

Use of otolith chemistry and shape to assess - stock structure of blue grenadier - (*Macruronus novaezelandiae*) in the Commonwealth Trawl and Great - Australian Bight fisheries -

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Use of otolith chemistry and shape to assess the stock structure of blue grenadier (*Macruronus novaezelandiae*) in the Commonwealth Trawl and Great Australian Bight fisheries

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NON-TECHNICAL SUMMARY -

2007/030 - Use of otolith chemistry and shape to assess stock structure of blue grenadier (*Macruronus novaezelandiae*) in the Commonwealth Trawl and Great Australian Bight fisheries

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Objectives:

1. - Use stable isotope, elemental chemistry and shape analyses of otoliths to assess stock structure of blue grenadier across the Commonwealth Trawl and Great Australian Bight Trawl Sectors of the SESSF.

Non Technical Summary: -

OUTCOMES ACHIEVED -

Comparison of otolith shape, elemental chemistry and stable isotopes of blue grenadier from three key regions of the Southern and Eastern Scalefish and Shark Fishery (SESSF) (western Tasmania, eastern Bass Strait, Great Australian Bight) has provided new information that will be utilised by management and industry to improve stock assessments and develop appropriate spatial management of the fishery. Specifically, the comparisons supported that the blue grenadier fisheries in the Commonwealth Trawl and Great Australian Bight Sectors of the SESSF are based on separate stocks. This new information, combined with earlier studies of larval distributions and dispersal processes, will provide a basis for consideration of independent management arrangements for blue grenadier in these two sub-fisheries of the SESSF. An additional outcome was the provision of evidence for structuring of blue grenadier populations within the Commonwealth Trawl Sector of the SESSF. Demonstration of variation in otolith elemental and stable isotope chemistry between the western Tasmanian and eastern Bass Strait fishery regions indicates the potential value of further application of these otolith techniques to provide clarification of the links between these fisheries. The project has provided timely information given the recent emphasis on the need to clarify the relationship between grenadier fisheries to the east and west of Bass Strait and Tasmania and both industry and fisheries resource managers have expressed keen interest in the outcomes.

Non technical summary

Blue grenadier (*Macruronus novaezelandiae*), is one of the most commercially important fish species in southern Australia. The landed value of blue grenadier from the Southern and Eastern Scalefish and Shark Fishery (SESSF) was \$13.9 million in 2006–07 (ABARE 2008). The species is targeted by trawlers along the shelf slope at depths between 300 and 600 m. The major fishing areas in the Commonwealth Trawl Sector (CTS) of the SESSF are off western Tasmania and eastern Bass Strait/eastern Tasmania, but there is a developing fishery in the Great Australian Bight Trawl Sector of the SESSF. A large proportion of the annual catch is derived from spawning adults that aggregate off western Tasmania during the winter months. There is considerable uncertainty over how fishing of the west Tasmanian spawning aggregation may impact replenishment and production in other regions of the CTS and also the developing grenadier fishery in the Great Australian Bight. Industry and management have recognised the need for clarification of whether or not the fisheries in different regions of the CTS and Great Australian Bight are independent of the west Tasmanian fishery, and can therefore be treated as separate management units. Earlier genetic studies indicated subtle variation between blue grenadier from the eastern and western Tasmanian fisheries, but were largely inconclusive in relation to levels of mixing among these fishery regions and did not include fish from the Great Australian Bight.

Several characteristics of fish otoliths (earbones) including: shape, elemental chemistry, and stable isotope ratios can be influenced by variation in environmental conditions, food composition, physiology, metabolism and growth of fish. Because these factors can vary geographically, the above-mentioned characteristics of otoliths can indicate spatial separation of fish for parts of, or all of, their lives and have become valuable indicators of stock structure, particularly in situations where genetic indicators have provided inconclusive results. This project involved comparisons of otolith shape, otolith elemental chemistry, and otolith stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) for two cohorts/age groups (the 2002 cohort age 4 years, and the 1993 cohort age 13 years) of blue grenadier sampled in 2007 from the three major regions of the SESSF; western Tasmania (WTAS), eastern Bass Strait (EBS) and the Great Australian Bight (GAB). The two cohorts that formed the basis for this study were identified from age composition data as the dominant cohorts across all three regions, indicating broad scale consistency of these major recruitment events. The primary objective of the project was to provide information to industry and management that could help determine whether or not the developing fishery in the GAB sector can be treated as a separate management unit to the WTAS fishery within the CTS. A secondary objective involved providing new information that could contribute to clarification of the relationship between the WTAS and EBS regions of the CTS.

Otolith shape varies dramatically among species due largely to genetic differences. Variation of otolith shape among individuals of a species is, however, predominantly influenced by variation in a variety of environmental factors that influence otolith growth, with genetic variation playing only a more minor role. Comparisons of otolith shape for the age 4/2002 cohort indicated that the samples from the GAB region differed from those collected in the WTAS region. No differences among regions were detected for the age 13/1993 cohort. The reason for the lack of significant variation in otolith shape among regions for the age 13/1993 cohort is unclear, but may relate to changes in the sensitivity of the otolith shape technique with age.

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) was used to analyse the elemental chemistry of the otolith cores (representing the first 1–3 months of life) and the otolith margins (representing the recent period of life, assumed representative of the area of capture). Comparison of the otolith core chemistry is most important in relation to assessing stock structure. Differences in otolith core chemistry among adult fish sampled from different geographic regions indicate that the adults were spatially separated during the early life stages. Furthermore, the fact that adult fish have significant opportunity to mix among sampling regions prior to collection (i.e. 4 and 13 years in this study) indicates that separation of the early life stage is maintained at adulthood. Regional differences in otolith core chemistry of adult fish can therefore indicate that replenishment of fisheries in different regions is not dependent on the same source (i.e. spawning area), supporting the treatment of the different fishery regions as components of different stocks for management purposes.

For both cohorts the otolith core chemistry varied significantly among regions for magnesium, copper and barium. Previous studies have clearly demonstrated that variation of barium levels in otoliths can be closely related to variation in the concentration in the water, and there is some evidence that Cu levels in

otoliths can also vary in response to concentration in the water. The reasons for variation in the levels of magnesium in otoliths remain unclear, but are unlikely linked to variation in water chemistry and more likely linked to growth and or metabolic effects. The key results were; the consistent differences, for each cohort, in otolith core chemistry between the GAB and WTAS regions, and between the EBS and WTAS regions. There was also significant variation in otolith margin chemistry between the GAB and WTAS regions, and the EBS and WTAS regions for the age 4/2002 cohort. Significant overlap occurred between the GAB and EBS regions for the otolith margin chemistry of the age 4/2002 cohort when it was highly unlikely that the fish from these widely separated regions had co-occurred in the same geographic area during the recent period prior to capture. The observed overlap of the otolith margin chemistry between these two regions along with considerations of previous larval sampling and dispersal modelling, and the geographic distance between the two regions, would suggest that the overlap was likely the result of regional similarity of the environmental and or biological influences on otolith chemistry rather than mixing of populations between the EBS and GAB regions. Overall, the results of the otolith chemistry component of the project do not support the existence of one widely mixing population along the southern Australian continental slope or that the populations in each of the three regions are solely dependent on a common spawning area. While it is impossible to rule out some mixing among the fishery regions, the otolith chemistry data indicated that any mixing, particularly between the WTAS/EBS regions (i.e. the CTS) and the GAB region, is likely of limited importance in relation recruitment to the GAB fishery.

Values $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ were determined for whole otolith sections, and therefore provided an integrated value for the entire life of the individual. In situations where salinity shows little variation, regional differences in $\delta^{18}\text{O}$ of otolith carbonate will be primarily linked to variation in water temperature (higher $\delta^{18}\text{O}$ of otolith indicates lower water temperature). Variation in the $\delta^{13}\text{C}$ of otolith carbonate is thought to be related to a combination of environmental conditions (including temperature), diet, physiology and metabolic effects. $\delta^{13}\text{C}$ differed significantly between samples from the GAB region, and those from both the WTAS and EBS regions for the age 4/2002 cohort. Furthermore, although differences were not statistically significant for the age 13/1993 cohort, the pattern of $\delta^{13}\text{C}$ variation among regions was similar between cohorts. $\delta^{18}\text{O}$ did not vary significantly among regions for the age 4/2002 cohort, but for the age 13/1993 cohort, the WTAS samples displayed significantly higher $\delta^{18}\text{O}$ than both the EBS and GAB samples. This indicated that the WTAS fish had experienced lower water temperatures for a significant period of their life compared to the EBS and GAB fish. The difference between the EBS and WTAS regions was particularly interesting, and may indicate different depth and or latitudinal movement patterns with age for fish from these two regions. Further analysis of $\delta^{18}\text{O}$ variation with age is recommended to develop a greater understanding of the relationships between the blue grenadier populations in the EBS and WTAS regions.

$\delta^{18}\text{O}$ values for otoliths were converted to water temperature using relationships from the literature. The temperature conversions were compared to 'actual' water temperature data collected at various depths in each region. Temperature estimates from $\delta^{18}\text{O}$ of otoliths accurately matched the actual water temperatures at the depths at which most blue grenadier are captured. Furthermore, the actual water temperature data indicated negligible variation among the three regions at the capture depths of blue grenadier (300–600 m). The limited variation in $\delta^{18}\text{O}$ of otoliths among regions for the age 4/2002 cohort was a result of the low of variation in water temperature, contrary to what would have been predicted from consideration of surface water temperatures. The accuracy with which the $\delta^{18}\text{O}$ values of blue grenadier otoliths indicated water temperature bodes well for their further application to study differences in latitudinal and or depth related migration histories.

In summary, the otolith indicators provided support for separate stocks of blue grenadier in the Great Australian Bight Trawl and Commonwealth Trawl Sectors of the SESSF for the purposes of management and assessment. They also indicated that blue grenadier from the western Tasmanian and eastern Bass Strait regions of the CTS were unlikely part of one highly mixed south eastern Australian stock. The results from this study will provide a basis for future consideration of spatial management of the blue grenadier resource in southern Australia and indicate the need for further consideration of the degree of connectivity between blue grenadier populations to the east and west of Tasmania and Bass Strait.

Keywords:

Stock assessment, otolith, fishery management, stable isotope, elemental chemistry, shape analysis

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FINAL REPORT -

2007/030 - Use of otolith chemistry and shape to assess the stock structure of blue grenadier (*Macruronus novaezelandiae*) in the Commonwealth Trawl and Great Australian Bight fisheries

Background

The Southern Australian fishery

Blue grenadier (*Macruronus novaezelandiae*) (Fig. 1) is an important component of the demersal and mid-water trawl fisheries in both southern Australia and New Zealand. The overall annual blue grenadier catch from southern Australian waters over the past 3 years has ranged from 6652 to 3857 tonnes with a market value ranging from \$11,907,000 to \$14,271,000 (www.abareconomics.com, ABARE 2008). In south eastern Australia, blue grenadier are mainly caught under quota within the Commonwealth Trawl Sector (CTS) of the Southern and Eastern Scalefish and Shark Fishery (SESSF) which had a TAC of 4,368 tonnes in 2008. Within the CTS, the species is largely targeted by trawlers off western Tasmania where the bulk of the catch is taken during winter spawning aggregations (the spawning sub-fishery) (Fig. 2). The other significant fishing area within the CTS is off eastern Bass Strait/eastern Tasmania where the species is also targeted during the winter spawning period (Fig. 2). Most of the blue grenadier taken in the SESSF is from the CTS, but the species is also taken in the Great Australian Bight Trawl Sector (GABTS) of the SESSF where there is interest in further developing the fishery, particularly in the central region of the Bight (Fig. 2). Recent catches (last 3 years) in the GABTS have ranged from 100 to 340 tonnes per annum with a market value per annum ranging from \$375,000 to \$688,000 (www.abareconomics.com).

While most of the catch is taken during the winter spawning period, the species is also taken as by-catch and occasionally targeted outside this period (the 'non-spawning fishery'). The blue grenadier fishery in the GABTS is currently managed under catch limits which are independent of the SESSF quota. Under this system when the GABTS catch reaches 400 tonnes for the year a review of any data collected and further assessment occurs, and if the catch reaches 500 tonnes, fishing ceases unless a spawning aggregation is found, in which case an acoustic survey and collection of samples occurs (Anon. 2007). The lack of understanding and assumptions about the relationship between the blue grenadier harvested in the GABTS region and those harvested around western Tasmania and eastern Bass Strait in the CTS is currently impeding the development of a more spatially structured quota management plan for blue grenadier in southern Australia, and is the major impetus for this project.

Distribution and recruitment

Australian blue grenadier are distributed from mid-New South Wales to southern Western Australia including Tasmania (Smith 2000). Adult blue grenadier are found mostly at depths ranging from ~200 to 700 m on the shelf slope, but can occur to depths of 1000 m, and young juveniles (< 4 years age) are found predominantly between 200 to 400 m (Kuo and Tanaka 1984a; Smith 1994). Most blue grenadier taken commercially in Australian waters are captured from depths between 300 and 600 m with the majority of the catch taken from the 450 to 550 m depth range (this report). Catch rates in Australia are highest off the west coast of Tasmania on the shelf slope during winter where the species aggregates to spawn, and this region has been confirmed as a major spawning ground for the species (Gunn *et al.* 1989; Bulman *et al.* 1999). Although catch rates are highest from the west Tasmanian spawning aggregation, the fish do not stay densely aggregated on the spawning ground for the whole year (Smith 2000). On the west Tasmania spawning grounds the length-frequency distribution and sex-ratio change throughout the winter fishing

season, suggesting movement of fish to and from the area; however the rates and dynamics of these movements are poorly understood (Smith 1994; Bulman *et al.* 1999).

The Australian blue grenadier fishery over the past two decades has been characterized by episodic recruitment and currently only two strong year classes (cohorts) dominate the catch (Krusic-Golub *et al.* 2007). The factors that drive episodic recruitment are unknown for Australian blue grenadier. Correlative studies involving New Zealand blue grenadier stocks have indicated links with the southern oscillation index, winds and sea temperature, suggesting higher recruitment is more likely in cooler autumn/winter conditions, although mechanisms behind these relationships with climate are unclear (Livingston 2000).

Biology, age and growth

Age at maturity has been estimated at 4 and 5 years for males and females respectively, and this is also the age at which most individuals recruit to the fishery (Sullivan and Cordue 1990). Blue grenadier spawn in winter to early spring, mostly from June to September, however spawning has been observed as early as May (Smith 1994). On the western Tasmanian spawning grounds, the abundance of blue grenadier eggs is highest on the edge of the continental shelf (Bulman *et al.* 1999), where adult blue grenadier are observed to aggregate in canyons to spawn (P. McCoy, pers. comm.). In New Zealand, blue grenadier grow to about 27–30 cm TL (total length) in their first year of growth, 42–47 cm TL in the second year, and 55–58 cm TL in the third year (Horn and Sullivan 1996). Similar patterns of age and growth occur for southern Australian populations (Krusic-Golub *et al.* 2007). Further, length-at-age estimates overlap for both the Tasmanian and Great Australian Bight fisheries (Krusic-Golub *et al.* 2007). Maximum age in New Zealand is at least 20 years for females and 15 years for males (Horn and Sullivan 1996). In Australia, the maximum age appears to be at least 24 years, and in both New Zealand and Australia maximum length is at least 110 cm (Krusic-Golub *et al.* 2007).

Blue grenadier are thought to feed mainly at night, with important prey species including lantern fish, royal red prawns, krill and squid (Bulman and Blaber 1986). Predators of juvenile blue grenadier may include adult blue grenadier and pink ling (Bulman and Blaber 1986).

Stock structure and spawning areas

In Australia, blue grenadier are currently considered as a single interbreeding population based on earlier studies of genetic variation (electrophoretic analyses of isozyme variation) (Milton and Shaklee, 1987). Blue grenadier in Australian waters are, however, genetically distinct and considered reproductively isolated from New Zealand populations (Milton and Shaklee 1987). While there is evidence of genetic homogeneity across the south-eastern Australian populations, the earlier genetic comparisons did not extend to the west of the western Tasmanian spawning area (Milton and Shaklee 1987). Genetic homogeneity can be maintained over broad spatial scales even in situations where reproductive exchange is in fact limited over the same scales (Waples 1998). In these situations genetically homogeneous populations may in fact be comprised of a number of 'phenotypic stocks' based on geographic variation of other vital rate parameters (e.g. growth rate, size at maturity, fecundity, recruitment variation etc.) that are critical for understanding fishery dynamics and managing impacts of fishing on population processes (Begg and Waldman 1999). Importantly, the major sources of population replenishment (i.e. spawning/nursery areas) may vary among populations irrespective of genetic homogeneity. Understanding whether or not the major sources of replenishment for discrete localised fisheries are different is critical for determining whether or not they can be considered as separate units for management purposes. This understanding is clearly lacking for blue grenadier in Australian waters and this presents difficulties for catch allocation of across the SESSF and evaluation of the need for a more refined spatial assessment and management approach.

Earlier studies of reproductive condition and larval distributions have indicated that at least one major spawning ground occurs off western Tasmania (Gunn *et al.* 1989; Bulman *et al.* 1999). Furthermore, hydrodynamic dispersal modelling has indicated that dispersal of larvae from this spawning area is likely to be predominantly south around the southern tip of Tasmania, possibly extending northward to the east coast of Tasmania (Thresher *et al.* 1988; Bruce *et al.* 2001). There is, however, evidence that another smaller spawning area may exist along the edge of the continental shelf in eastern Bass Strait (Bruce *et al.* 2001). Bruce *et al.* (2001) reported blue grenadier larvae in coastal waters off eastern Victoria and southern New South Wales in August. Back calculated spawning dates indicated that these larvae were generally spawned

earlier than the larvae collected during the same period off western and southern Tasmania (Bruce *et al.* 2001). Furthermore, otolith increment widths were significantly wider in larvae caught off eastern Victoria and southern NSW than those from western Tasmania suggesting that they experienced faster growth, possibly related to exposure to warmer surface water temperatures than the western Tasmanian larvae (Bruce *et al.* 2001). Both these lines of evidence suggest that the larvae sampled by Bruce *et al.* (2001) off eastern Victoria and southern New South Wales did not originate from the west Tasmanian spawning area. Hydrodynamic modelling of larval dispersal also suggested that larvae collected off southern New South Wales and eastern Victoria were likely spawned on the shelf edge in eastern Bass Strait (Bruce *et al.* 2001). Beside the major spawning area off western Tasmania and the likely smaller spawning area off eastern Bass Strait, there are also anecdotal reports of ripe blue grenadier being captured in winter in canyons along the continental shelf slope off Portland (western Victoria) and off Kangaroo Island (South Australia) (P. McCoy, pers. comm.).

While it appears likely that a considerable proportion of the blue grenadier in the waters around Tasmania are derived from the major western Tasmanian spawning area, blue grenadier captured along the shelf edge in eastern Bass Strait are possibly derived from both the western Tasmanian and the smaller eastern Bass Strait spawning areas, however, the relative importance of both these replenishment sources is unclear. Unlike the Tasmanian and eastern Bass Strait fishing areas, there is nothing known in relation to the origins of blue grenadier recruiting to the Great Australian Bight region. There have been no confirmed locations of major spawning aggregations in the GAB, and it is unclear whether blue grenadier captured in the GAB fishery are replenished from the same stock that spawns off western Tasmania. As a result, it has been unclear whether blue grenadier caught in the GABTS and CTS should be managed as separate stocks or not. Clarifying these uncertainties is important for developing future management arrangements for the blue grenadier fisheries in southern Australia, and is central to this project.

Understanding stock structure for fisheries management – information from otoliths

Defining what constitutes a 'stock' is a critical precursor for determining the relevant approaches for actually resolving 'stock structure'. Importantly, fisheries managers and fisheries scientists need to understand and agree on the definition before research into resolving stock structure is planned. The definition of stock proposed by (Hilborn and Walters 1992) is relevant for the current study, "stocks are arbitrary groups of fish large enough to be essentially self-reproducing, with members of each group having similar life history characteristics". This definition does not infer genetic separation but allows for limited exchange between stocks such that losses from a stock due to emigration and additions due to immigration are negligible compared to reproduction, growth and mortality rates within the region of the stock. As such, the impacts of fishing on a stock should have negligible impact on the dynamics of other stocks. This study is focused on comparing different fishing regions, with each region viewed as a potentially distinct management unit. In order to justify separation of the fishing regions into separate management units, managers require evidence that the different regions are indeed relatively independent of each other (i.e. each region is part of a different stock), they do not necessarily need to define the actual spatial boundaries of each stock, or indeed identify every stock within the species range.

The two broad approaches to studying stock structure involve either genotypic (genetic) or phenotypic (physical) variation (Begg *et al.* 1999). Genotypic variation is useful for indicating long-term reproductive isolation and evolutionary differences among stocks, whereas phenotypic variation can involve shorter-term, environmentally induced differences in life history and/or physical traits such as age and growth, migration pathways, fecundity and morphometrics. It is recommended that where possible, both genotypic and phenotypic information be utilized in determining stock structure (Begg and Waldman 1999). Previous genetic analysis has failed to yield clear stock structure information due to apparent genetic homogeneity of blue grenadier across the Tasmanian and eastern Bass Strait/NSW fisheries (Milton and Shaklee 1987). In the current study we have focused on using variation in otolith shape, elemental and stable isotope chemistry as phenotypic indicators of stock structure (Campana and Casselman 1993; Begg and Waldman 1999; Campana 1999; Campana and Thorrold 2001).

Otolith shape

Comparison of otolith shape provides one possible tool for assessing stock structure (Campana and Casselman 1993). On an inter-species level, otolith shape can be extremely variable, enabling the identification of species (i.e. for diet analyses, palaeontology studies etc.). At the species level, subtle differences in otolith shape can be used as a phenotypic indicator of stock structure. The mechanisms causing differences in otolith shape within a species are poorly understood, but are generally thought to reflect differences in environmental conditions, particularly conditions that influence feeding and growth; and or subtle variation in genetics (Campana and Casselman 1993; Gauldie and Crampton 2002; Gagliano and McCormick 2004).

Otolith shape has been used throughout the world as an aid to assessment of stock structure for species as diverse as Atlantic cod, orange roughy, Atlantic mackerel, king mackerel and blue and silver warehou (Castonguay *et al.* 1991; Campana and Casselman 1993; DeVries *et al.* 2002; Smith *et al.* 2002; Talman *et al.* 2003). Fourier analysis of the shape of sagittal otoliths appears to be the most promising technique for discrimination of fish stocks, with previous studies demonstrating the capacity for highly accurate discrimination among fish collected from different regions (DeVries *et al.* 2002). Fourier analysis in conjunction with randomisation tests that are robust to deviations from assumptions of normality provides a powerful and robust method for the analysis of otolith shape differences between sample groups (Efron and Tibshirani 1993). The technique reduces the matrices of complex numbers to a pair-wise probability of a 'one stock' relationship hypothesis. This technique was further extended in Smith *et al.* (2002), where a method to determine the appropriate number of harmonics for use in further analyses was introduced.

All of the Fourier shape analysis techniques use coordinate data to represent the shape of the otolith. Traditionally, polar coordinate data has been used to calculate the Fourier series. Using this method, the centroid (geometric centre) of the otolith is treated as the origin for a series of radiating lines, and distance from the centroid to the edge of the otolith is used to calculate the Fourier series (Campana and Casselman 1993; Gagliano and McCormick 2004). Unfortunately, software commonly used to collect polar Fourier data (e.g. Optimas™) does not account for multiple boundary crossings of the radiating lines. This can often lead to an incorrect assessment of the otolith shape. Where otolith shape is more complex (or margins are irregular) a more robust form of analysis must be used. This problem is resolved by using the Cartesian coordinates as data for the Fourier analysis, and involves using the x-y coordinate data at equi-distant points around the perimeter of the otolith as complex numbers for calculating the Fourier series. This technique has been used and refined in a number of studies on stock delineation (Smith *et al.* 2002; Talman *et al.* 2003) and is employed in the current study.

Otolith chemistry

Trace elements

Otoliths are composed predominantly of calcium carbonate within a protein matrix (Degens *et al.* 1969; Campana 1999), with a small proportion of the otolith (approximately 1% by weight) comprised of elemental impurities (i.e. non calcium carbonate) (Campana 1999). These elemental impurities can be derived either directly from the ambient water across the gills, or potentially indirectly from the diet or ingested water (Campana 1999). The amounts and rates of incorporation of particular chemical impurities into otoliths can be influenced by variation in exogenous environmental factors such as: water chemistry, temperature and salinity, and or endogenous factors such as diet, growth rate and physiology/metabolism (Kalish 1989; Sadovy and Severin 1994; Campana 1999; Campana and Thorrold 2001; Elsdon and Gillanders 2002; Elsdon and Gillanders 2003b; Morales-Nin *et al.* 2005; Hamer and Jenkins 2007a). Importantly, otoliths are metabolically inert, meaning that once impurities are incorporated into the otolith crystal matrix they remain there for the life of the fish (Campana 1999). Furthermore, because otoliths grow continuously throughout a fish's life, they record variation in trace elemental and isotopic composition for the entire life-history of each fish (Campana 1999). These properties have allowed variation in otolith chemistry to be used to infer geographic migration behaviour, environmental history, and population structure (Campana *et al.* 1994; Swearer *et al.* 1999; Bath *et al.* 2000; Campana *et al.* 2000; Thorrold *et al.* 2001; Gillanders 2002a; Elsdon and

Gillanders 2003a; Hamer *et al.* 2003; Patterson *et al.* 2004; Dorval *et al.* 2005; Elsdon and Gillanders 2005; Fowler *et al.* 2005; Hamer *et al.* 2005; McCulloch *et al.* 2005; Stransky *et al.* 2005).

The application of otolith chemistry to investigate fish 'stock structure' dates back to the late 1980's (Edmonds *et al.* 1989). One of the earlier studies to employ this technique involved an analysis of blue grenadier stock structure in New Zealand waters (Kalish *et al.* 1996). While the New Zealand study was inconclusive in relation to stock structure (i.e. lack of regional variation in otolith chemistry, see below) this may have been due to several limiting factors, in particular, the high limits of elemental detection and resultant limited element list available from the analytical technique available at the time (inductively coupled plasma-atomic emission spectroscopy, ICP-AES) (Kalish *et al.* 1996). The elements detectable in this earlier study (Na, S, P, K, Ca, Cu, Sr, Zn) were generally physiologically important or highly regulated with their incorporation into otoliths not clearly related to environmental variation. Further, some of these elements are thought not to be bound tightly within the otolith crystal lattice making them subject to post-mortem and handling effects (Proctor and Thresher 1998; Thresher 1999). In short, the lack of variation detected in this study may have been due to the poor sensitivity of the elemental variables analysed to environmental variation. The inconclusive results of this earlier study highlight an important limitation to the interpretation of otolith chemistry data in relation to inferring stock structure. Low or insignificant variation in otolith chemistry among sampling regions could occur irrespective of stock structure if environmental variation among regions has negligible influence on variation of otolith chemistry. Alternatively, low or insignificant variation of otolith chemistry among sampling regions could result from a highly dispersed completely mixed unit stock, irrespective of the influences of regional environmental variation on otolith chemistry.

Clearly, where the otolith chemistry technique does not detect significant variation among sampling regions it will be difficult to make inferences in relation to stock structure. It is therefore imperative that methodologies are employed that maximise the chances of detecting environmental influences on otolith chemistry. Many earlier stock structure studies involving otolith chemistry compared the chemistry of whole otoliths dissolved in solution (i.e. bulk analysis) (Edmonds *et al.* 1992; Begg *et al.* 1998). This approach not only averages out chemical variation over the entire life, thus removing any potentially informative variation among life-history stages, but even if clear differences in bulk chemistry are detected among regions, it is still possible that significant mixing among regions may have occurred at a particular period(s) during the life-history (Begg *et al.* 1998). Finally, it is now clear that just as spatial environmental variation can influence otolith composition at a variety of scales, so too can temporal variation in environmental conditions (Campana *et al.* 2000; Gillanders 2002b; Hamer *et al.* 2003). This recognition means that spatial comparisons of otolith chemistry should remove the potential confounding influence of temporal variation in environmental factors by restricting comparisons to the same cohorts (Gillanders 2002b; Talman *et al.* 2003; Hamer *et al.* 2005).

Recent developments in analytical technologies, particularly, inductively coupled plasma-mass spectrometry (ICP-MS), have provided scientists with higher limits of elemental detection and sensitivity, and an increased capability to detect a broader range of elements in otoliths. Furthermore, the coupling of laser ablation (LA) sampling with ICP-MS has allowed for high resolution sampling within otolith sections (Fowler *et al.* 1995; Russo *et al.* 2002) so that elemental analysis can now target particular life-history stages. Furthermore, a variety of studies have investigated the role of environmental and physiological processes in influencing incorporation of impurity elements into otoliths (Kalish 1989; Bath *et al.* 2000; Elsdon and Gillanders 2002; Elsdon and Gillanders 2003b; Elsdon and Gillanders 2004; De Vries *et al.* 2005; Martin and Thorrold 2005; Walther and Thorrold 2006; Hamer and Jenkins 2007a), providing a basis for designing and interpreting spatial comparisons of otolith chemistry in relation to stock structure hypotheses. In the current study we employ LA-ICP-MS to compare otolith trace element chemistry of different life-history stages (i.e. otolith cores - early life and otolith margins - recent life) among blue grenadier sampled from the three major fishing grounds in southern Australia (Great Australian Bight, West Tasmania and Eastern Bass Strait, Fig. 2) and from two cohorts (1993 cohort sampled at age 13+ years and the 2002 cohort samples at age 4+ years).

Stable isotopes

Otolith trace element chemistry is not the only chemical technique available for investigating stock structure. The carbon ($^{13}\text{C}/^{12}\text{C}$, i.e. $\delta^{13}\text{C}$) and oxygen ($^{18}\text{O}/^{16}\text{O}$, i.e. $\delta^{18}\text{O}$) isotopic ratios of otolith carbonate have also been

utilized in studies of fish stock structure and environmental history (Campana, 1999; Campana and Thorrold, 2001). $\delta^{18}\text{O}$ has been shown to be a useful indicator of temperatures experienced by fish due to the fact that the fractionation of $^{18}\text{O}/^{16}\text{O}$ during deposition of otolith carbonate is influenced by temperature (Iacumin *et al.* 1992; Thorrold *et al.* 1997; Dufour *et al.* 1998; Edmonds *et al.* 1999; Gao and Beamish 1999; Weidman and Millner 2000; Begg and Weidman 2001; Gao *et al.* 2001; Stephenson *et al.* 2001; Hoie *et al.* 2004). $\delta^{18}\text{O}$ of seawater is, however, largely a function of salinity, although the relationship can vary among ocean regions (LeGrande and Schmidt 2006). For offshore oceanic species where salinity variation is minimal, most of the variation in $\delta^{18}\text{O}$ of otolith aragonite will be related to temperature variation (Campana 1999). Unlike $\delta^{18}\text{O}$, the $\delta^{13}\text{C}$ of otolith is not in equilibrium with the surrounding water, and it is thought that much of this disequilibrium is related to the influence of diet composition and metabolic effects (Thorrold *et al.* 1997). Variation in the $\delta^{13}\text{C}$ of otolith carbonate is thought to be related to a combination of environmental conditions, diet, physiology and metabolic effects (Kalish 1991a; Kalish 1991b; Iacumin *et al.* 1992; Thorrold *et al.* 1997; Schwarcz *et al.* 1998; Campana 1999; Weidman and Millner 2000; Solomon *et al.* 2006). In the case of $\delta^{18}\text{O}$ of otolith, where the influence of temperature is clearly established, it is possible to make some a-priori predictions as to regional differences in $\delta^{18}\text{O}$. In the current study samples are being compared among fishing regions separated over a latitudinal range of $\sim 10^\circ$ and with surface temperature differences of several $^\circ\text{C}$ expected.

Project Overview

In this project we use a combination of otolith shape, trace element, and stable isotope ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) analyses to investigate fishery stock structure of blue grenadier in southern Australian waters. More specifically, this project assesses the degree to which fish caught in three regions of the SESSE; the Great Australian Bight Trawl Sector, and the western and eastern Bass Strait regions of the Commonwealth Trawl Sector (Fig. 2), can be separated using these three otolith-based stock structure indicators. Furthermore, independent comparisons are made for two cohorts to assess whether patterns of stock discrimination are consistent across time.

Need -

In July 2006, the Great Australian Bight Industry Association (GABIA) discussed the need for further research to assess the stock structure of blue grenadier between the Great Australian Bight Trawl Sector (GABTS) and other fishing areas, particularly the west Tasmanian region of the Commonwealth Trawl Sector (CTS) of the SESSF. With total allowable catches for blue grenadier in the CTS set under quota each year and separate catch limits in the GABTS, GABIA commented that it is very important for both the GABTS operators and the Australian Fisheries Management Authority (AFMA) to better understand blue grenadier stock structure. GABIA suggested that assessing the stock structure of blue grenadier was a high priority if techniques were available that would have a high likelihood of indicating stock structure if it existed, and in particular, indicate whether or not the fisheries in the GAB and west Tasmanian regions are based on separate stocks for fisheries management purposes. This view was consistent with the views of, and noted by, the Great Australian Bight Trawl Sector Management Advisory Committee (GABMAC). AFMA also support the need for research into stock structure of blue grenadier by indicating that 'determining the stock structure of blue grenadier is a very high priority for management', and 'results of stock structure research will influence the future management of blue grenadier in the GABTS, with the two most likely options being a global TAC apportioned between the GABTS and South East Trawl Fishery (SETF) or separate management of stocks in each zone. In short, both industry and management have indicated the need for research to assess the stock structure of blue grenadier between the GABTS and CTS to direct the future management of this valuable fisheries resource.

Objectives -

1. Use stable isotope, elemental chemistry and shape analyses of otoliths to assess stock structure of blue grenadier across the Commonwealth Trawl and Great Australian Bight fisheries.

Methods

Sample collections

Blue grenadier (Fig. 1) were collected from two regions within the Commonwealth Trawl Sector (CTS) identified as western Tasmania (WTAS), and eastern Bass Strait (EBS); and within the Great Australian Bight Trawl Sector (referred to as GAB) (Table 1. Fig. 2). Samples were collected opportunistically by onboard observers from commercial trawl catches taken in each of the regions between January and November 2007.



Figure 1. Blue grenadier, *Macruronus novaezelandiae* (image at top from New Zealand Seafood Industry Council – SeaFIC, image at bottom courtesy of Paul McCoy).

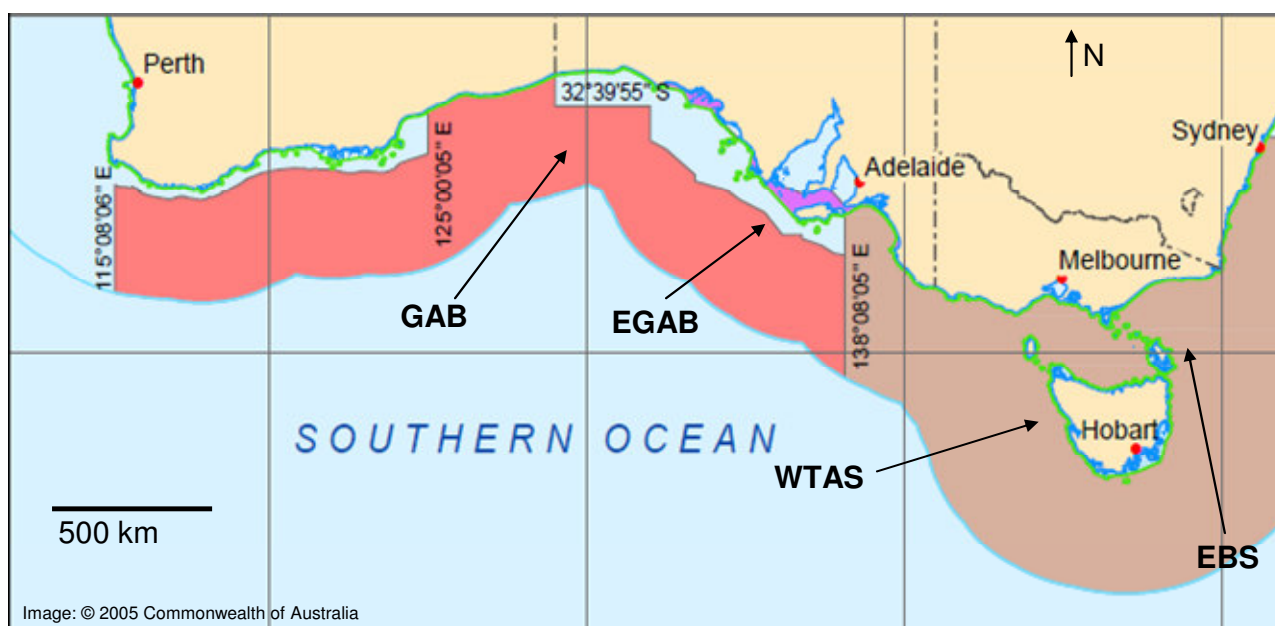


Figure 2. Map showing the two sampling regions within the Commonwealth Trawl Sector (CTS) of the Southern and Eastern Shark and Scalefish Fishery (SESSF) (highlighted in brown): western Tasmania (WTAS), and eastern Bass Strait (EBS); and two sampling regions within the Great Australian Bight Trawl Sector fishery (highlighted in red): central Great Australian Bight (GAB) and eastern Great Australian Bight (EGAB). Image from Australian Fisheries Management Authority (www.afma.gov.au).

Table 1. Summary of the total number of samples (n) collected, sex ratio (F:M), number with sex unknown or immature (U), number of sampling events (Events), first (Start) and last (End) sampling dates, and the minimum (L_{min}), maximum (L_{max}), modal (L_{mode}) and average (L_{avg}) total lengths of blue grenadier collected in each of the CTS and GABTS sampling regions. SD = standard deviations. Region codes are as for Fig. 2 caption.

Region	n	F:M	U	Events	Start	End	L_{min} (cm)	L_{max} (cm)	L_{mode} (cm)	$L_{avg} \pm SD$ (cm)
EBS	206	2:1	59	7	20/3/07	8/11/07	37	102	77	78 ± 13
WTAS	1029	1.2:1	16	24	23/1/07	26/10/07	27	110	72	85 ± 11
GAB	252	3.5:1	21	3	27/6/07	4/11/07	53	106	76	81 ± 11

NB: Of the 252 samples collected from the GAB for ageing, only 19 were collected from the EGAB region and these were all from the age 13+/1993 cohort. The EGAB samples are included in the GAB ageing data.

Age composition

Where possible one sagittal otolith from each pair with intact/undamaged margins was set aside for shape analyses and selection for otolith chemistry analysis (below). The remaining otolith was used for ageing. Ages were determined by counting validated annual opaque increments (Horn and Sullivan 1996) using standard procedures developed by the Central Ageing Facility (below). All ageing was completed by technicians with experience in ageing blue grenadier. The resulting age compositions were compared among sampling regions and the data were used to select individuals from the same cohorts (year-classes) and ages for the otolith shape and chemistry components. This was essential to ensure that comparisons among sampling regions were not temporally confounded by differences in the ages and or cohorts comprising the comparison groups.

Otolith preparation for ageing

Otoliths were transversely sectioned using a 3-stage process; embedding, sectioning and mounting. To embed the otoliths, a thin layer of clear polyester resin was poured onto the base of a silicon mould and left to partially cure. Otoliths were arranged in 2 rows of 5 on the resin. Resin blocks were labelled and coated with another layer of resin. Blocks were then oven cured at 55°C for 24 hr. Otolith sections were cut using a Gemmasta™ lapidary saw fitted with a diamond-impregnated blade. From each row of otoliths, 4 sections were taken (approximately 350 µm in thickness). This was to ensure that the primordium of each otolith was included. Sections were cleaned using alcohol and stored in vials. For identification, each vial contained a sample identification label consisting of batch and fish number. A small amount of resin was poured onto a glass slide (50 x 75 mm). Otolith sections were immersed in the resin and the identification label placed at the top of the slide. Once the resin had semi-cured, further resin was applied to the preparations and coverslips applied. Slides were oven cured at 30°C for 3 hrs.

Ageing protocol

Sections were viewed with transmitted light at 10x or 16x magnification. Higher magnification was sometimes required for the examination of growth increments near the otolith margin. All sections from each sample were examined and the section closest to the primordium with the clearest microstructure was used. Ages were estimated by counting the number of opaque increments from the primordium to the posterior edge on either the dorsal or ventral side of the otolith sulcus, depending on increment clarity (Fig. 3). To avoid potential bias, all counts were made without knowledge of fish size, otolith mass or area of capture.



Figure 3. Image of a transverse section of a sagittal otolith from a 13+ year old *Macruronus novaezelandiae*. Annual opaque increment zones are marked by the white dots (14th increment forming on otolith margin), arrow indicates core region. Viewed with transmitted light at 16x magnification.

Otolith shape analysis

Otolith samples were dominated by individuals of age 4+ and 13+ years (see results), and most were collected during June/July 2007. These two cohorts would have originated from spawning in winter 2002 and 1993, respectively. The shape analyses focussed on samples only from these two cohorts (Table 2). Sufficient numbers of otoliths with intact margins were available for robust pairwise comparisons among all three regions for the age 4+/2002 cohort, but only between the GAB and WTAS region for the age 13+/1993 cohort (Table 2).

An initial comparison between otoliths of the 4+ and 13+ age groups from the same region (WTAS) revealed highly significant differences in otolith shape parameters between the two age groups ($p < 0.001$, $n=484$). It was therefore not appropriate to pool samples across age groups for shape comparisons among sampling regions, and all analyses of regional differences in otolith shape are cohort/age specific.

Table 2. Sample numbers used for shape analyses by area, collection date and age, along with the number of sampling events from which the otoliths were derived.

Region	Age (years)	Collection Date			Number of sampling events
		March 2007	June/July 2007	November 2007	
EBS	4	3	48	9	4
WTAS	4	0	226	16	4
GAB	4	0	93	0	2
WTAS	13	0	185	57	5
GAB	13	0	66	14	3

Image analysis

Images of each otolith were collected using Optimas™ software and an M3C type-s Wild Leitz dissecting microscope, with samples illuminated by transmitted light (Fig. 4). A Pulnix TMC-6 CCD colour camera was used to acquire each image. An image of each otolith was saved to tagged image file format (tiff) in 24-bit colour at a magnification of $2.54 \times (6.4 \times \text{magnification}, 0.63 \times \text{secondary objective and a } 0.63 \times \text{c-mount objective})$. Images were converted to joint photographic group 8-bit black-and-white format (jpg) for analysis.

Three traces of the outline of the otolith were made. The first collected the otolith area measurement in pixels. The second collected the perimeter measurement. The third collected the x-y coordinates at 128 equidistant points around the perimeter. A scalar shape index (circularity) was also calculated as the perimeter squared over the area, and recorded. The x-y coordinates were used by the image analysis software to calculate the Fourier series. The resultant Fourier series was exported to MS™ Excel for further analyses. The real and complex components of the Fourier series were used to calculate the Fourier descriptor (Harmonic) by taking the absolute value of the complex number:

$$\text{Fourier descriptor (FD)} = \sqrt{a + bi}$$

Where a = real component of complex number, and bi = imaginary component of complex number

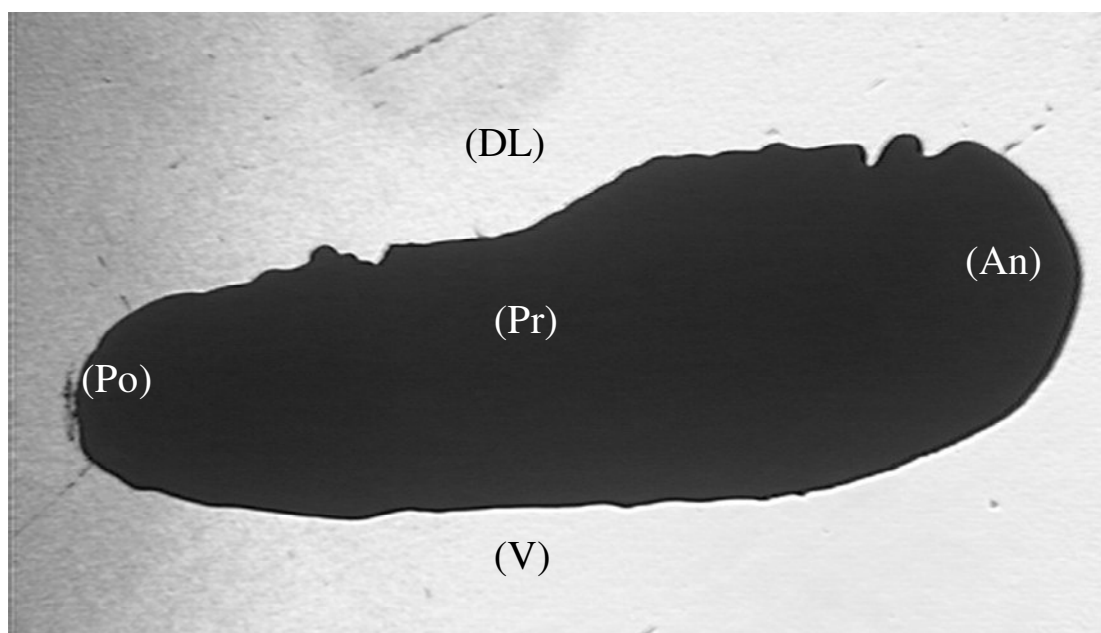


Figure 4. Shape of a whole otolith (distal surface up) from a *Macruronus novaezelandiae* as viewed using transmitted light. (DL=dorsal, V=ventral, Po=posterior, An=anterior and Pr=primordium)

Further tests using Fourier descriptors (harmonics)

Determination of appropriate numbers of harmonics

To determine the appropriate number of harmonics (Fourier descriptors) which would adequately describe the shape, a range finding test was performed. This involved reconstructing the shape from each of the complex numbers in the Fourier series and calculating error with respect to the reconstructed shape using the complete Fourier series (Fig. 5). To standardise for otolith position on the screen, the zeroth Fourier descriptor was set to $0 + 0i$. The Fourier series was also standardised for otolith size by dividing all subsequent Fourier descriptors by the first complex number in the series. Otolith shape before and after standardisation are shown in figures 6 & 7, respectively. The maximum error possible was the difference between a reconstruction of the otolith using the zeroth (and the 127th) complex numbers and the full series of complex numbers expressed as a percentage. Thirty samples (age 13+ GAB) were randomly selected and the shape was reconstructed using additional complex numbers from each end of the complex array. This process was continued until the otolith shape had been reconstructed 30 times (Fig. 8). The mean error and minimum and maximum values at each harmonic are shown in figure 5. As the maximum error at the 30th harmonic was greater than 5% (Fig. 5), all harmonics were used in subsequent analyses.

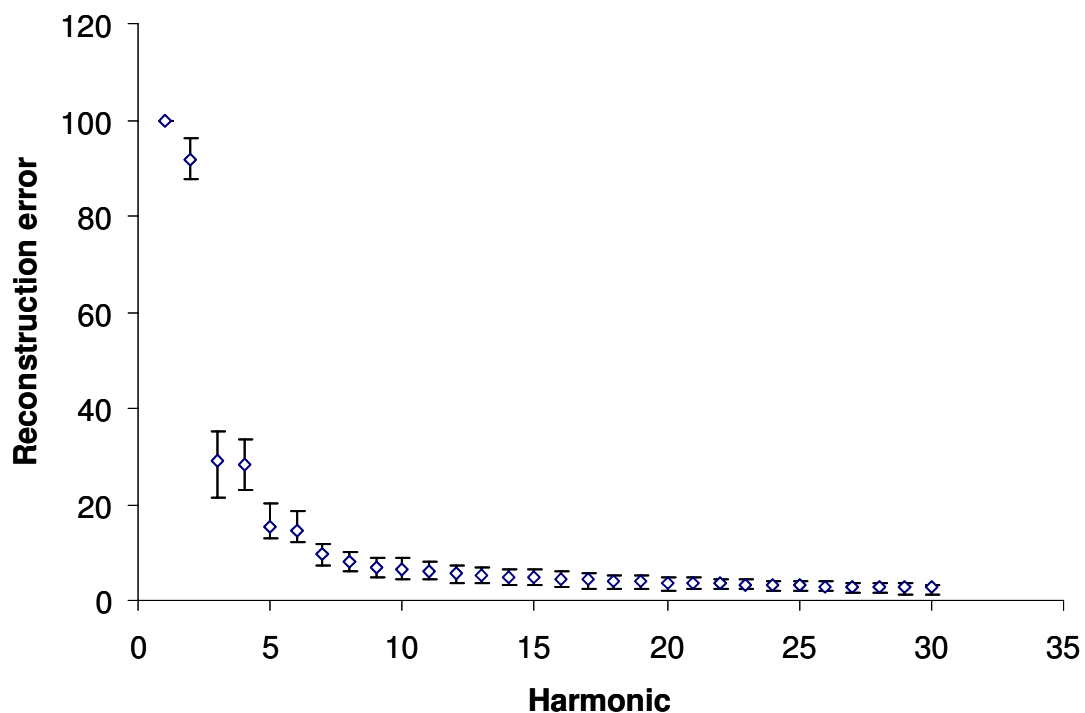


Figure 5. Percentage reconstruction error during range finding test. Error bars represent minimum and maximum reconstruction error at each harmonic.

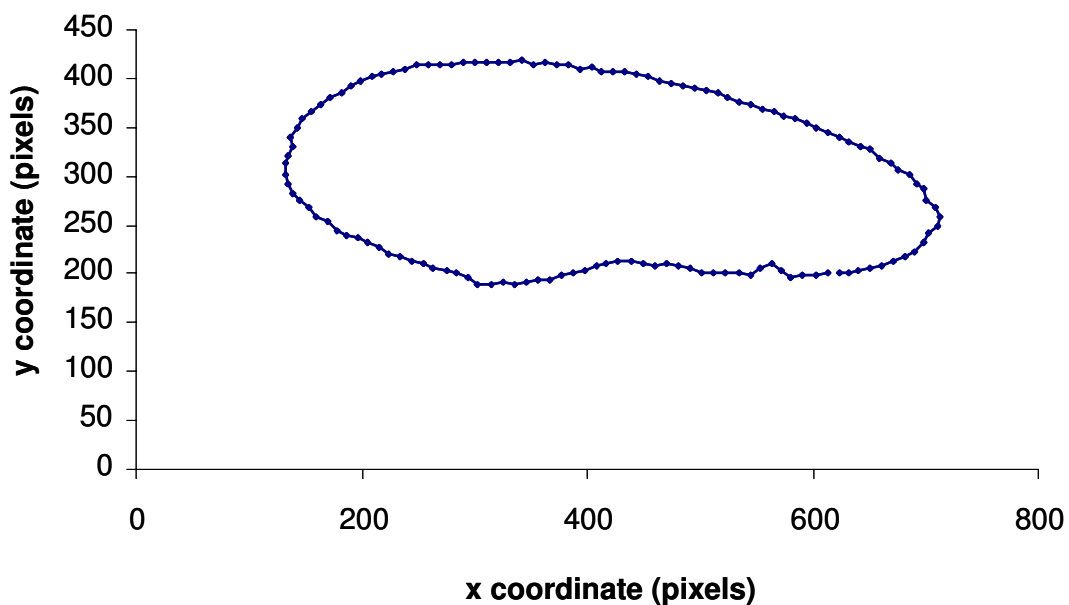


Figure 6. Sampled points from an age 13+ year old blue grenadier otolith.

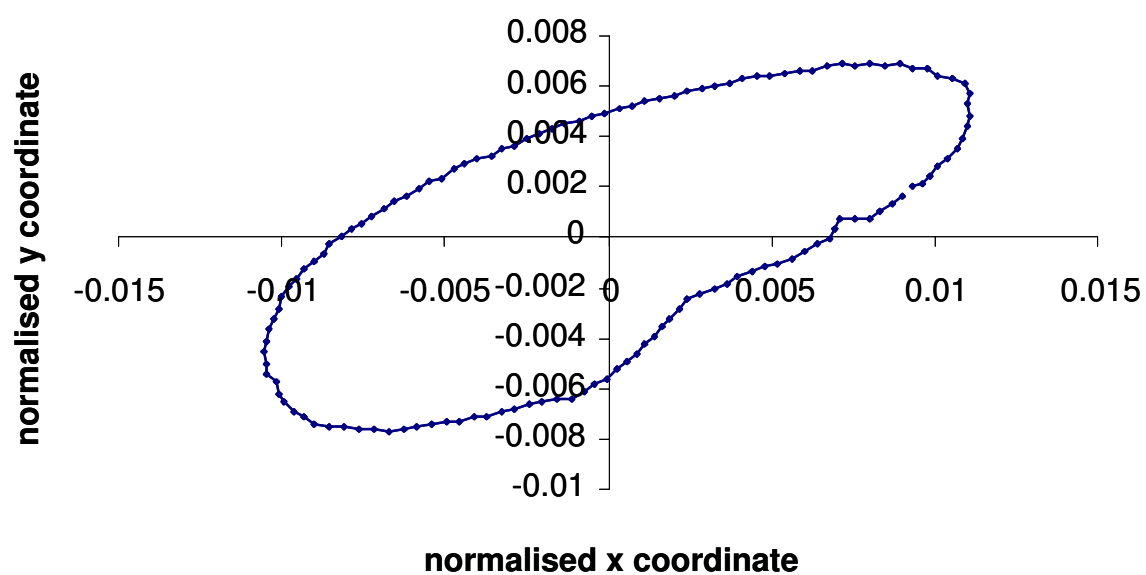


Figure 7. Sampled points from an age 13+ year old blue grenadier otolith after standardisation.

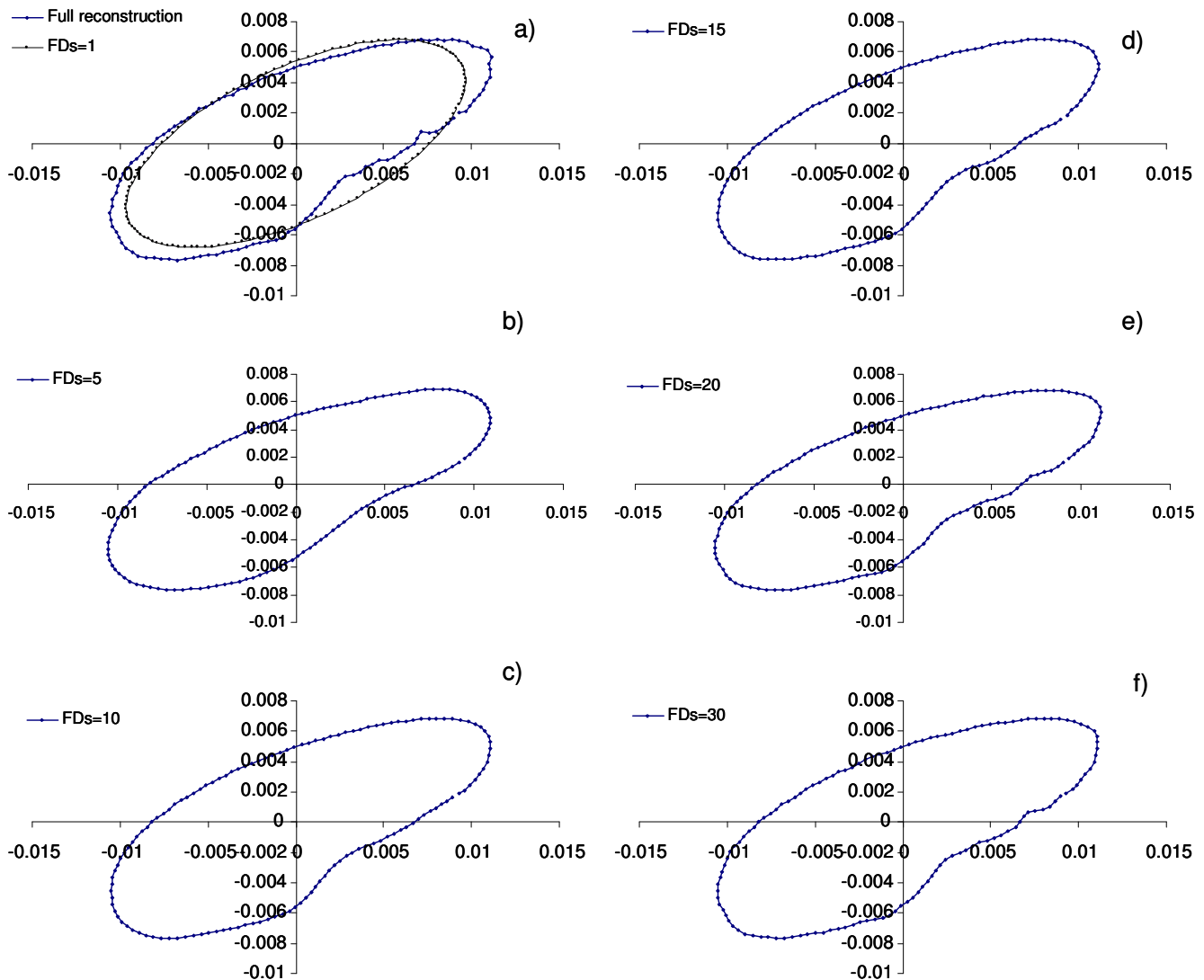


Figure 8. Reconstruction of otolith shape using a) first pair of complex numbers (overlay is the reconstruction from the full series), b) five pairs of complex numbers, c) 10 pairs of complex numbers, d) 15 pairs of complex numbers, e) 20 pairs of complex numbers, f) 30 pairs of complex numbers. A pair of complex numbers is the n^{th} complex number and the $128-n^{\text{th}}$ number. All reconstructions included the zeroth $(0+0i)$ Fourier descriptor.

Data analysis

To determine the probability of a one-stock membership between the two groups of otoliths, a randomisation test was used. To reduce biases caused by the weighting of different sample sizes, a balanced design was used for the randomisation test. All of the samples were used from the area that had the lowest sample numbers; samples were randomly selected without replacement from the comparison group until sample numbers were equal. The squared difference between the means for each of the harmonics was calculated. The observed harmonic distance (OHD) was calculated as the square root of the sum of the squared differences:

$$OHD_{jk} = \sqrt{\sum (\bar{H}_{ij} - \bar{H}_{ik})^2}$$

Where OHD_{jk} = observed harmonic distance between area j and area k , $\bar{H}_{ij}$ and $\bar{H}_{ik}$ = i th mean harmonic from area j , and i th mean harmonic from area k , respectively.

The matrix of Fourier descriptors for samples from the two areas being compared was thus reduced to one harmonic distance value. To calculate the randomised harmonic distance (RHD), the array of harmonics was randomly sorted and the same series of calculations performed. This process was repeated 5,000 times with each new RHD stored. The array was then sorted and a distribution of RHDs calculated. Bootstrapping with 5,000 randomised re-samplings (sample size equivalent to minimum number of samples available from smallest of the two comparison groups, re-sampling with replacement) of the data from each of the comparison groups was used to determine a distribution of the OHD between the two groups. The location of the mean OHD on the distribution of RHDs was used to determine the probability that the difference between sampling groups (i.e. fishery regions) was indicative of a significant difference in otolith shape between regions. A difference in otolith shape was considered significant if the mean OHD of the bootstrapped distribution was greater than the 95th percentile (right tail) of the distribution of the RHDs (i.e. $p < 0.05$).

Otolith chemistry

Similar to the otolith shape analyses, regional comparisons of otolith chemistry were restricted to the two dominant age groups/cohorts present in the samples. Otoliths for chemistry and stable isotopes were randomly selected from those available. A summary of the samples included in the otolith chemistry component is provided in Table 3.

Table 3. Summary of otolith samples analysed for elemental and stable isotope chemistry. Total number of samples analysed (n), sex ratio (F:M), number with sex unknown or immature (U), number of sampling events (Events), and the minimum (Lmin), maximum (Lmax), modal (Lmode) and average (Lavg) total lengths of blue grenadier collected in each of the CTS and GABTS sampling regions. SD = standard deviations. Region codes are as for Fig. 2. The majority of the samples were collected in June/July 2007, except for samples from the EGAB region that were collected in November 2007.

Region	Age (y)	n	F:M	U	Events	Lmin (cm)	Lmax (cm)	Lmode (cm)	Lavg $\pm$ SD (cm)
EBS	4+	27	0.5:1	15	6	65	79	74	73 $\pm$ 4
	13+	37	1.3:1	5	5	76	102	85	88 $\pm$ 7
WTAS	4+	27	0.9:1	0	14	67	97	73	74 $\pm$ 6
	13+	26	1:1	0	17	78	100	84	89 $\pm$ 7
EGAB	13+	17	1.1:1	0	1	79	103	89	92 $\pm$ 6
GAB	4+	26	1.7:1	10	2	53	87	62	67 $\pm$ 9
	13+	11	1.8:1	0	2	84	93	85	87 $\pm$ 3

Elemental chemistry

Two regions of the otoliths were focused on for elemental analysis; the core and the margin. The core region was analysed to assess whether individuals from the different sampling regions may have originated from different spawning/nursery areas (i.e. independent replenishment sources). Furthermore, if clear discrimination of otolith core chemistry among regions was detected for adult fish it would suggest that mixing of fish derived from different juvenile source areas was minimal, supporting the existence of stock structure. The margins were analysed to assess whether elemental chemistry of recently deposited otolith material varied among regions. The results of these analyses were compared to the patterns observed from the comparisons of the core chemistry, and also provided a test of whether otolith chemistry was sensitive to regional variation in the ocean environment. This is important for interpreting the regional variation or lack thereof observed for chemistry of the otolith cores. We made the assumption that the marginal otolith chemistry would be representative of the broad geographic regions where the samples were collected (i.e. group structure was assumed).

Preparation and analytical procedure

Otoliths were embedded in epoxy resin (AKA-Resin and AKA-Cure, Pacific Laboratory Supplies). Two transverse sections of 300 and 400 μ m thickness respectively were taken through the otolith primordial region using a slow speed diamond blade saw (Buehler) lubricated with Milli-Q water. The first section was used for trace element analyses and the second section was set aside for stable isotope analyses.

Otolith transverse sections were mounted onto microscope slides using epoxy resin and polished with lapping film (Ultralap 30 μ m) lubricated with Milli-Q water to remove cutting effects. Mounted otolith

sections were sonicated in Milli-Q water for three minutes to remove any surface contaminants, liberally rinsed in Milli-Q water, air dried, and stored in sealed polypropylene containers until subsequent analysis.

Trace element analyses were undertaken using a New Wave Research UP-213 Nd:YAG ultraviolet laser microprobe operated in Q-switched mode coupled to a Thermo Finnigan Element 2 high resolution inductively coupled plasma mass spectrometer (HR-ICP-MS) situated at Department of Primary Industries, Fisheries Research Branch, Queenscliff. Data were collected for the isotopes ^{25}Mg (magnesium), ^{55}Mn (manganese), ^{65}Cu (copper), ^{66}Zn (zinc), ^{88}Sr (strontium), ^{85}Rb (rubidium), ^{138}Ba (barium), and ^{208}Pb (lead), along with ^{43}Ca (calcium) which was used as the internal standard with a concentration in otolith of $388,000 \mu\text{g g}^{-1}$ (Yoshinaga *et al.* 2000). Calibration was achieved with the National Institute of Standards (NIST) 612 glass wafer using standard methods described in (Lahaye *et al.* 1997; Hamer *et al.* 2003). Resolved concentrations were expressed as ratios to Ca ($\mu\text{mol mol}^{-1}$). A laser spot diameter of $80 \mu\text{m}$ was used, repetition rate of 6 Hz and fluence of $\sim 10 \text{ J cm}^{-2}$. Replicate calibration standards were analysed after every sequence of 12 otolith ablations. Each sequence generally consisted of 4 ablations on one randomly selected otolith from each of the three regions. The 4 ablations on each transverse section consisted of two ablations adjacent to, but not overlapping, the proximal margin of the otolith section and two adjacent ablations over the otolith core region (Fig. 9). One otolith margin ablation was made adjacent to the ventral side of the sulcal groove, and the other toward the ventral tip of the transverse section (Fig. 9). The ablations over the core region would have approximated the first 1 - 3 months of life, and the margin region approximately the last 6 months and 1 year of life for the 4+ and 13+ year old fish respectively. Otolith surfaces were pre-ablated prior to actual data acquisition to remove any residual surface contamination. Each laser ablation spot sample consisted of 15 blank scans of the above isotopes, followed by an ablation period of 25 scans, of which the first 10 scans were excluded from data integration to allow for signal stabilisation. The sample data were blank subtracted before being integrated to provide the actual counts used for the calculation of element:calcium ratios. Detection limits were calculated for each sample based on three standard deviations of blank gas samples taken at the beginning and end of each analysis day and were adjusted for ablation yield of each sample (Lahaye *et al.* 1997). Detection limits, LOD are reported in Table 4. Precision and recovery were estimated for the NIST 612 standard and were: recovery, mean %/SD, Mg – 100/3, Mn – 100/3, Cu – 100/7, Zn – 100/10, Rb – 100/6, Sr – 100/4, Ba – 100/4, Pb – 99/6; precision % RSD, mean/SD from 9 separate analysis days, Mg – 6/3, Mn – 3/1, Cu – 7/4, Zn – 10/6, Rb – 6/3, Sr – 4/1, Ba – 4/1, Pb – 6/4.

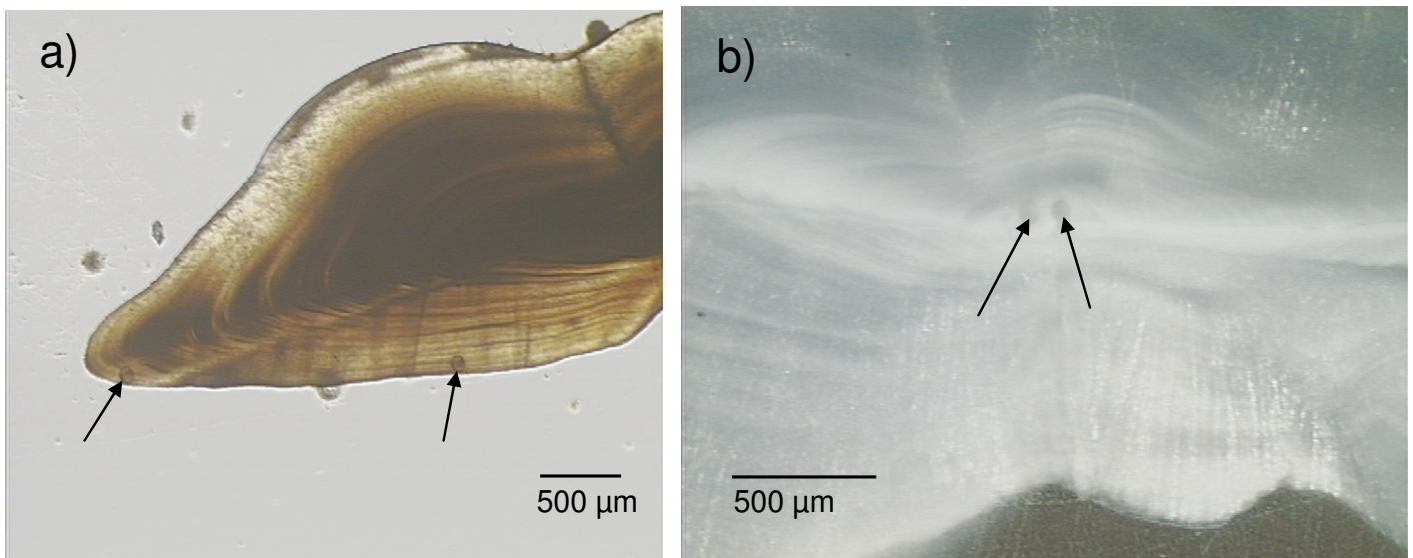


Figure 9. Images of, a) the ventral tip (transmitted light) and b) the core region (reflected light) of a transverse section of a sagittal otolith from a *Macruronus novaezelandiae*. Positions of laser ablation craters ($80\mu\text{m}$ diameter) are indicated by the arrows.

Table 4. Limits of detection (LOD) for each element in otoliths and percentages of otolith ablations that were above LOD and blank gas levels for core and margin ablations.

Element	LOD (mean $\pm$ SD) ($\mu\text{mol mol}^{-1}$ Ca)	% ablations >LOD cores	% ablations >LOD margins	% ablations >blank cores	% ablations >blank margins
Mg	9.31 ($\pm$ 2.29)	100	100	100	100
Mn	0.37 ($\pm$ 0.12)	100	21	100	98
Cu	0.33 ($\pm$ 0.14)	84	41	99	98
Zn	0.38 ($\pm$ 0.15)	73	25	99	96
Rb	0.11 ($\pm$ 0.03)	6	2	99	99
Sr	1.14 ($\pm$ 0.29)	100	100	100	100
Ba	0.06 ($\pm$ 0.02)	100	100	100	100
Pb	0.01 ($\pm$ 0.01)	38	6	100	99

Data analysis

Initially the raw resolved element:Ca ratios were examined for data that were determined as being below the instrument LOD and the gas blanks. Table 4 summarizes the extent to which data for individual elements fell below the LOD and gas blanks for the core and margin ablations.

Although not reported in detail here, there were clear differences in the levels of Mg, Mn, Cu, Zn, Ba (higher in core region) and Sr (higher at otolith margins) between the core and margins of otoliths (compare Figs. 13 & 15). These differences are reflected in the differences in the percentages of ablation samples that fell below LOD for the core and margin ablations, particularly for Mn, Cu and Zn. There is no strict protocol for dealing with data that fall below instrument LODs in comparative otolith chemistry studies. LODs are typically used to reject data that could potentially be the result of instrument noise. While below LOD data would typically not be reported in situations where high confidence in reported 'accuracy' levels is required (eg. environmental compliance, medical research etc.), below LOD data may still be useful in indicating variation among sample groups in comparative otolith chemistry studies (Ben-Tzvi *et al.* 2007). Although it should be recognised that the closer the determined levels are to the LOD the more the unexplained variation (noise) in the data, and therefore the more difficult it will be to detect statistically significant differences among groups. Where data consistently fall below blank levels it is assumed that any positive signals are most likely due to instrument noise or sporadic contamination. Data not consistently above blank levels were excluded from further analyses. To maximise the amount of information extracted from the elemental data for the statistical analysis of otolith chemistry (Ben-Tzvi *et al.* 2007) we chose to retain all elements except for Rb and Pb. We considered that significant differences among regions, irrespective of some of the data falling below the LOD, were meaningful.

The data for the individual ablations were averaged across the two core region ablations (cores) and the two margin region ablations (margins) to provide the core and margin elemental:Ca ratios used for all statistical analyses. Prior to formal statistical analyses, box plots of raw and log transformed data were examined to identify extreme outliers, defined as data that were >3 interquartile ranges either above the 75th percentile or below the 25th percentile (Wilkinson *et al.* 1996). Extreme outliers are likely to be inaccurate data and were detected for Mg (8 samples), Mn (2 samples), Cu (2 samples), and Zn (10 samples). These data were disregarded from all further statistical analyses and graphs.

For the core and margin otolith regions, we conducted the same series of analyses. Firstly we conducted univariate analysis of variance (ANOVA) to assess whether individual element:Ca ratios differed significantly among regions and cohorts, and also whether there were any interactions between regions and cohorts. Regions and cohorts were treated as fixed factors and fish as replicates. Tukey's HSD *post hoc* tests

were used to determine the sources of any significant interactions and the nature of significant differences among regions for each cohort.

Secondly, for each cohort we conducted multivariate analysis of variance (MANOVA) to determine whether multivariate elemental:Ca ratios differed significantly among regions, followed by pairwise comparisons to determine which regions differed from each other. Thirdly, we conducted quadratic discriminate function analysis (QDFA) with a cross validation leave-one-out jackknife classification procedure (i.e. the observation being classified is removed from the baseline data set and treated as an unknown), to assess how accurately individual fish could be assigned to their collection region based on the elemental composition of their otoliths. For MANOVA and QDFA we only included elements for which the univariate ANOVA indicated a significant region main effect ($p < 0.05$). For all ANOVA, MANOVA and QDFA; Mg, Mn, Cu, Zn and Ba data were $\ln(x+1)$ transformed. QDFA was chosen over linear discriminant function analysis due to some inequality of covariance matrices indicated from qualitative comparisons of within-group scatterplot matrices among the three groups. QDFA does not require homogeneity of within-group covariance matrices and is therefore less sensitive to any deviations from multivariate normality (Quinn and Keough 2002). The Pillai trace ($p < 0.05$) statistic was used to test for significance of MANOVA as it is the most robust to any deviations from multivariate normality (Quinn and Keough 2002). F-to-remove statistics, which provide a measure of the contribution that individual variables make to discrimination, were used to assess which elements contributed most to discrimination, and therefore to classification accuracy (Wilkinson *et al.* 1996). Canonical discriminant function plots, with 95% confidence ellipses around the data for each group were used to display variation in the multi-elemental otolith chemistry within and among regions. Separate QDFA and classification analyses were conducted for each cohort and the core and margin ablation zones.

Comparison of EGAB and GAB

For the 13+/1993 cohort we obtained samples from two regions in the GABTS; an eastern region (EGAB) and the same central GAB region as for the 4+/2002 cohort (Fig. 2). To assess how these sub-regions within the Great Australian Bight compared with each other and the other regions sampled in CTS, we also conducted univariate ANOVA, and Tukey's HSD *post hoc* test with samples from the eastern and central Great Australian Bight collection areas separated. If there were no significant differences between these two sub-regions within the Great Australian Bight, it would justify pooling the samples from the EGAB with the those collected from the main collection region in the central Great Australian Bight.

NB: For all multivariate analyses we have referred to the Great Australian Bight fishery as GAB, although in the 13+/1993 cohort, samples were obtained from two regions (central and eastern) within the Great Australian Bight fishery (Fig. 2).

Stable isotopes

Preparation and analytical procedure

To remove the mounting resin from the embedded otolith sections, the sections were heated to approximately 60 °C for a short period to soften the surrounding resin. The resin was then teased away from the otolith sections under a dissecting microscope while still warm. The otolith sections were then placed in plastic vials with Milli-Q water and sonicated for 5 mins, followed by three liberal rinses in Milli-Q water. The sections were dried overnight at 40 °C and were then ground to a fine powder using an agate mortar and pestle, the resulting powder stored in plastic vials and weighed. Protein was not removed from otolith powder for stable isotope analysis (Gao and Beamish 1999).

Stable isotope analyses were conducted by Iso-Analytical (UK). For analysis, sample powder (ca. 2 mg) was placed in clean glass septum capped vials. The vials were then placed in a drying oven for 24 hours prior to the caps being fitted to ensure no moisture was present. The vials then had their headspaces flushed with pure helium (99.995%). After flushing, ~0.5 ml of pure phosphoric acid was injected into the vials and mixed with the sample powder. The samples were left to react with the acid for 24 hours at ambient temperatures then heated for 40 minutes to 80° C to ensure complete conversion to carbon dioxide. Phosphoric acid suitable for isotopic analysis of carbonate samples was prepared according to the procedure published by Coplen *et al.* (1983).

The CO₂ gas was then analysed by continuous flow, isotope ratio mass spectrometry (IRMS). In brief, the CO₂ is flushed from the septum vial using a double holed needle and resolved on a packed column gas chromatograph. The carbon dioxide then enters the ion source of a Europa Scientific 20-20 IRMS and is ionised and accelerated. Here, gas species of different mass are separated in a magnetic field and then simultaneously measured using a Faraday cup collector array at m/z 44, 45, and 46.

The reference material used for this analysis was calcium carbonate standard IA-R022 ($\delta^{13}\text{C}_{\text{V-PDB}}$ -28.63 ‰ and $\delta^{18}\text{O}_{\text{V-PDB}}$ -22.69 ‰), which is traceable to NBS-19 (Limestone, $\delta^{13}\text{C}_{\text{V-PDB}}$ +1.95 ‰ and $\delta^{18}\text{O}_{\text{V-PDB}}$ -2.2 ‰). During analysis, NBS-19, IA-R022 and NBS-18 ($\delta^{13}\text{C}_{\text{V-PDB}}$ -5.01 ‰ and $\delta^{18}\text{O}_{\text{V-PDB}}$ -23.20 ‰) were analysed as check samples for assessment of accuracy (see Table 5). The International Atomic Energy Agency, Vienna, distributes NBS-18 and NBS-19 as international reference standards. Finally, 31 randomly selected samples were analysed in duplicate to assess precision of the methods (Table 5).

Data analysis

Statistical comparisons of stable isotope ratios among regions followed the same format as described above for trace elements. Based on the known influence of temperature on oxygen isotopic fraction during otolith deposition (lower temperature = higher $\delta^{18}\text{O}$) (Weidman and Millner 2000; Hoie *et al.* 2004), and the latitudinal and estimated temperature variation among the sampling regions, we expected that samples from WTAS should exhibit the highest $\delta^{18}\text{O}$, followed by EBS, and that GAB samples should have the lowest $\delta^{18}\text{O}$. Because of the likely multiple influences of diet, temperature and physiology on $\delta^{13}\text{C}$ in otoliths (Weidman and Millner, 2000) we did not make any a-priori predictions of regional variation in $\delta^{13}\text{C}$.

Based on the $\delta^{18}\text{O}$ of the otoliths and a constant salinity of 35 across the study region we estimated the average water temperature experienced by individuals from the relationship published by Weidman and Millner (2000) for North Atlantic cod. Similar relationships have been reported for other species (Thorrold *et al.* 1997).

$$\delta^{18}\text{O}_{\text{Otolith (PDB)}} = -0.22 + 4.39 + \delta^{18}\text{O}_{\text{water (SMOW)}} \text{ (Weidman and Millner 2000)}$$

The PDB and SMOW subscripts indicate the international standard measurement scales for $\delta^{18}\text{O}$ for carbonate and water respectively. The $\delta^{18}\text{O}_{\text{water (SMOW)}}$ was estimated based on a salinity (S) of 35 and the empirical relationship for the conversion of salinity to $\delta^{18}\text{O}_{\text{water (SMOW)}}$ developed by Ganssenn and reported in Witbaard *et al.* (1994).

$$\delta^{18}\text{O}_{\text{water (SMOW)}} = -14.555 + 0.417 \text{ S}$$

Temperature estimates derived from $\delta^{18}\text{O}$ in otoliths were compared with actual water temperature data obtained for bottom waters at various depths in each of the three fishing regions (sourced from the CSIRO; CARS - Climatology of Australasian Regional Seas program).

Table 5. Summary of quality control data for stable isotope analyses, a) standard reference materials, b) replicate samples of blue grenadier otolith powder.

a)

IA-R022 calcium carbonate (n=20)		NBS-18 calcite (n=8)		NBS-19 calcium carbonate (n=6)	
$\delta^{13}\text{C}_{\text{V-PBD}} (\text{‰})$	$\delta^{18}\text{O}_{\text{V-PBD}} (\text{‰})$	$\delta^{13}\text{C}_{\text{V-PBD}} (\text{‰})$	$\delta^{18}\text{O}_{\text{V-PBD}} (\text{‰})$	$\delta^{13}\text{C}_{\text{V-PBD}} (\text{‰})$	$\delta^{18}\text{O}_{\text{V-PBD}} (\text{‰})$
True value = -28.63	True value = -22.69	True value = -5.01	True value = -23.02	True value = 1.95	True value = -2.23
Determined value: mean $\pm$ SD, (range)	Determined value: mean $\pm$ SD, (range)	Determined value: mean $\pm$ SD, (range)	Determined value: mean $\pm$ SD, (range)	Determined value: mean $\pm$ SD, (range)	Determined value: mean $\pm$ SD, (range)
-28.63 $\pm$ 0.06 (-28.74 : -28.55)	-22.69 $\pm$ 0.11 (-22.90 : -22.52)	-5.08 $\pm$ 0.05 (-5.17 : -4.99)	-23.02 $\pm$ 0.05 (-23.10 : -22.94)	1.89 $\pm$ 0.03 (1.85 : 1.94)	-2.23 $\pm$ 0.19 (-2.46 : -1.96)

b)

$\delta^{13}\text{C}_{\text{V-PBD}} (\text{‰})$ (n=31)	$\delta^{18}\text{O}_{\text{V-PBD}} (\text{‰})$ (n=31)
Replicate 2 as % of replicate 1 : mean $\pm$ SD, (range)	Replicate 2 as % of replicate 1: mean $\pm$ SD, (range)
99.31 $\pm$ 4.31 (88.32 : 108.32)	99.24 $\pm$ 5.30 (87.15 : 112.27)

Results

Age composition

The age composition of blue grenadier sampled from southern Australian waters in 2007 was clearly dominated by two age groups; 13+ and 4+ years of age (Fig. 10). Fish from these two dominant age groups would have originated from spawning in the winters of 1993 and 2002 respectively (i.e. 1993 and 2002 cohorts).

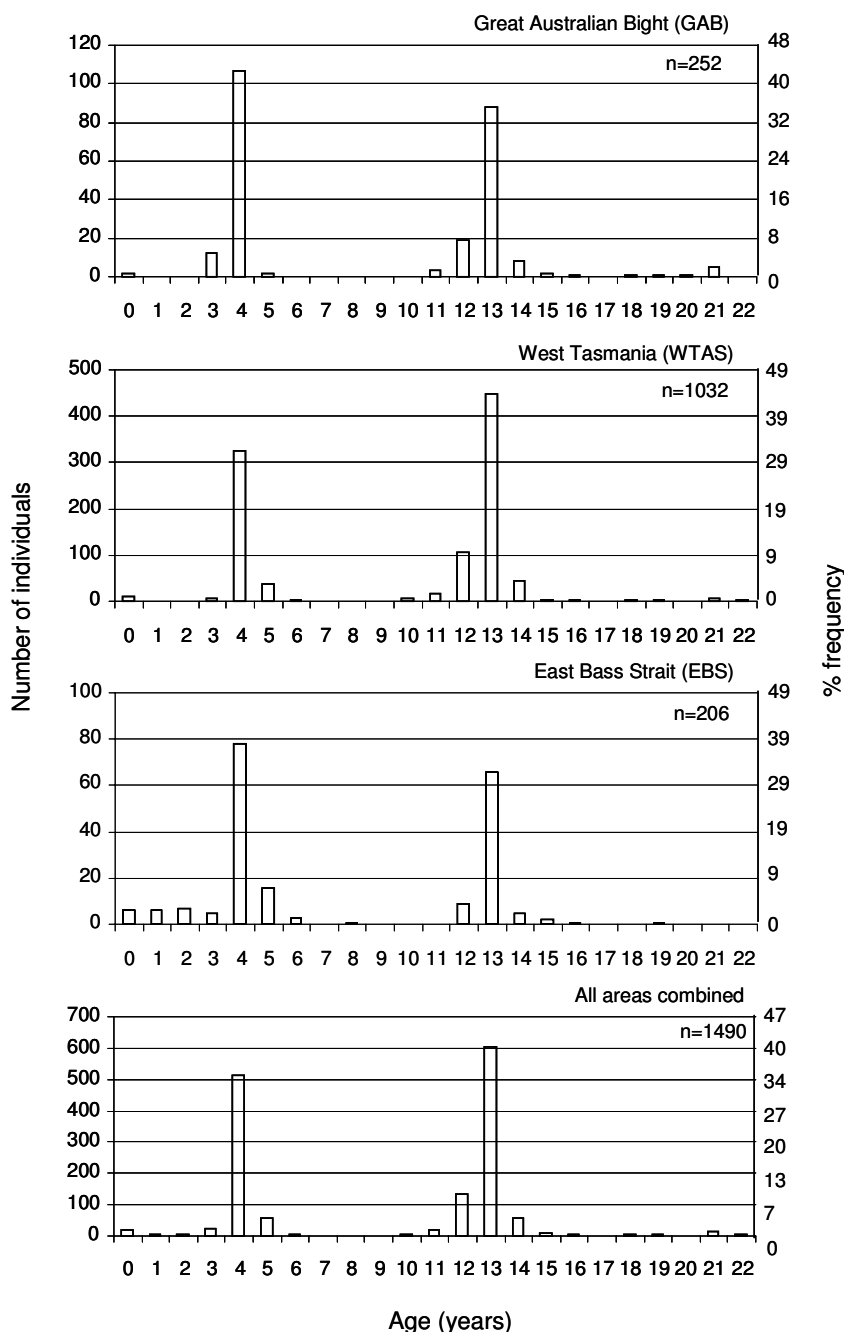


Figure 10. Age compositions of blue grenadier collected in 2007 from trawl catches in three regions of southern Australia (refer to Fig. 1, Table 1).

The oldest fish present in the samples were 22 years of age collected off west Tasmania, and 21 years of age collected in the Great Australian Bight, possibly indicative of an earlier strong cohort spawned in 1985 (Fig. 10).

Otolith shape analysis

Age 4+/2002 cohort

Randomisation tests of pairwise comparisons of otolith shape among the three sampling regions indicated significant differences between the GAB samples and those from the WTAS region; however, the comparisons of WTAS with EBS, and EBS with the GAB, were not significant (Table 6). The distributions of the OHDs derived from bootstrapping indicated complete overlap with the randomised distributions for both the EBS v GAB and EBS v WTAS comparisons (Fig. 11). For the WTAS v GAB comparison the majority of the bootstrap OHDs were greater than the 95th percentile of the randomised distribution, indicating a highly significant difference in otolith shape between these regions (Fig. 11).

Age 13+/1993 cohort

Due to the low sample sizes available for the 13+/1993 cohort from the EBS region, statistical comparisons of otolith shape were only possible between the GAB and WTAS regions for this cohort. In contrast to the younger cohort, the comparison between the GAB and WTAS regions was not significant (Table 6). Although the mean OHD was less than the 95th percentile, the distribution of the bootstrapped OHDs was situated to the right of the randomised distribution indicating some variation between the regions above that expected based on random sampling (Fig. 11).

Table 6. Pairwise results matrix for comparisons of otolith shape among three fishery regions for; a) age 4+/2002 cohort, and b) age 13+/1993 cohort. GAB = Great Australian Bight, WTAS = western Tasmania, EBS = eastern Bass Strait. NS = non-significant difference: $p > 0.05$, S = significant difference: $p < 0.05$, NA = not analysed due to insufficient sample size for EBS.

a) age 4+/2002 cohort		
	WTAS	EBS
GAB	S	NS
WTAS		NS
b) age 13+/1993 cohort		
	WTAS	EBS
GAB	NS	NA
WTAS		NA

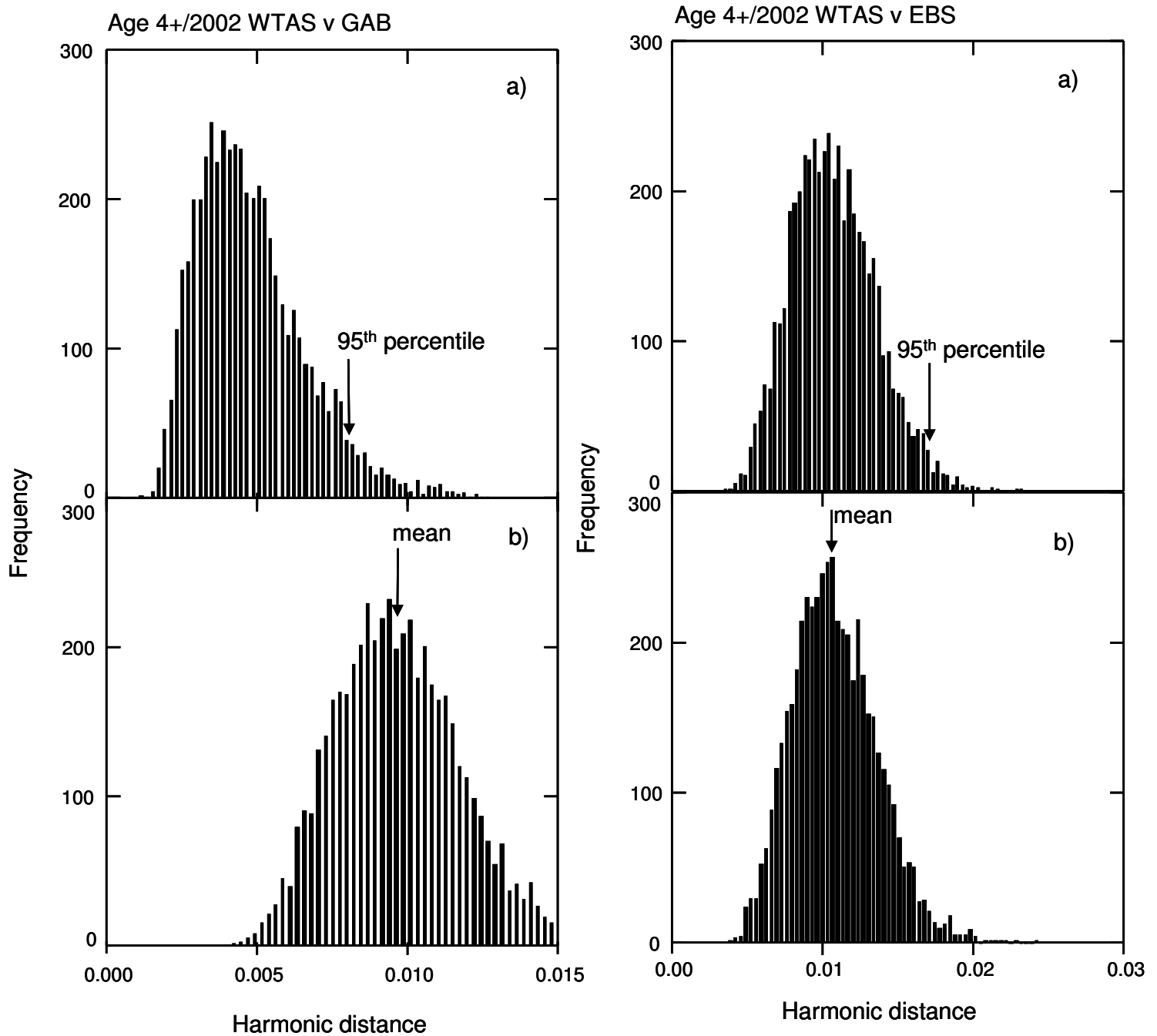


Figure 11. Comparisons of distributions of randomised harmonic distances (a, b), with the distributions of observed harmonic distances (OHDs) among regions obtained from bootstrapping (b, d).

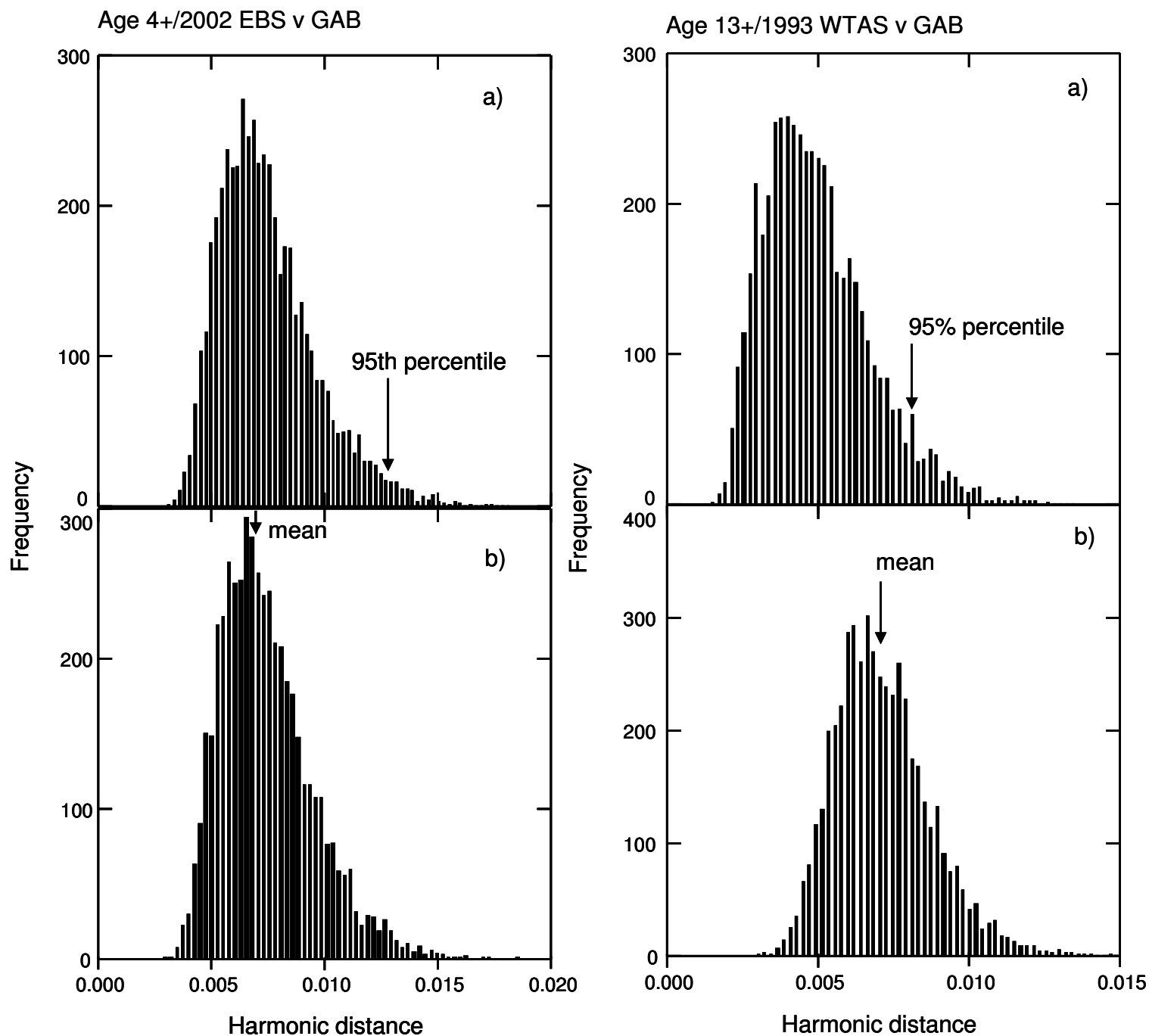


Figure 11 continued. Comparisons of distributions of randomised harmonic distances (a, b), with the - distributions of observed harmonic distances (OHDs) obtained from bootstrapping (b, d). -

Otolith elemental chemistry

Individual elements - cores

Magnesium: Univariate ANOVA indicated significant differences in Mg:Ca of otolith cores among the three sampling regions ($p < 0.05$), but also significant differences between age groups/cohorts ($p < 0.001$), and a significant interaction between regions and age groups/cohorts ($p < 0.05$) (Fig. 12, Table 7). There was a trend for higher Mg:Ca ratios in the cores of the 4+/2002 cohort, however the difference between cohorts appeared to be greater for the WTAS and ETAS samples than the GAB samples (Fig. 12). The significant interaction between cohort and region was due to the Mg:Ca ratios of otolith cores of the 4+/2002 cohort being higher for EBS than GAB samples (Tukey's, $p < 0.05$), whereas for the 13+/1993 cohort, Mg:Ca ratios were significantly higher for EBS than WTAS samples (Tukey's, $p < 0.02$) (Fig. 12, Table 7). Analysis of the 13+ cohort with GAB and EGAB samples treated separately indicated that the EGAB samples had significantly higher Mg:Ca than the WTAS samples (Tukey's, $p < 0.05$), but were not significantly different to the GAB samples (Tukey's $p > 0.05$) (Table 7).

Manganese: Univariate ANOVA did not indicate any significant differences in Mn:Ca ratios of otolith cores among regions or between age groups/cohorts ($p > 0.05$ all tests) (Fig. 12, Table 7).

Copper: Univariate ANOVA indicated highly significant differences in Cu:Ca of otolith cores among the three sampling regions ($p < 0.001$) (Fig. 12, Table 7). For both the 4+/2002 and 13+/1993 cohorts Cu:Ca in otolith cores was significantly higher in samples from WTAS than both EBS (Tukey's, age 4+ $p < 0.001$, age 13+ $p < 0.05$) and the GAB (Tukey's, age 4+ $p < 0.001$, age 13+ $p < 0.05$). There was also a significant interaction between cohorts and regions ($p < 0.05$) which was due to Cu:Ca in otolith cores from WTAS being higher in the 4+/2002 cohort than the 13+/1993 cohort (Fig. 12), but negligible variation between the cohorts for the GAB and EBS regions (Fig. 12). Analysis of the 13+/1993 cohort with the EGAB and GAB samples treated separately indicated that the WTAS samples had significantly higher Cu:Ca than the GAB samples (Tukey's, $p < 0.05$), but were not significantly different to the EGAB samples (Tukey's, $p > 0.05$), and there was no significant difference between the EGAB and GAB regions (Table 7).

Zinc: Univariate ANOVA did not indicate any significant differences in Zn:Ca ratios of otolith cores among regions or between cohorts/ages ($p > 0.05$, all tests) (Fig. 12, Table 7).

Strontium: Univariate ANOVA did not indicate any significant differences in Sr:Ca ratios of otolith cores among regions ($p > 0.05$, all tests) (Fig. 12, Table 7). However, there was a highly significant difference between cohorts ($p < 0.001$) with the 13+/1993 cohort exhibiting higher Sr:Ca in otolith cores. There was also a significant interaction between cohort and region ($p < 0.05$) which appeared to be due to Sr:Ca variation being greater between cohorts for the GAB and EBS regions than the WTAS region (Fig. 12).

Barium: Univariate ANOVA indicated significant ($p < 0.01$) differences among sampling regions for Ba:Ca in otolith cores (Fig. 12, Table 7). There was also a significant difference between cohorts ($p < 0.05$) with the 13+/1993 cohort displaying slightly higher Ba:Ca in otolith cores than the 4+/2002 cohort (Fig. 12). The interaction between cohorts and region, was not significant ($p > 0.05$) (Fig. 12, Table 7), however, *post hoc* Tukey's tests indicated that while for both cohorts there was a trend for slightly higher Ba:Ca in otolith cores from WTAS compared to the GAB region (Fig. 12) the difference was only significant for the 4+/2002 cohort (Tukey's, $p < 0.05$) (Table 7). Separate analysis of the 13+/1993 cohort with the EGAB and GAB samples treated separately indicated no significant differences among these regions (Table 7).

Table 7. Results of univariate ANOVA and *post-hoc* Tukey's tests comparing individual element:Ca ratios in otolith cores of blue grenadier collected in winter/spring 2007 from three regions; the Great Australian Bight (GAB), western Tasmania (WTAS) and eastern Bass Strait (EBS), and two cohorts; age 4+ years (2002 cohort) and age 13+ years (1993 cohort). All element:Ca ratios $\ln(x+1)$ transformed except for Sr:Ca, * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$. Results of *post-hoc* Tukey's tests for the age 13+/1993 cohort include separation of the GAB and EGAB samples.

Source	df	MS	p-value	<i>Post-hoc</i> Tukey's differences by regions
Magnesium:Calcium				
Region	2	0.428	0.022*	4+ - EBS>GAB*
Cohort	1	3.437	<0.001***	13+ EBS>WTAS*, EGAB>WTAS*
Region x Cohort	2	0.414	0.025*	
Error	161	0.110		
Manganese:Calcium				
Region	2	0.007	0.891	4+ - no significant differences
Cohort	1	0.012	0.656	13+ - no significant differences
Region x Cohort	2	0.040	0.525	
Error	164	0.062		
Copper:Calcium				
Region	2	5.369	<0.001***	4+ - WTAS>ETAS***, WTAS>GAB***
Cohort	1	0.001	0.951	13+ - WTAS>ETAS*, WTAS>GAB*
Region x Cohort	2	1.041	0.013	WTAS>GAB*
Error	165	0.234		
Zinc:Calcium				
Region	2	0.096	0.101	4+ - no significant differences
Cohort	1	0.022	0.470	13+ - no significant differences
Region x Cohort	2	0.011	0.775	
Error	161	0.041		
Strontium:Calcium				
Region	2	66,413	0.636	4+ - no significant differences
Cohort	1	4,143,737	<0.001***	13+ - no significant differences
Region x Cohort	2	467,080	0.043*	
Error	165	146,143		
Barium:Calcium				
Region	2	0.486	0.005**	4+ - WTAS>GAB*
Cohort	1	0.473	0.022*	13+ - no significant differences
Region x Cohort	2	0.079	0.414	
Error	165	0.089		

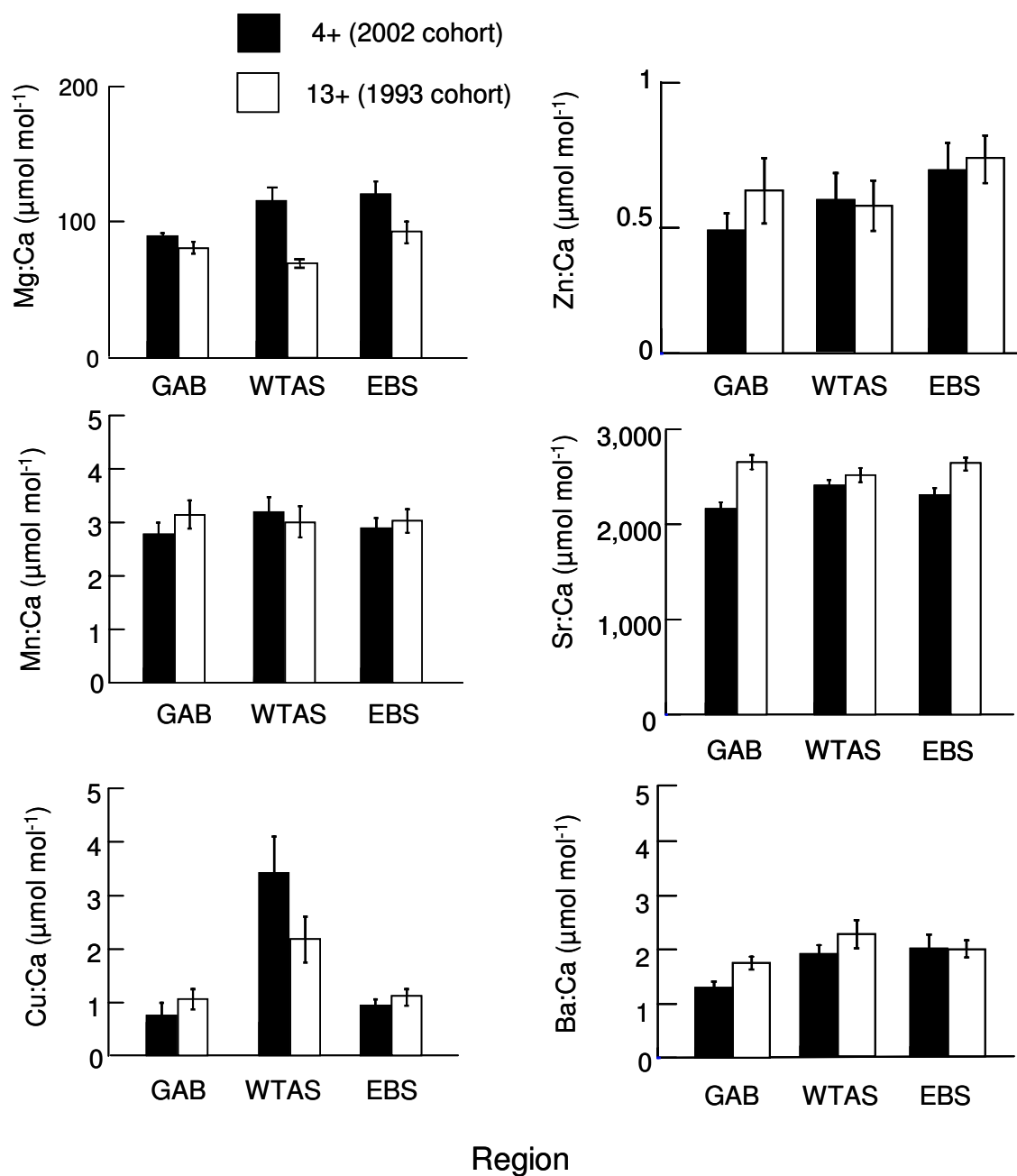


Figure 12. Comparisons of individual element:Ca ratios (mean $\pm$ SE) in otolith cores of blue grenadier collected in winter/spring 2007 from three regions; the Great Australian Bight (GAB), western Tasmania (WTAS) and eastern Bass Strait (EBS), and two cohorts; age 4+ years (2002 cohort) and age 13+ years (1993 cohort).

Multi-elemental chemistry - cores

4+/2002 cohort

Sr:Ca, Mn:Ca and Zn:Ca were excluded from the MANOVA, QDFA and classification analyses of otolith core chemistry. MANOVA of Mg:Ca, Cu:Ca and Ba:Ca was highly significant (Pillai Trace, $df_{6, 150}$, $p < 0.001$). The pairwise comparisons indicated that all regions differed significantly from the each other (Hotelling's T-Square, WTAS v EBS, WTAS v GAB - $p < 0.001$, GAB v EBS - $p < 0.05$).

The canonical variate plot (Fig. 13a) indicated that there was significant overlap among individual samples from all three regions, however, the samples from the WTAS and EBS regions were more variable than the GAB samples, and a large proportion of samples from the GAB and EBS regions were separated from the WTAS region along canonical variate 1. This separation was due to the tendency for higher Mg:Ca and lower Cu:Ca and Ba:Ca ratios in the cores of otoliths from the GAB and EBS regions (Fig. 12). F-to-remove values indicated that the order of importance for discrimination was Cu:Ca > Mg:Ca > Ba:Ca.

The jackknifed classification procedure with all regions included was moderately accurate in assigning individuals to their collection region based on the Cu:Ca, Mg:Ca and Ba:Ca ratios of their otolith cores (Table 8a). Classification accuracy was highest for the GAB samples at 67%, followed by EBS at 63% and then WTAS at 44% (Table. 8a). Classification errors for GAB samples were mostly due to samples being misclassified as EBS, and for EBS, classification errors were almost entirely due to samples being misclassified to GAB (Table 8a). For the WTAS region classification errors were evenly divided between the EBS and GAB regions (Table 8a). It was likely that overlap between the GAB and EBS regions was due to similarity in environmental conditions rather than mixing among regions (see discussion) and that misclassification among these regions was confusing interpretation of classifications between these two regions and the important spawning area of WTAS. The classification procedure was therefore repeated with EBS and GAB samples compared separately to WTAS (Table 8b). These additional analyses achieved higher accuracy for both the EBS and GAB regions, and a significant improvement for the WTAS region (Table 8b).

Table 8. 4+/2002 cohort cores - Results of jackknife classification of individuals based on $\ln(x+1)$ transformed Mg:Ca, Cu:Ca and Ba:Ca in otolith cores. Data are percentages of the samples from each collection region (indicated in left column) classified to each region. a) all regions included, b) GAB and EBS compared separately with WTAS. Order of importance of element:Ca ratios (F-to-remove) to discrimination is indicated in table footnotes.

a)

Region of collection	Region classified to (% of sample)		
	EBS	GAB	WTAS
EBS (n=27)	63	33	4
GAB (n=27)	22	67	11
WTAS (n=25)	28	28	44

Cu:Ca>Mg:Ca>Ba:Ca

b)

Region of collection	Region classified to (% of sample)		Region of collection	Region classified to (% of sample)	
	GAB	WTAS		EBS	WTAS
GAB (n=27)	78	22	EBS (n=27)	89	11
WTAS (n=25)	36	64	WTAS (n=25)	36	64

Cu:Ca>Ba:Ca>Mg:Ca

Cu:Ca>Mg:Ca>Ba:Ca

13+/1993 cohort

MANOVA of Mg:Ca, Cu:Ca and Ba:Ca was highly significant (Pillai Trace, $df_{6, 170}$, $p < 0.01$). Similar to the 4+/2002 cohort, the pairwise comparisons indicated that both the EBS and GAB regions differed significantly from the WTAS region (Hotelling's T-Square, $p < 0.01$), however for the 13+/2002 cohort, these two regions were not significantly different from each other (Hotelling's T-Square, $p > 0.05$). The canonical variate plot again indicated significant overlap among samples from the three regions (Fig. 13b). Similar to the 4+/2002 cohort, the samples from the WTAS and EBS regions were more variable than the GAB region, even though for this cohort the GAB samples were collected from two areas within the Great Australian Bight fishery (Fig. 13b). Similar to the 2002 cohort there was also greater overlap between EBS and GAB region samples than between these two groups and the WTAS samples (Fig. 13b). F-to-remove values indicated that the order of importance for discrimination was Mg:Ca > Cu:Ca > Ba:Ca.

The jackknifed classification procedure involving all regions was moderately accurate in assigning individuals collected from the GAB and WTAS region based on the Mg:Ca, Cu:Ca and Ba:Ca ratios of their otolith cores, but performed poorly for the EBS region (Table 9a). Classification accuracy was again highest for the GAB samples at 61%, followed by WTAS at 50% and then EBS at only 20% (Table. 9a). Classification errors for the EBS samples were mostly due to their misclassification to the GAB region (Table 9a). However, unlike the 4+/2002 cohort, classification errors for GAB samples were more evenly distributed between the EBS and WTAS regions (Table 9a). For the WTAS region, classification errors were mostly due to misclassification to the GAB region (Table 9a). The classification analyses repeated with EBS and GAB compared separately to WTAS achieved higher accuracy for both the EBS and GAB regions, but only a marginal improvement for the WTAS region (Table 9b).

Table 9. 13+/1993 cohort cores - Results of jackknife classification of individuals based on $\ln(x+1)$ Mg:Ca, Cu:Ca and Ba:Ca in otolith cores. Data are percentages of the samples from each collection region (indicated in left column) classified to each region. a) all regions included, b) GAB and EBS compared separately with WTAS. Order of importance of element:Ca ratios (F-to-remove) to discrimination indicated in table footnotes.

a)

Region of collection	Region classified to (% of sample)		
	EBS	GAB	WTAS
EBS (n=35)	20	57	23
GAB (n=28)	21	61	18
WTAS (n=26)	15	35	50

Mg:Ca>Cu:Ca>Ba:Ca

b)

Region of collection	Region classified to (% of sample)		Region of collection	Region classified to (% of sample)	
	GAB	WTAS		EBS	WTAS
GAB (n=28)	79	21	EBS (n=35)	63	37
WTAS (n=26)	35	65	WTAS (n=26)	46	54

Cu:Ca>Mg:Ca>Ba:Ca

Mg:Ca=Cu:Ca>Ba:Ca

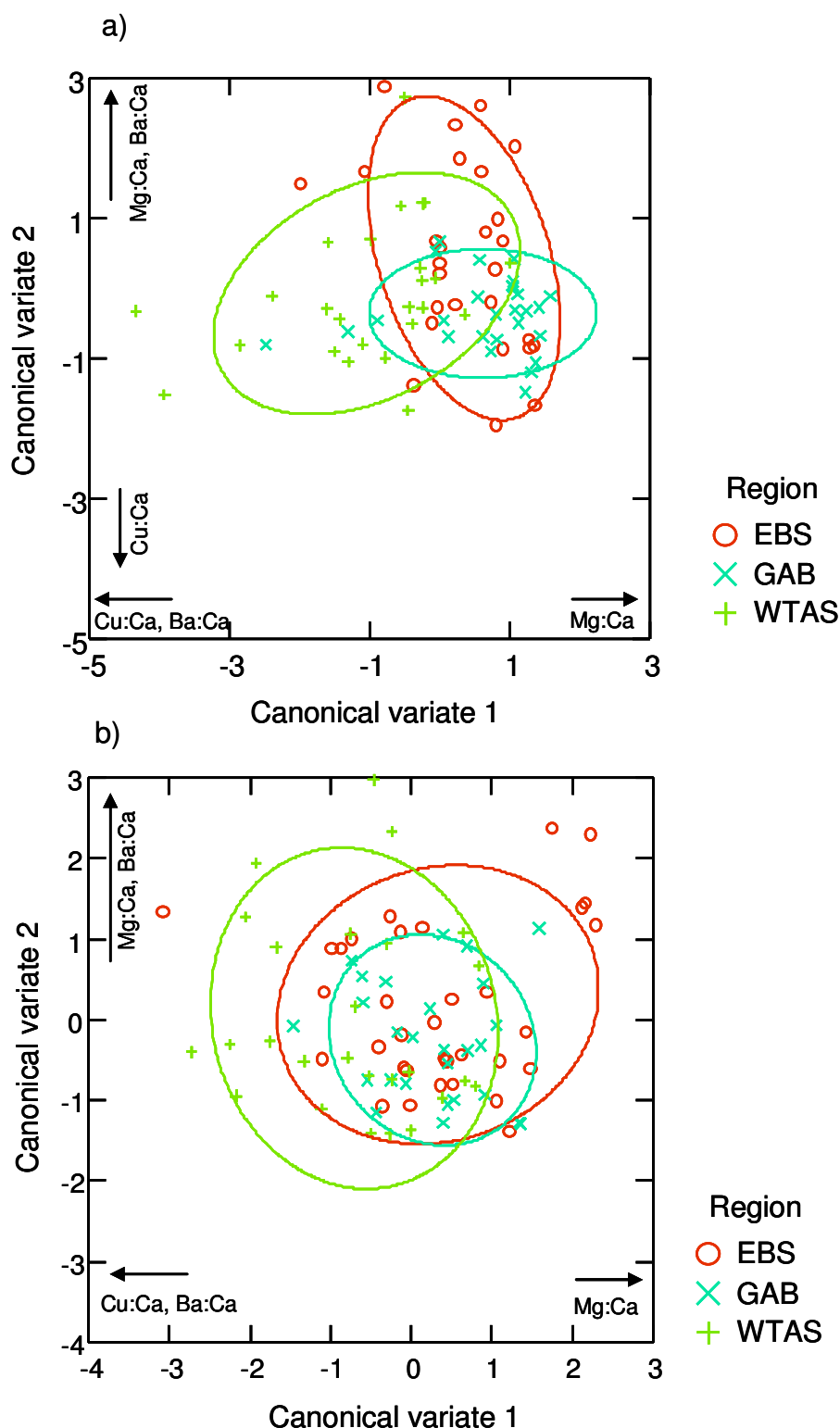


Figure 13. Canonical variate plots comparing multi-element chemistry (Mg:Ca, Cu:Ca, Ba:Ca) of otolith cores of blue grenadier among three fishery regions; the Great Australian Bight (GAB), western Tasmania (WTAS) and eastern Bass Strait (EBS), and for two cohorts; a) age 4+ years (2002 cohort), b) age 13+ years (1993 cohort). Data are individual fish and ellipses represent 95% confidence intervals around the data for each group.

Individual elements - margins

Magnesium: Univariate ANOVA indicated significant differences in Mg:Ca of otolith margins among the three sampling regions ($p < 0.001$), and a highly significant difference between age groups/cohorts ($p < 0.001$) (Fig. 14, Table 10). Mg:Ca ratios were higher in the margins of the 4+/2002 cohort (Fig. 14, Table 10). For the 4+/2002 cohort the region by cohort interaction was not significant, however, Mg:Ca ratios were significantly higher for the EBS than WTAS samples (Tukey's, $p < 0.01$) (Fig. 14, Table 10), but for the 13+/1993 cohort, EBS was significantly higher than the GAB (Tukey's, $p < 0.01$), but not the WTAS region. Analysis of the 13+/1993 cohort with the EGAB and GAB samples treated separately demonstrated that the EGAB samples had significantly lower Mg:Ca than the EBS samples (Tukey's, $p < 0.05$), but were not significantly different to the GAB samples (Tukey's, $p > 0.05$) (Table 10).

Manganese: Univariate ANOVA did not indicate a significant region main effect on Mn:Ca ratios, however, there was a significant region by cohort interaction ($p < 0.001$) (Table 10). The interaction was due to the GAB samples having significantly higher Mn:Ca than the WTAS samples for the 4+/2002 cohort (Tukey's, $p < 0.01$), however, there was no significant variation among regions for the 13+/1993 cohort (Fig. 14, Table 10). There was also a highly significant age/cohort effect due to the 4+ age group having higher Mn:Ca in the otolith margins than the 13+ age group (Fig. 14, Table 10). Analysis of the 13+/1993 cohort with the EGAB and GAB samples treated separately indicated no significant differences among regions (Table 10).

Copper: Univariate ANOVA indicated significant differences in Cu:Ca of otolith margins among the three sampling regions ($p < 0.001$), however, the region by cohort interaction was also significant ($p < 0.05$) (Fig. 14, Table 10). For the 4+/2002 cohort Cu:Ca in otolith margins was significantly higher in samples from WTAS than both EBS (Tukey's, $p < 0.01$) and the GAB (Tukey's, $p < 0.01$), however, differences among regions were not significant for the 13+ cohort (Fig. 14, Table 10). Variation between cohorts was not significant (Fig. 14, Table 10). Analysis of the 13+/1993 cohort with the EGAB and GAB samples treated separately indicated no significant differences among regions (Table 10).

Zinc: Univariate ANOVA did not indicate any significant variation in Zn:Ca ratios of otolith margins among regions for either cohort ($p > 0.05$ all tests), although there was a highly significant difference between cohorts with the 4+/2002 cohort having higher Zn:Ca of otolith margins (Fig. 14, Table 10). Analysis of the 13+/1993 cohort with the EGAB and GAB samples treated separately also indicated no significant differences among regions (Table 10).

Strontium: Univariate ANOVA did not indicate any significant variation in Sr:Ca ratios of otolith margins among regions (Fig. 14, Table 10). However, there was a highly significant difference between age groups/cohorts ($p < 0.001$) with the 13+ age group exhibiting higher Sr:Ca in the otolith margins than the 4+ cohort (Fig. 14, Table 10). Analysis of the 13+/1993 cohort with EGAB and GAB samples treated separately indicated no significant differences among regions (Table 10).

Barium: Univariate ANOVA indicated highly significant ($p < 0.001$) differences among the three sampling regions for Ba:Ca in otolith margins (Fig. 14, Table 10). There was also a significant difference between cohorts ($p < 0.001$) with the 13+/1993 cohort displaying higher Ba:Ca in otolith margins than the 4+/2002 cohort (Fig. 14). The interaction between cohort and region was not significant ($p > 0.05$) (Fig. 14, Table 10), however, *post-hoc* Tukey's tests indicated that while there was a trend for higher Ba:Ca in otolith margins from the GAB region than the WTAS and EBS regions (Fig. 14), the differences were only significant for the 4+/2002 cohort (Tukey's, $p < 0.05$) (Table 10). Analysis of the 13+/1993 cohort with the EGAB and GAB samples treated separately indicated no significant differences among regions (Table 10).

Table 10. Results of univariate ANOVA and *post-hoc* Tukey's tests comparing individual element:Ca ratios in otolith margins of blue grenadier collected in winter/spring 2007 from three regions; the Great Australian Bight (GAB), western Tasmania (WTAS) and eastern Bass Strait (EBS), and two cohorts; age 4+ years (2002 cohort) and age 13+ years (1993 cohort). All element:Ca ratios $\ln(x+1)$ transformed except for Sr:Ca, * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$. Results of *post-hoc* Tukey's tests for the age 13+ years (1993 cohort) include separation of the EGAB and GAB samples.

Source	df	MS	p-value	<i>Post-hoc</i> Tukey's differences by regions
Magnesium:Calcium				
Region	2	1.681	<0.001***	4+ - EBS>WTAS**
Cohort	1	12.418	<0.001***	13+ - EBS>GAB**, EBS>EGAB*
Region x Cohort	2	0.462	0.101	
Error	158	0.198		
Manganese:Calcium				
Region	2	0.003	0.183	4+ - GAB>WTAS**
Cohort	1	0.132	<0.001***	13+ - no significant differences
Region x Cohort	2	0.012	<0.003**	
Error	165	0.002		
Copper:Calcium				
Region	2	0.051	<0.001***	4+ - WTAS>GAB**, WTAS>EBS**
Cohort	1	0.0001	0.867	13+ - no significant differences
Region x Cohort	2	0.014	0.026*	
Error	158	0.004		
Zinc:Calcium				
Region	2	0.002	0.342	4+ - no significant differences
Cohort	1	0.160	<0.001***	13+ - no significant differences
Region x Cohort	2	0.002	0.650	
Error	155	0.005		
Strontium:Calcium				
Region	2	128,416	0.141	4+ - no significant differences
Cohort	1	21,582,294	<0.001***	13+ - no significant differences
Region x Cohort	2	10,430	0.851	
Error	165	64,723		
Barium:Calcium				
Region	2	0.061	<0.001***	4+ - GAB>EBS*, GAB>WTAS***
Cohort	1	1.315	<0.001***	13+ - no significant differences
Region x Cohort	2	0.013	0.185	
Error	165	0.007		

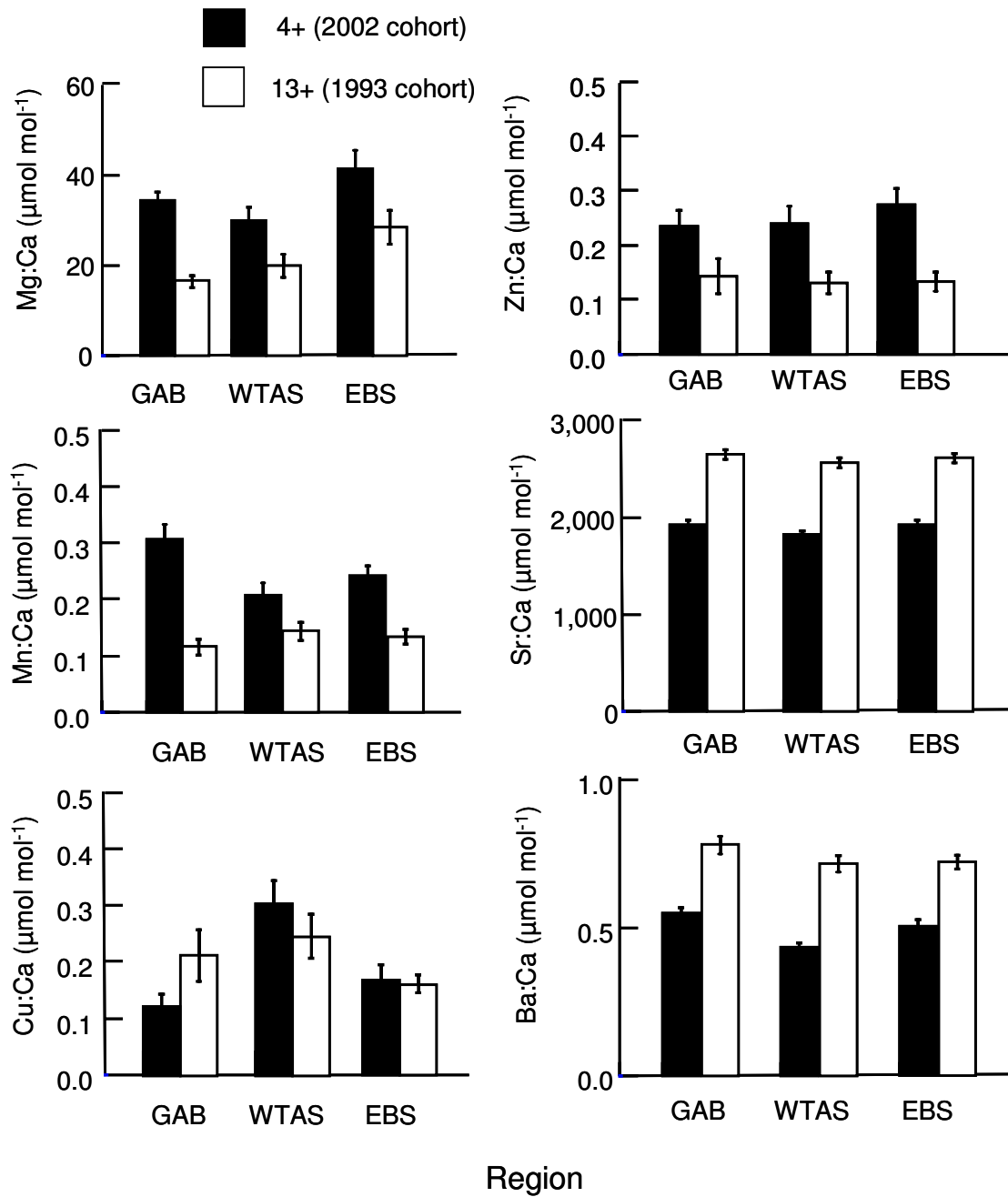


Figure 14. Comparisons of individual element:Ca ratios (mean $\pm$ SE) in otolith margins of blue grenadier collected in winter/spring 2007 from three regions; the Great Australian Bight (GAB), western Tasmania (WTAS) and eastern Bass Strait (EBS), and two cohorts; age 4+ years (2002 cohort) and age 13+ years (1993 cohort).

Multi-elemental chemistry – margins

4+/2002 cohort

MANOVA of Mg:Ca, Cu:Ca and Ba:Ca was highly significant (Pillai Trace, $df_{6,148}$, $p < 0.001$). The pairwise comparisons indicated that both the EBS and GAB regions were significantly different to the WTAS region (Hotelling's T-Square, $p < 0.001$), however, they were not significantly different to each other ($p > 0.05$). The canonical variates plot (Fig. 15a) indicated that there was significant overlap among samples from all three regions, however, the samples from the WTAS and GAB regions were separated along canonical variate 1 (Fig. 15a). This separation was due to the tendency for lower Ba:Ca and Mg:Ca, but higher Cu:Ca in the margins of otoliths from WTAS compared to the GAB region (Fig. 14). Similar to the otolith core data, there was also greater variability in the EBS otolith margin data, and the EBS data overlapped both the WTAS and GAB data (Fig. 15a). F-to-remove values indicated that the order of importance for discrimination was Ba:Ca > Cu:Ca > Mg:Ca.

For the WTAS and GAB regions the jackknifed classification procedure including all regions was moderate to highly accurate in assigning individuals to their collection region based on Mg:Ca, Cu:Ca and Ba:Ca ratios of their otolith margins (Table 11a). Classification accuracy was highest for individuals collected from WTAS at 80%, followed by GAB at 74% and then EBS at 31% (Table 11a). Classification errors for the WTAS and GAB regions were generally evenly distributed among the other regions (Table 11a). The EBS samples showed poor classification accuracy reflecting the high variability of the samples from this group and their considerable overlap with both the WTAS and GAB sample groups (Fig. 15a). The classification procedure repeated with EBS and GAB compared separately to WTAS achieved significantly higher accuracy for the EBS region, and small improvements for the GAB and WTAS regions (Table 11b).

Table 11. 4+/2002 cohort margins - Results of jackknife classification of individuals based on $\ln(x+1)$ transformed Mg:Ca, Cu:Ca, Ba:Ca in otolith margins. Data are percentages of the individuals from each collection region (indicated in left column) classified to each region. a) all regions included, b) GAB and EBS compared separately with WTAS. Order of importance of element:Ca ratios (F-to-remove) to discrimination indicated in table footnotes.

a)

Region of collection	Region classified to (% of sample)		
	EBS	GAB	WTAS
EBS (n=26)	31	46	23
GAB (n=27)	11	74	15
WTAS (n=25)	12	8	80

Ba:Ca>Cu:Ca>Mg:Ca

b)

Region of collection	Region classified to (% of sample)		Region of collection	Region classified to (% of sample)	
	GAB	WTAS		EBS	WTAS
GAB (n=27)	85	15	EBS (n=26)	73	27
WTAS (n=25)	12	88	WTAS (n=25)	20	80

Ba:Ca>Cu:Ca>Mg:Ca

Cu:Ca>Mg:Ca>Ba:Ca

13+/1993 cohort

MANOVA of Mg:Ca, Cu:Ca and Ba:Ca was highly significant (Pillai Trace, $df_{6, 166}$, $p < 0.01$). The pairwise comparisons indicated that only the EBS and GAB regions differed significantly from each other (Hotelling's T-Square, $p < 0.01$). The canonical variates plot indicated significant overlap among samples from the three regions; however, there was some separation of GAB and EBS samples along canonical variate 1 due to the trend for higher Cu:Ca and Ba:Ca, but lower Mg:Ca in GAB than EBS samples (Fig. 15b). Similar to the otolith core data for both cohorts, and the otolith margins for the 4+/2002 cohort, the samples from the EBS region were more variable than the other two regions (Fig. 13 & 15). F-to-remove values indicated that the order of importance for discrimination was Mg:Ca > Cu:Ca > Ba:Ca.

The jackknifed classification procedure was moderately accurate in assigning individuals collected from the GAB region, but performed poorly for the EBS and WTAS regions (Table 12a). Classification accuracy was highest for the GAB samples at 67%, followed by EBS at 47% and then WTAS at 46% (Table 12a). Classification errors for the EBS samples were evenly spread between the GAB and WTAS regions (Table 12a). The poor classification success for WTAS samples was due to their overlap with the GAB samples, and most misclassified GAB samples were classified to WTAS (Table 12a, Fig. 15b). The classification procedure repeated with EBS and GAB compared separately to WTAS achieved significantly higher accuracy for the WTAS region, but only minor improvements for the GAB and EBS regions (Table 12b).

Table 12. 13+/1993 cohort margins - Results of jackknife classification of individuals based on $\ln(x+1)$ transformed Mg:Ca, Cu:Ca and Ba:Ca in otolith margins. Data are percentages of the samples from each collection region (indicated in left column) classified to each region. a) all regions included, b) GAB and EBS compared separately with WTAS. Order of importance of element:Ca ratios (F-to-remove) to discrimination indicated in table footnotes.

a)

Region of collection	Region classified to (% of sample)		
	EBS	GAB	WTAS
EBS (n=36)	47	25	28
GAB (n=27)	7	67	26
WTAS (n=24)	17	37	46

Mg:Ca>Cu:Ca>Ba:Ca

b)

Region of collection	Region classified to (% of sample)		Region of collection	Region classified to (% of sample)	
	GAB	WTAS		EBS	WTAS
GAB (n=27)	67	33	EBS (n=36)	58	42
WTAS (n=24)	37	63	WTAS (n=24)	29	71

Ba:Ca>Mg:Ca>Cu:Ca

Mg:Ca>Cu:Ca>Ba:Ca

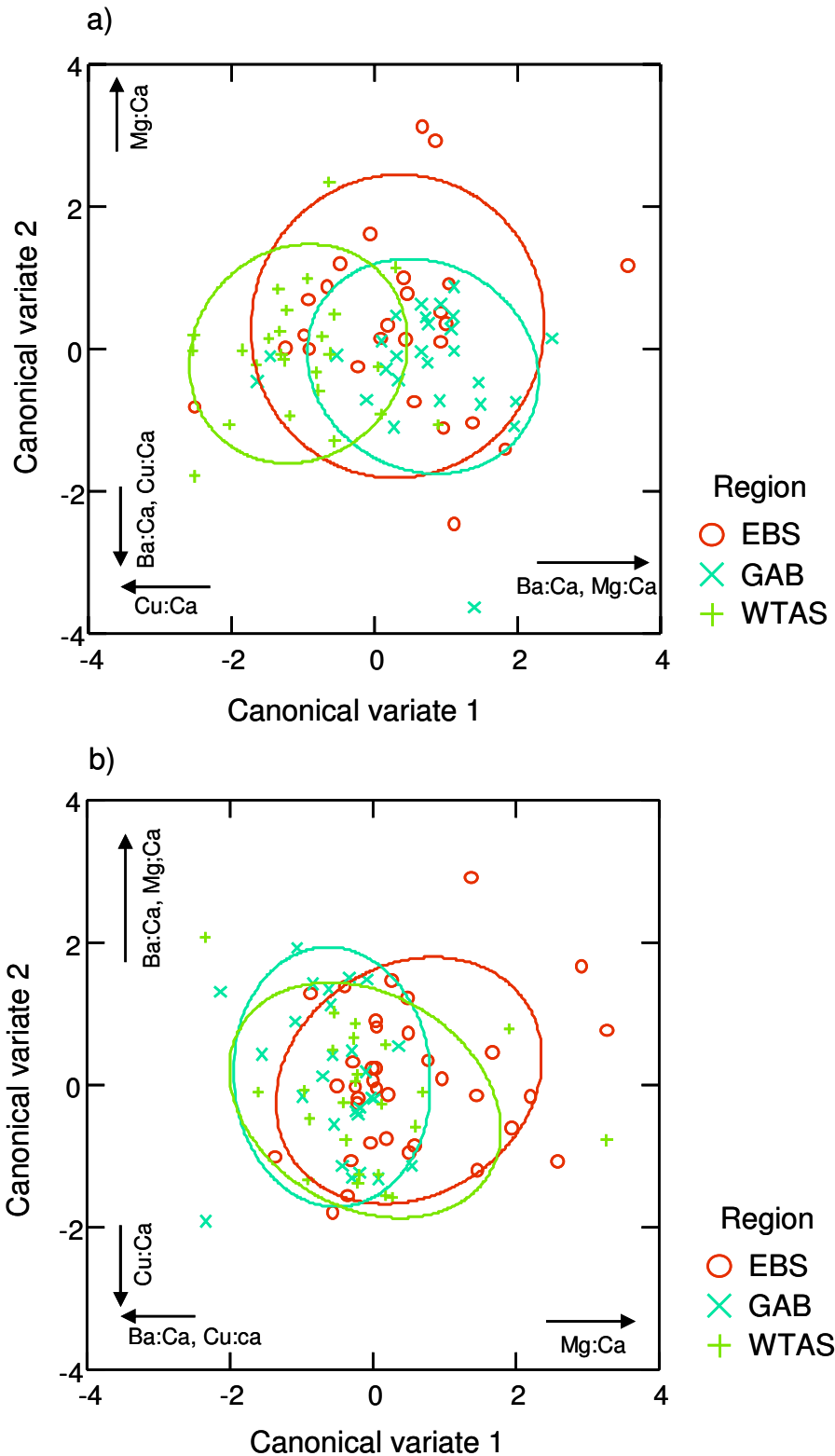


Figure 15. Canonical variate plots comparing multi-element chemistry (Mg:Ca, Cu:Ca, Ba:Ca) of otolith margins of blue grenadier among three fishery regions; the Great Australian Bight (GAB), western Tasmania (WTAS) and eastern Bass Strait (EBS), and for two cohorts; a) age 4+ years (2002 cohort), b) age 13+ years (1993 cohort). Data are individual fish and ellipses represent 95% confidence intervals around the data for each group.

Stable isotopes -

Oxygen - $\delta^{18}\text{O}$

Univariate ANOVA indicated significant differences in $\delta^{18}\text{O}$ of otoliths among the three sampling regions ($p < 0.001$), and a significant difference between cohorts/ages ($p < 0.001$) (Fig. 16a, Table 13). However, the region by cohort interaction was also significant ($p < 0.01$) (Table 13). The interaction is attributed to the lack of significant variation among regions for the 4+/2002 cohort, but highly significant differences among regions for the 13+/1993 cohort due to the WTAS samples having significantly higher $\delta^{18}\text{O}$ than both the GAB and EBS samples (Fig. 16a, Table 13). $\delta^{18}\text{O}$ was generally higher for the 13+ age group, but this effect was more pronounced for the samples from WTAS than the other regions (Fig. 16a). Analysis of the 13+/1993 cohort with the EGAB and GAB samples treated separately indicated that $\delta^{18}\text{O}$ of samples from both EGAB and GAB were significantly lower than WTAS, but not different to each other or EBS (Table 13).

Carbon - $\delta^{13}\text{C}$

Univariate ANOVA indicated significant differences in $\delta^{13}\text{C}$ of otoliths among the three sampling regions ($p < 0.001$), and also significant differences among cohorts ($p < 0.001$) (Fig. 16b, Table 13). The interaction between cohort and region was not significant ($p > 0.05$) (Fig. 16b, Table 13). There was a highly significant age group/cohort effect due to the 13+ age group having higher (i.e. lower negative values) $\delta^{13}\text{C}$ than the 4+ age group (Fig. 16b). The pattern of variation among the sampling regions was the same for each cohort, with both WTAS and EBS samples displaying generally higher $\delta^{13}\text{C}$ than the GAB samples, however, these differences among regions were only statistically significant for the 4+/2002 cohort (Fig. 16b, Table 13). Analysis of the 13+/1993 cohort with the EGAB and GAB samples treated separately indicated no significant differences among these regions (Table 13).

Isotopes combined

For the 4+/2002 cohort, scatter plots of $\delta^{18}\text{O}$ versus $\delta^{13}\text{C}$ indicated separation of EBS samples from GAB samples due to differences in $\delta^{13}\text{C}$, with WTAS samples having $\delta^{13}\text{C}$ values intermediate between these two groups (Fig. 17a). There was little separation among groups due to variation in $\delta^{18}\text{O}$ (Fig. 17a).

For the 13+/1993 cohort the scatter plots of $\delta^{18}\text{O}$ versus $\delta^{13}\text{C}$ indicated separation of WTAS samples from both EBS and GAB samples due to differences in $\delta^{18}\text{O}$ (Fig. 17b), however, similar to the 4+/2002 cohort, separation of the EBS and GAB samples was mostly due to differences in $\delta^{13}\text{C}$ (Fig. 17a,b).

MANOVA of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ indicated significant variation among regions for both cohorts (age 4+/2002 cohort, Pillai Trace, $df_{4, 152}$, $p < 0.05$; age 13+/1993 cohort, Pillai Trace, $df_{4, 178}$, $p < 0.001$). Pairwise comparisons indicated for the 4+/2002 cohort that only the GAB and EBS regions differed significantly (Hotelling's T-Square, $p < 0.01$). For the 13+/1993 cohort the WTAS region was significantly different than both the GAB and EBS regions (both comparisons Hotelling's T-Square, $p < 0.001$), but these two regions were not significantly different from each other (Hotelling's T-Square, $p > 0.05$).

For the age 4+/2002 cohort there was a significant overlap among samples from all regions in the canonical variate plot (Fig. 18a). Jackknifed classification accuracy was moderate for the EBS and GAB regions (59% and 54% respectively), but was poor for the WTAS region (35%) (Table 14a). Misclassified WTAS and EBS samples were mostly due to being classified to EBS and WTAS respectively, however, misclassified GAB samples were distributed equally between the EBS and WTAS regions (Table 14a). When the GAB and WTAS regions were analysed without inclusion of the EBS, there was an increase in classification accuracy for the WTAS region (73%), but no change for the GAB region (Table 14b). When the EBS and WTAS regions were analysed without inclusion of the GAB, there was negligible change in classification accuracy for either region (Table 14b).

For the age 13+/1993 cohort there was significant overlap among samples from the EBS and GAB regions, but the WTAS samples were separated to a greater degree along canonical variate 1 (i.e. higher $\delta^{18}\text{O}$) (Fig. 18b). Jackknifed classification accuracy was moderate for the GAB and WTAS regions (64% and 52% respectively), but was poor for the EBS region (35%) (Table 15a). Misclassified EBS and WTAS samples were mostly due to being classified to the GAB, however, misclassified GAB samples were again distributed relatively evenly between the EBS and WTAS regions (Table 15a). When the GAB and WTAS regions were analysed without inclusion of the EBS, there was an increase in classification accuracy for the GAB region (79%), but no change

for the WTAS region (Table 15b). When the EBS and WTAS regions were analysed without inclusion of the GAB, there was a significant increase in classification accuracy for the EBS region (78%), and also an increase for the WTAS region (63%) (Table 15b).

Table 13. Results of univariate ANOVA and *post-hoc* Tukey's tests comparing isotopic ratios in otolith margins of blue grenadier collected in winter/spring 2007 from three regions; the Great Australian Bight (GAB), western Tasmania (WTAS) and eastern Bass Strait (EBS), and two cohorts; age 4+ years (2002 cohort) and age 13+ years (1993 cohort). * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$. Results of *post-hoc* Tukey's tests for the 13+/1993 cohort include separation of the EGAB and GAB samples.

Source	df	MS	p-value	<i>Post-hoc</i> Tukey's differences by regions
$\delta^{18}\text{O}$				
Region	2	0.144	<0.001***	4+ - no significant differences
Cohort	1	0.710	<0.001***	13+ - WTAS>GAB***, WTAS>EBS***
Region x Cohort	2	0.104	0.002**	WTAS>EGAB***, WTAS>GAB*
Error	165	0.016		
$\delta^{13}\text{C}$				
Region	2	0.710	0.001**	4+ - EBS>GAB***, WTAS>GAB*
Cohort	1	7.203	<0.001***	13+ - no significant differences
Region x Cohort	2	0.021	0.802	
Error	165	0.095		

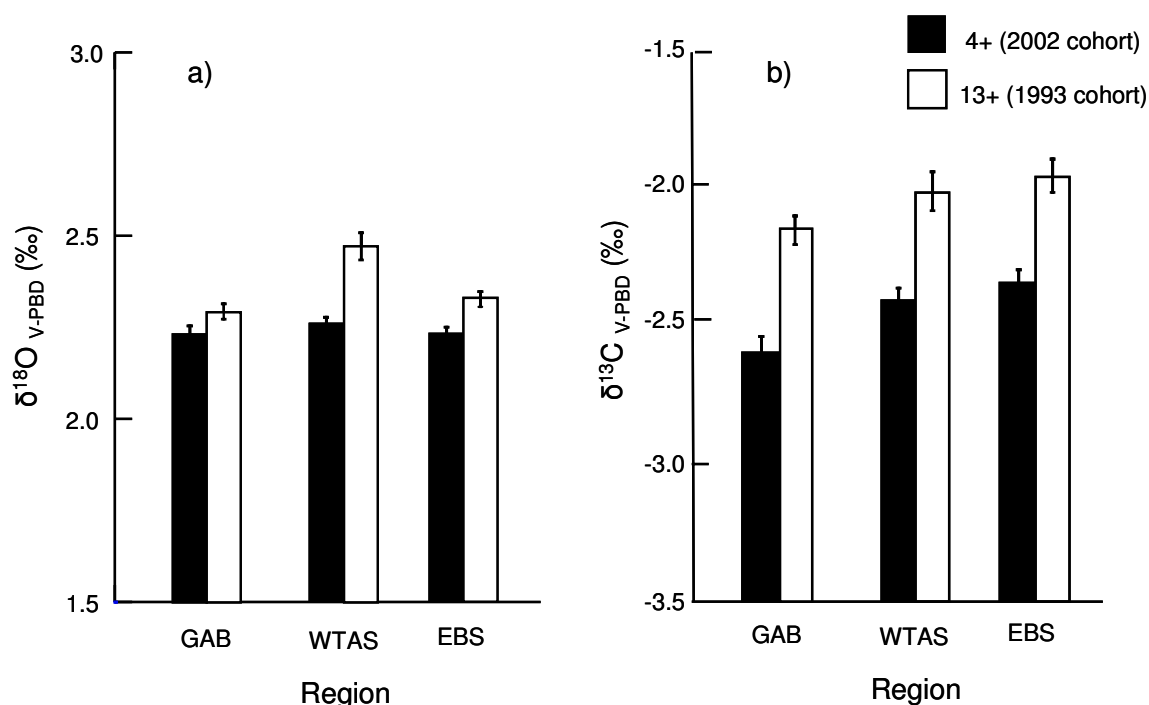


Figure 16. Comparisons of; a) $\delta^{18}\text{O}$ and b) $\delta^{13}\text{C}$ (mean $\pm$ SE) in whole otolith sections of blue grenadier collected in winter/spring 2007 from three regions; the Great Australian Bight (GAB), western Tasmania (WTAS) and eastern Bass Strait (EBS), and two cohorts; age 4+ years (2002 cohort) and age 13+ years (1993 cohort).

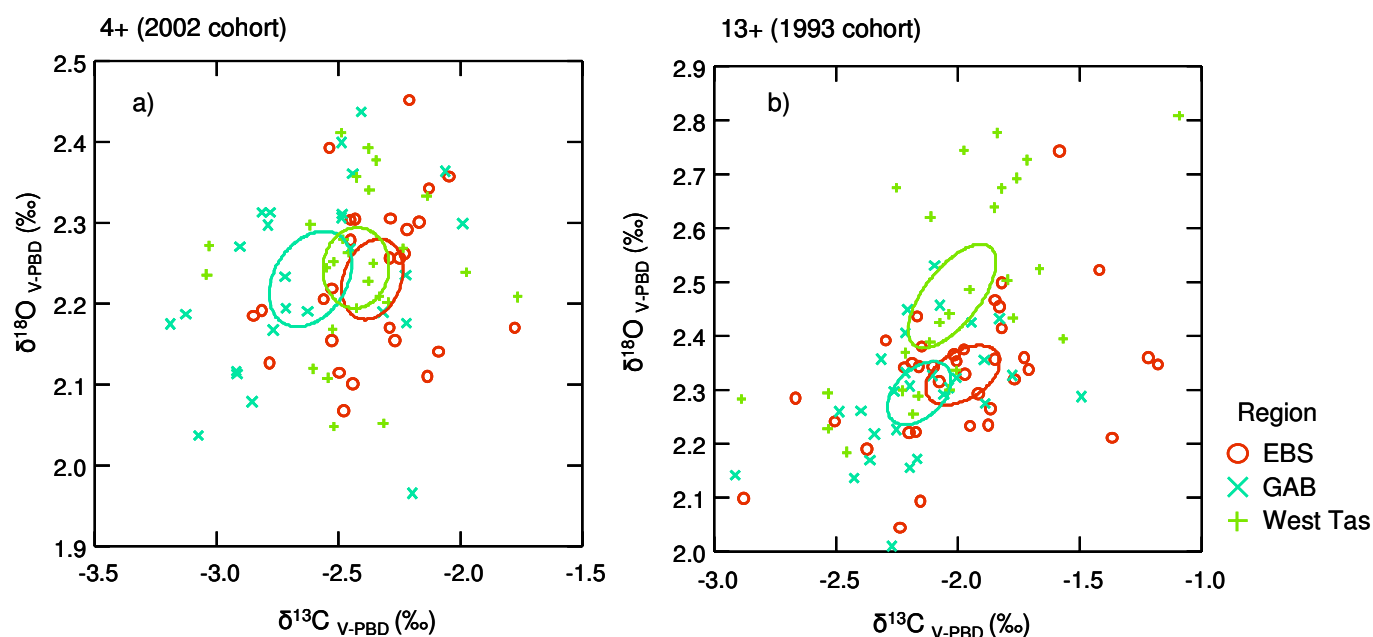


Figure 17. Scatterplots of $\delta^{18}\text{O}$ against $\delta^{13}\text{C}$ of whole otolith sections from blue grenadier collected in winter/spring 2007 from three regions; the Great Australian Bight (GAB), western Tasmania (WTAS) and eastern Bass Strait (EBS), and two cohorts; a) age 4+ years (2002 cohort) and b) age 13+ years (1993 cohort). Data are values for individual fish and ellipses indicate 95% confidence intervals around the group centroids.

Table 14. 4+/2002 cohort - Results of jackknife classification of individuals based on $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ in whole otolith sections. Data are percentages of the samples from each collection region (indicated in left column) classified to each region. a) all regions included, b) GAB and EBS compared separately with WTAS. Order of importance of isotope ratios (F-to-remove) to discrimination indicated in table footnotes.

a)

Region of collection	Region classified to (% of sample)		
	EBS	GAB	WTAS
EBS (n=27)	59	15	26
GAB (n=26)	23	54	23
WTAS (n=26)	54	11	35

 $\delta^{13}\text{C} > \delta^{18}\text{O}$

b)

Region of collection	Region classified to (% of sample)		Region of collection	Region classified to (% of sample)	
	GAB	WTAS		EBS	WTAS
GAB (n=26)	54	46	EBS (n=27)	59	41
WTAS (n=26)	27	73	WTAS (n=26)	54	46

 $\delta^{13}\text{C}$ $\delta^{13}\text{C} > \delta^{18}\text{O}$

Table 15. 13+/1993 cohort - Results of jackknife classification of individuals based on $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ in whole otolith sections. Data are percentages of the samples from each collection region (indicated in left column) classified to each region. a) all regions included, b) GAB and EBS compared separately with WTAS. Order of importance of isotope ratios (F-to-remove) to discrimination indicated below tables.

a)

Region of collection	Region classified to (% of sample)		
	EBS	GAB	WTAS
EBS (n=37)	35	51	14
GAB (n=28)	22	64	14
WTAS (n=27)	11	37	52

 $\delta^{18}\text{O} >> \delta^{13}\text{C}$

b)

Region of collection	Region classified to (% of sample)		Region of collection	Region classified to (% of sample)	
	GAB	WTAS		EBS	WTAS
GAB (n=28)	79	21	EBS (n=37)	78	22
WTAS (n=27)	48	52	WTAS (n=27)	37	63

 $\delta^{18}\text{O} >> \delta^{13}\text{C}$ $\delta^{18}\text{O} >> \delta^{13}\text{C}$

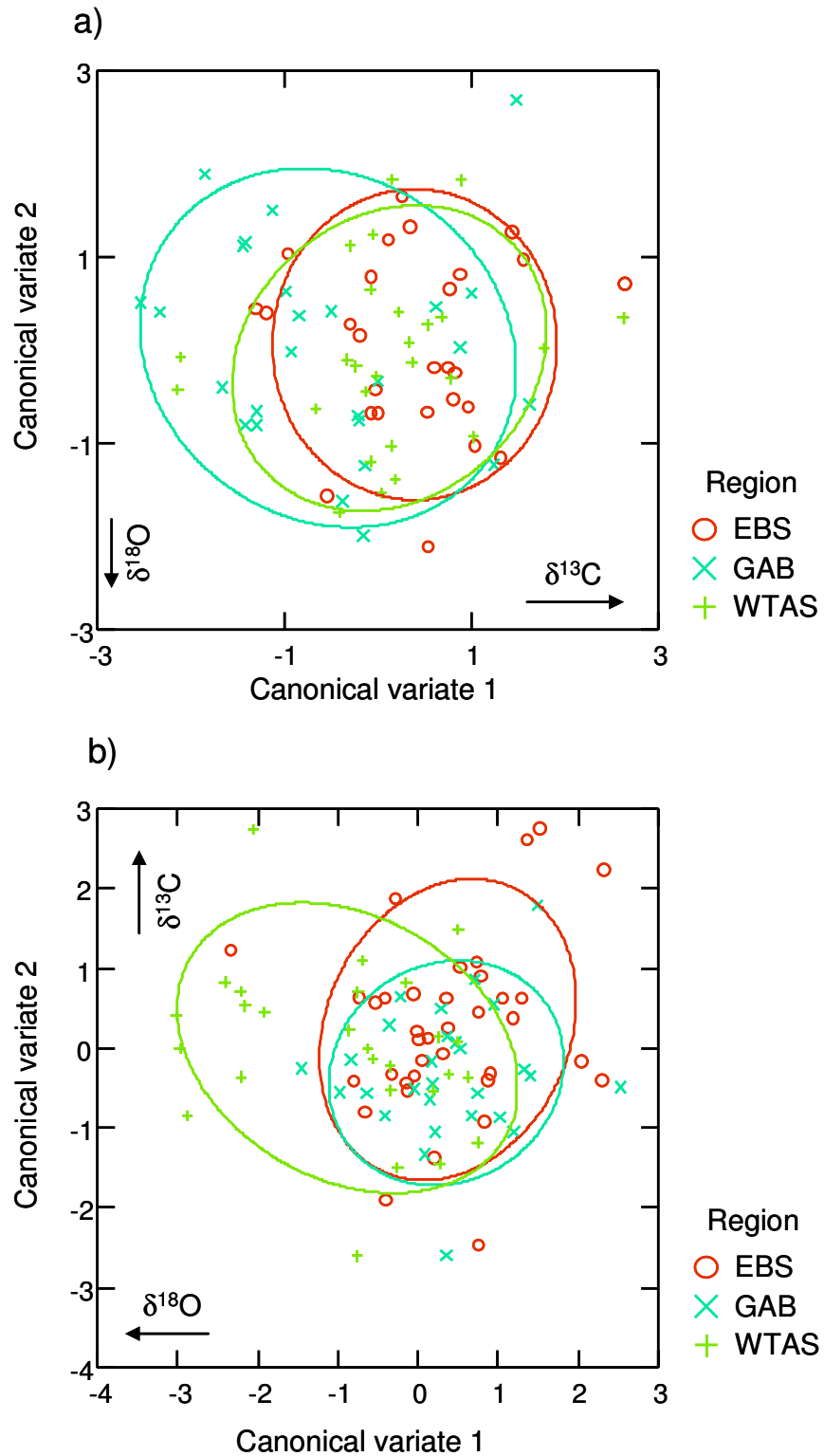


Figure 18. Canonical variate plots comparing combined $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ of whole otolith sections from blue grenadier among three fishery regions; the Great Australian Bight (GAB), western Tasmania (WTAS) and eastern Bass Strait (EBS), and for two cohorts; a) age 4+ years (2002 cohort), b) age 13+ years (1993 cohort). Data are individual fish and ellipses represent 95% confidence intervals around the data for each group.

$\delta^{18}\text{O}$ otolith and water temperature

$\delta^{18}\text{O}$ from whole otolith sections provided water temperature estimates integrated over the entire life history of individuals. For the 4+/2002 cohort, water temperatures estimated from $\delta^{18}\text{O}$ in otoliths were similar across all regions (mean/SD, °C: GAB = 10.0/0.5, WTAS = 9.9/0.4, EBS = 10.0/0.4) (Fig. 19). For the 13+/1993 cohort, water temperatures estimated from $\delta^{18}\text{O}$ otolith were similar for the EBS and GAB regions, but were lower for the WTAS region (mean/SD, °C: GAB = 9.7/0.5, EBS = 9.6/0.6, WTAS = 8.8/0.8) (Fig. 19). The estimated temperatures for the 4+/2002 cohort were only slightly higher than those estimated for the 13+/1993 cohort for the GAB and EBS regions, but were approximately 1°C higher in the 4+/2002 cohort for the WTAS region (Fig. 19).

The depth distribution of blue grenadier was estimated from NetSond mean depth data for individual trawl shots collated from over 3000 records by the onboard observer program. These data are displayed in figures 20a-d. Unfortunately, few data (18 records) were available for the GAB region, but the available data indicated that capture depths ranged from 150–900 m, with most of the recorded capture depths being within the 400–600 m range, similar to that observed for the WTAS and EBS regions (Fig. 20 a-d). The available temperature data for the three fishing regions indicated that at the 400–600 m depth range where most blue grenadier are captured, temperature ranged from ~10.5°C at 400 m to ~8.2°C at 600 m (Fig. 21a). Therefore, the actual measured water temperature range across the depths where blue grenadier are mostly captured clearly overlapped the water temperatures estimated from the $\delta^{18}\text{O}$ values of otoliths, confirming that conversion of $\delta^{18}\text{O}$ otolith to water temperature was accurate.

Finally, the quadratic curves fitted to the available water temperature data for each region between 0 and 600 m indicated that while the surface waters in the GAB region were significantly warmer (by up to 5°C) than those in both the WTAS and EBS regions, at 350 m depth the water temperatures converged for all three regions (Fig. 21b). Therefore, contrary to our initial expectations, differences in $\delta^{18}\text{O}$ otolith among regions would be expected to be small if fish remained in the 400–600 m depth range in each of the three regions, consistent with the $\delta^{18}\text{O}$ otolith data for the 4+/2002 cohort (Fig. 16a). The variation between cohorts and the higher $\delta^{18}\text{O}$ values for the WTAS samples in the 13+/1993 cohort is possibly related to a shift in mean residence depth with age and or more time spent at more southerly latitudes with age.

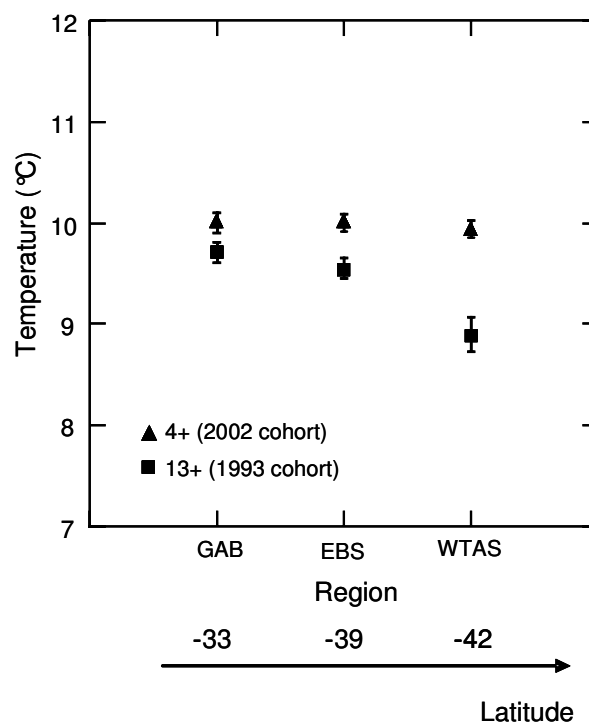


Figure 19. Mean water temperature ($\pm$ SE) experienced by blue grenadier of two age groups/cohorts estimated from otolith $\delta^{18}\text{O}$ values. GAB = Great Australian Bight, EBS = Eastern Bass Strait, WTAS = Western Tasmania (see methods for further details of conversion equations).

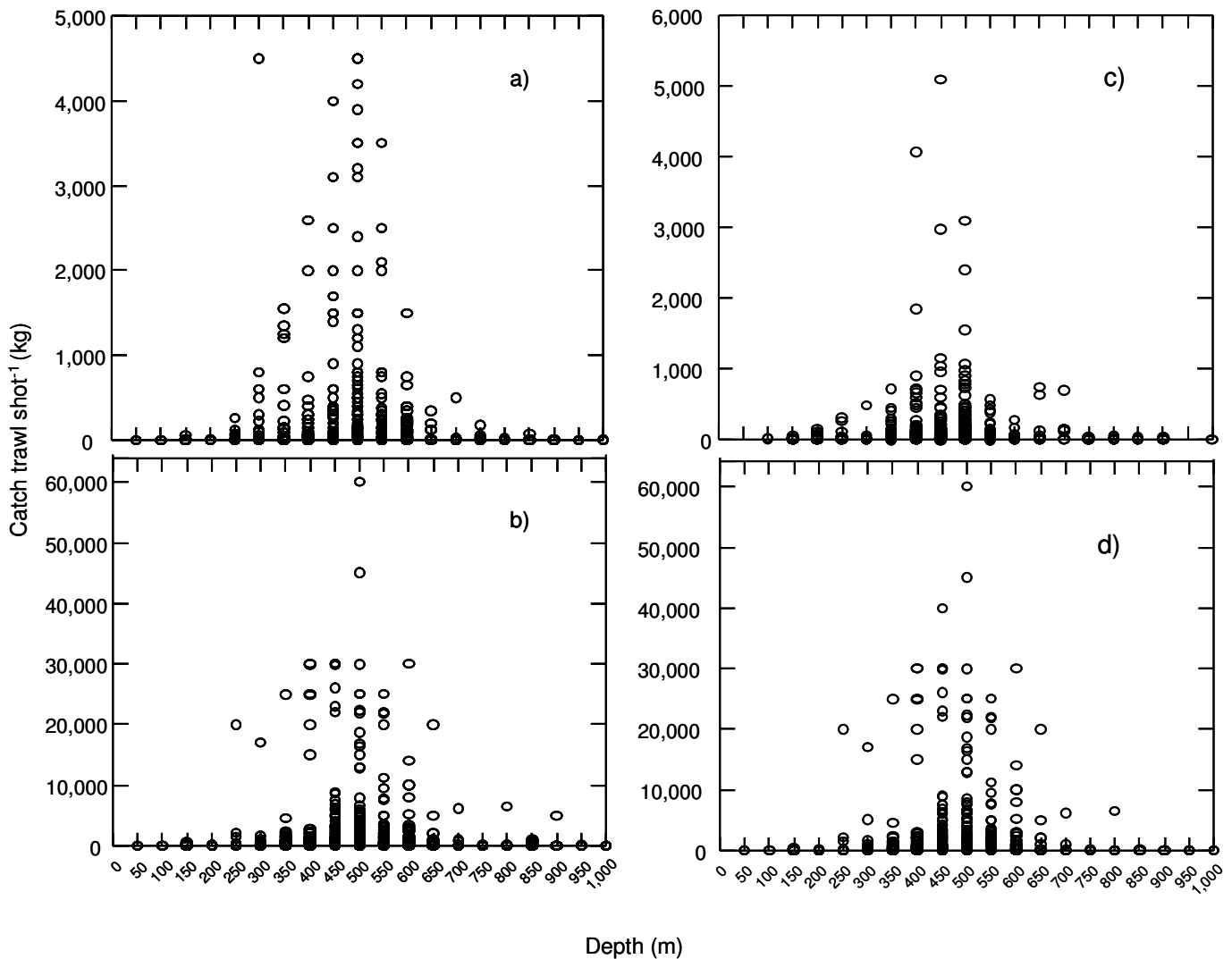


Figure 20. Depth distribution of blue grenadier retained and discarded catches recorded from approximately 3000 trawl shots; a) WTAS region discards, b) WTAS region retained, c) EBS discards, d) EBS retained. Insufficient records were available for the GAB region, but see text. Data (circles) represent individual trawl shots.

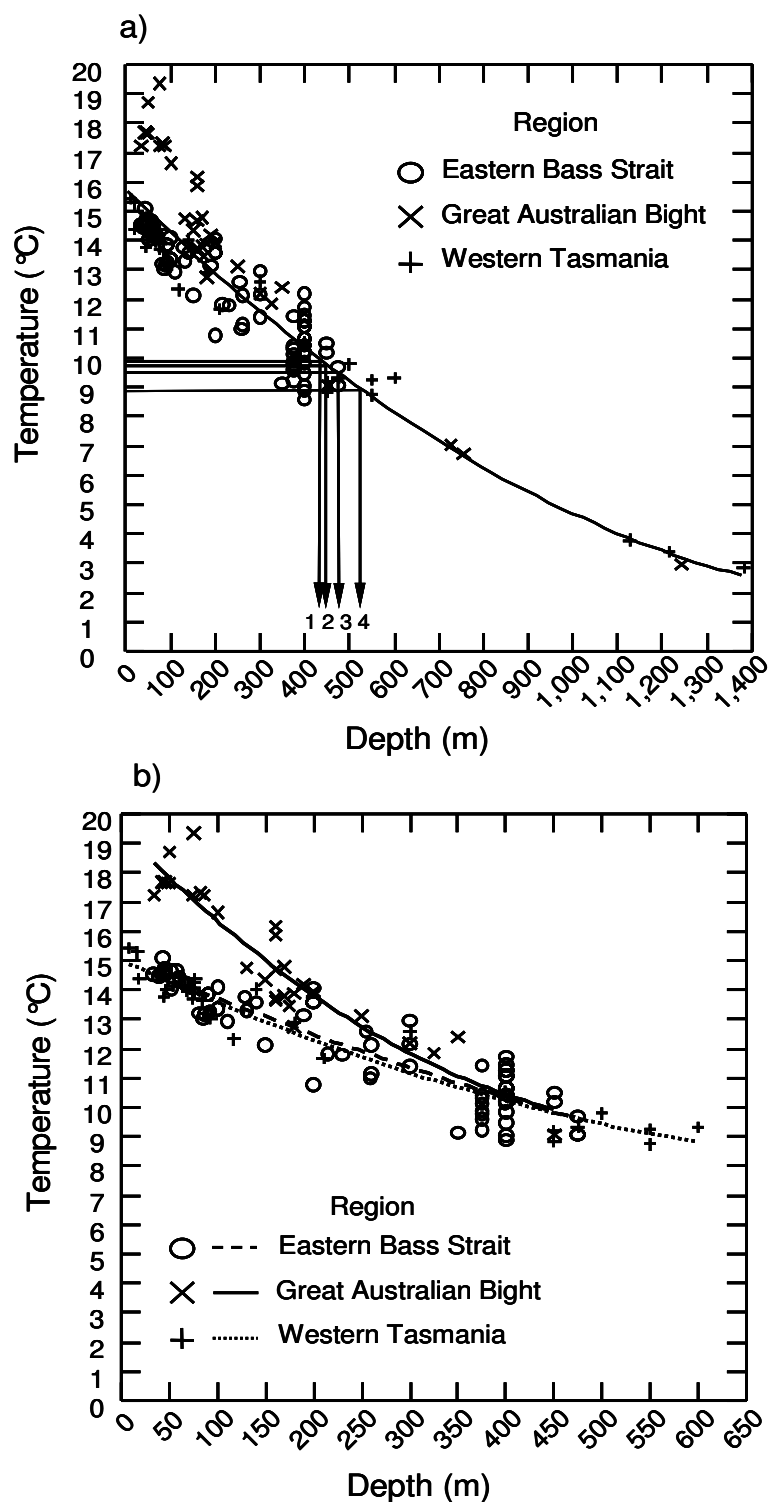


Figure 21. Sea temperature variation with depth. Temperature data is for near bottom waters obtained from the CSIRO - Climatology of Australasian Regional Seas data base. Each point represents a yearly average. EBS refers to the East Bass Strait region between 147.5°E–148.5°E/38.5°S–45.0°S, WTAS refers to the west Tasmanian region between 144.5°E–145.5°E/38.0°S–44.0°S, GAB refers to the Great Australian Bight region between 127.5°E–128.5°E/32.0°S–33.5°S. a) All data, arrows indicate the residence depths predicted from the mean $\delta^{18}\text{O}$ of otoliths converted to temperature (i.e. Fig. 19); 1 = age 4+/2002 cohort pooled across regions, 2 = GAB 13+/1993 cohort, 3 = EBS 13+/1993 cohort, 4 = WTAS 13+/1993 cohort. b) actual water temperature data restricted to the 0–600 m depth range. Quadratic curves are fitted to the data.

Table 16. Summary matrices indicating the otolith parameters that were significantly different between regions. E-cores = elemental chemistry of otolith cores, E-margins = elemental chemistry of otolith margins.

a) Age 4+/2002 cohort

	GAB	WTAS
WTAS	Shape, E-cores, E-margins, $\delta^{13}\text{C}$	
EBS	E-cores, $\delta^{13}\text{C}$	E-cores, E-margins

b) Age 13+/1993 cohort

	GAB	WTAS
WTAS	E-cores, $\delta^{18}\text{O}$	
EBS	E-margins	E-cores, $\delta^{18}\text{O}$

Discussion -

The objective of this study was to determine whether otolith-based techniques (shape, elemental chemistry, and stable isotopes) could provide useful information for assessing whether blue grenadier fisheries in different regions of southern Australia should be treated as components of separate stocks for management purposes. Of particular importance to this project was determining whether or not the winter spawning fishery off western Tasmania should be managed separately to that of the developing fishery in the Great Australian Bight. While this was the major impetus for this project, sample availability allowed three important blue grenadier fishing regions in the SESSF (western Tasmania - WTAS, eastern Bass Strait – EBS, and the Great Australian Bight - GAB) to be included in the study. The 2007 age structure of the populations in all three regions was dominated by two major cohorts indicating similar timing of major recruitment events across the entire southern Australian fishery. One cohort consisted of fish of 13 years of age that were derived from spawning in winter 1993, and the other cohort consisted of fish of 4 years of age that were derived from spawning in winter 2002. To maintain temporal overlap and age consistency of the comparisons among regions, we conducted separate analyses of otolith parameters for each of these cohorts. The results indicated, for both cohorts, statistically significant variation in otolith shape, elemental chemistry and stable isotope (both $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) ratios among the three fishery regions. Patterns of variation among regions, however, depended to a certain extent on the parameters being compared and the particular cohort (Table 16). Samples from the GAB region differed significantly from those from the WTAS and EBS regions for some trace elements, stable isotopes and otolith shape. There were also significant differences in some of the measured otolith parameters between the WTAS and EBS regions. Interpretation of results for each technique is discussed in more detail below, however, there was general support for fish collected from the GAB region differing from those collected from the WTAS region. Although there was generally more overlap of the data between the EBS and GAB regions, the distance between these regions (>2000 km), including the shallow water barrier of Bass Strait, suggests that the overlap was more likely the result of similarity in the factors influencing otolith shape and chemistry rather than a high level of connectivity between these two regions.

Before detailed discussion and interpretation of the results, it is important to firstly consider the nature of the sample collections in space and time in relation to the interpretation of stock structure. Sample collections were restricted to the three important fishing grounds (i.e. sampling was structured around the fishery), and therefore much of the distribution of the species along the southern Australian continental slope was not sampled. This means that while differences among the fishing regions may indicate that the different regions are components of different stocks, we cannot delineate the boundaries or spatial extent of individual stocks under the sampling regime and analytical approaches employed in the current study. This would require a far more detailed, spatially structured sampling design. Further, the sampling was also focussed on the winter when blue grenadier aggregate in certain areas to spawn. While the winter sampling period was based around the nature of the fishery, it is possible that differences detected among samples collected during the spawning period could break down outside the spawning period if fish disperse and mix away from and or between spawning regions, but then separate and migrate back to their original spawning regions during the spawning period (Campana *et al.* 1999). The extent of such mixing outside the winter spawning period, if any, is not clearly assessed in the current study, and would require sampling both during and outside of the spawning season (i.e. winter aggregated versus summer dispersed/mixed (Campana *et al.* 1999). However, the majority of blue grenadier fishing is focussed on the winter spawning period, and any mixing among regions outside that period is likely to be of limited consequence to the spatial management of the fishery, unless the 'non-spawning' fishery is to be expanded in the future.

A further important consideration for interpreting the results of all three otolith methods is that they all rely on detecting variation in the measured otolith parameters among regions. The 'top down' methodology employed in this study relies on the assumption that regional variation in the measured otolith parameters will occur if stock separation exists (Thresher 1999). If no variation is detected among the regions of interest the data are largely inconclusive in relation to stock structure. This is because a lack of variation could be the result of a lack of variation in the important environmental and or physiological processes that influence otolith shape and chemistry rather than a lack of stock separation. Lack of significant variation in any of the otolith parameters will merely indicate a failure of the techniques and or sampling approach to reject the

null hypothesis of no differences (i.e. no separation) among regions. In this situation other techniques may be required to provide a clearer understanding of stock structure, if it indeed exists.

Otolith shape

Significant variation in otolith shape was detected between the GAB and WTAS regions for the 4 year old samples (2002 cohort). Significant variation in the shape primarily indicates variation in features of the otolith margin. The significant difference in otolith shape of 4 year olds between the GAB and WTAS regions provide supporting evidence that the GAB samples had been separated from the WTAS samples for a significant period of their life. Otolith shape comparisons between the WTAS and EBS region proved inconclusive in relation to identifying stock separation among these regions.

It was interesting that the difference between the GAB and WTAS regions detected for the 4+/2002 cohort was not replicated to the same degree in the 13+/1993 cohort. This difference could be due to several reasons. Firstly, it is possible that otolith shape variability among regions is more pronounced for younger ages/smaller otoliths. Otolith shape variation within a species is suggested to be more influenced by variation in growth rate than genetics (Campana and Casselman 1993). While growth rate effects on otolith shape might be expected to accumulate with age, leading to greater variability of otolith shape with age, young fish grow faster than older fish, and growth rates of young fish are generally more sensitive to environmental variation than older fish (Maceina 2006). Therefore it is possible that environmentally-driven variation of otolith shape is more pronounced for younger than older fish. Secondly, the precision of otolith shape measurements could be lower for older fish with more complex otolith margins, potentially resulting in age dependent sensitivity of the statistical analyses to detect minor shape differences among regions. The previous two possibilities can be considered as 'age' influences on otolith shape comparisons. Thirdly, the difference between the cohorts/age groups may reflect differences in the extent and type of environmental variation experienced by each of the cohorts over their lifetime. This is a temporal effect due to the two cohorts developing over different chronological time periods. Separation of age (ontogenetic) from cohort (temporal) effects on otolith shape comparisons requires comparison among regions for the same cohorts sampled at different ages (i.e. multi-year samples of the same cohort).

Irrespective of the reason(s) for the different results for each cohort, the variation in otolith shape for the 4-year old fish indicates that the GAB fish had experienced different environmental conditions than the WTAS samples for a significant proportion of their life. Although genetic effects cannot be ruled out, we would have expected that a genetic effect should have been detected for both cohorts. Although earlier studies have indicated little genetic variation between the WTAS and EBS regions (Milton and Shaklee 1987), there is no information on genetic variation between the GAB and WTAS/EBS regions. The need for further genetic comparisons involving the GAB region is discussed below.

Finally, it should be recognised that otolith shape differences are a morphological effect integrated over the entire life history. Differences in shape do not necessarily provide evidence that mixing of early life stages among regions has not occurred, or that fish residing in the different regions were not derived from the same spawning source(s). In order to indicate separation of early life stages different approaches are required that specifically target the early life history period of the otolith (i.e. elemental chemistry of otolith cores).

Elemental chemistry

The two ablations over the otolith core region would have provided an elemental signature for a period of time within the first 1-3 months of life when individuals would have been approximately 5–20 cm total length, and pelagic in the upper water column (Horn and Sullivan, 1996). The chemical signature of the core region may therefore have encompassed both larval drift and the early juvenile stage. The locations and preferred depths of the early life stage of blue grenadier are poorly known in Australian waters, however the information on larval distributions and sporadic collections of small juveniles indicates that they are likely to occur in shelf waters and at shallower, and more inshore, depths than the adults (Thresher *et al.*, 1988; Gunn *et al.* 1989; Livingston 1990; Thresher *et al.* 1992; Horn and Sullivan 1996; Bruce *et al.* 2001). The comparison of the otolith core chemistry is the most important in relation to assessing stock structure. Significant differences in otolith core chemistry among groups of adult fish sampled in different regions indicate that

the early life histories of fish from the different groups had occurred in different environments. While it is difficult to confidently state where the early life history period actually occurred in space without baseline location chemical tags specific to the larval/juvenile stage (Thorrold *et al.* 2001; Hamer and Jenkins 2007b), the fact that the variation among groups was detected from samples of adult fish that had significant prior opportunity to mix, indicates that separation at the juvenile stage is maintained at adulthood. This provides evidence that the groups of adults sampled from the different regions were derived from different replenishment sources, which is critical information for developing models of stock structure.

As mentioned previously, a significant drawback of the otolith chemistry 'top down' approach to stock structure studies is that lack of significant variation among sample groups can be largely inconclusive. The elemental chemistry of the otolith margin was compared with the aim of detecting whether 'recently' deposited otolith material differed among fishing regions, assuming that the fish from the different regions had remained separated for the recent period of time over which the otolith margin material was precipitated (i.e. group separation is assumed). Significant differences in elemental composition for the otolith margins would indicate that otolith elemental chemistry was sensitive to regional variation in environmental conditions and or other biological effects on otolith chemistry (food, physiology, growth etc.). While this in itself is not a sufficient indicator of stock separation, it indicates whether a lack of variation in the otolith core comparisons is likely the result of a lack of sensitivity of the technique. Furthermore, significant variation in otolith margins (i.e. proof of sensitivity of the technique), but not in otolith cores, when combined with the other information on the distribution of the early life history stages, could indicate that the adults were derived from a similar replenishment source(s) but had become separated later in life (Fowler *et al.* 2005).

Three elements; Mg, Cu and Ba, demonstrated variation among the three fishing regions. However, the only element that showed relatively consistent variation among regions for both cohorts and both otolith sampling positions was Cu. The levels of Cu determined in blue grenadier otoliths were within the ranges reported for otoliths of other species (Campana 1999; Hanson and Zdanowicz 1999; Thresher 1999; Yoshinaga *et al.* 1999; Forrester 2005). For both cohorts, Cu levels were higher in otolith cores of the WTAS samples than those from both the EBS and GAB regions. The source of the significant regional variation in Cu levels of the otolith cores is unclear. There is some evidence that variation in Cu incorporation into otoliths is linked to variation in the amounts of Cu in the water and or the sediments (Milton and Chenery 2001; Forrester 2005). Milton and Chenery (2001) demonstrated, in the laboratory, that higher Cu levels in the water resulted in increased Cu levels in barramundi otoliths, but that higher Cu levels in food did not result in increased levels in the otoliths. The same authors in an earlier paper also suggested that Cu incorporation in otoliths may be related to proteins more so than as substitution for Ca in the aragonite matrix (Milton and Chenery 2000). While there is evidence that ambient Cu levels in the water can influence Cu levels in otoliths, Cu is an essential element for fish health, and due its toxicity, levels of Cu in the bloodstream are highly regulated by the liver (Bury *et al.* 2003). Due to this high level of physiological regulation, Cu incorporation into otoliths is not expected to be tightly linearly related to levels in the ambient water or the food (Campana 1999).

Interestingly, an earlier study by Thresher *et al.* (1992) indicated that the food chain supporting blue grenadier larvae off western Tasmania was not based on phytoplankton, but on microbial decomposition of seagrass detritus that is suspended from coastal seagrass beds and or flushed out of major estuaries along the western Tasmanian coast during winter storms. The largest estuary along the west Tasmanian coast is Macquarie Harbour, which discharges at Strahan immediately adjacent to the major blue grenadier spawning area (Thresher *et al.* 1992). The rivers that drain into Macquarie Harbour include the King River that has received high Cu loads from the Queenstown Cu mine for 100 years (McQuade *et al.* 1995). The water and sediments of Macquarie Harbour itself are known to have exceptionally high Cu levels as a result of the long-term Cu contamination from the King River (Stauber *et al.* 1996; Teasdale *et al.* 1996). Although we have no ambient Cu data for western Tasmanian marine waters there is a potential source of Cu enrichment immediately adjacent to the western Tasmanian spawning grounds. It is plausible that the elevated Cu levels in otoliths of blue grenadier sampled off western Tasmania are related to higher Cu levels in the water or the food chain that are linked to the King River/Macquarie Harbour source, or the catchment area in general.

The consistency of the patterns of Cu variation in otolith cores for both cohorts indicates temporal consistency of the effect, and suggests that the early life history of adult blue grenadier collected in WTAS

occurred in a different environment than the adults sampled from the EBS and GAB areas. While the Cu data were less reliable for the otolith margins due to the lower levels (i.e. large proportion of data below LOD), the pattern of variation among regions was remarkably similar to that observed for the cores, indicating persistently higher Cu levels in the WTAS region than the EBS and GAB regions, and also an ontogenetic effect on incorporation (lower incorporation with age) of this element into blue grenadier otoliths.

Similar to Cu, despite the lack of any clear links between environmental variation and Mg incorporation into otoliths (Martin and Wuenschel 2006; Hamer and Jenkins 2007a), significant variation in otolith Mg was detected among regions. Interpretation of variation in otolith Mg was complicated due to the differing patterns of variation among regions depending on the cohort, and otolith sampling position. For the otolith cores of the 4 year olds there were generally higher Mg levels for the WTAS and EBS samples than the GAB samples, but for the 13 year olds there was less variation, and the largest difference was between WTAS and EBS regions. For the otolith margins of both cohorts Mg was generally higher in the EBS samples, than the other two regions.

Similar to Cu, Mg is an essential element for fish health, but is super abundant in seawater compared to the requirements of fish, and therefore its uptake and levels in body fluids and flesh are also highly regulated (Watanabe *et al.* 1997). Laboratory and field studies have failed to provide evidence that variation in ambient Mg levels is an important influence on variation of levels in otoliths (Martin and Wuenschel 2006; Hamer and Jenkins 2007a). Mg incorporation into otoliths is known to vary independently of variation in water chemistry and or temperature and there is evidence to suggest a link between Mg incorporation and otolith precipitation/growth rate (Martin and Thorrold 2005; Hamer and Jenkins 2007a). The levels of Mg in the otolith core region were more than double those of the otolith margins, irrespective of cohort, and the differences between cores and margins were greater for the 13 year old samples. This pattern clearly indicated ontogenetic variation in incorporation of Mg into otoliths, possibly related to changes in growth and or metabolic rate with age. Subtle variations in otolith growth and or metabolic rates may have contributed to the variation in otolith Mg detected among regions in the current study.

Unlike Mg and Cu, there is substantial evidence across multiple species that variation in otolith Ba is related to variation in the amount of Ba in the water (Bath *et al.* 2000; Milton and Chenery 2001; Elsdon and Gillanders 2003b; Martin and Thorrold 2005; Martin and Wuenschel 2006; Walther and Thorrold 2006; Hamer and Jenkins 2007a). The differences in otolith Ba can therefore be assumed to be indicative of variation in the ambient Ba levels that the fish were exposed to. Ba in marine waters typically shows a nutrient-type profile with higher levels closer to landmasses, freshwater inputs, upwelling zones and in deeper ocean waters, and is generally considered an indicator of productivity (Dehairs *et al.* 1990; Coffey *et al.* 1997; Lea *et al.* 1989; Jacquet *et al.* 2004). Interestingly, while the patterns of otolith Ba variation among regions were very similar between the cohorts for both the otolith core and margin comparisons, the patterns among regions actually differed between the core and margin comparisons. For the otolith cores, mean Ba was higher in the WTAS and EBS samples than the GAB samples, but for the margins it was slightly higher in the GAB samples than the WTAS and EBS samples. Variation among regions was however low, indicative of low variation of ambient Ba levels across the oceanic sampling regions at the residence depths of blue grenadier. Similarity between cohorts in the patterns of variation for the cores, even though the core otolith material for each cohort was deposited 9 years apart, suggests temporal consistency of the variation in ambient Ba levels across the three sampling regions. We therefore hypothesise that the different patterns of Ba variation among regions between the cores and otolith margins are somehow related to shifts in the spatial and or depth distribution between the early juvenile and adult life stages.

While significant ANOVA and MANOVA comparisons among regions would indicate in a 'statistical' sense that the samples from each region were derived from different populations, there was significant overlap in the distributions of the individual and multi-elemental data. The jackknife classification procedure was moderately accurate at assigning individuals to their collection region for the GAB samples irrespective of cohort or otolith sampling position (61% and 67% accuracy for the otolith cores for each cohort, and 74% and 67% accuracy for otolith margins for each cohort). However, classification accuracy varied for the other two regions depending on which cohort and otolith sampling zone was compared. For the EBS region highest classification accuracy (63%) was for the otolith cores of the 4+ age group, and for the WTAS region highest classification accuracy (80%) was for the otolith margins of the 4+ age group.

The interpretation of jackknife classification accuracy rates from otolith chemistry data in relation to inferring stock structure is somewhat problematic. While high levels of classification accuracy (>80%) based on reasonable sample sizes (>20–30 individuals per group) provide strong evidence that the sample groups represent groups of fish with different environmental histories, moderate levels of classification accuracy and/or low sample sizes can make interpretation more difficult. Jackknife classifications are particularly sensitive to low sample sizes when there is overlap among sample groups, and if separation of groups is clear but sample sizes are low, confidence in the degree of 'true' group separation will be low. Another issue often overlooked when interpreting jackknifed classification results in stock structure studies is where individuals from group A are classified with high accuracy, but large numbers of individuals from another group B are also classified to group A (i.e. overall classification accuracy is low). Classification analyses involving otolith chemistry data will commonly involve overlap among individuals from different groups and misclassifications will occur irrespective of highly significant statistical differences (ANOVA, MANOVA). Determining whether misclassified samples are due to mixing among groups or similarities in the environmental histories of individuals, even though they may have been spatially separated, is difficult without other information. When interpreting the classification accuracy rates it is therefore important to consider the distribution of the data for individual fish within and between each group (Campana 2005).

The canonical variate plots of the otolith core chemistry indicated tighter clustering of the samples from the GAB region compared to those from the EBS and WTAS region. This tighter clustering would have been responsible for the higher classification accuracy of the GAB samples (i.e. the individual GAB samples were closer to their group centroid) even though there was overlap with the other regions, particularly EBS. The tighter clustering of the GAB samples may indicate lower environmental variability across the GAB region and/or that the samples came from groups of fish that had remained more closely associated in space and time resulting in individuals experiencing more similar environmental conditions. We find it highly unlikely that the high rates of misclassification and data overlap between the EBS and GAB samples was an indication of mixing between these two regions. This is particularly so for the otolith margin comparisons of the age 4 cohort where 46% of EBS samples were classified to the GAB. Given the young age of the fish and the width of the marginal increment relative to the ablation diameter, sampling during the winter spawning season would mean that for the misclassifications to be indicative of mixing between these regions individuals that had spent their recent summer/autumn in the GAB region would have had to migrate around southern Tasmania to EBS (~2000 km) in a short enough time so that their marginal otolith chemistry remained dominated by material deposited in the GAB region. Such a large-scale migration between the GAB and EBS regions over a short time period is highly unlikely. We suggest that the overlap of marginal otolith composition between these two regions indicates similarity of the environmental conditions influencing otolith chemistry rather than recent mixing of individuals between regions, and that this justified repeating the classification procedure with each of these regions compared separately against WTAS. For these reduced classification analyses there were general improvements in classification accuracy for each region. This was particularly evident for the otolith cores, with high classification accuracy of both GAB and EBS samples against WTAS (78% and 89% respectively 4+/2002 cohort, 79% and 63% 13+/1993 cohort). Classification accuracy of WTAS samples was moderately improved when compared separately to the GAB and EBS regions (54% to 65%). For the margins the reduced classification analysis resulted in higher classification accuracy for all regions for the 4+/2002 cohort (ranging from 73% to 88%), although improvement was less pronounced for the 13+/1993 cohort.

Overall, the results of the otolith chemistry component do not support the existence of one widely-mixing population along the southern Australian continental shelf as has been suggested by earlier genetic studies (Milton and Shaklee 1987). While it is impossible to rule out some mixing among fishery regions, the otolith chemistry data suggests that any mixing, particularly between the WTAS/EBS regions (i.e. the Commonwealth Trawl Sector) and the GAB region, is likely of limited importance in relation to interdependence of replenishment of these fisheries.

Stable isotopes

The comparisons of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ revealed some of the most interesting results. In particular, the consistent pattern of higher $\delta^{13}\text{C}$ in the EBS and WTAS samples than the GAB samples, and the higher $\delta^{18}\text{O}$ in the otoliths of the 13+/1993 cohort from the WTAS region compared to both the EBS and GAB regions.

It has been estimated that approximately 70–80% of the carbon in fish otoliths is derived from dissolved inorganic carbon (i.e. from the water), and that the remaining 20–30% is derived from metabolic sources (i.e. food) (Kalish 1991a; Thorrold *et al.* 1997; Weidman and Millner 2000; Solomon *et al.* 2006). Therefore, although we have no data on $\delta^{13}\text{C}$ in the water in the various regions or in the diet of blue grenadier, the differences observed are indicative of different sources of metabolic carbon (i.e. trophic effects), and or greater enrichment of ^{13}C in the waters around western Tasmania and eastern Bass Strait, than in the GAB region. Temperature variation may have also had a minor effect on the regional variation in $\delta^{13}\text{C}$ of otoliths due to its minor direct influence on carbon isotopic fractionation during deposition of otolith carbonate (Weidman and Millner 2000). It is also plausible that the higher $\delta^{13}\text{C}$ in the Tasmanian otolith samples could be partly related to the influence of seagrass detritus on the planktonic food chain, particularly off western Tasmania, as seagrass has a higher $\delta^{13}\text{C}$ than marine phytoplankton (Thresher *et al.* 1992).

It was interesting that the 13+ age group had higher $\delta^{13}\text{C}$ than the 4+ age group in all regions. Several previous studies have indicated that the $\delta^{13}\text{C}$ of otoliths shows an increasing trend with age that is linked to ontogenetic shifts in diet to higher trophic levels, and changes in the contribution of respired carbon to the otolith carbonate as the metabolic rate declines (Weidman and Millner 2000; Begg and Weidman 2001; Sako *et al.* 2007). There is little information on the diet of juvenile and adult blue grenadier other than the adults in the Tasmanian region are thought to feed predominantly on myctophid fish (Koslow, 1994), and diet of blue grenadier in New Zealand may also include significant amounts of lower trophic level groups such as decapods and euphausiids (Kuo and Tanaka 1984b; Clark 1985). Given the limited temperature variation among regions at the residence depths of blue grenadier, variation in $\delta^{13}\text{C}$ between the GAB and WTAS/EBS regions may be related to differences in the amount of fish versus crustaceans in the diet, and that the differences in $\delta^{13}\text{C}$ between age groups were due to changes in diet or metabolism with age.

We hypothesised that $\delta^{18}\text{O}$ of otoliths would provide an indicator of population structure due to its well documented relationship with water temperature, the constant salinity, and the latitudinal and sea surface temperature variation among the three fishery regions. However, when we collated water temperature data at the actual depths where blue grenadier predominantly occur, it became clear that variation among the three regions was much lower (almost negligible) than expected from initial consideration of the surface water temperatures. While the $\delta^{18}\text{O}$ of the otolith sections appeared to provide an accurate proxy of water temperature integrated over the life of the fish, the regional consistency of the water temperature at depths below 350 m meant that significant variations in $\delta^{18}\text{O}$ otolith among the sampling regions would actually be unlikely assuming similar depth distributions of blue grenadier across the three regions, which was supported by the fishery catch at depth data.

The minimal variation in $\delta^{18}\text{O}$ among regions for the age 4+/2002 cohort was consistent with this expectation, with only slightly, but statistically insignificant, higher $\delta^{18}\text{O}$ otolith (indicative of lower water temperature) for WTAS samples. However, for the 13+/1993 cohort there was a highly significant difference in the $\delta^{18}\text{O}$ of the WTAS samples compared to those from the GAB and EBS regions. The higher $\delta^{18}\text{O}$ in the otoliths of 13+/1993 cohort for WTAS indicated an integrated temperature history of approximately 1°C cooler than the age 4+ samples from all regions, including WTAS, and of approximately 0.6 to 1°C cooler than the age 13+ samples from the GAB and EBS regions respectively. The general trend across all three regions for the older fish to have higher $\delta^{18}\text{O}$ than the younger fish indicated greater time spent in cooler waters with age. While the differences in $\delta^{18}\text{O}$ of otoliths provided strong evidence that the 13+ fish from WTAS had been subject to a different integrated temperature history than the 13+ fish from the other regions, it is difficult to conclude whether this difference was due to the WTAS fish spending more time in deeper waters, but remaining resident off WTAS, or alternatively spending more time at slightly cooler more southerly latitudes. Increased spatial and depth coverage of water temperature data could help clarify which of these two scenarios is most likely.

The approach of analysing $\delta^{18}\text{O}$ from a whole otolith section provides a temperature value that represents an integrated value for the entire life of the individual, and would be influenced by the relative proportions of otolith material deposited at different periods of the life history. Observation of otolith microstructure indicated that for the 4 year old fish the otolith material analysed for stable isotopes would have been dominated by material deposited during the first 1-2 years of life, however, for the 13 year old fish the otolith material would have been dominated by material deposited after maturity (i.e. from age 4-13 years). The fact that the values of $\delta^{18}\text{O}$ otolith are averaged across the life span of individuals would mean that difference in $\delta^{18}\text{O}$ among the two age groups due to shifts in temperature exposure with age would have

been attenuated, and variation with age is likely to be greater than indicated from our comparison of whole otolith sections. High resolution (micro-mill) sampling across the otolith sections is required to compare chronological/ontogenetic $\delta^{18}\text{O}$ variation among fish from the three regions (Weidman and Millner 2000; Begg and Weidman 2001). High resolution $\delta^{18}\text{O}$ sampling across otolith sections could provide information on depth and or spatial movement behaviour of blue grenadier with age that may provide additional information on migratory contingents and population structure within the southern Australian fishery. This additional information could be particularly useful for further clarification of the relationship between the WTAS and EBS fishery regions (see Further Development).

Implications for management

The differences in otolith shape, elemental and stable isotope chemistry between the WTAS and GAB regions support the contention that blue grenadier populations in the Commonwealth Trawl and Great Australian Bight Sectors of the SESSF are likely derived from different stocks and would benefit from separate management arrangements. Importantly, the results of the otolith comparisons are supported by previous studies of larval distributions, drift card releases and modelling of larval advection pathways from the major west Tasmanian spawning grounds. These previous early life history studies indicated that most larval stages are likely to be transported southwards or be retained relatively close to the west Tasmanian spawning area, and in support of the modelling predictions, few eggs or larvae have been sampled immediately north of the west Tasmanian spawning area (Thresher *et al.* 1988; Gunn *et al.* 1989; Bulman *et al.* 1999; Bruce *et al.* 2001; Neira *et al.* 2000). Thresher *et al.* (1988) conducted extensive ichthyoplankton surveys extending continuously from eastern Bass Strait around southern Tasmania to the eastern Great Australian Bight and failed to find blue grenadier larval stages to the north west of King Island, with the greatest concentration occurring off west Tasmania. Similarly, Neira *et al.* (2000) did not report blue grenadier larvae in ichthyoplankton samples collected from Port Campbell (western Victoria) to Port McDonnell (eastern South Australia) in late July 1998. This is despite fishery catch records indicating sporadic catches from various locations extending from western Tasmania along the shelf edge to the western Great Australian Bight. The differences in otolith core chemistry support these previous studies in indicating that blue grenadier in the GAB region are unlikely to be derived from the same spawning (replenishment) source as those off the west Tasmanian coast. Furthermore, the differences in otolith shape and stable isotopes provided supporting evidence that the GAB samples had remained separated from the WTAS samples. Based on the otolith techniques applied in this study and the previous early life history studies there is considerable support for the GABTS to be treated as a separate management unit to the WTAS region of the CTS of the SESSF.

The results of the previous research on larval distributions and dispersal mentioned above, particularly the work by Bruce *et al.* (2001), indicate that it is highly unlikely that replenishment of the GAB region population is dependent on the same spawning source as supports the EBS region. While we found significant variation in some of the otolith parameters among the GAB and EBS regions, there was generally more overlap in the data between these regions than between the WTAS and GAB samples, even though the EBS and GAB regions are geographically further apart. Given the large separation between the EBS and GAB sampling regions, and the unlikely chance of larval/juvenile mixing between these regions we suggest that the overlap of the otolith core chemistry data was most likely due to similarity in environmental conditions rather than mixing between regions (also supported by the overlap of otolith margin chemistry). Based on the water temperature estimates and similar depth distributions of blue grenadier from catch records in these two regions, lack of significant difference in $\delta^{18}\text{O}$ was not surprising. Any link between these two regions would appear to require active migration of older individuals, and due to the shallow nature of Bass Strait relative to the depth distribution of the species, the migration route would apparently require individuals to migrate around southern Tasmania, bypassing the WTAS region, and covering a distance of over 2000 km.

Finally, the relationship between blue grenadier caught in the WTAS and EBS regions is less clear in relation to development of appropriate spatial management arrangements. Otolith chemistry and $\delta^{18}\text{O}$ did show statistically significant variation between the WTAS and EBS sample groups. The difference in $\delta^{18}\text{O}$ between samples of the age 13 cohort for these two regions, but similarity between the EBS and GAB regions was most interesting. If the WTAS and EBS fish were from the same population we would have expected a consistent similarity of $\delta^{18}\text{O}$ among these two regions for both age groups. The higher $\delta^{18}\text{O}$ in the otoliths of

the 13+ age group from WTAS compared to EBS indicates that the WTAS 13+ samples spent more time in cooler waters than the EBS samples. This may either indicate different degrees of movement to cooler more southerly latitudes in the non-spawning season or movement of WTAS fish to deeper waters than the majority of EBS fish. Either way the difference clearly indicates a different environmental history. The variation in both $\delta^{18}\text{O}$ and otolith core chemistry between the WTAS and EBS samples indicate that a simple one stock model for the WTAS and EBS fisheries may also not be appropriate, and points to the need for further research to clarify the degree of connectivity between the fisheries in these two regions.

Earlier larval sampling and dispersal modelling has indicated that spawning likely occurs in the EBS region (Gunn *et al.* 1989; Bruce *et al.* 2001) and genetic studies have indicated some differences between samples collected off eastern and western Tasmania during the spawning season (Milton and Shaklee 1987). However, it is also possible that larvae spawned in west Tasmania could disperse to eastern Tasmania and possibly even to eastern Bass Strait, albeit in low numbers (Thresher *et al.* 1988). Discrimination accuracy between EBS and WTAS was moderate to high based on otolith core chemistry, however, because the laser sampling was not highly specific to the larval phase, differences in core otolith chemistry may still have occurred among WTAS and EBS samples if transport from WTAS to EBS was relatively rapid (i.e. weeks-month). It is possible the adult fish in both areas may originate from the west Tasmanian spawning area, but that migratory contingents develop with age with some adults taking up a migratory route that involves migration to spawning areas off eastern Bass Strait where as others migrate to the main spawning area off western Tasmania. In this situation, while adults recruiting to the WTAS spawning fishery may be largely comprised of fish that originated there, adults recruiting to the EBS fishery may be a mixture of both locally spawned fish and vagrants originating from the major WTAS spawning ground.

While the data from this study support the contention, for management purposes, that the EBS and WTAS fishing regions are not part of one highly mixed stock that extends from west Tasmanian to eastern Bass Strait, the actual level of mixing between the EBS and WTAS regions, and the degree of dependency of the EBS region on replenishment from the WTAS spawning area, requires further clarification. Further study involving high resolution sampling of $\delta^{18}\text{O}$ and trace elements across otolith sections, to produce life-history profiles of chemical variation would be valuable to investigate whether migratory contingents exist, and to develop a clearer understanding of the contribution of spawning in the WTAS region to the fishery in EBS (see Further Development).

Benefits -

The information reported herein will provide fisheries managers and industry with a basis for future consideration of management of blue grenadier in different regions of the SESSF. In particular, the results will help industry and management bodies in refining their data collection and assessment processes and will assist in decision making regarding appropriate management of blue grenadier in the Great Australian Bight Trawl and Commonwealth Trawl Sectors of the SESSF. The results will be of benefit to fisheries scientists and modellers by providing a basis for spatial structuring of modelling and population assessments leading to improved understanding of production trends and impacts of fishing in each of these sectors of the fishery. The industry will benefit by having a clearer understanding that fishing in the Commonwealth Trawl Sector will likely have negligible impact on production in the Great Australian Bight Trawl Sector and vice versa. Finally, the data presented here indicate that connectivity between the western Tasmanian and eastern Bass Strait regions within Commonwealth Trawl Sector may be less than currently thought. The relationship between these two regions is a current concern of industry and managers and the results of this study should provide further support for additional investigation of this issue. Finally the results are of benefit to fisheries researchers through the demonstration of the application of a variety of otolith based measures to improving understanding of fishery stock structure of a deep water oceanic species.

Further Development -

There are three key areas for further development in relation to understanding blue grenadier stock structure. First, we recommend further otolith chemistry and, particularly, $\delta^{18}\text{O}$ investigations of the western Tasmanian and eastern Bass Strait samples. This additional study should involve life-history laser ablation ICP-MS transects of elemental chemistry, and importantly, the use of micro-mill sampling to construct life-history profiles of $\delta^{18}\text{O}$ across otoliths. This additional data could be obtained relatively economically from the samples collected and prepared during the current study. The information from these additional analyses would be valuable in further understanding the relationship between the western Tasmanian and eastern Bass Strait regions of the CTS and also provide information on life history movement patterns in relation to water temperature and depth. Secondly, extension of the earlier genetic studies by additional comparisons between the Great Australian Bight region and the eastern Bass Strait /west Tasmanian regions could help clarify the nature of the stock separation between the GABTS and CTS fisheries. There is also scope to revisit comparisons between the western Tasmanian/eastern Bass Strait regions using more advanced genetic analyses (i.e. mitochondrial DNA). Thirdly, while it appears unlikely that spawning on the west Tasmanian spawning grounds contributes significantly to recruitment into the Great Australian Bight region, there is no information on stock distribution and the locations and extent of spawning in the Great Australian Bight region, and therefore whether or not this region could be important for replenishment of populations to the east, such as the eastern Great Australian Bight region and western Victoria. Despite anecdotal reports of ripe fish across the western Victorian and Great Australian Bight regions there has been no histological study of gonads to confirm the occurrence and extent of spawning across this region and this warrants further investigation. Finally, we were unable to obtain sufficient otolith samples for a thorough investigation of the eastern region of the GABTS, further efforts to collect otolith and gonad samples from this region would allow more detailed comparisons between this region and both the main fishery region in the central GAB and the CTS fishery regions.

Planned Outcomes -

The key planned outcome was to provide new information to assist in assessment of stock structure among the major blue grenadier fishing areas in southern Australia, particularly the Commonwealth Trawl and the Great Australian Bight Trawl sectors of the SESSF. The comparisons of otolith shape, elemental chemistry and stable isotopes all provided evidence that the Great Australian Bight Trawl Sector and Commonwealth Trawl Sector of the SESSF harvest blue grenadier from separate stocks and should be managed accordingly. An additional outcome was the provision of evidence for structuring of blue grenadier populations within the Commonwealth Trawl Sector of the SESSF.

Conclusion -

Otolith based indicators of population structure: otolith shape, stable isotope and elemental chemistry; provided valuable information for assessing fishery stock structure of blue grenadier in southern Australian waters. Variation in each of these parameters was detected among three important regions in the SESSF: western Tasmania, eastern Bass Strait, and the Great Australian Bight. The otolith indicators provided support for separation of the assessment and management of blue grenadier in the Great Australian Bight Trawl Sector and the Commonwealth Trawl. The comparisons also indicated that the western Tasmanian and eastern Bass Strait regions of the SESSF were unlikely to be part of one highly mixed, south eastern Australian stock. The results from this study will provide a basis for future consideration of spatial management of the blue grenadier resource in southern Australia and indicate the need for further consideration of the degree of connectivity between blue grenadier populations to the east and west of Tasmania and Bass Strait.

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Appendix 1: Staff

Otolith elemental chemistry, stable isotopes, data analyses and report writing

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