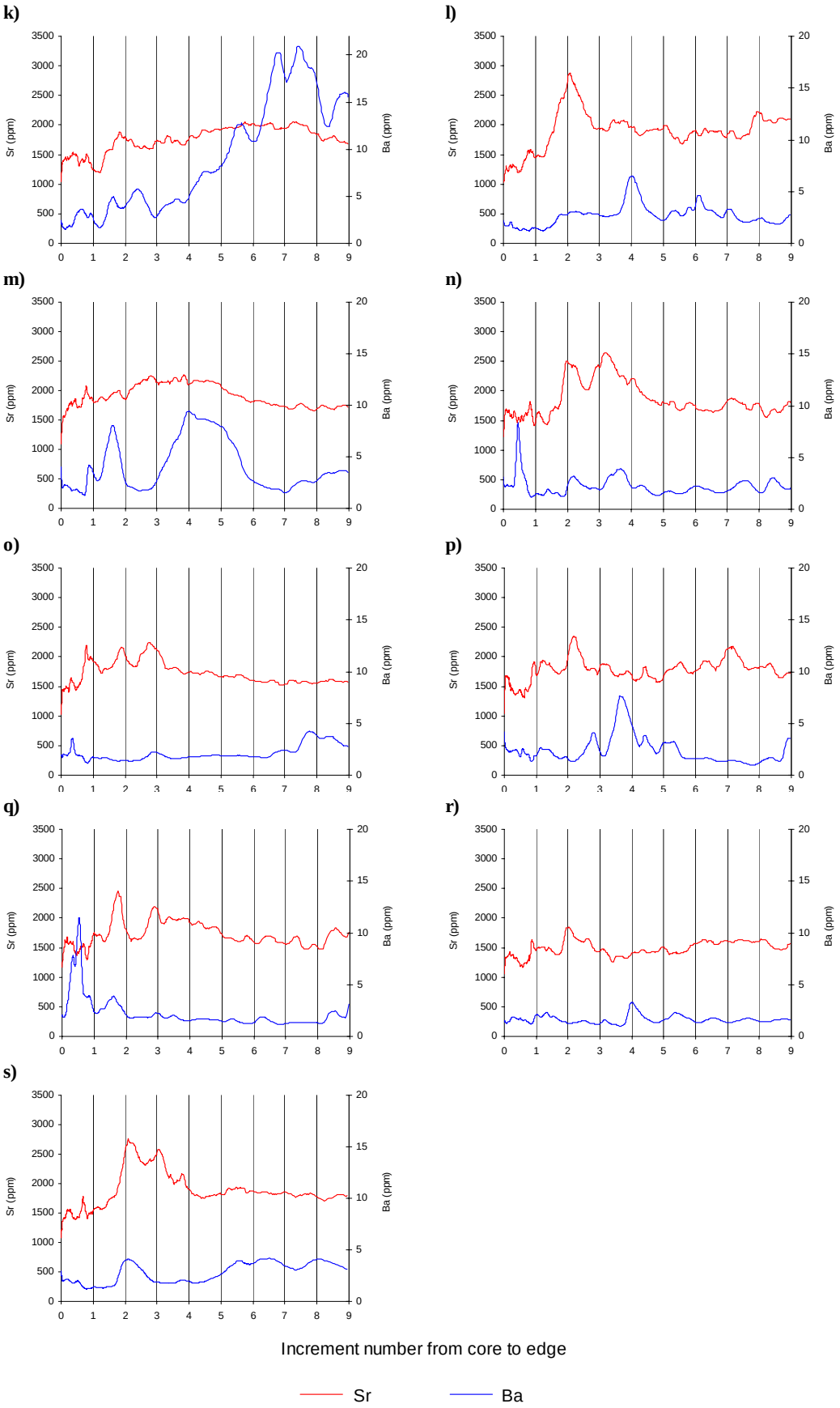

Figure A.4 cont…..

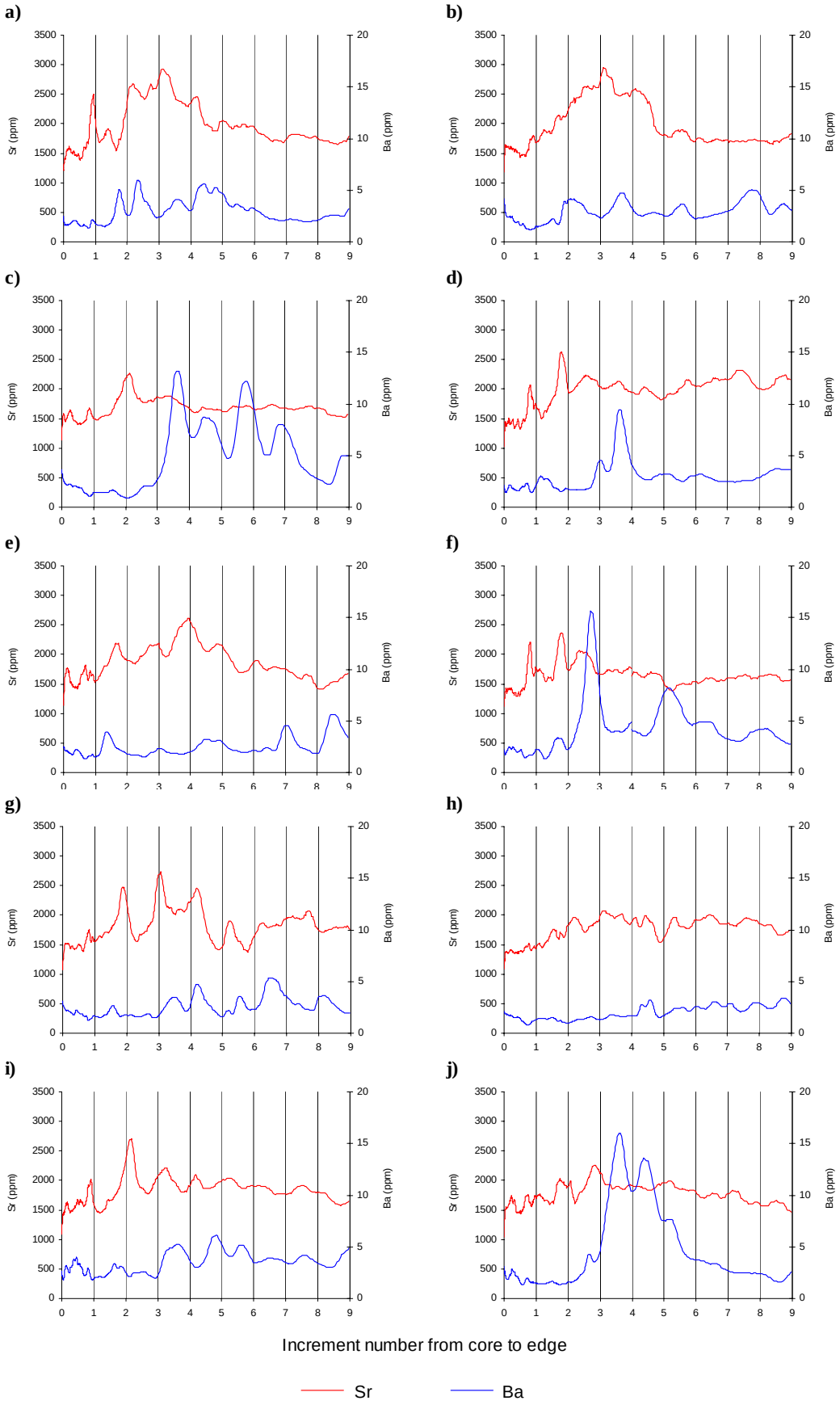

Figure A.5 Individual elemental profiles for Sr and Ba for otoliths from fish taken from WC.

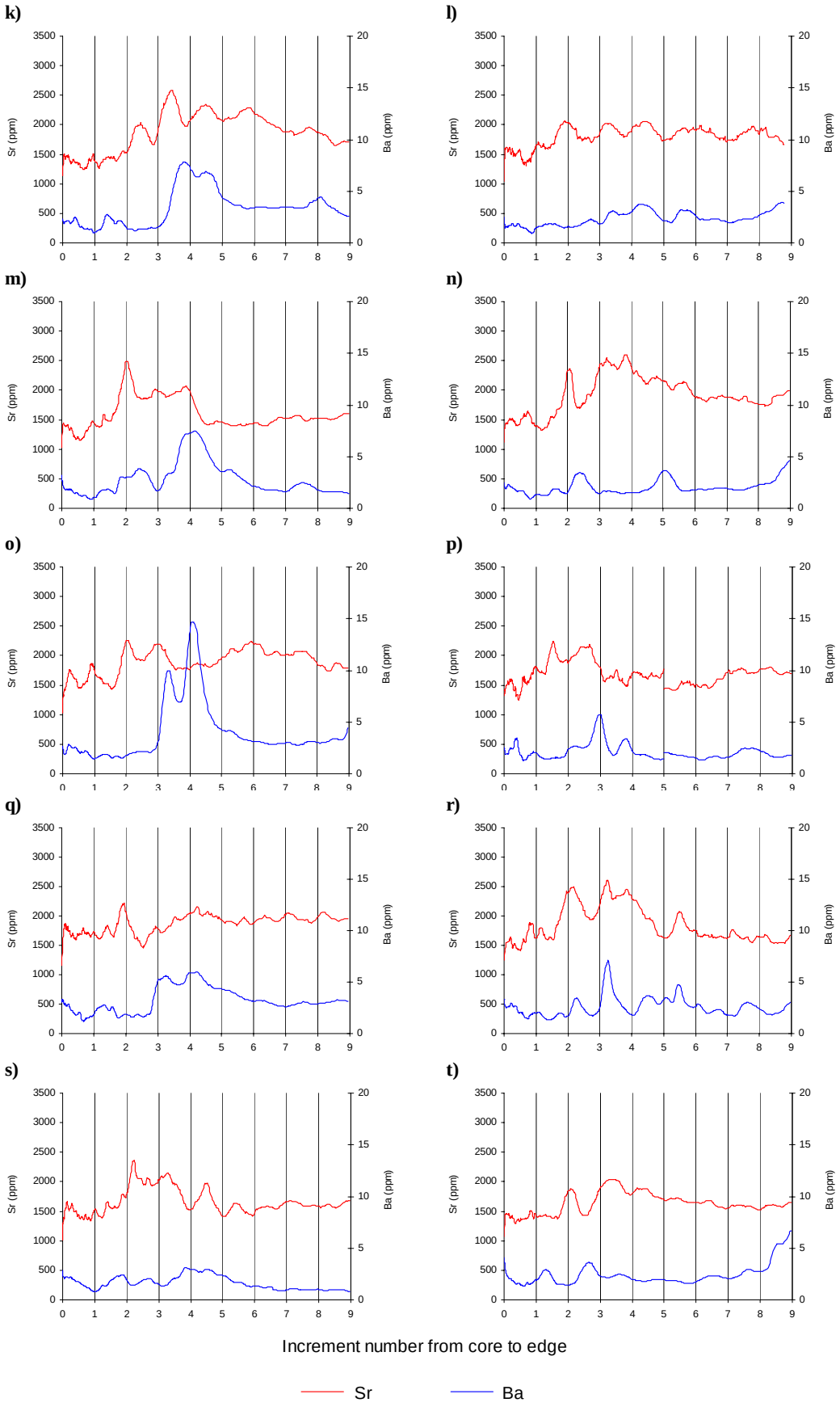

Figure A.5 cont…..

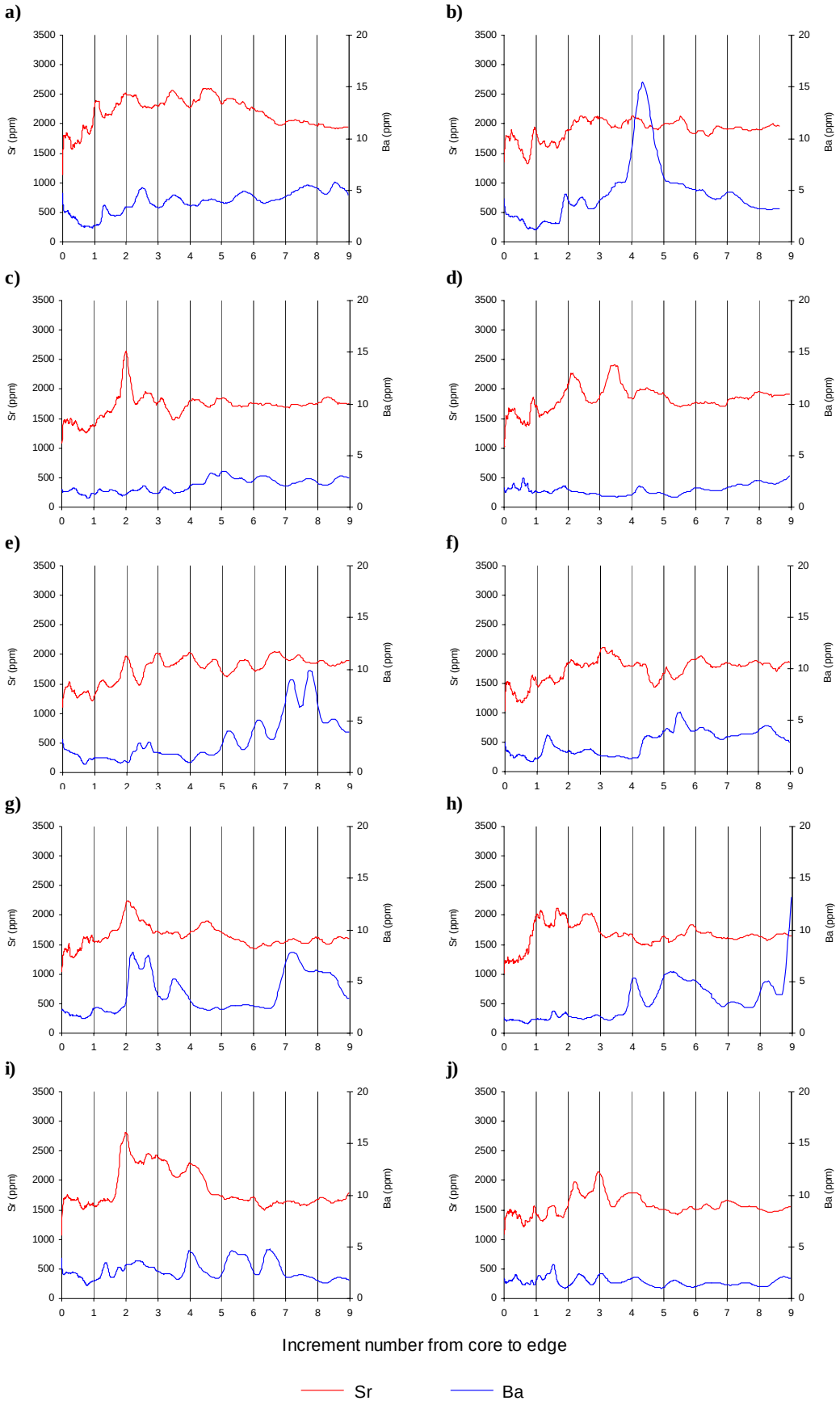

Figure A.6 Individual elemental profiles for Sr and Ba for otoliths from fish taken from SE.

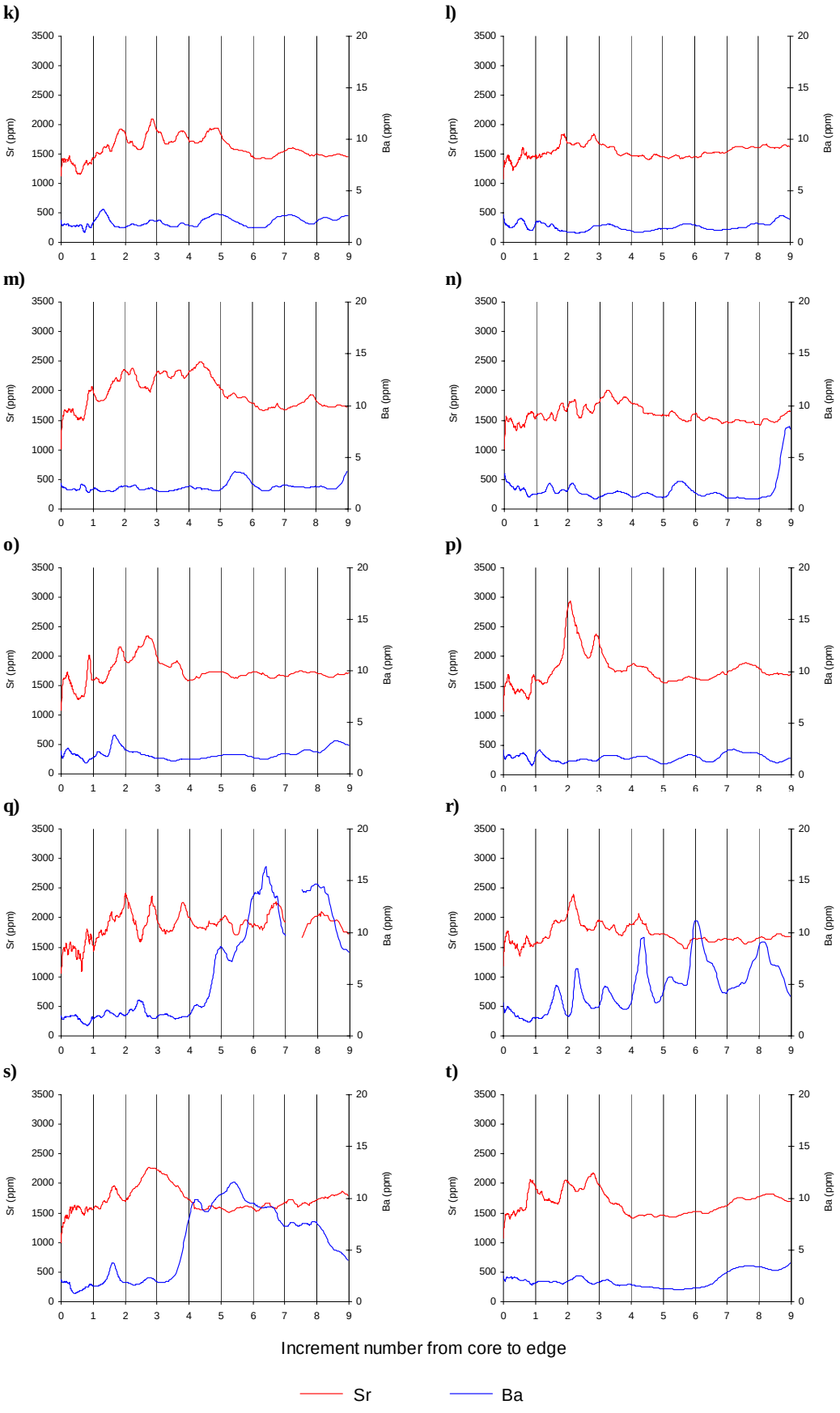

Figure A.6 cont…..