Spatial structuring and patterns of connectivity among Antarctic toothfish (*Dissostichus mawsoni*) stocks in the Southern Ocean: a view through otolith chemistry

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Abstract

An important prerequisite of sustainable fisheries management is knowledge about the spatial structure of fish populations. Such information provides a basis for understanding population dynamics and connectivity as well as posing questions around a species' resilience to ongoing fishing pressure. For Antarctic toothfish (*Dissostichus mawsoni*), a bentho-pelagic fish species with a spatial distribution that encompasses much of the Southern Ocean south of about (60°S), aspects around population structure and connectivity are still uncertain. The basis of this study was to gain a better understanding of Antarctic toothfish population structuring across spatially discrete fishing areas located around the Antarctic continent. The primary aims were therefore to determine whether patterns of connectivity between these areas were evident and whether source or sink areas for toothfish existed across its Southern Ocean distribution which would be a key understanding towards effective management of toothfish populations. To that end, this study used fish otoliths (ear bones) and laser ablation inductively coupled mass spectrometry (LA-ICP-MS) to determine life history aspects of Antarctic toothfish.

The first research chapter (Chapter 2) tests the efficacy of otolith microchemistry techniques by determining if otolith edge chemistry (corresponding to recent capture) could distinguish toothfish fishery grounds. Fish otoliths were obtained by scientific observers on board longline vessels operating across spatially discrete fishing areas in the Ross Sea (RS), Amundsen Sea (AMS), Southern Atlantic Ocean (SAO) and Southern Indian Ocean (SIO). Based on four elements (Al, Mg, Ba and Sr), significant spatial heterogeneity was shown among most regions indicating the water masses were quite different. The strongest patterns of separation were between the RS and SAO where

significantly lower Sr compositions in the Ross Sea corresponded with a lower salinity water regime consistent with large scale freshening events within the Ross Sea. Conversely, Al. compositions were significantly lower in toothfish from the SAO (Tukey's HSD, $P < 0.001$) than in the Ross Sea, consistent with fish exposure to the Al-depleted source waters in the Weddell Sea, whereas differences in Mg were possibly reflective of physiological effects linked to recent spawning in these regions. Spatial heterogeneity was further evident through quadratic discriminant function analyses (QDFA) with jack-knife classification rates for the Ross Sea (81%) and Southern Atlantic Ocean (79%). However, areas adjacent to the RS and SAO did not show sufficient otolith elemental differences to separate them, possibly the result of regional scale gyres and the Antarctic Circumpolar Current (ACC) mixing the water mass within and across adjoined areas. These findings suggest otolith chemistry can discriminate Antarctic toothfish populations across several spatially discrete fishing grounds throughout the Southern Ocean. It also has the potential to aid in future investigations aimed at discerning the spatial structure of toothfish stocks throughout the Southern Ocean and the extent to which stocks may be connected.

In the second research chapter (Chapter 3), the spatial structure of Antarctic toothfish populations was examined from the otolith nuclei of toothfish collected from the Ross Sea (RS) Amundsen Sea (AMS) Southern Atlantic Ocean (SAO) and Southern Indian Ocean (SIO). ANOVA trace element analyses of the otolith nuclei of 10-year and 14-year-old toothfish indicated the water mass during early growth was quite different at least between the RS and SAO. That the age of fish was the same among group analyses limited any potential for overlapping noise in elemental tags between fish that may have spawned in different years (Elsdon et al 2008).Spatial heterogeneity of Antarctic toothfish during

early growth was consistent with otolith edge compositions where low Al reflected fish exposure to Al-depleted source waters in the Weddell Sea and Mg which was higher in the closely adjoined RS and AMS compared to the SAO and SIO. However, spatial visualisations of the otolith nucleus compositions and agglomerative hierarchical cluster (AHC) analyses using Al, Mg, Sr and Li revealed separation between the RS and SAO that was not as clearly defined as in the otolith edge chemistry of the same fish (Chapter 2). Some individuals, primarily from adjoining areas downstream of the RS (AMS) and SAO, showed patterns of connectivity consistent with transport of larvae from upstream of these areas through the Antarctic Circumpolar Current. This would diminish any unique elemental signatures between areas similar to patterns observed in the otolith edge chemistry of the same fish and suggest the population structure of Antarctic toothfish is more spatially complex than the spatial management units of the fishery. Nonetheless, the indication of spatial heterogeneity between the RS and SAO was still evident suggesting larvae from these areas had close affinities to their respective capture locations highlighting these regions as important source areas for Antarctic toothfish. Overall, these findings provide supporting evidence to the existence of separate Antarctic toothfish populations between the Ross Sea and Southern Atlantic that is supplementary to genetic evidence between these regions. This will provide stronger grounds for fisheries management decisions for Antarctic toothfish stocks within these areas and throughout the Southern Ocean.

In the third study (Chapter 4) otolith natal chemistries of Antarctic toothfish were evaluated to determine whether fish from two small scale research units (SSRU) 88.1C in the Ross Sea and 88.2H in the Amundsen Sea fishery could be differentiated by the trace elemental concentrations in the otolith edge. As a follow on to this, the potential of otolith

nucleus signatures to distinguish stocks among these regions was also investigated. For the elements Mg, Al and Sr, patterns of spatial heterogeneity in otolith edge chemistry was shown. This resulted in the correct classification of 63% of fish overall to their original capture sites. However, discrimination of otolith natal signatures for adult age 17 toothfish using Al and Zn showed greater classification success (79%), compared to the otolith edge, which suggested that the majority of toothfish from SSRU 88.1C and 88.2H show similar patterns of structuring consistent with their known capture location, indicating they may have used different spawning habitats between areas. That adult toothfish in these analyses were of the same age, and likely subject to the same environmental conditions during early growth reduced the influence of indifferent chemistries associated with fish that may have been spawned in different years (Elsdon et al 2008). However, given these analyses only included adult toothfish, further investigations using a larger sample base of both adults and juveniles collected from shelf and slope regions within Ross Sea and Amundsen Sea would provide stronger evidence of structuring between these regions.

In the final research chapter (Chapter 5), life history chronologies of Antarctic toothfish were examined from fish otoliths obtained in consecutive seasons (2012 –2013) from two longline operations within the Ross Sea. Specific chronologies were acquired using laser ablation ICP-MS to determine whether spatial variability in otolith chemistry could differentiate capture location and population structure of Antarctic toothfish in consecutive years. The otolith edge chemistries of adult Antarctic toothfish showed no significant spatial heterogeneity between the Pacific Antarctic Ridge (PAR) and continental slope in either season.

The lack of spatial heterogeneity in the otolith edge chemistry of the same adult age classes contributed to the low discriminatory power and overall classification success between these areas in 2012 (47%) and 2013 (67%). This indicates the environmental conditions within the Ross Sea may be similar between years in these areas. This may have been due to regional scale hydrographic features driving the water mass from the PAR over the Slope and onto the continental Shelf (Locarnini 1994; Budillon et al. 2003) diminishing any distinguishable elemental profiles within these areas and in the otoliths of toothfish.

Nevertheless, despite a lack of spatial heterogeneity among adult otolith edge compositions, significant spatial differences were strongly evident at least among the same subadult age classes in otoliths from 2012. This underscores patterns of variability in environmental conditions between years and age classes for toothfish in the Ross Sea. However, significant spatial heterogeneity in otolith edge chemistry between Slope and Shelf areas for Mg and Ba did not correspond with higher discriminatory power with classification success rates overall in 2012 (47%) and 2013 (55%) further evidence that Slope and Shelf areas are reasonably homogeneous with some seasonal variability. Such a finding will confound the ability to identify the contribution of recruits in one year from adults of unknown provenance in another year (Gillanders 2002) promoting data misinterpretation (Reis-Santos et al. 2012).

This finding would indicate the use of otolith nucleus signatures in assessing connectivity of Antarctic toothfish over different years would be untenable if the same habitat markers associated with spawning areas vary over time. However, smaller sample sizes among 2013 collections may have contributed to the lack of statistical power and further analyses across a broader array of elements is perhaps recommended. Similarly, although fishing

operations across small-scale research units (SSRU) were visited in both years, the same fishing areas themselves were not. This may have diminished more representative sampling comparisons. Also, the otolith nucleus chemistry of adult and subadult toothfish within the Ross Sea showed no significant spatial variability among elements (Al, Mg, Sr and Ba) in either year. This indicated larval growth among both toothfish age classes was much the same within the Ross Sea. Similarly, nonmetric multidimensional scaling visualisations showed no clear separation among adults suggesting that the majority of Antarctic toothfish from the Ross Sea fishery use a common spawning ground. However, one individual from the Slope in 2012 and two from the PAR in 2013 had nMDS distance measures outside the 95% confidence ellipses suggesting these fish may have utilised different spawning grounds.

While these findings support the notion of a single spawning population for Antarctic toothfish within the Ross Sea over consecutive years, the lack of representative sample sizes in 2013, along with the inability to sample the same locations in each year (due to fishing restrictions or variable sea ice conditions) poses some uncertainty around elemental tags, and further investigation is required. Nevertheless, the findings are in line with genetic evidence and similar otolith microchemistry approaches (Smith & Gaffney 2005; Hanchet & Rickard 2008; Kuhn & Gaffney 2008; Ashford et al. 2012). A small proportion of individuals had otolith nucleus chemistries that suggest they utilised different spawning areas outside of the Ross Sea indicating the population structure of Antarctic toothfish is more spatially complex than the more confined fisheries management areas of the Ross Sea.

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1. Introduction

Sustainable fisheries management requires knowledge of the spatial structure of fish populations (Jones 1999). Such information provides a basis for understanding population dynamics and connectivity as well as posing questions around a species' resilience to ongoing fishing pressure (Thorrold et al. 2001). Marine fish populations range from broad and homogeneous to complex and interconnected units (Papetti et al. 2013; Ruzzante et al. 2006). Population units that mix at different life stages in their life cycle pose problems for providing accurate management advice (Stephenson, 1999; Kell and Bromley, 2004; Campbell et al. 2007; Kell et al. 2009). This is largely because there is often a mismatch between actual biologically discrete populations and fishery management units (Iles and Sinclair, 1982; Stephenson, 1999; Frank and Brickman, 2000; Reiss et al. 2009; Cope and Punt, 2011; Ulrich et al. 2013).

The mechanisms that determine population differences and connectivity within marine environments are wide ranging. They include physical forcing (via oceanic currents, fronts, eddies, gyres or salinity and temperature gradients) bio-physical attributes associated with egg buoyancy and larvae swimming capabilities (Iles and Sinclair, 1982; Jørgensen et al. 2005; Cowen et al. 2006) fish behaviour (e.g. spawning site fidelity or straying) and ontogeny (Petitgas et al. 2010, 2013; Secor, 2015). A range of approaches (e. g., morphometrics, tag-recapture, and otolith microchemistry) have proven useful for stock identification (Ihssen et al. 1981, Pawson and Jennings 1996), and seek to identify units that are self-sustaining (Begg and Waldman 1999) and thus directly or indirectly assess the degree of connectivity or genetic dispersal.

This thesis focused on Antarctic toothfish (*Dissostichus mawsoni* – Norman 1937) a large bentho pelagic fish harvested as an exploratory fishery throughout the Southern Ocean south of about 60°S (Gon & Heemstra 1990). The spatial structure of Antarctic toothfish stocks and their connectivity to underlying populations around Antarctica remain uncertain. The present thesis aimed to improve the current understanding around Antarctic toothfish population structure using otolith microchemistry approaches. In the present introduction, an overview of the general biology, ecology, and spatial distribution of Antarctic toothfish within the primary fishing grounds of the Ross Sea are outlined. Then fisheries management and bounded areas of the exploratory fishery throughout the Southern Ocean are then described and underlying gaps around life history and population structure defined. The general concepts of otolith microchemistry principles and application in stock assessment studies are also introduced. Finally, the structure and objectives of the thesis using this approach are revealed.

1.1 Biology and ecology of toothfish *(Dissostichus mawsoni)*

Antarctic toothfish (*Dissostichus mawsoni* Norman, 1937) are a large-bodied bentho-pelagic fish endemic to the sub-zero waters of the Southern Ocean south of about 60°S (Gon & Heemstra 1990), (Fig 1.1). Fish can reach lengths of up to 200 cm, weigh, over 100 kg, swim to depths > 2000 metres, but are mostly found at 1000–1600 m along continental slope areas (Hanchet et al. 2015). Although lacking a swim bladder, adult toothfish are able to occupy pelagic habitats through morphological and physiological adaptations associated with reduced skeletal ossification (Eastman et al. 2014) and increased lipid accumulation (Eastman 1988).

However, juvenile toothfish at a length of about 15 cm are primarily benthic (not buoyant) because they are not buoyant (Roshchin 1997; Near et al. 2003).

Only when Antarctic toothfish reach a standard length (SL) greater than 81 cm do they obtain sufficient lipid stores to become neutrally buoyant enabling them to utilise wider ranges of the water column (Near et al. 2003). Given the differences in habitat between juveniles and adult toothfish, Near et al. (2003) hypothesised that the diets of juveniles and adults should differ significantly as a result of this ontogenetic shift in buoyancy. Consequently, significant differences in toothfish diet over offshore oceanic features (banks, ridges and seamounts) compared to continental slope habitats have been found (Stevens et al. 2014). Although grenadiers (*Macrourus* spp.) appear to be important prey species of toothfish, they are present on oceanic features and over the slope. Other species of importance to adult toothfish on oceanic features, include violet cod (*Antimora rostrata*) and cephalopods with occasional mesopelagic to epipelagic fish and jellyfish. On the continental slope grenadiers, icefish *Chionobathyscus dewitti*), eel cods (*Muraenolepis* spp.), ophiuroids, small coral fragments and stones (accidentally ingested), reflect patterns of benthic foraging (Stevens et al. 2014). For juveniles, over the continental shelf, crustaceans are more common prey items.

The reproductive biology of Antarctic toothfish has been difficult to determine (Parker & Grimes 2010a; Parker & Marriott 2012), but histological assessments on mature toothfish from the Ross Sea indicate the size at which Antarctic toothfish become reproductively mature is around 16 years (132 cm) for females and 12 years (120 cm) for males (Parker & Grimes 2010a; Sutton et al. 2012). Differences in length frequency distribution, sex ratio, fish condition and reproductive

development among toothfish from the northern (north of 70ºS) and southern Ross Sea have been shown (Fenaughty 2006). Length modal distributions in toothfish from the north average ~141 cm for males and 152 cm for females (Fenaughty 2006). Conversely, fish from the south differ from the north showing multimodal distributions that include higher levels of sexually immature fish (Fenaughty 2006). In the north, sexually mature toothfish > 120 cm are more conspicuous with few fish < 100 cm present.

The proportion of females to males varies between regions. Generally, a lower proportion of females to males is evident in the population north of 70°S and a higher ratio of females to males from 70°S southwards (Fenaughty 2006). . With similar unimodal trends and the prominence of adults in developing condition of sexual maturity, and the low proportion of smaller fish (< 100 cm TL) for both sexes, toothfish populations found over the BANZARE Bank in the east Antarctic show comparable patterns to the Ross Sea (Yates et al. 2019). Similarities regarding mean GSI values for males (6.52) and females (7.73) reported in the northern Ross Sea in February (Fenaughty 2006), are also consistent to those on the BANZARE Bank around the same season (Yates et al. 2019).

1.2 Size distribution of Antarctic toothfish in the Ross Sea

Size distribution of Antarctic toothfish within the Ross Sea (Convention Area 88) reflect ontogenetic shifts (Hanchet et al. 2003; Hanchet & Rickard 2008). Juvenile toothfish (< 80 cm) show localised distributions to shallow waters (< 800 m) in areas around the Balleny Islands (north of Cape Adare), along the southeastern Ross Sea shelf (of the Amundsen Sea) and over shelf areas to the west of the Glomar Challenger Basin (Hanchet et al. 2003; Hanchet & Rickard 2008). However, in

thsoutheast Amundsen Sea, juveniles and sub-adults (50–100 cm) are more common along the continental slope and shelf habitats in deeper water (1000–1500 m), (Fig 1.1).

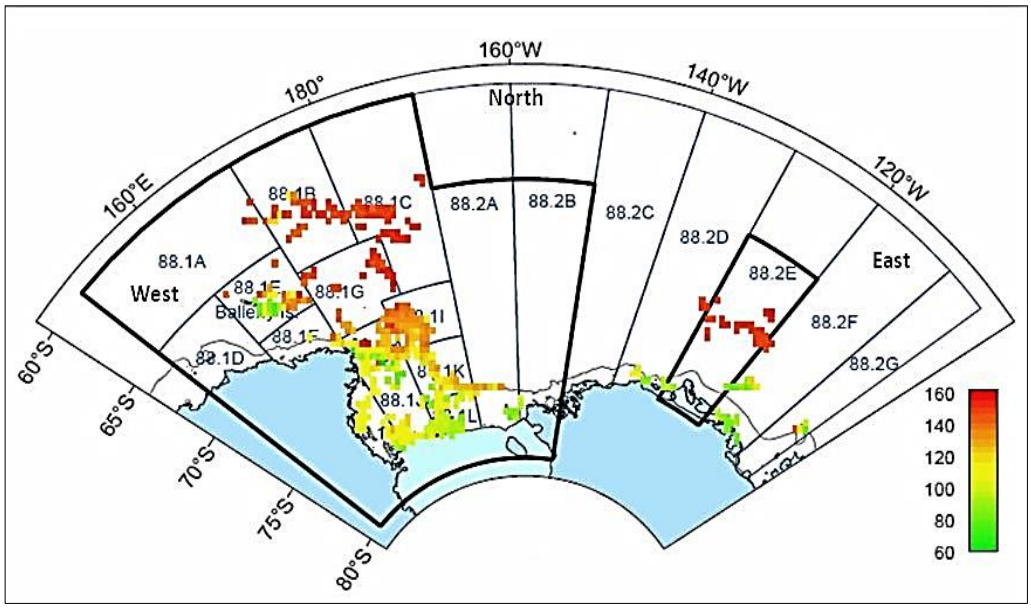

Figure 1.1 Size frequency distribution showing median length (cm) categories (collated data 1997–2010) of Antarctic toothfish caught in Subareas 88.1 and 88.2 (bounded regions), with the grey line representing the 1000 m depth contours (image source: Hanchet et al 2010).

Sub-adult toothfish (80–100 cm) are mostly found on the Ross Sea shelf and around the Balleny Islands. They are also prominent on the Ross Bank (88.1L) Scott Island and in Terra Nova Bay (88.1J) where they are the main size group. Along the continental slope 1000 m depth contour, subadults are spread across the Iselin Bank (88.1H) and Pennell Bank (88.1K), with small pockets of sub-adults found further east in the Amundsen Sea (88.2G). Adult toothfish (100–120 cm) are mostly found in deeper water (> 1000 m) on the continental slope although, some large fish (160 cm) have been caught in shallow depths (600 m) in McMurdo Sound nearer the continent (Ainley et al. 2013). The largest adults are more often collected further

north of the continental slope over the banks, ridges, and hills of the Pacific–Antarctic Ridge (PAR), (Hanchet & Mormede 2010), (Fig 1.1).

1.3 Antarctic toothfish fishery and management

The exploratory bottom longline fishery for Antarctic toothfish has been operating out of the Ross Sea since 1997 (Hanchet & Rickard 2008). New Zealand first established small-scale fishing operations in the region with up to three vessels per year (between 1997–2002) and catches < 1 tonne in 1997 to 1333 tonnes in 2000 (Hanchet et al. 2003). The fishery has since expanded with 13 licensed fisheries and 23 vessels from nine countries (i.e., Australia, Chile, Japan, Spain, Republic of Korea, Russian Federation, United Kingdom, Ukraine, New Zealand, and Uruguay) now targeting toothfish in management areas across most of the subantarctic oceans (CCAMLR 2020a) (Fig 1.2). Fishing operations typically start around December and end around February (austral summer) where the remainder of the year is mostly covered by sea ice limiting access.

Since 1997, Antarctic toothfish catches in the Ross Sea (Area 88.1) had been gradually increasing before stabilising in 2005 at around 3000 tonnes per year (CCAMLR 2013). In 2019, catches in the Ross Sea of 3046 tonnes have been taken by 19 vessels (CCAMLR 2020a), whereas, in the Amundsen Sea (Area 88.2) a total of 753 tonnes were caught by 13 vessels (CCAMLR 2020b) and 376 tonnes in the Southern Atlantic Ocean (Area 48.6) by two vessels (CCAMLR 2020c). The most recent fishing operations for Antarctic toothfish in the Southern Indian Ocean sector (Division 58.4.3b) has been in 2012, where 4 tonnes of Antarctic toothfish were taken as no legal fishing in Division 58.4.3b has occurred since 2012.

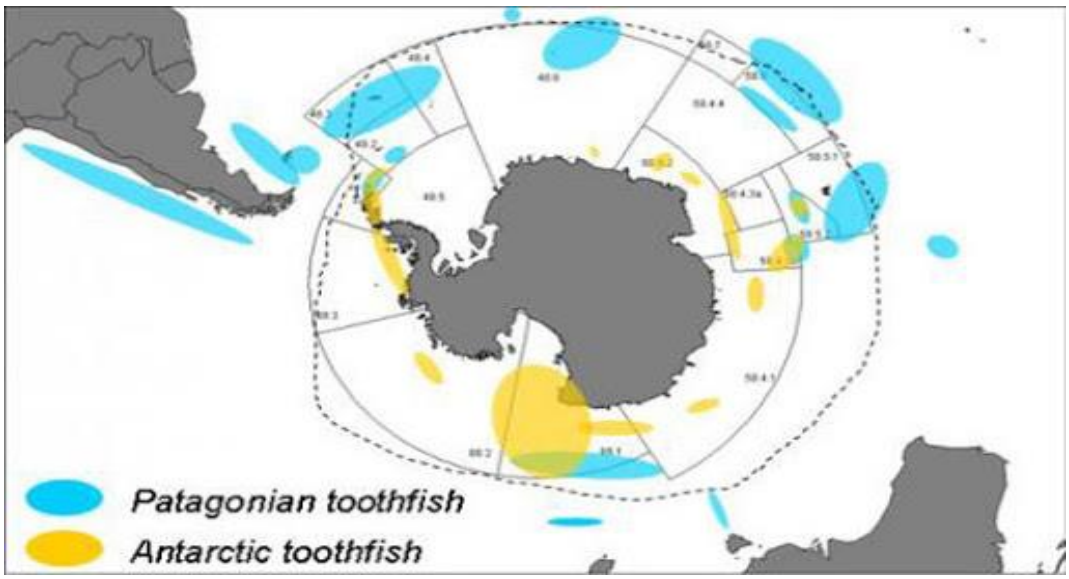

Figure 1.2 Distribution of Antarctic toothfish in relation to fishery management convention areas convention area (Area 48, 58 and 88), including seven exploratory fisheries (map source; NIWA - https://niwa.co.nz/fisheries/research-projects/antarctic-fisheries-research/the-toothfish).

Toothfish fisheries in Antarctic waters include both Antarctic toothfish (*Dissostichus mawsoni*) and its sister species Patagonian toothfish (*Dissostichus eleginoides*), which has a similar Southern Ocean distribution to Antarctic toothfish albeit along a more northerly distribution 55ºS (Fig 1.2), although in some regions both species are known to overlap (Hanchet & Mormede 2010). Toothfish species and mackerel icefish (*Champsocephalus gunnari*) are largely managed under the jurisdiction of the Conservation of Antarctic Marine Living Resources (CCAMLR) established under the umbrella of the Antarctic Treaty (1959) to conserve the marine life of the Southern Ocean surrounding Antarctica. Management of these fisheries follows an ecosystem-based and precautionary approach, and management objectives that balance 'conservation' and 'rational use' of living resources while maintaining existing ecological relationships.

The current CCAMLR rules allow for a long-term reduction of up to 50% over the next 35 years. The status and management of the toothfish fishery is reviewed annually under CCAMLR's Working Group on Fish Stock Assessment (WG-FSA) and the Scientific Committee. The WG-FSA and Scientific Committee draw on available scientific information around harvesting levels and management issues from research outcomes across national programmes (of member countries) and existing data collection programmes.

Monitoring of the fisheries is performed using information reported to the Secretariat during the fishing season under the CCAMLR Scheme of International Scientific Observation (SISO). Member countries involved in the fishery maintain complementary management strategies in areas under their jurisdiction (of the Convention Area), as well as in waters adjacent to the Prince Edward and Marion Islands and Crozet and Kerguelen Islands. The Convention Area where fisheries operate, generally cover the area south of the Antarctic Convergence varying from 60°S in the Pacific Sector to 45°S in the western Indian Ocean Sector), (Fig 1.3).

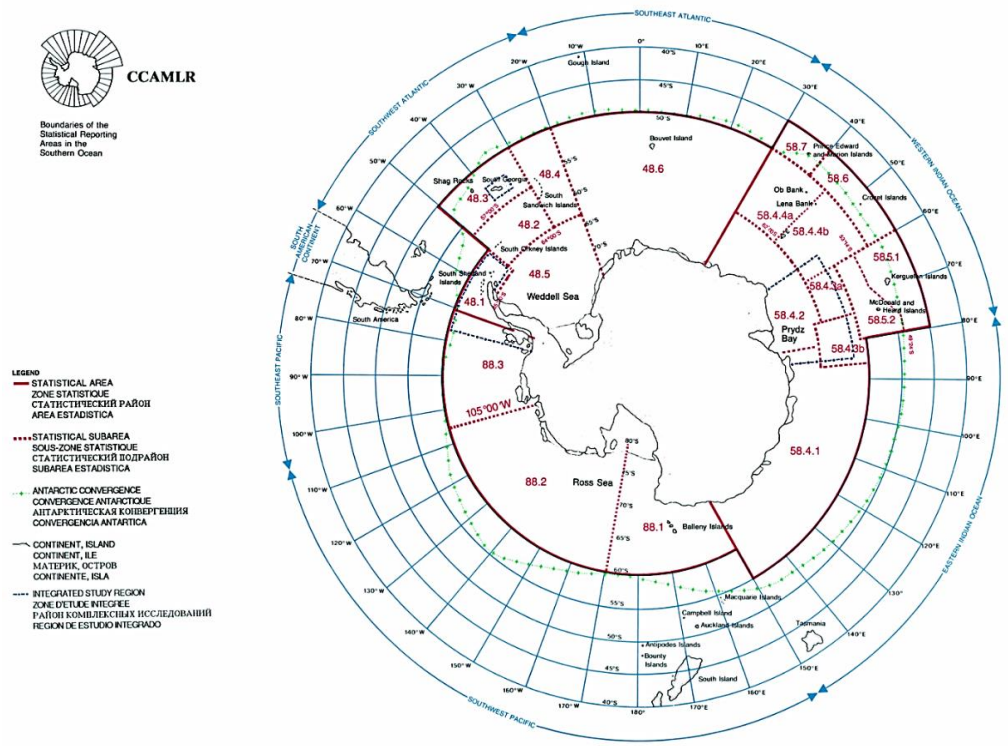

Figure 1.3 CCAMLR convention area management boundaries with small scale statistical sub areas and divisions of the Ross Sea (Area 88) the Southern Atlantic (Area 48) and the Indian Ocean (Area 54). Green dotted line depicts the Antarctic Convergence. Map source: (https://www.ccamlr.org/en/organisation/convention-area).

1.4 Life history of Antarctic toothfish

Knowledge on early the life history of Antarctic toothfish has been particularly elusive (Eastman & DeVries 2000; Fenaughty 2006; Hanchet & Rickard 2008). The challenge in characterising the life history of Antarctic toothfish across its Southern Ocean distribution is that the number and precise spawning locations are not known (Fenaughty & Stevens 2003; Fenaughty et al. 2008; Hanchet & Rickard 2008). However, a hypothetical life history for Antarctic toothfish drawing on reproductive evidence (gonadosomatic data) and a considerable amount of length and age distribution data collected within the Ross Sea fishery (since 1997), was proposed by Hanchet and Rickard (2008) which suggests spawning occurs on the ridges and

banks of the Pacific–Antarctic Ridge during winter and spring from June to November, peaking around September. Depending on the precise location of spawning, eggs and larvae become entrained within the Ross Sea gyres and be exported either west settling out around the Balleny Islands north of Cape Adare and adjacent Antarctic continental shelf, south onto the Ross Sea shelf, or eastwards with the eastern Ross Sea gyre settling out along the continental slope and shelf to the east of the Ross Sea in the Amundsen Sea (Subarea 88.2). As juveniles grow, they move west back towards the Ross Sea shelf and into deeper water (> 600 m) while gradually moving northwards as they mature, feeding in the slope region in depths of 1000–1500 m, where they gain condition before moving north onto the Pacific–Antarctic ridge to start the cycle again (Hanchet & Rickard 2008). Spawning fish may remain in the northern area for up to 2–3 years (Hanchet & Rickard 2008) before moving southwards back onto the shelf and slope where productivity is higher, and where food abundance is greater to regain condition before moving back north to spawn (Fig 1.4).

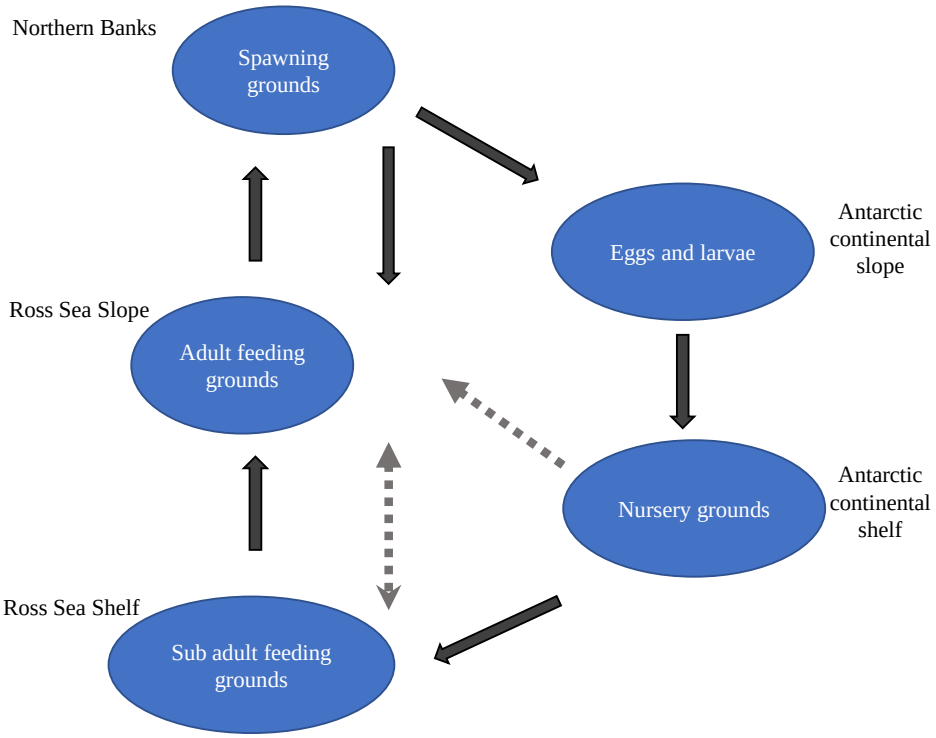

Figure 1.4 Hypothetical life history of Antarctic toothfish (*Dissostichus mawsoni*) in the Ross Sea region redrawn from Hanchet et al (2008).

1.5 Population structure

Several studies have reported uncertainty around the population structure of Antarctic toothfish around the Antarctic continent (Smith & Gaffney 2005; Kuhn & Gaffney 2008; Mugue & Petrov 2014). For example, using a combination of mitochondrial DNA (mtDNA) analyses and nuclear DNA introns, Smith and Gaffney (2005), found no population differentiation between Antarctic toothfish collected from the South Shetland Islands (Subarea 48.1), the Ross Sea Dependency (Subarea 88.1), and Australian Antarctic Territory, division 58.4. However, Kuhn and Gaffney (2008), used mitochondrial and nuclear single nucleotide polymorphism (SNP) techniques on fish collected from the same regions. In their study, genetically distinct populations of Antarctic toothfish were reported between the South Shetland Island, Ross Dependency, and the Australian Antarctic

Territory. Structuring between these areas were attributed to cyclonic circulations associated with the Weddell Sea and Ross Sea Gyres acting as physical barriers to migration (Parker et al. 2002; Smith & Gaffney 2005) as a well as patterns of natal site fidelity which would facilitate population subdivision in Antarctic toothfish (Kuhn & Gaffney 2008).

Following on from these studies, Mugue et al. (2014) found methodological inconsistencies in the assessment of polymorphism at a number of loci reported by Kuhn and Gaffney (2008) such that conclusions about genetic isolation of Antarctic toothfish in the Ross Sea should be considered with caution. More recently, chemical markers in otoliths (fish ear bones) have been used as a means to resolve indifferences between genetic approaches (Ashford et al. 2006; Rooker et al. 2007). Moreover, the combination of both genetics and otolith chemistry has highlighted their potential as powerful tools for fisheries management (Milton & Chenery 2001; Miller et al. 2005; Ashford et al. 2006; Feyrer et al. 2007; Rooker et al. 2007), with otoliths enabling particular temporal or spatial events that have occurred throughout a fish's lifetime to be determined (i.e., spawning, migration and growth), (Edmonds et al. 1992; Gillanders & Kingsford 1996; Swearer & Shima 2010).

1.6 Otolith microchemistry

The incidence of studies that have used fish otoliths to assess population structure has notably increased over the past decade (Edmonds et al., 1989; Kalish, 1990; Thresher et al., 1994, Shima and Swearer 2009; Thorrold and Swearer, 2009). The basis of these studies is founded on the principle understanding that dissolved trace elements in the water may become chemically bound to the otolith matrix in chronological sequence (Campana and Thorrold, 2000), and are not altered or

resorbed during periods of low growth (Campana and Nielson 1985). The composition of trace elements in otoliths can therefore be a reflection of the water chemistry experienced by fish throughout their life (Fowler et al., 1995; Gallahar and Kingsford, 1996). However, a growing number of studies have revealed that some elements (i.e., potassium and iron) are subject to physiological constraints (Kalish, 1991, Sinclair 2005; Trudel et al. 2010; Sturrock et al. 2012) or include minor contributions from diet, demonstrating that their relative contribution to otolith chemistry will vary among elements (Walther and Thorrold 2006; Doubleday et al. 2013). For this reason, knowledge of how exogenous and endogenous factors affect otolith chemistry is essential to reconstruct the life history events and the migration pattern of fish species (Elsdon et al. 2008; Reis-Santos et al. 2013). Otoliths provide chemical markers that can be a useful stock discriminator for marine and estuarine fish (Edmonds et al., 1989; Kalish, 1990; Thresher et al., 1994), or identify the contribution of individual fish to different nursery habitats as adults (Edmonds et al., 1992; Gillanders and Kingsford, 1996; Rooker et al., 2001; Swearer and Shima, 2010).

1.7 Research aims and rational

The aim of this research was to use otolith chemistry techniques and laser ablation inductively coupled mass spectrometry (LA-ICP-MS), to assess the population structure of Antarctic toothfish across fishing areas in the Southern Ocean. This study aimed to determine (i) if trace elements in Antarctic toothfish otoliths can be suitable for discriminating among possible stocks of this species across spatially discrete fishing areas around the Antarctic continent and whether they can be useful in identify patterns of connectivity between fishing areas. At finer spatial scales, a preliminary investigation on the structure of populations and patterns of

connectivity between the primary fishing ground in the Ross Sea and adjoining Amundsen Sea were evaluated. Similarly, to assess the efficacy of otoliths as environmental habitat markers and population identifiers across different years, a temporal study (iv) of Antarctic toothfish in main fishery of the Ross Sea was conducted.

1.8 Thesis outline

This thesis comprises four research chapters (Chapters 2–5), all of which follow a common goal of better understanding aspects of Antarctic toothfish population structure and connectivity and at identifying the variability of otolith chemical compositions at finer spatial and temporal scales.

To that end, the first chapter provides a general introduction to Antarctic toothfish biology and ecology followed by an outline of how the chapters contribute to the overall research aims. Chapter 2 presents findings around the utility of otolith elemental signatures corresponding to recent growth (otolith edge) in discriminating the capture locations of Antarctic toothfish verifying the efficacy of this approach. Chapter 3 retrospectively examines the otolith nucleus signatures of the same fish from Chapter 2 and identifies whether fish collected from the same sample areas shared common spawning grounds. Chapter 4 was a preliminary investigation of adult Antarctic toothfish from two adjoining regions, the Ross Sea and Amundsen Sea to determine the relative composition of individuals that were likely to have shared common spawning areas. In Chapter 5, the temporal variability of otolith elemental compositions among Ross Sea fish collected in different years was examined to test the efficacy of otoliths as consistent habitat markers, and therefore useful population markers. Chapter 6 presents a discussion around

primary outcomes of each chapter with regards to understandings around the spatial structure and connectivity of Antarctic toothfish populations around the Antarctic continent.

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2. Otolith chemistry reveals spatial structuring among Antarctic toothfish (Dissostichus mawsoni) populations in the Southern Ocean.

2.1. Abstract

Knowledge of the spatial structure of fish populations and connectivity among early life stages is important to understand recruitment and provides a geographical context of dispersal from spawning areas. To examine the spatial structure of Antarctic toothfish (*Dissostichus mawsoni*) populations from discrete fishing areas around the Antarctic continent, otolith micro–chemical assays using laser ablation inductively coupled mass spectrometry (ICP-MS) was used to determine if trace element-Ca compositions in the otolith edge (corresponding to recent capture), could distinguish toothfish capture locations.

Fish otoliths were collected by scientific observers onboard CCAMLR sanctioned longline vessels operating in the Ross Sea (RS), Amundsen Sea (AMS), Southern Atlantic Ocean (SAO) and Southern Indian Ocean. Based on four elements (Mg, Al, Sr and Ba), significant univariate ANOVA analyses that were independent of growth effects, revealed significant spatial variability in element-Ca compositions of Mg, Al and Sr in toothfish between locations. Tukey's HSD pairwise comparisons showed significant differences in Mg between Ross Sea and SIO fish compared to fish from the AMS and SAO, may be linked to physiological changes consistent with mature fish caught near spawning grounds over the PAR whereas significantly lower compositions of Al in SAO fish compared to all other locations

reflected exposure to Al depleted source waters of the AAIW which forms in the Weddell Sea. Significantly high compositions of Sr in SAO and SIO toothfish were explained by lower salinity in Ross Sea and AMS fish due to decadal freshening events and the prominence of low salinity shelf water in these regions. The discriminatory power of multi-element signatures between these areas was supported by high jack-knife classification rates for the Ross Sea (81%) and Southern Atlantic Ocean (79%) with moderate classification for the AMS (57%). The SIO did not reflect sufficient spatial variability in elemental signatures to enable meaningful discrimination of toothfish from this area. This was possibly due to hydrographic features (oceanic gyres) and the Antarctic Circumpolar Current (ACC) mixing within and across these regions. These results suggest otolith chemistry is an effective tool in discriminating Antarctic toothfish populations across several spatially discrete fishing grounds throughout the Southern Ocean. This finding will aid in future investigations aimed at discerning the structure of toothfish stocks and extent to which they may be connected using otolith microchemistry techniques.

2.2. Introduction

The chronological properties of fish otoliths as chemical recorders of habitat use throughout a fish's life is a characteristic that fish ecologists often exploit to better understand individual life histories or population linkages (Patterson et al. 2004; Longmore et al. 2014; Morat et al. 2014). The utility of otoliths in this regard is promising given the limitations of conventional tagging approaches in estimating connectivity rates among life stages throughout time and space (Gillanders 2005). This is particularly true in fully marine species where larval, or nursery origins of adults are seldom known. Instead, the chemical constituents in fish otoliths derived from the water mass that fish occupied (Campana et al. 1999) provide an unaltered elemental fingerprint (Campana et al. 1999; Campana 2001; Grønkjær 2016; Chung et al. 2019).

Although the use of otoliths as habitat markers is dependent on each spatially separated population sampled displaying unique elemental signatures (Gillanders 2005; Elsdon et al. 2008) it is not necessary to understand the processes generating such differences provided they are distinguishable among locations of interest (Elsdon et al. 2008). As a consequence, many studies have shown that differentiation of fish from geographically separated areas is possible (Edmonds et al. 1992; Patterson et al. 1999; Rooker et al. 2001), or that habitat utilisation and early life history movements can be traced (Ashford & Duhamel 2005; Elsdon & Gillanders 2005; Ruttenberg et al. 2008; Longmore et al. 2014).

Antarctic toothfish (*Dissostichus mawsoni* Norman, 1937), are a large bodied bentho-pelagic predator of the Antarctic waters typically found along continental

shelf, and slope areas south of about 60ºS (Gon & Heemstra 1990; Eastman & DeVries 2000). Age at maturity for Antarctic toothfish is 13 years for males and 17 years for females, with mature fish not spawning every year (Eastman & DeVries 2000; Fenaughty et al. 2008; Parker & Grimes 2010b). While aspects of Antarctic toothfish's size distribution (Roshchin 1997; Hanchet & Mormede 2010; Petrov & Tatarnikov 2010), biology (Yukhov 1971; Kiss et al. 2004; Nicodemus-Johnson et al. 2011; Gordeev et al. 2014; Petrov & Gordeev 2015), feeding ecology (Petrov & Tatarnikov 2011; Roberts et al. 2011; Stevens et al. 2014) and genetic structure (Smith 2001; Parker et al. 2002; Smith & Gaffney 2005; Kuhn & Gaffney 2008) have been well studied, information pertaining to their early life history and population structure has been particularly elusive (Eastman & DeVries 2000; Fenaughty 2006; Levin 2006; Hanchet & Rickard 2008). This is largely due to spawning occurring during winter when the grounds are covered in sea ice (Hanchet & Rickard 2008).

The basis of this study was that variations in water chemistry across the different Southern Ocean basins where Antarctic toothfish occur would exist and therefore yield spatially distinct and locally stable patterns in otolith microchemistry. The utility of this approach requires knowledge on whether distinct habitat signatures exist. If it does, then quantifying the relative contribution of natal sources to adult populations may then be possible as a stock identifier, which in turn would have important implications regarding management of this fishery.

2.3. Materials and methods

2.3.1 Fish otolith collections

Antarctic toothfish otoliths were collected by scientific observers onboard bottom longline vessels operating in CCAMLR Statistical Subareas (SS) SS.88.1 of the Ross Sea (RS) and SS.88.2 of the Amundsen Sea (AMS) during the 2011–2012 austral summer (December–February) and in SS.48.6 of the Southern Atlantic Ocean (SAO) and Division (D) D.58.4.3 of the Southern Indian Ocean during May 2012 (Fig. 2.1, Table 2.2). Fishing operations were conducted within the RS and AMS by three New Zealand vessels (Aotea II, San Aspiring and Janas).

Collections from the Astrid and Khonen Ridges and within the Ekstrom Basin of the SAO were carried out by a Japanese vessel the Shinsei Maru III which also fished in Division 54.4.3B over the BANZARE Bank southwest of Heard Island in the Southern Indian Ocean (Fig. 2.1C & D). Fishing operations around the same time between oceanic basins was not possible given only a small number of vessels were operating around Antarctica and had to travel great distances between fishing areas (Table 2.2). Similarly, sea ice melt dynamics between areas limited or delayed access to fishing grounds, further adding to time lags in sampling. As a result, it is possible that temporal differences in sampling may lead to discrepancies in the ability of otolith edge chemistry to distinguish capture sites.

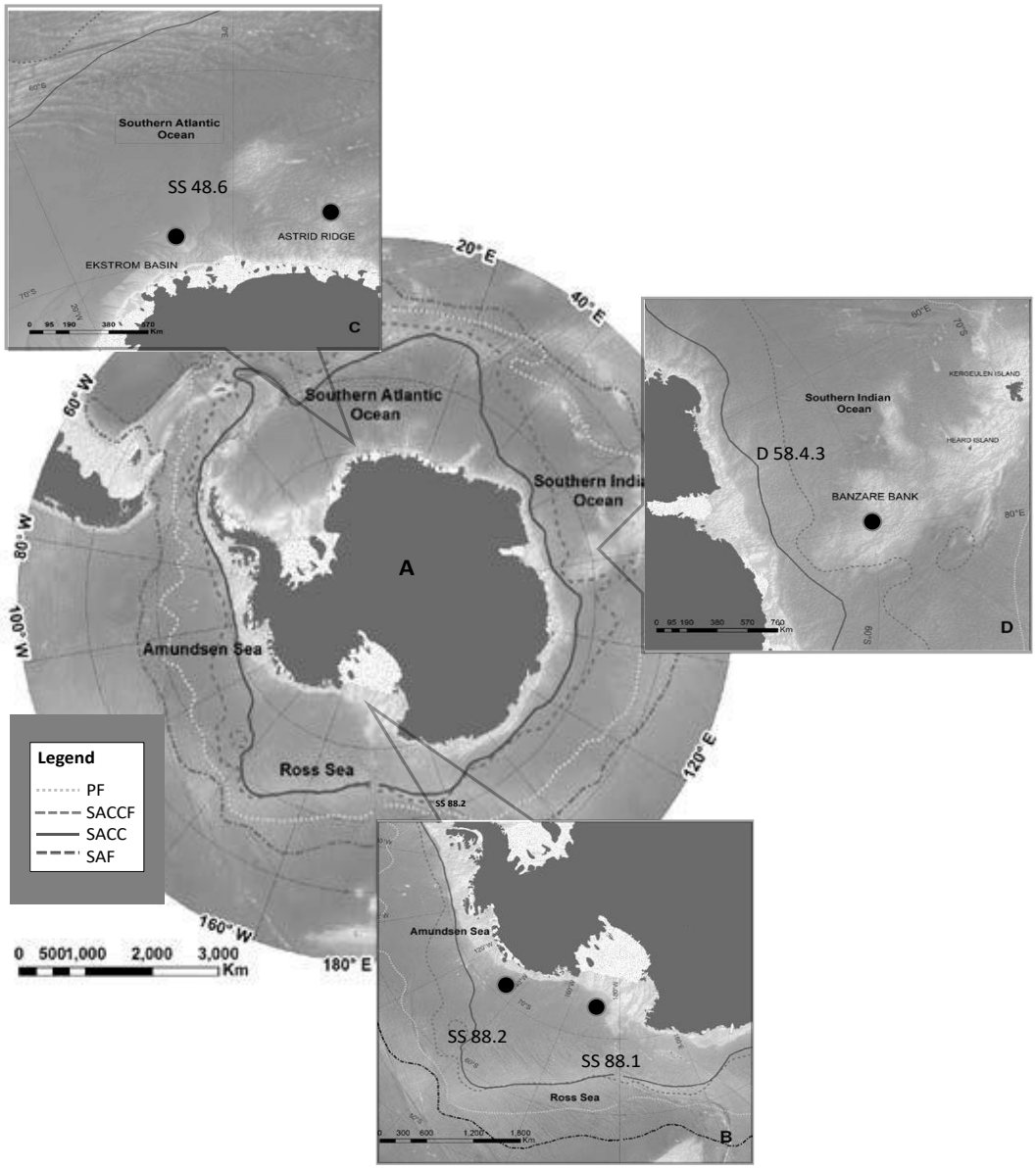

Figure 2.1 CCAMLR convention areas (A) where fishing was carried out within (B) the Ross Sea (SS.88.1), (C) Amundsen Sea (SS.88.2), (D) Southern Atlantic Ocean (SS.48.6) and (D) BANZARE Bank in Division 58.4.3 during the 2011–2012 austral summer. Lines depict oceanic circulations around Antarctica including the Polar Front (PF), Southern Antarctic Circumpolar Current Front (SACCF) Southern Antarctic Circumpolar Current (SACC) and Sub-Antarctic Front (SAF). Black circles represent sample areas.

Nevertheless, the expectation here is that within region differences in environmental conditions will be strong enough such that differentiation of toothfish would still be applicable at broad circumpolar scales. Fish otoliths were stored in paper envelopes

and labelled with catch data for each fish including total length (TL) measurements (cm), weight (g) and sex before being couriered to the University of Waikato.

2.3.2 Otolith cleaning and preparation

Otolith handling and preparation was carried out using acid washed plastic utensils soaked in a 10% HNO_3 over 24 h. Sagittal otolith pairs were cleaned in 19 mm glass scintillation vials topped up with ultrapure Milli-Q water and run through an ultrasonic bath for 4 min. Vials were drained and a 3% solution of H_2O_2 was added, left to sit for ~3 min to remove connective tissue, triple rinsed in Milli-Q water and sonicated for 3 min. Otoliths were dried in a laminar flow cabinet over 24 h prior to mounting. Cleaned fish otolith collections across all sample locations for the 2012 fishing season were randomly selected for mounting on petrographic slides. Only right sagittal otoliths were used for chemical analyses except where only one otolith was available (Fig 2.2).

Otoliths were embedded in silicon moulds using Nuplex® K36 epoxy resin with the distal surface facing up. Resin blocks were air dried for 24 h prior to sectioning. Transverse sections were made through the primordia using a low speed Isomet diamond cutting saw (Buehler–USA) fitted with two blades spaced 250–300 µm apart. Each thin section was polished submerged within Milli-Q water to expose the otolith nucleus using a sequence of 2400 then 4000 µm grit wetted carbide paper (Struers), after which the Milli-Q water was replaced. Accuracy of polishing was determined using a Nikon SMZ645 (C - W 10 x A/22) stereo microscope whereby ring structures radiating from the outer growth zones were gradually removed exposing the nucleus just short of the otolith surface. Sectioned otoliths were glued to small 50 x 25 mm petrographic slide with 20-30 sectioned otoliths mounted per

slide using Crystalbond^{TM}509 thermo-setting adhesive, rinsed in Milli-Q water, then sonicated for 4 min, before a 2% HNO_3 acid solution was added and left to rest for ~15 s. The acid was drained, and the slide triple rinsed in Milli-Q water and sonicated for 2 min, dried in a laminar flow cabinet, and stored in cover slide container ready for laser ablation.

2.3.3 Laser ablation ICP-MS optimisation

Acquisition of trace elements in the otolith edge of Antarctic toothfish were assayed using a system consisting of a Perkin Elmer DRCII ELAN 6000 ICP-MS (Waltham, MA) coupled to a New Wave Research UP-213 nm Nd-YAG laser ablation system (Fremont, CA), which was operated at the University of Waikato, New Zealand. Analytical settings, running conditions and selection of trace elements for this study are summarised in Table 2.1, and were determined through a preliminary analysis of Antarctic toothfish (*Dissostichus mawsoni*) and Patagonian toothfish (*Dissostichus eleginoides*) otolith samples collected by NZ longline vessel operating in the Ross Sea during the 2009–2010 austral summer (Fig 2.2). Laser ablation analyses using a broad suite of elements and operating conditions (spot size, power settings, repetition rates etc) were carried out to determine optimum yields and baseline detection as well as utilising elements from similar studies (Ashford et al 2010, 2007).

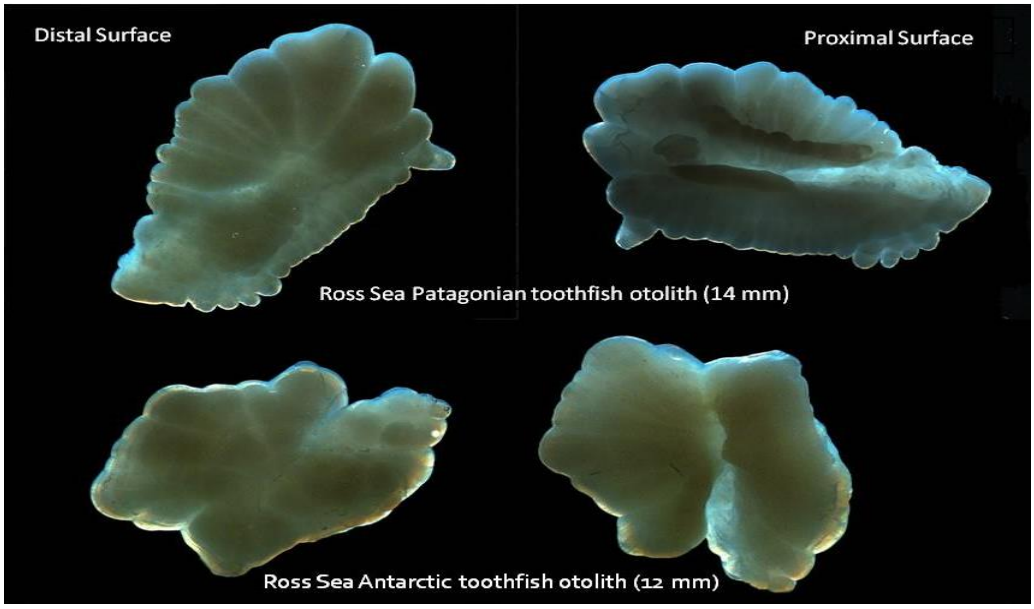

Figure 2.2 Images of cleaned Patagonian toothfish (*Dissostichus eleginoides*) and Antarctic toothfish (*Dissostichus mawsoni*) otoliths extracted from toothfish from the Ross Sea fishery (Photo R Tana).

Prior to sample analyses, the ICP-MS was optimised using a helium (He) and argon (Ar) carrier gas configuration. A suite of 12 isotopes were initially analysed both in otoliths and National Institute of Standards and Technologies standard reference material (NIST612) within which the concentration values of 50 elements have been reported but see Pearce et al. (1997). However, based on preliminary analysis, only eight of the 12 elements were consistently above the minimum detection limits (MDL) with remaining elements excluded. To account for elemental interferences produced by the presence of oxygen in the ICP-MS, carrier gas transport lines or on samples, oxide production in the ICP-MS was monitored by measuring ThO^+/Th^+ ratios during continuous ablation of NIST612 standard reference material. The same NIST standard was analysed with a single slide of 20–30 samples (randomly selected from a block of slides) using a continuous line scan set at a repetition rate of 20 Hz, output power of 60%, spot size of 60 µm for an acquisition time of ~4

min. As Th^+ has a high affinity for oxygen, the ICP-MS was tuned by manually adjusting the nebuliser gas flow to give a ThO^+/Th^+ ratio of approximately 1%.

Table 2.1 Laser ablation ICP-MS instrument and operating conditions as well as data acquisition parameters for multi-element analysis of Antarctic toothfish otoliths collected from the RS, AMS, SAO and SIO during 2011–2012 collections.

Description	Value
ICP-MS System	ELAN Perkin Elmer 6000
Nebulizer Gas Flow [NEB]	0.66 litres min^{-1}
Gas configuration	Argon–Helium
Auxiliary Gas Flow	1.2 litres min^{-1}
Plasma Gas Flow	15
Makeup Gas Flow	1.0 litres min^{-1}
Lens Voltage	7.25
ICP RF Power	1350 watts
Dwell time	15 ms^{-1}
Mass range	3–200
RF power	1350 watts
Laser probe System	New Wave Research
Laser type	Nd:YAG probe
Acquisition mode	Q–switched
Wavelength frequency	213 nm
Analog Stage Voltage	–1850
Pulse Stage Voltage	1100
Scan type	raster
Repetition rate	20 Hz
Beam diameter	25 µm
Output power	60%
Isotopes monitored	^{7}Li, ^{27}Al, ^{25}Mg, ^{42}Ca, ^{43}Ca, ^{55}Mn, ^{66}Zn, ^{88}Sr, ^{137}Ba
Calibration	
Standard reference material	NIST612
Internal standard	^{42}Ca

Once this was achieved it was assumed that all other oxide interferences were negligible (Lichte et al. 1987) and otolith sample analysis could begin. Similarly, sensitivity checks were carried out by monitoring Th^+/U^+ ratios until counts were > 20,000 cps. For optimal sensitivity, nebuliser gas flow rates as well as the lens

position were adjusted manually on the ICP-MS and were generally acceptable around 0.66 litres min^{-1} (Table 2.1). Elemental concentrations (ppm) from NIST612 reference material were internally standardised using ^{42}Ca by way of GLITTER data reduction software (Version 4.4.1, Macquarie Research Limited© 1991–2000). The laser was operated in Q-switched time resolved mode with an average energy reading of 0.0734 mJ, a scan speed of 15 μm s^{-1} to enable scanning of the full suite of elements more closely in time and a repetition rate of 20 Hz (Table 2.1). Beam diameter was set at 25 μm with the laser fired at 60% power, while travelling in a rastar configuration along the otolith edge for a distance of ~700 μm.

Prior to each sample ablation background intensity readings of residual material settled within the sample chamber, transport lines, and ICP-MS were recorded and subtracted online using GLITTER. Variability in laser signal intensities over prolonged periods of use for elements were accounted for by analysing NIST 612 standard reference material twice before otolith sample analysis, once every seven samples and twice at the end of the run for each individual slide. Similarly, once a sample slide was completely assayed, it was removed and replaced with another slide and the sample chamber was purged for 60 min to clean any residual material from the ablation chamber/lines and ICP-MS before standard and sample analyses could begin. Replicate standard analyses were interpolated by GLITTER across samples enabling correction within mounted sample slides and between day variations in instrument sensitivity (drift) across the atomic mass range. Agreement of elements in each standard run were monitored in real time against reported NIST612 values for each element and where readings were not in good agreement

standards were run again. Relative standard deviations (RSD%) of elements from repeat NIST612 standard analyses (N = 81), within and between days were 3.8% (^{7}Li), 3.7% (^{25}Mg), 1.3% (^{27}Al), 1.09% (^{43}Ca), 1.6% (^{55}Mn), 4.1% (^{66}Zn), 1.1% (^{88}Sr), and 4.7% (^{137}Ba).

Similarly, energy output readings (mJ) for each otolith and standard ablation were recorded and assessed against real time trace plots to determine if fluctuations in power might correspond to changes in concentration yields or via artefacts of the laser. Any major fluctuations resulted in re-runs of the analysis. This approach differs from other data reduction software packages (e.g., Iolite) in that review of data immediately after ablation as concentrations ppm with background subtracted was possible, whereas for other programs this is only possible after offline processing when the entire run is complete. Final output data from individual ablations were acquired for each element as an average reading of the peak laser signal (elemental concentrations in ppm). Mean minimum detection limits (MDL) at 99% confidence were calculated within GLITTER using background readings and Poisson counting statistics (Griffin et al. 2008). Similarly, data selection software in GLITTER were used to review and select time-resolved laser signals for each element enabling 100% readings for all elements used in this study.

2.3.4 Age estimation

Age estimates were conducted on the left sagittal otolith for the purpose of consistency in reading similarly orientated otoliths within a resin block. This was in accordance with standardised protocols developed by Sutton and Horn (2010) for Antarctic toothfish as part of the Scientific Committee CAMLR work plan for 2010

(SC-CAMLR-FSA 2009, paragraphs 9.4–9.8). Similarly, the manual represents protocols used by the National Institute of Water and Atmospheric Research Limited (NIWA) for estimating age of Antarctic toothfish collected from the Ross Sea (Sutton & Horn 2010). Otoliths were baked in a preheated Contherm Thermotec 2000 oven at 285 ºC for approximately 15–20 min until a dark brown colour, left to cool at room temperature then embedded in Nuplex® K36 epoxy resin with the distal surface facing up.

Transverse sections were made down the primordium of each otolith using a low speed (3–4) Isomet diamond cutting saw (Buehler-USA). Sectioned otolith blocks were then polished using a sequence of SIC-2400 followed by 4000 µm grit wetted carbide paper (Struers) to expose the primordium for age reading. Otolith age readings were carried out under a stereo microscope (40x magnification), with additional incident light from an external source. Prior to reading, a thin layer of paraffin oil was brushed over the otoliths to hide fine scratches for improved readability. A sequence of opaque and translucent zones constitutes a single annulus (Campana 1999; Sutton & Horn 2010), with multiple annuli radiating from the primordium to the otolith edge reflecting overall age. All areas of the otolith were examined to find the clearest patterns of zonation. However, readings were generally made on the ventral portion of the otolith, on the proximal surface or near the sulcus. Otolith readings were conducted twice by a single reader with precision between readings assessed using the average per cent error (APE) (Beamish & Fournier 1981) and mean coefficient of variation (Chang 1982) approach.

2.3.5 Data analysis

Ontogenetic changes can result in morphological and physiological modifications within fish that can influence otolith elemental compositions among some fish species (Fowler et al. 1995; Macdonald et al. 2020a), but not others (Daverat et al. 2005). To account for growth effects, univariate analysis of covariance (ANCOVA) using a general linear model approach was carried. This was conducted only after the homogeneity of slopes assumption ($P > 0.05$) was met. A significant length effect was identified for Sr:Ca among capture locations (ANCOVA, $P < 0.001$) and was corrected for by subtracting the common linear slope (Edmonds et al. 1992). The correction AC = C-L, where AC = the correlated concentration, adjusted for fish length; C = the concentration of a given element (ppm) for a fish of fork length L and r = the regression coefficient or the "common slope" for the covariate length. After correction of the length effect, all size classes were pooled in analyses. Quadratic discriminant function analysis (QDFA) was used to explore the utility of multivariate normal otolith edge signatures in correctly classifying Antarctic toothfish to their capture locations taking into account prior probabilities (the proportion of each category in the training set). QDFA was preferred over linear models because the null hypothesis that observed variance/covariance matrices of the dependent variables be equal across groups was not met (Box test, $\chi 2 = 43.7$, df =30, $P < 0.0001$).

Prior to using QDFA, multivariate outliers were removed using plotted robust squared Mahalanobis distances of the residuals (D^2) against the corresponding quantiles (Q-Q plot) of the $\chi 2$ distribution. The selection of elements in the QDFA model that contributed most to the discrimination of Antarctic toothfish to their

respective capture locations was based on standardised discriminant canonical coefficients for each element of the first two factors (factors > 0.50), and spatial variability in significant elemental univariate ANOVA means tests. Similarly, multicollinearity was evaluated for all elements included in the discriminant model Al, Mg, Sr and Ba with tolerance levels ranging between 0.800–0.896 and variable inflation rates (VIF) between 1.116–1.250. Classification accuracy of fish to their known capture location was determined using cross-validated 'leave-one-out' jack-knife classification. Analyses were performed in STATISTICA version 11 and XLSTAT-Pro version 2015.2.

2.4. Results

2.4.1 Size and age of Antarctic toothfish

The composition of Antarctic toothfish size and age classes varied between locations (Table 2.2). Only adult Antarctic toothfish were represented across all regions ranging from 124– 68 cm TL in the RS (mean age 16 years), 125–170 cm TL from the AMS (mean age 14 years), and 130–170 cm TL in the SAO (mean age 13 years) and SIO (mean age 14 years), (Table 2.2). Sub-adults from the RS (mean age 10 years) and SAO (mean age 10 years) were much the same ranging from 66–100 cm TL and 69–98 cm TL respectively. Only two sub-adults were collected in the SIO (mean age 13 years) and ranged from 76–94 cm TL with no sub-adults among AMS collections (Table 2.2). The majority of juveniles were taken from the RS (mean age 8 years) at 58–65 cm TL, with one fish (60 cm TL) sampled from the SAO (age 10 years).

Table 2.2 Details of Antarctic toothfish otolith collections including number of juveniles, sub-adult and adults, minimum and maximum sizes (TL cm) sex, average age and fishing dates in the RS and AMS during the austral summer (Dec 2011–Feb 2012) and the eastern Antarctic SAO and SIO during May 2012. (--) refers to missing data.

			Length (cm)		Age	Sex	
Location	Size	N	Min	Max	Mean	M	F
RS	Juveniles	22	58	65	8	8	14
	Sub adults	24	66	100	10	15	9
	Adults	18	124	168	16	9	9
	Total	64	--	--	--	32	32
AMS	Adults	23	125	170	14	15	8
	Total	23	--	--	14	15	8
SAO	Juveniles	1	60	--	10	1	1
	Sub adults	15	69	98	10	5	10
	Adults	31	130	170	13	19	12
	Total	48	--	--	12	25	23
SIO	Sub adults	2	76	94	13.5	--	2
	Adults	20	130	170	14.4	9	11
	Total	22	--	--	14.3	9	13

2.4.2 Spatial variability in otolith edge chemistry

With the inclusion of length corrected Sr, ANCOVA analyses of otolith edge signatures revealed no effect differences between , sex or location, locations which may confound interpretations of elemental compositions as site-specific discriminators (Table 2.3). Subsequently, univariate ANOVA analyses showed significant differences in otolith edge chemistry between capture locations (Table 2.4), independent of any interaction effects (Table 2.3). In the instance of Al concentrations, significant differences were revealed among fish from the different

fishing grounds with the greatest separation between Ross Sea and the AMS, SAO and SIO fish (Table 2.4, Fig 2.3).

Table 2.3 Univariate ANCOVA tests of effect differences among (ln) transformed element-Ca compositions of Mg, Al, Sr and Ba otolith edge chemistries, between, location, sex and fish length. Length (cm) aswas set as a a covariate and sex and location set as fixed factors.

			ANCOVA			
Effect	Element	df	SS	MS	F	P
Location	Mg	3	0.006	0.002	0.91	0.44
Sex		1	0.003	0.003	1.30	0.26
Length		1	0.005	0.005	2.47	0.12
Location*Sex		3	0.007	0.002	1.04	0.38
Location*Length		3	0.010	0.003	1.56	0.20
Sex*Length		1	0.002	0.002	1.10	0.30
Location*Sex*Length		3	0.009	0.003	1.38	0.25
Error		141	0.299	0.002		
Total		156	0.37			
Location	Al	3	0.006	0.002	0.55	0.65
Sex		1	0.0004	0.0004	0.09	0.76
Length		1	0.002	0.002	0.23	0.63
Location*Sex		3	0.003	0.001	0.26	0.85
Location*Length		3	0.007	0.002	0.61	0.61
Sex*Length		1	0.001	0.001	0.13	0.72
Location*Sex*Length		3	0.0027	0.0009	0.23	0.87
Error		141	0.539	0.004		
Total		156	0.894			
Location	Sr	3	23.52	7.84	1.79	0.15
Sex		1	13.10	13.10	2.99	0.09
Length		1	6.81	6.81	1.55	0.21
Location*Sex		3	38.41	12.80	2.92	0.04
Location*Length		3	25.12	8.37	1.91	0.13
Sex*Length		1	11.76	11.76	2.68	0.10
Location*Sex*Length		3	30.78	10.26	2.34	0.08
Error		141	618.61	4.39		
Total		156	1003.21			
Location	Ba	3	0.011	0.004	1.19	0.32
Sex		1	0.001	0.001	0.45	0.50
Length		1	0.0004	0.0004	0.14	0.71
Location*Sex		3	0.008	0.003	0.87	0.46
Location*Length		3	0.007	0.002	0.80	0.50
Sex*Length		1	0.001	0.001	0.47	0.49
Location*Sex*Length		3	0.003	0.001	0.33	0.80
Error		141	0.420	0.003		
Total		156	0.500			

More specifically, otolith Al compositions of Antarctic toothfish captured off the continental slope in the AMS, the shelf and slope of the Astrid Ridge and Ekstrom Basin

(SAO) and from the BANZARE Bank (SIO) were significantly lower than in RS fish (Table 2.4 Fig 2.3).

Table 2.4 Univariate ANOVA analyses testing for spatial differences among individual (ln) transformed trace element compositions (Al Mg, Sr, and Ba) in the otolith edge of fish between capture locations.

			ANOVA			
Effect	df	Element	SS	MS	F	P
Region	3	Mg	0.04	0.01	7.00	0.0002
Error	153		0.33	0.002		
Total	156		0.37			
Region	3	Al	0.31	0.10	26.54	0.0001
Error	153		0.59	0.004		
Total	156		0.89			
Region	3	Sr	218.69	72.90	14.22	0.0001
Error	153		784.51	5.13		
Total	156		1003.21			
Region	3	Ba	0.01	0.005	1.43	0.24
Error	153		0.49	0.003		
Total	156		0.50			

High Al in RS fish revealed some of the strongest patterns of structuring between the RS compared to the AMS, SAO and SIO regions (Fig. 2.3). Similarly, fish taken off the continental slope in the AMS and eastwards from the SAO overlying the Astrid Ridge and Ekstrom Basin shelf areas had significantly lower concentrations of Mg than fish from the Ross Sea (Fig. 2.3). However, no significant differences in Mg were indicated between toothfish from the Ross Sea and the BANZARE Bank in the SIO despite these regions being separated at much larger spatial scales (Fig. 2.2). Significant differences in Sr indicated strong discriminatory potential showing notable variability among capture locations (Table 2.4, Fig. 2.3).

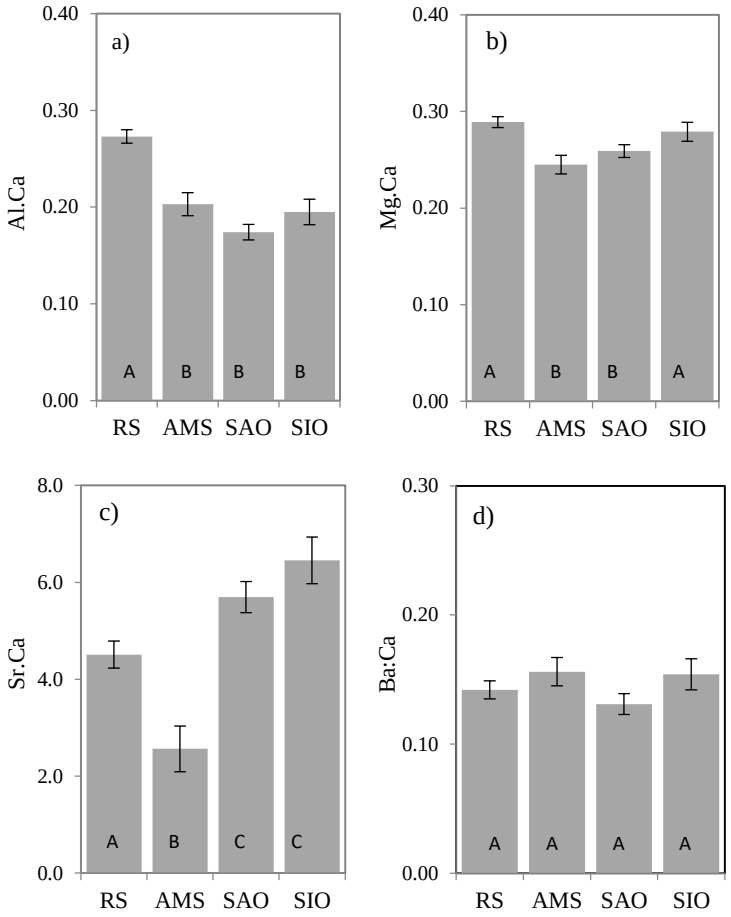

Figure 2.3 Mean (± SE) elemental compositions (a) Al (b) Mg (c) Sr and (d) Ba as ln transformed (mmol mol^{-1}) in the otolith edge of Antarctic toothfish collected in 2011–2012 from the RS, AMS, SAO and SIO. Letters that differ between locations represent significant differences through Tukey's unequal (HSD) pairwise comparisons, alpha $P < 0.05$.

Pairwise differences were largely attributed to higher Sr in fish from the Astrid Ridge and Ekstrom Basin in the SAO and BANZARE Bank in the SIO compared to lower Sr in fish from the Ross Sea (Fig 2.3). The lowest concentrations of Sr overall, were highlighted in fish taken from the continental slope in the AMS which differed significantly in pairwise comparisons between the, SAO and SIO. However, elemental concentrations of Sr in fish from the Astrid Ridge and Ekstrom Basin SAO and BANZARE Bank in the SIO showed only marginal differences (Fig 2.3).

2.4.3 Classification of Antarctic toothfish to capture location

Based on the significance of elemental compositions among Antarctic toothfish capture locations the same elements were used to construct a discriminant model. The model also included Ba as collectively these signatures contributed the most to the discrimination of toothfish (Table 2.4). Subsequently, a QDFA model (accounting for unequal covariances) was constructed in which all size classes were pooled, and multivariate normal elements Al, Mg, Ba and Sr were used as predictors to discriminate Antarctic toothfish to their capture location. The model was significant (QDFA, Wilk's Lambda = 0.39; $F_{13, 397} = 1.77$; $P < 0.0001$), indicating that otolith chemistry may be a useful tool in distinguishing group membership of Antarctic toothfish between their capture locations (Fig 2.4). Chemical compositions of Mg, Al and Ba from the otolith edge explained 69% of the total variability in the first factor and separated Ross Sea and AMS fish with moderate distinction from fish captured on the Astrid Ridge and Ekstrom Basin in the SAO and over the BANZARE Bank in the SIO (Fig. 2.4).

Table 2.5 Standardised canonical discriminant function coefficients showing the best otolith elemental predictors (> 0.50) for use in the QDFA analyses according to the first and second functions.

Elements	F1	F2
Mg	–0.027	0.548
Al	0.833	0.411
Sr	–0.870	0.697

For the second factor, the remaining 28% of explained variability was correlated with Sr, which showed overlapping trends in elemental signatures with the SAO and

SIO. However, poor separation between SAO and SIO did enable greater distinction of these areas from the more distant Ross Sea (Fig. 2.4 and Table 2.4).

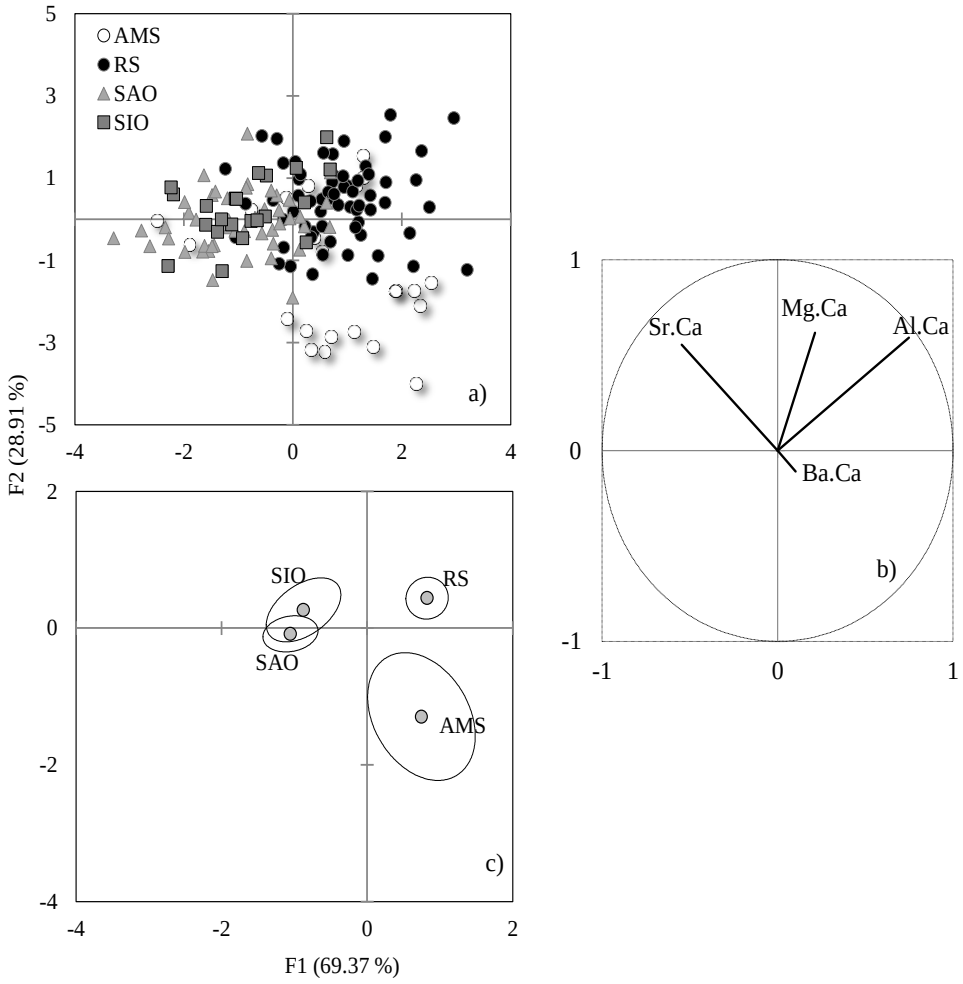

Figure 2.4 QDFA canonical factor plot (a) and contributing elements to the discrimination factor plot (b) of the otolith edge signatures analysed using ln transformed Al, Mg, Sr and Ba including mean group centroids and surrounding 95% confidence ellipses (c) showing discrimination of Antarctic toothfish to capture locations in RS, AMS, SAO and SIO.

Closer examination of mean centroid plots with 95% confidence ellipses indicate clearer separation between the group means of Antarctic toothfish from all capture locations (Fig. 2.4). The moderate degree of discrimination in fish among capture locations was reflected in cross validated "leave-one-out" results with an overall classification success of 68% (Table 2.6). Of these, the highest classification success

went to fish from the Ross Sea (81%) followed by fish taken along Astrid Ridge and Ekstrom Basin SAO (79%) whereas Antarctic toothfish from the AMS (57%) were moderately classified and fish captured from the BANZARE Bank in the SIO were the most poorly classified (14%) (Table 2.65).

Table 2.6 Cross validated (jack-knife) classification matrix of QDFA analyses using otolith edge element-Ca ratios of Al, Mg, Sr and Ba from 2011–2012 collections to assign Antarctic toothfish to capture locations. Rows represent original capture location columns represent predicted capture location. Analyses were weighted to group size.

	Predicted location					
Actual location	RS	AMS	SAO	SIO	Total	% Correct
RS	52	4	7	1	64	81%
AMS	5	13	4	1	23	57%
SAO	7	1	38	2	48	79%
SIO	6	1	12	3	22	14%
Total	70	19	61	7	157	68%

In the Ross Sea, four toothfish were classified east to the AMS and seven fish were misclassified to the SAO with only one individual assigned to the SIO (Table 2.5). In the SIO, the majority of fish misclassified were sent to the SAO (12), to the west with six fish going to the Ross Sea which in an easterly trajectory along the ACC would be the furthest distance overall (Fig. 2.1). The exception to closest neighbour misclassifications were fish taken from the SAO (38) which were misclassified to the Ross Sea (7), only a few fish assigned to the SIO (2), and one fish misclassified to the AMS (Table 2.5).

2.5. Discussion

Knowledge of the spatial structure of fish populations is an important prerequisite for management efforts, as it provides a geographic context by which areas of

interest can be the focus of fisheries management effort (Chittaro et al. 2010). Increasingly, chemical constituents in fish otoliths are used to identify spatially discrete populations, because physicochemical differences in the water mass can imprint themselves in otoliths chronologically (Campana et al. 1999; Arai et al. 2003) and therefore be useful for tracing fish movements (Zlokovitz et al. 2003; Brazner et al. 2004; Whitledge 2009) or as stock identifiers (Bath et al. 2000; Patterson et al. 2004; Ashford et al. 2006; Chittaro et al. 2006; Thorisson et al. 2011; Soeth et al. 2019).

In the present study, analysis of four trace elements (Al, Mg, Sr and Ba) in the otolith edge, highlighted significant spatial variability in Antarctic toothfish between several distinct oceanographic regions. Although overlapping patterns in elemental concentrations were evident among most basins, separation was significantly stronger between fish from the Ross Sea and Weddell Sea in the Southern Atlantic sector. The Ross Sea is the primary fishing ground for Antarctic toothfish classed as an 'exploratory' fishery that began in 1996 – 1997 (Hanchet et al 2015). The Weddell Sea in the Southern Atlantic Ocean, however, has had limited fisheries research since the founding of CCAMLR (Petrov and Gordev 2015), indicating that otolith chemistry can successfully assign fish to their region of capture where current information gaps and fishing effort exist.

Moreover, the strong discriminatory power of multi–element signatures between regions was supported by high classification rates for the Ross Sea (81%) and Southern Atlantic Ocean (79%) with moderate classification for the AMS (57%) suggesting that for future investigations assignment of transient adults to locations using juvenile sources may be possible.

2.5.1 Comparison with previous studies

These findings show similar trends to Patagonian toothfish (*Dissostichus eleginoides*), a more northerly distributed relative which demonstrated similar patterns of structuring around Antarctica using largely the same elements (Ashford et al. 2006; Ashford et al. 2012). Moreover, the similarity in regional structuring of otolith Mg, Sr, and Ba in this study using different micro–probe instruments, further highlights the utility of this approach. Importantly, the consistency of the same elemental constituents in separating toothfish from the Ross Sea fishery and the Southern Atlantic sector over different years suggests geo-chemical signatures in otoliths between these regions over time maybe relatively stable. The only other study of Antarctic toothfish otolith chemistry was by Ashford et al. (2012). In their study, finer scale structuring of toothfish populations within the Ross Sea were determined with spatial differences in element-Ca concentrations attributed to deep-water mixing processes, physiological changes and life history movements.

2.5.2 Regional structuring in otolith chemistry

In the present study, Sr was significantly higher in Antarctic toothfish from the SAO and SIO compared to the RS and AMS sectors, indicating strong environmental gradients in salinity between these regions. In the RS and AMS, lower Sr could be due to the prominence of Low Salinity Shelf Water (LSSW) occurring at intermediate depths within the central eastern Ross Sea (Jacobs et al. 1985; Locarnini 1994; Russo et al. 1999), and the Bellingshausen and Amundsen Seas where increased basal ice melting occurs (Jacobs & Giulivi 1985; Jacobs 2002; Gerringa et al. 2012). These inputs have been attributed to decadal freshening in the Ross Sea with salinity decreases in the westward coastal and slope front currents,

consistent with increased melting of continental ice upstream in the Amundsen Sea (Jacobs & Giulivi 2010). Furthermore, with ongoing freshening (Jacobs et al. 2002; Smith et al. 2014) and the influence of the Ross Sea Gyre, extending northwards to the winter sea ice edge (Locarnini 1994; Budillon et al. 2003; Rickard et al. 2010) and mixing across the continental shelf break (Locarnini 1994; Budillon et al. 2003), lower otolith Sr in fish from the RS and AMS might be expected. Conversely, high Sr in fish from the SAO and SIO may be due to less freshening in the Weddell Sea with reduced sea ice formation and melt, decreasing freshwater inputs (Meredith & King 2005) and because Weddell Sea ice shelves are sheltered from the influence of the ACC by the Weddell Sea Gyre (Purkey & Johnson 2013). Practical applications of otolith Sr analyses with diadromous fish species have been successful in characterising migratory life histories between fresh and saltwater environments (e.g., Limburg et al. 2003; Yang et al. 2006; Brown et al. 2007). These studies have been founded on the basis of experimental tank studies showing positive correlations between the salinity of ambient water and the resulting otolith Sr concentration (e.g., Secor et al. 1995; Farrell and Campana 1996; Zimmerman 2005).

However, otolith Sr in marine fish cannot be explained by ambient water concentrations alone and may be governed by ontogeny (Fuiman & Hoff 1995; Clarke & Friedland 2004; Brown & Severin 2009) or temperature and physiological effects (Kalish, 1991; Elsdon & Gillanders, 2004; Brown & Severin, 2009; Miller, 2011). Among some marine species, growth rate and reproduction appear to have a greater effect on otolith Sr uptake than temperature (Kalish, 1989, 1991). However, the documented effects of temperature on Sr partitioning between otolith and water

has been somewhat ambiguous, with evidence of positive, negative, and non-significant relationships reported among several controlled experiments (Townsend et al. 1992; Elsdon & Gillanders 2002; DiMaria, Miller & Hurst 2010; Reis-Santos et al. 2013). Such inconsistencies have been attributed to variability in fish physiology, biochemistry, and otolith morphology (S¨ollner et al., 2003; Popper & Fay, 2011) highlighting interspecific differences in otolith Sr uptake particularly among marine fish (Kalish, 1989; Hamer & Jenkins, 2007; Swearer et al., 2003; Rooker et al., 2004).

Subsequently, a unified theory of Sr incorporation in fish otoliths is not applicable to all fish because the factors influencing Sr uptake in otoliths will differ in effect or magnitude among species, families, and life history types (Brown & Severin, 2009). To validate otolith Sr uptake in Antarctic toothfish controlled laboratory experiments using an array of age classes across a range of salinity or temperature regimes would be required. The temperature regimes at which Antarctic toothfish occur 0.7 – 1.95°C (Lam et al 2018) are at the extreme threshold at which tank experiments have been conducted for marine species (Brown and Severin 2009).

Such an approach was beyond the scope of the present study and to my knowledge no experimental studies have been conducted on Antarctic toothfish. To successfully record individual migration pathways, environmental signals would need to outweigh physiological noise (Brown and Severin 2009). In this regard, the use of Sr compositions in this study incorporated individual size classes below the ontogenetic threshold (< 120 cm), (Eastman & DeVries 2000; Fenaughty et al. 2008; Parker & Grimes 2010b). Similarly, with the indication of a size effect for Sr, the data were length corrected to account for growth differences. The use of Sr in

statistical analyses were also carried out with multi-element combinations for discrimination models enabling distinct spatial classification proxies for toothfish. Spatial structuring according to Al, Mg and Ba from the otolith edge separated Ross Sea and AMS fish with moderate distinction from fish captured in the SAO and SIO. The composition of Al in the Southern Ocean maybe the result of atmospheric inputs (Duce & Tindale 1991; Gao et al. 2001; Kramer et al. 2004; Measures et al. 2005), or sediment remobilisation (Measures & Edmond 1990; Moran et al. 1992).

Fish from the SAO showed the lowest concentrations of Al overall regions which likely reflect exposure to Al-depleted source waters of the Atlantic Antarctic Intermediate Water (AAIW) which is known to form in the Weddell Sea (Measures & Edmond 1990). Moreover, the prominence of this Al-depleted water-mass in the Weddell Sea is regarded as a source area of Al, detected in the North Pacific (Moran et al. 1992). Similarly, the proximity the Weddell Sea Gyre between the ACC and the Antarctic Continental Shelf, would enable mixing to adjoining sample areas of the present study, may have constituted low Al in fish from the SAO and to a lesser extent the SIO.

In contrast, higher concentrations of Al in Antarctic toothfish from the Ross Sea maybe the result of increased sea ice production and release as meltwaters (Fitzwater et al. 2000). In terms of Mg, spatial variations indicated significant regional differences in otolith edge chemistry for Ross Sea and SIO fish compared to fish from the AMS and SAO, which may be linked to physiological changes (Bath et al. 2000; Dorval et al. 2007; Hamer & Jenkins 2007). Ashford et al. (2007) suggested otolith Mg in *D. eleginoides* may be associated with spawning around the Burdwood Bank south of the Falkland Islands, whereas off the Pacific–Antarctic

Ridge near spawning areas of the Ross Sea (Fenaughty et al. 2008; Hanchet & Rickard 2008), high otolith Mg was evident in Antarctic toothfish. While tank experiments on Mg uptake in freshwater species appear to reflect physiology and not the environment (Melancon et al. 2009; Woodcock et al. 2012), actual rates of incorporation can be species specific (Hamer & Jenkins 2007).

2.5.3 Overlapping elemental signatures

For regions where overlapping signatures confounded discrimination, misclassifications were reasonably consistent with their nearest neighbour**.** For example, fish taken over the BANZARE Bank in the SIO were misclassified to closely adjoined areas in the SAO, while fish from the AMS were misclassified to adjoining regions east of the Antarctic Peninsula in the SAO and west to the Ross Sea. Consequently, two hydrographic features in the Southern Ocean and their interaction with the Antarctic Circumpolar Current (ACC) may obscure distinct elemental signatures of adjoining regions consistent with observed misclassifications.

The Ross Sea Gyre and the Weddell Sea Gyre are hydrographic features with distinct boundaries in the Southern Ocean of which the otolith chemistry of Antarctic toothfish were shown to be more strongly structured within. Both gyres play an important role in coupling the west to east flowing Antarctic Circumpolar Current (ACC) consisting of a mixture of deep water from all of the world's oceans to shelf waters close to the Antarctic continent (Orsi et al. 1995). The proximity of the AMS and SIO to these gyres and their downstream location along the ACC (Orsi et al. 1995), might enable mixing between water masses, limiting the ability of

otolith edge chemistry to distinguish between closely connected areas. Alternatively, temporal differences at when fish were collected across the different oceanic basins may have confounded the ability of otolith edge chemistry to distinguish capture locations. Temporal lags were due to the spatial distances vessels needed to travel between fishing areas and by delays in sea ice melt limiting access to some fishing grounds over the continental shelf. This may have resulted in different elemental exposures in otolith edge chemistry overtime corresponding to discrepancies in habitat markers among fish. However, that other regions were still able to be distinguished (i.e. RS and SAO) through otolith edge chemistry suggests that at regional scales environmental conditions were still sufficient to imprint distinct elemental fingerprints in toothfish.

Additional processes which may explain mis-classifications between some areas could also occur if toothfish recently moved between adjoining areas eroding the utility of site-specific signatures (Elsdon et al. 2008). In this study, temporal lags due to the spatial distance's vessels needed to travel between fishing areas and by delays in sea ice melt limiting access to some fishing grounds over the continental shelf probably had some bearing on misclassifications. However, that other regions were still able to be distinguished (i.e., RS and SAO) through otolith edge chemistry suggests that at regional scales environmental conditions were still sufficient to imprint distinct elemental fingerprints in toothfish.

Tag information on Antarctic toothfish and Patagonian toothfish exists for much of Antarctica including the Falkland Islands and South Georgia in the Southern Atlantic Ocean (Marlow & Agnew 2003; Agnew & Clark 2006; Brown et al. 2013) off Heard Island in the Southern Indian Ocean (Williams & Tuck 2002) and

throughout the Ross Sea (Parker et al. 2014; Parker et al. 2016). These studies suggest that large scale movements of toothfish from release sites > 50 km is limited (Williams & Tuck 2002; Marlow & Agnew 2003; Parker et al. 2014; Parker et al. 2016). Movements of Antarctic toothfish over shorter distances appear consistent with a physiology of white muscle and high myotomal composition (Eastman & DeVries 1981). Similarly, with reduced ossification and high lipid content aiding neutral buoyancy, this would also limit swimming capacity (Eastman & DeVries 1982; Near et al. 2003). Nevertheless, although movements appear limited in Antarctic toothfish the potential for movement across distant waterbodies has previously been documented between the Ross Sea and AMS (Parker et al. 2014) which would obscure elemental signatures that may exist between these regions. Similarly, one fish has been traced 1900 km in the Southern Indian Ocean off Heard Island (Williams & Tuck 2002) whereas another tagged fish from the Falkland Islands was recaptured 1000 km from its release site (Laptikhovsky et al. 2006). Additionally, poor discrimination between the SIO and AMS regions may be resolved by the use of other chemical markers (i.e., Mn, Sn, B, Li, Zn and Ni) which were not able to be utilised in this study because of statistical violations.

In summary, this study demonstrates the utility of elemental fingerprints in distinguishing Antarctic toothfish sampled within a single season and across a spatial range not previously recorded for this species. Spatial structuring was most evident between the main fishing area within the Ross Sea fishery and the Weddell Sea Southern Atlantic sector highlighting the utility of otolith microchemistry in identifying toothfish habitat leading towards future investigations were population differences at least between the Ross Sea fishery and SAO maybe possible. This

was supported by similar patterns of structuring identified in previous studies using the same signatures (Sr, Mg and Ba) over different years suggesting that otolith geochemistry sources between these regions over time maybe relatively stable. However, to test this assumption, further investigations based over consecutive seasons would be necessary. Adjacent areas to the Southern Atlantic (Southern Indian Ocean) and to a lesser extent the Ross Sea (Amundsen Sea) did not reflect sufficient otolith geo-chemical differences that would enable meaningful discrimination of these areas. Consequently, poor discrimination was attributed to hydrographic processes (oceanic gyres) and the ACC circumpolar current mixing within and across adjoined areas.

The spatial structure of Antarctic toothfish throughout the Southern Ocean as evidenced by elemental fingerprints revealed two spatially discrete areas. This finding will aid in future investigations aimed at discerning the structure of Antarctic toothfish stocks and extent to which they may be connected. This will be useful to fisheries managers as areas outside of the main Ross Sea fishery are exploratory receiving limited fishing effort and knowledge about Antarctic toothfish life history characteristics.

2.6 References

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3. Large-scale population structuring and connectivity of Antarctic toothfish in the Southern Ocean based on otolith nucleus chemistry

3.1 Abstract

To understand the spatial extent to which Antarctic toothfish (*Dissostichus mawsoni*) populations were structured, otolith elemental assays using laser ablation inductively coupled mass spectrometry (LA-ICP-MS) were conducted using age 10 and age 14-year-old fish collected by observers in the Ross Sea, Amundsen Sea, Southern Atlantic and Southern Indian Oceans. Univariate ANOVA analyses of trace element signatures in the otolith nuclei indicated significant spatial differences in Sr, Al and Mg in toothfish captured from the Ross Sea and the Southern Atlantic, indicating significant differences in environmental conditions between these regions. Spatial visualisation of non-metric multidimensional scaling and agglomerative hierarchical cluster (AHC) analyses also revealed broad-scale spatial heterogeneity between the same areas.

This analysis suggested that among adult and sub-adult toothfish, two distinct spawning populations with close affinities to the Ross Sea and Southern Atlantic were evident, indicating these regions were important source areas for Antarctic toothfish. However, a small portion of individuals from the Amundsen Sea and the Southern Indian Ocean had natal signatures that overlapped with Ross Sea and the Southern Atlantic fish indicating they had dispersed from their original spawning grounds, through large-scale oceanic currents during early growth. These findings provide supporting evidence to the existence of separate Antarctic toothfish stocks

between the Ross Sea and Southern Atlantic. However, the Ross Sea and Southern Atlantic Ocean may also contribute disproportionately to recruitment in neighbouring areas of the Southern Indian Ocean and Amundsen Sea. This would indicate that fisheries management effort of Antarctic toothfish in the Ross Sea and Southern Atlantic should be considered as separate stocks.

3.2 Introduction

An important prerequisite of sustainable fisheries management is knowledge about the spatial structure of fish populations (Jones et al. 1999). Such information not only forms the basis for understanding population dynamics and connectivity but also poses questions about a species resilience to ongoing harvesting (Thorrold et al. 2001). Among marine species, population connectivity is more often viewed with regards to larval recruitment processes, where larvae are exported from the pelagic zone to benthic environments (Underwood & Fairweather 1989; Fairweather 1991). Moreover, the pelagic stage represents an important phase of a fish's life, as larvae may be widely dispersed from natal spawning areas through advective currents that determine the replenishment or export of locally spawned populations (Doherty & Williams 1988).

Not surprisingly, quantifying larval dispersal in marine environments is considerably challenging given the limited ability to track larvae *in situ* using conventional tagging methods (Leis & Carson-Ewart 1998; Cowen & Sponaugle 2009). Similarly, among many marine species the natal and nursery origins of adults are often unknown (Jones et al. 1999; Cowen & Sponaugle 2009). Increasingly, fish otoliths have been used as habitat markers to distinguish the spawning origins of adult or juvenile fish stocks based on their ability to record trace element compositions reflective of the environment fish inhabit throughout their life (Campana 1999; Campana & Thorrold 2001; Martin & Wuenschel 2006; Macdonald & Crook 2010; Mirasole et al. 2017). The premise for using otoliths as natural habitat markers is that as fish grow, elemental constituents from the surrounding water mass are incorporated (daily) onto the otolith matrix (Fowler et

al. 1995; Campana 1999) and once deposited are neither reworked or re-mineralised (Campana 1999). With the exception of a few trace elements that may be subject to physiological or genetic influences (Kalish 1989; Proctor & Thresher 1998; Campana 1999; Sturrock et al. 2015; Macdonald et al. 2020b) otoliths have proven to be useful at delineating population differences among marine species (Thorrold et al. 2001; Ashford et al. 2006; Swan et al. 2006; Ashford et al. 2008; Vasconcelos et al. 2008; Svedäng et al. 2010; Ashford et al. 2012; Papetti et al. 2013; Tanner et al. 2016; Wheeler et al. 2016).

Notably, the use of otolith natal signatures in identifying population structure has equal relevance to genetic differentiation between fish stocks, as the maintenance of genetic differentiation among sexually mature fish relies on their tendency to return to the same natal spawning areas (Parker et al. 2002; Robichaud & Rose 2002; Kuhn & Gaffney 2008; Rooker et al. 2008). In this regard, otolith chemistry approaches enable questions around natal origins, or natal homing, to be addressed (Thorrold et al. 2001). For Antarctic toothfish (*Dissostichus mawsoni*), a large bodied bentho-pelagic fish species that constitutes an important commercial longline fishery, quantifying larval dispersal patterns and population structure poses significant challenges to fishery managers. The fishery as a whole encompasses much of the Southern Ocean and is managed by the Commission for Conservation of Marine Living Resources (CCAMLR) with quota determined biennially among spatially designated fishing areas of the Southern Ocean south of 60°S (Hanchet et al. 2015). The aim of this study was to examine elemental signatures in the otolith nucleus of Antarctic toothfish to assess differences in spawning areas. It was envisaged that this would enable the spatial structure of Antarctic toothfish spawning populations to be identified providing insight on the

extent to which populations within these oceanic basins were connected. This would help to ensure that the approach by CCAMLR managing these fisheries is ecologically sustainable.

3.3 Materials and methods

3.3.1 Fish otolith collections

Antarctic toothfish otolith collections were obtained by scientific observers onboard bottom longline vessels operating in CCAMLR Convention areas around Antarctica (Fig 3.1) Fishing was conducted in the Ross Sea (RS) and Amundsen Sea (AMS) over continental shelf and slope areas by three New Zealand vessels (Aotea II, San Aspiring and Janas) during the austral summer (December 2011–February 2012). Whereas in the Southern Atlantic (SAO) over the Astrid and Khonen Ridges and on the BANZARE Bank in the Southern Indian Ocean, a single Japanese vessel (Shinsei Maru III) collected toothfish samples during May 2012 (Fig. 3.1).

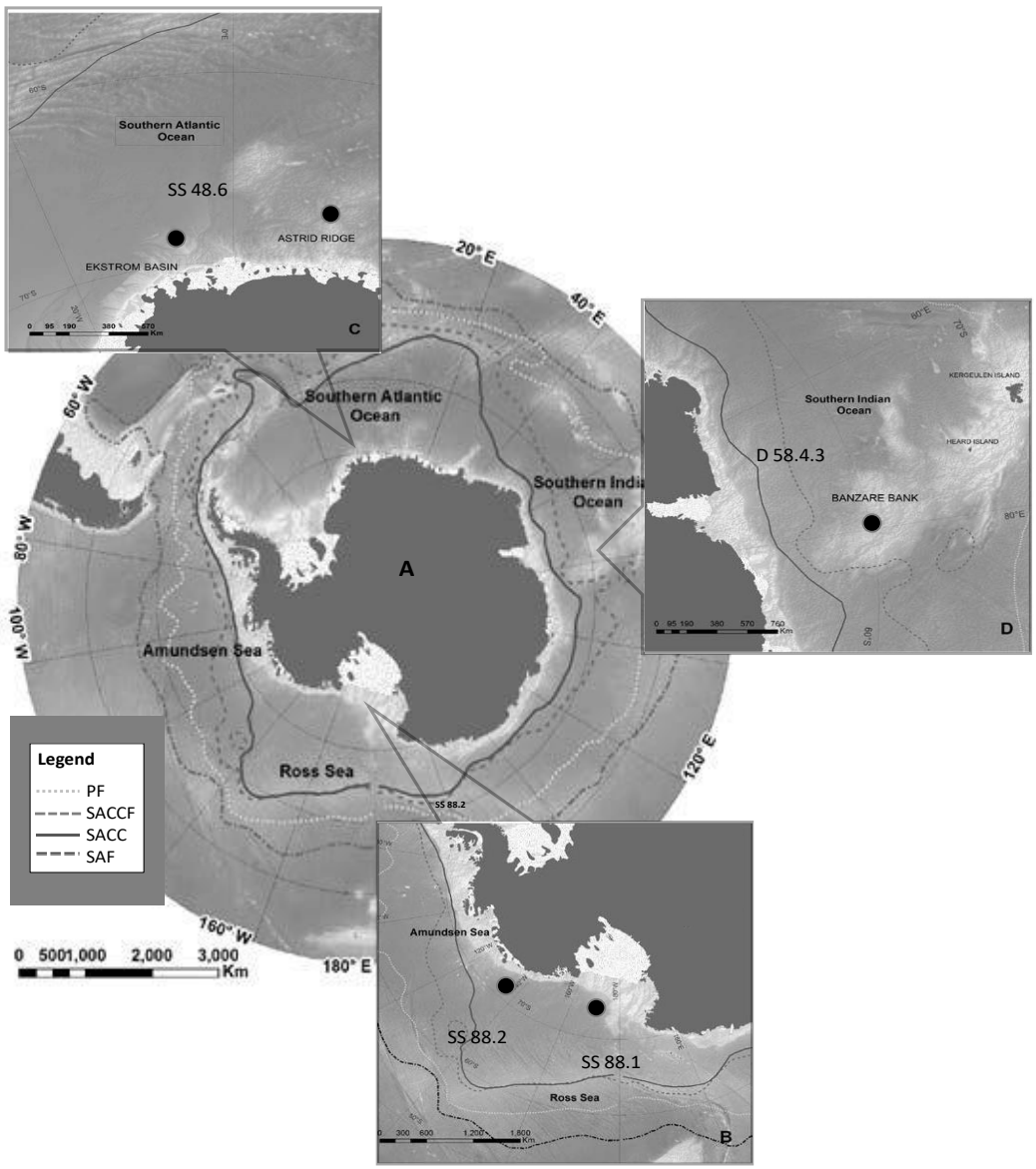

Figure 3.1 Convention areas (A) where fishing was carried out within the Ross Sea (SS.88.1), Amundsen Sea (SS.88.2) (B) the Southern Atlantic Ocean (SS.48.6) over the Ekstrom Basin and Astrid Ridge (C) and over the BANZARE Bank in Division 58.4.3 (D) during the 2011–2012 austral summer. Lines represent hydrographic features around Antarctica including the Polar Front (PF), Southern Antarctic Circumpolar Current Front (SACCF) Southern Antarctic Circumpolar Current (SACC) and Sub-Antarctic Front (SAF). Circles represent sample areas

3.3.2 Otolith preparation

Otolith handling and preparation was carried out according to protocols and processes outlined in Chapter 2 (Section 2.3.2). Otolith extraction and handling was

carried out using acid washed plastic utensils. Sagittal otolith pairs were cleaned in glass scintillation vials topped up with ultrapure Milli-Q water and sonicated for 4 min., then dried in a laminar flow cabinet prior to mounting. Cleaned fish otolith collections across all sample locations for the 2012 fishing season were randomly selected for mounting on petrographic slides. Only right sagittal otoliths were used for chemical analyses except where only one otolith was available (Chapter 2, section 2,3.2).

Otoliths were embedded in silicon moulds using Nuplex® K36 epoxy resin with the distal surface facing up. Transverse sections were made through the primordia using a low speed Isomet diamond cutting saw (Buehler–USA) Thin sections were polished submerged within Milli-Q water to expose the otolith nucleus using a sequence of 2400 then 4000 µm grit wetted carbide paper (Struers), after which the water was replaced. Accuracy of polishing was determined using a Nikon SMZ645 (C - W 10 x A/22) stereo microscope. Ring structures radiating from the outer growth zones were gradually removed exposing the nucleus just short of the otolith surface. Sectioned otoliths were glued to small 50 x 25-mm petrographic slide with 20-30 sectioned otoliths mounted per slide using Crystalbond^{TM}509 thermo-setting adhesive, rinsed in Milli-Q water, then sonicated for 4 min, dried in a laminar flow cabinet, and stored in cover slide container ready for laser ablation.

3.3.3 Laser ablation analysis

Trace element analyses of the otolith nuclei of Antarctic toothfish was conducted using laser ablation inductively coupled mass spectrometry (LA-ICP-MS). The system consisted of a Perkin Elmer DRCII ELAN 6000 ICP-MS (Waltham, MA) coupled to a New Wave Research UP-213 nm Nd-YAG laser ablation system

(Fremont, CA) held at the University of Waikato, New Zealand. Analytical settings, running conditions and selection criteria of trace elements for this study are reported in chapter 2 (section 2.3.3). Moreover, the ICP-MS optimisation protocols, standard analysis, oxide interference corrections and sensitivity checks employed in this study are discussed in more detail in Chapter 2 (section 2.3.3) alongside operational conditions shown in Table 2.1.

In general, beam diameter settings for ablations were set at 25 μm with the laser fired at 60% power and a repetition rate of 20 Hz, while travelling an equivalent distance (~700 μm) in a raster mode grid formation (150 μm x 150 μm) centred within the first annual increment. The incubation period of Antarctic toothfish eggs and time of passive drift is not known precisely (Behrens et al 2021). However, hatching dates for Antarctic toothfish estimated from juveniles from the South Shetland Islands by counting micro-increments between hatching checks and sampling date in the core region of sagittal otoliths (La Mesa, 2007) suggest hatching of Antarctic toothfish eggs during winter spawning would be followed by a 3–4 months incubation and larval period in early spring for the Ross Sea, Southeast Pacific Basin, and Amundsen Sea regions (Hanchet et al., 2008; Parker et al., 2019). By 9-12 months Antarctic toothfish would reach a size of around 4-8cm before developing free swimming schools and remaining in the surface waters for another 6-9 months before settling out on the shelf at around 15cm (Hanchet et al 2008). In sampling within the first annulus (but not to the first annulus edge) of Antarctic toothfish larval growth signatures would consist of spawning and incubation period (3-4 months) as well as larval drift (5 months). Across the known spatial range of Antarctic toothfish this would presumably enable distinct regional scale natal signatures to be incorporated in otoliths. For each day's analyses, mean

minimum detection limits (MDL) at 99% confidence (ppm) were calculated for individual elements with GLITTER using background readings and Poisson counting statistics (Griffin et al. 2008). Similarly, GLITTER was used to review and select time-resolved peak laser signals for each element enabling 100% readings for all elements used in this study.

3.3.4 Age estimation

As per previous sections age estimates of Antarctic toothfish were carried out in accordance to the preparation methods and ageing protocols outlined in Chapter 2 (section 2.3.4).

3.3.5 Data analysis

To meet assumptions of multivariate normality among element-Ca ratios, Mardia's multivariate skewness and kurtosis measures were used (Khattree & Naik 2000) alongside Shapiro Wilks normality tests. Element-Ca concentrations of Al, Mg, and Sr and Zn did not meet normality assumptions and were (ln) transformed. Although Zn element-Ca ratios showed departure from normality (Shapiro Wilk, $P < 0.03$) it was still included in parametric ANOVA analyses the RS, AMS, SAO and SIO, mean differences in element-Ca concentrations were assessed using univariate and multivariate ANOVA analyses. Where significant differences were identified, Tukey's pairwise comparisons identified where *posteriori differences* ($\alpha = 0.05$) were located. In order to visualise spatial relationships among individual fish, non-metric multidimensional scaling (nMDS) ordinations (measuring dissimilarity between data points) were obtained using Euclidean distances (Kruskal & Wish 1978). How well derived configurations represented the original dissimilarities was

determined by the lowest stress coefficient (Kruskal stress < 0.1). Ordinations were constructed in two dimensions using standardised (mean = 0, SD = 1) element-Ca compositions to account for any scale differences, while the selection of elements was based on those that showed significant spatial variability among sample regions. The extent to which one or more populations might exist was investigated using Ward's minimum variance hierarchical cluster approach and the metric of Euclidean distances (Khattree & Naik 2000). The quality of clustering was evaluated using the within cluster variance with cluster truncation determined using Shannon's entropy (Shannon 1948) whereby the number of clusters were identified by the largest decrease between nodes.

3.4 Results

3.4.1 Size and age of Antarctic toothfish

Chemical constituents in fish otoliths corresponding to early growth were characterised among Antarctic toothfish sampled across four regions around Antarctica. Complete spatial coverage in representative size classes among fishing areas was only met for adult collections (mean 145.6 cm TL), (Table 3.1). The remaining size classes were limited only to age 8 juveniles (mean 63.5 cm TL) from the Ross Sea and age 10 sub-adults (mean 80.5 cm TL) from the Ross Sea and Southern Atlantic Ocean, with no juveniles or sub-adults taken from the Amundsen Sea or Southern Indian Ocean (Table 3.1). For otolith nucleus analyses, only age 14 adult toothfish (100–120 cm TL) were assayed by laser ablation being the only age class with sample sizes tenable from the RS (n = 17), SAO (n = 16) with slightly smaller numbers from the AMS (n =15) and SIO (n = 11). Analyses of sub-adults

(80–100cm) 11yr old toothfish were only possible for the RS and SAO with no sub-adults

Table 3.1 Details of Antarctic toothfish collections including number of samples, minimum and maximum fish size (total length, TL cm) for each size class and mean age, estimates for fish from the Ross Sea (RS), Amundsen Sea (AMS), Southern Atlantic Ocean (SAO) and Southern Indian Ocean. NB (--) = missing data.

			Sex		Length (cm)			
Location	Size	N	M	F	Min	Max	Age	Fishing dates
RS	Juveniles	22	8	14	58	65	8	25 Jan–12 Feb 2012
	Sub adults	24	15	9	66	100	10	23–29 Jan 2012
	Adults	18	9	9	124	168	16	2 Dec 2011–29 Jan 2012
	Total	64	32	32	--	--	--	--
AMS	Adults	23	15	8	125	170	14	26-Jan-12
	Total	23	15	8	--	--	14	--
SAO	Juveniles	2	1	1	60	--	10	18-Feb-12
	Sub adults	15	5	10	69	98	10	19-Feb-12
	Adults	31	19	12	130	170	14	6 Dec 2011–26 Feb 2012
	Total	48	25	23	--	--	12	--
SIO	Sub adults	2	--	2	76	94	13.5	8–16 May 2012
	Adults	20	9	11	130	170	14.4	8–16 May 2012
	Total	22	9	13	--	--	14.3	--

3.4.2 Spatial variability in natal chemistry

Univariate ANOVA analyses showed significant spatial differences in otolith nucleus signatures among regions (Table 3.2). Differences were the strongest among adults from the RS and SAO compared to other locations (Table 3.2, Fig. 3.2).

Table 3.2 Univariate ANOVA analyses tests for differences in (ln) Mg, Al, Sr and Zn in the otolith nucleus of adult Antarctic toothfish between capture locations.

			ANOVA			
Effect	df	Element	SS	MS	F	*P*
Location	3	Mg	0.028	0.009	3.67	*0.016*
Error	67		0.172	0.003		
Total	70		0.2			
Location	3	Al	0.015	0.005	7.43	*0.0002*
Error	67		0.045	0.001		
Total	70		0.06			
Location	3	Sr	0.049	0.016	0.73	0.538
Error	67		1.502	0.022		
Total	70		1.551			
Location	3	Zn	0.005	0.002	3.2	*0.027*
Error	67		0.036	0.001		
Total	70		0.041			

Similarly, Mg compositions were also significantly lower in toothfish from the SAO compared to the RS (Table 3.3, Fig. 3.2). Concentrations of Al were significantly lower (Tukey's HSD, $P < 0.001$) in fish from the SAO and SIO than in fish from the Ross Sea (Table 3.2, Fig. 3.2). Elemental differences were also evident in otolith compositions of Mg (ANOVA, $P < 0.001$), and Zn (ANOVA, $P < 0.01$), indicating a strong environmental gradient exists between fish from the RS and SAO. These patterns suggest that the availability of these elements in the water mass differs considerably between regions and appear to be higher in fish from the RS than in the SAO.

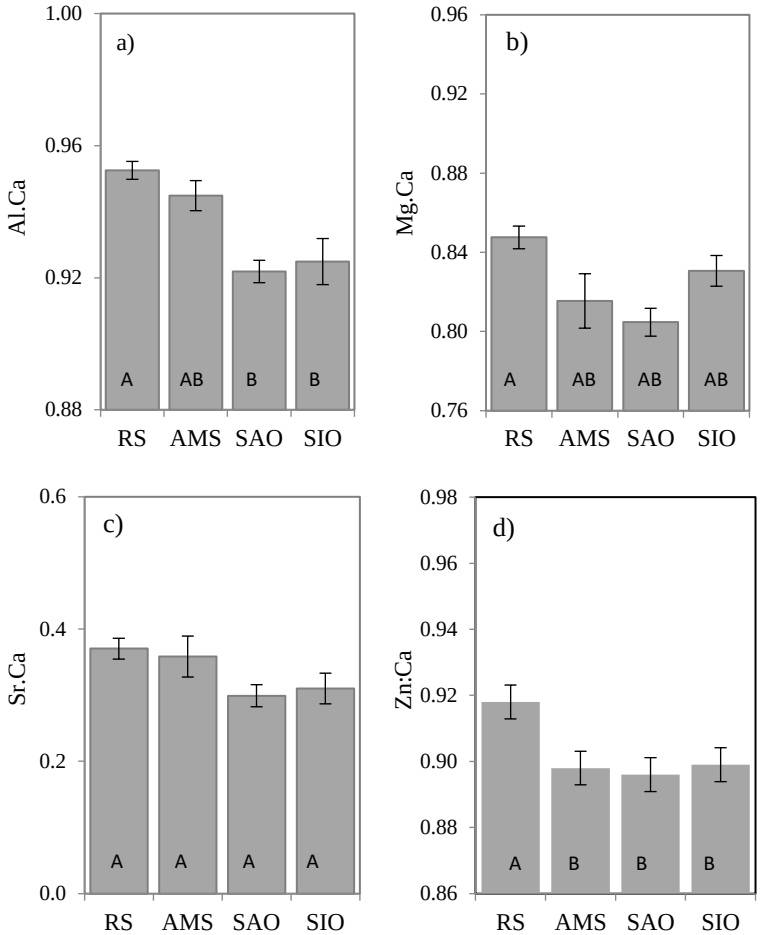

Figure 3.2 Mean (± SE) of (ln) transformed element-Ca compositions of (a) Al (b) Mg (c) Sr and (d) Zn in the otolith nucleus of Antarctic toothfish from the Ross Sea, Amundsen Sea, Southern Atlantic and Southern Indian Ocean. Letters that differ between locations represent significant differences (Tukey's $P < 0.05$).

3.4.3 Natal structuring of Antarctic toothfish

The spatial extent to which, the otolith nucleus of Antarctic toothfish were grouped, was visualised among age 14 (adult) and age 10 (sub-adult) collections only, to account for variations in water chemistry that may occur as a result of toothfish hatching across different years, and because representative sample sizes among some locations were only available for these age classes (Table 3.1).

Juvenile Antarctic toothfish, were only taken from the RS so were excluded from analyses. Using two dimensional nMDS ordinations and standardised compositions of Mg, Al and Zn, the otolith nucleus of Antarctic toothfish showed reasonable separation between some oceanic basins but not others (Fig. 3.3).

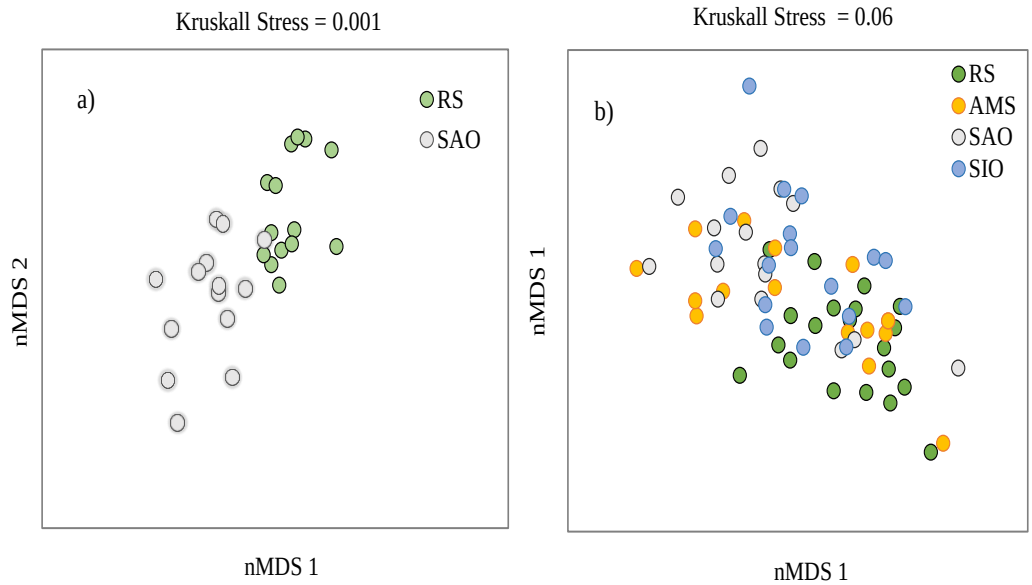

Figure 3.3 Two-dimensional ordination (nMDS) plots of otolith nucleus compositions of sub-adult (age 10) collections a) from the RS and SAO, and adults (age 14) collections b) from the RS, AMS, SAO and SIO. Convergence criterion for goodness of fit was Kruskal Stress (< 0.1).

Spatial differences in the otolith nucleus of sub-adult toothfish from the RS and SAO showed notable separation (Kruskal Stress = 0.00101) suggesting fish from these regions had close affinities to their capture locations. However, a small number of sub-adults from the RS had otolith nucleus chemistries similar to fish from the SAO indicating they may have shared a common spawning ground. Corresponding Ward's AHC cluster analyses revealed two primary groupings (clusters) with a third grouping only containing three sub-adults (Fig. 3.4). Of the

three groupings, two showed clear patterns of separation primarily between toothfish captured from the RS and SAO (Fig. 3.3).

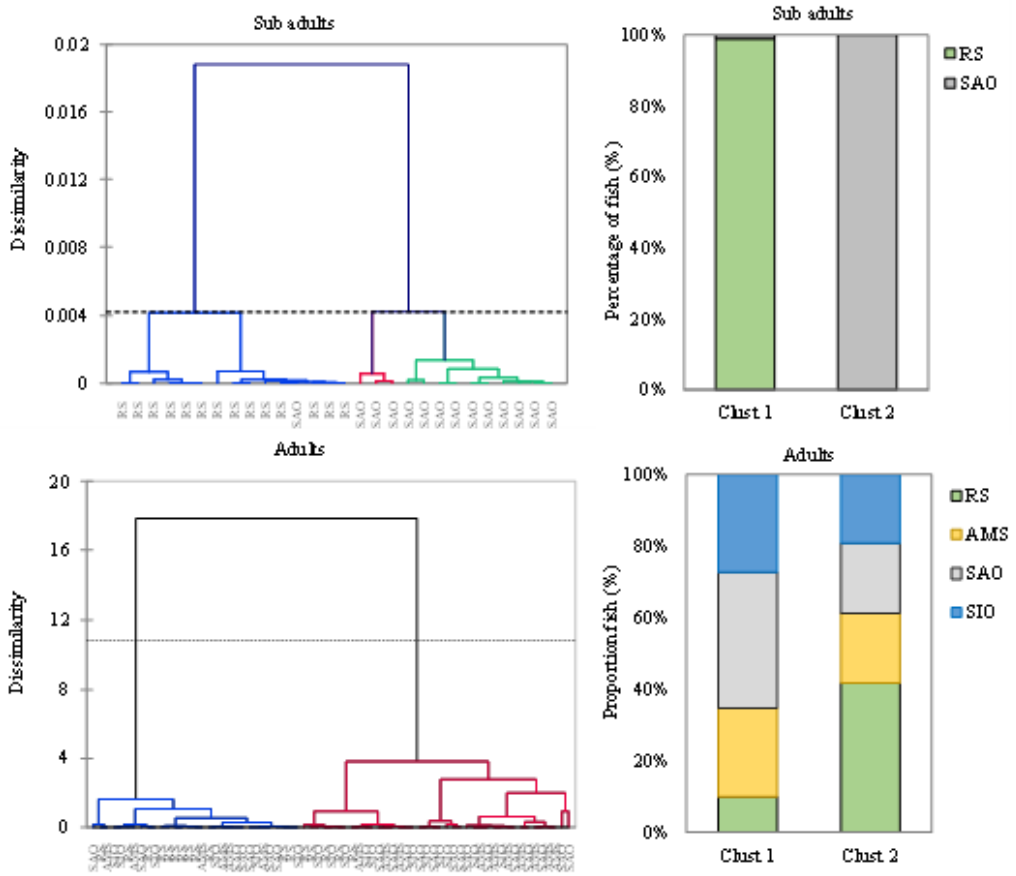

Figure 3.4 Ward's AHC cluster solution dendrograms (left) and class output histograms using otolith nucleus compositions (Mg, Al and Zn) from age 10 and age 14 Antarctic toothfish from the RS and SAO (top) and from the RS, AMS, SAO and SIO (bottom).

AHC cluster analyses suggested the first group was composed almost entirely of RS fish (97%) with a small contribution of SAO sub-adults (Fig. 3.4) indicating that larvae possibly mixed between these areas during early life. The second grouping was entirely composed of fish from the SAO (100%), suggesting these fish had spawning origins close to the SAO or had recently moved as a collective to this location (Fig. 3.4). Overlapping signatures in otolith nucleus may also result from the water mass among some locations being much the same, such that differences could not be distinguished among elemental compositions (Fig. 3.4,

bottom). Nevertheless, for the most part this suggests both the RS and SAO are important source areas for Antarctic toothfish. Among adult (age 14) natal compositions, where representative sample sizes from all oceanic basins were available only for a small number of adult age classes, spatial separation was still reasonably evident for the RS and SAO but not for other locations immediately downstream of these regions (Fig. 3.4). Two-dimensional nMDS visualisations showed considerable overlap in natal signatures of fish from the AMS and SIO with toothfish from the RS and SAO. Like sub-adult (age 10) natal compositions, nMDS visualisations of adult otolith nuclei revealed similar patterns of separation between the RS and SAO, albeit with overlapping mixtures from the AMS and SIO (Fig. 3.3). Along these lines, Ward's minimum variance AHC cluster analyses also identified two primary groupings (clusters), mostly composed of RS fish and SAO fish (Fig. 3.4) with the level of mixing still relatively high among locations. This suggests fish overall shared two common spawning grounds.

3.5 Discussion

3.5.1 Population structure of Antarctic toothfish in the Southern Ocean

The otolith nuclei of adult Antarctic toothfish varied sufficiently to distinguish two distinct geochemical areas one associated with the Ross Sea and another with the Southern Atlantic. These findings are consistent with otolith nucleus signatures of juveniles reported by Ashford et al. (2012) for Antarctic toothfish from the RS and SAO, further indicating the existence of two spawning populations between these regions. Even more promising is that the same patterns of spatial heterogeneity were also identified in the otolith nucleus of Patagonian toothfish (*D. eleginoides*), (Ashford et al. 2012), a sister species to Antarctic toothfish with a similar life cycle

that inhabits a close circum-sub-Antarctic distribution (45˚S and 62˚S), north of the polar front (DeWitt et al. 1990). While both species share a close phylogenetic relationship, their evolution in disparate thermal regimes demonstrate distinct genetic and physiological characteristics (Ghigliotti et al. 2007), yet both appear to incorporate element-calcium compositions in a way that distinguishes the same unique geographic areas (Ashford et al. 2008; Ashford et al. 2012) demonstrating the utility of otolith chemistry techniques as habitat tracers.

These results align closely with genetic differences that suggest limited gene flow may occur among Antarctic toothfish populations within the Australian Antarctic Territory, Ross Dependency and the South Shetland Islands (Kuhn & Gaffney 2008). Moreover, that the same age classes were examined across regions precludes any interferences that might occur from fish that were spawned in different years (Elsdon et al 2008). In this study, Ward's AHC cluster analyses and nMDS visualisations, showed larval mixtures were mostly affiliated with the Ross Sea and Southern Atlantic sectors indicating these were important source areas for larvae. This distinction was evident among the small number of sub-adult (age 10) compositions from these regions although the ability to distinguish may be confounded if sub-adult populations were sampled across more locations as was shown among adult natal signatures.

These patterns were driven by significant differences in spatial heterogeneity consistent with otolith edge chemistry (Chapter 2), and through current genetic evidence providing further credence to population subdivision. The importance of finer regional and broad scale circulations around the Antarctic continent also reflects the complexity of larval dispersal trajectories for Antarctic toothfish in

terms of facilitating the replenishment of localised populations to nursery grounds or advection of early life stages further downstream into adjoining areas. Ideally, using juvenile age classes would provide a stronger basis for these findings, although, for the given year of sampling in this study, representative age classes and sufficient sample sizes for these age classes were not available across all locations. Nevertheless, for adult (age 14) and sub-adult (age 10) age classes examined by 2D ordinations and cluster analyses, a notable portion of Antarctic toothfish from the Ross Sea and Southern Atlantic remained close to their respective spawning grounds.

The indication of a population boundary between the Ross Sea and Southern Atlantic was evident in the Southern Atlantic group, where natal contributions from the Ross Sea were relatively low, indicating the Ross Sea was not a principle source for the Southern Atlantic. Kuhn and Gaffney (2008) proposed that two major cyclonic clockwise circulations in the Southern Ocean, the Weddell Sea and Ross Sea gyres may exert a strong force in isolating toothfish populations between these regions. This population boundary would also be facilitated by homing tendencies among Antarctic toothfish which would further promote population subdivision between these regions (Parker et al. 2002).

3.5.2 Larval mixing between regions.

While most fish from the Ross Sea and Southern Atlantic showed close affinities to their respective capture areas, fish from the Amundsen Sea and Southern Indian Ocean had spatial aggregations that overlapped with the Ross Sea and Southern Atlantic Ocean during early growth. Predictable patterns of straying among fully marine fish may be discernible given information around ocean circulation

processes (Galindo et al. 2006; Treml et al. 2008; Ashford et al. 2010) larval behaviour and settlement cues (Hamilton et al. 2006), and where the proximity of neighbouring populations is known. In the case of Antarctic toothfish, the Antarctic Circumpolar Current (ACC) acts as a conduit for ocean scale transport of toothfish larvae outside of their respective spawning grounds (Ashford et al. 2005; Ashford et al. 2007; Ashford et al. 2008; Hanchet & Rickard 2008; Ashford et al. 2012). By similar means, finer scale cyclonic oceanic gyres can retain larvae within a region based on the proximity of these features to spawning grounds and maturing habitat (Ashford et al. 2007; Hanchet & Rickard 2008). For the Ross Sea, overlapping mixtures were primarily composed of fish from the Amundsen Sea, Southern Atlantic and Southern Indian Ocean.

Although the precise spawning locations of Antarctic toothfish are not known (Hanchet & Rickard 2008) reproductive evidence and body condition aspects from the Ross Sea fishery, suggest spawning may take place over the ridges, banks and sea mounts along the Pacific–Antarctic Ridge (PAR). Spawning would begin from May to early June, peaking during the winter months and continuing through to November (Hanchet et al. 2015). Based on Lagrangian particle simulations of Antarctic toothfish eggs and larvae by Ashford et al. (2012) and HiGEM ocean circulation models from (Hanchet & Rickard 2008), the precise location at which spawning occurs in relation to the cyclonic Ross Sea Gyre could lead to the retention or export of larvae out of the Ross Sea (Hanchet & Rickard 2008; Ashford et al. 2012; Hanchet et al. 2015), (Fig 3.5).

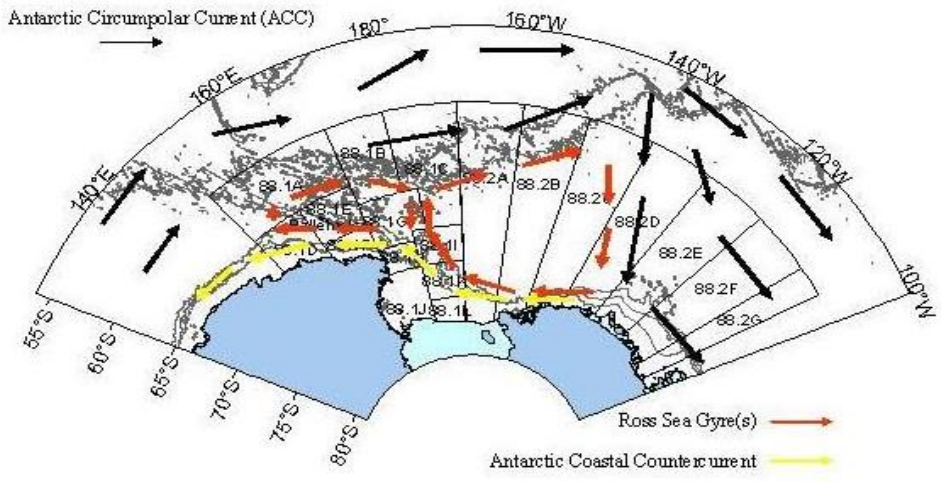

Figure 3. 5 Predicted Ocean circulation within the Ross Sea showing modelled locations of the Antarctic Circumpolar Current, Antarctic Coastal Counter current and Ross Sea gyres (Source Hanchet et al. 2008)

If spawning occurs south of the PAR, then eggs and larvae may be entrained by the western Ross Sea gyre and advected onto the highly productive continental shelf and slope where they can gain conditioning prior to spawning migrations back to the PAR (Hanchet & Rickard 2008). Physical dispersal of larvae from the PAR along this pathway would explain the high retention of recruits among sub-adult and adults from the Ross Sea that would facilitate close affinities to spawning grounds.

However, corresponding mixtures from the Southern Indian sector west of the Ross Sea could also be explained by dispersal pathways onto the continental slope and shelf via the Ross Sea Gyre. Ashford et al (2012) used Lagrangian particle simulations to predict potential transport pathways of Antarctic toothfish early life stages within the Ross Sea. This suggests that fish emerging from the Drygalski Trough near the continental slope may be entrained in downslope gravity currents leading to the Balleny Ridge and then into the deep western boundary and the

Antarctic Coastal Counter current along the continent coast into the southeastern Indian Ocean (Orsi et al. 1999), (Fig 3.5). This may account for the contribution of Ross Sea recruits in the Southern Indian sector, a trajectory more plausible than advection eastwards around the continent bypassing potential settlement areas in the Amundsen and Southern Atlantic.

For associated Amundsen Sea mixtures, larval contributions may have had natal origins to spawning grounds north of the PAR, near the eastward flowing ACC (Orsi et al. 1995). As a large-scale hydrographic feature that connects with all the Southern Ocean basins (Orsi et al. 1995) spawning events north of the PAR may see larvae advected eastward along the ACC to slope and shelf areas in the Amundsen Sea (Hanchet & Rickard 2008), (Fig 3.5). Based on tagging data, there is a possibility for movements of adults from the Amundsen Sea back onto the continental slope and shelf in the Ross Sea which would make reconciling spawning origins of individuals from the Ross Sea and the Amundsen Sea problematic. However, such movements may not be common given fish physiology and swimming capacity is not suited for long distance migration (Eastman & DeVries 1981, 1982; Hanchet & Rickard 2008; Parker et al. 2016) and mark recapture data indicate the movement of Antarctic toothfish rarely exceed 50–100 km from their release site (Parker et al. 2014).

Moreover, downstream export eastward along the ACC is consistent with model estimates by (Hanchet & Rickard 2008) where as much as 30% of eggs and larvae could be advected out of the Ross Sea. This would account for larval contributions to the Southern Atlantic. As post-larval drift is likely to occur over several months (Hanchet & Rickard 2008), advection from the Ross Sea to shelf areas on the

Southern Atlantic might be questionable given the distance between locations. Thus, Ross Sea contributions to the Southern Atlantic may be low and therefore may not be an important source to the Southern Atlantic. This sits favourably with population boundaries between the Ross Sea and Antarctic Peninsula where genetic evidence (Kuhn & Gaffney 2008) and the otolith nucleus chemistry of juvenile Antarctic toothfish For the Southern Atlantic mixtures, Ward's AHC cluster analyses and nMDS plots revealed two groups, one of which had natal compositions associated with the Amundsen Sea, west of the Southern Atlantic, while the other showed larval growth consistent with spawning near the BANZARE ridge in the Southern Indian Ocean east of the Southern Atlantic (Dunn et al. 2012). The west to east flowing Antarctic Circumpolar Current (ACC) connects the Atlantic, Pacific, and Indian Oceans, and serves as a principal pathway of exchange between deep water and shelf waters close to the Antarctic continent (Orsi et al. 1995). As a major geographic feature of the Southern Ocean the ACC may function as a conduit for larval exchange of toothfish outside of their respective spawning grounds (Ashford et al. 2005; Ashford et al. 2007; Ashford et al. 2008; Hanchet & Rickard 2008; Ashford et al. 2012) consistent with patterns of mixing shown in this study.

Along this pathway, recruits from the Amundsen Sea located upstream of the Southern Atlantic may be advected into the Southern Atlantic as was shown in edge chemistry (Chapter 2). Similarly, from the Southern Atlantic, larvae may also follow a similar pathway of exchange along the ACC east into the Southern Indian Ocean (Ashford et al. 2007). Mixtures from the Southern Indian Ocean to the Southern Atlantic might otherwise be limited against the prevailing ACC, however, some exchange along the Antarctic Coastal Current which flows westward and parallel to the Antarctic coastline (Tchernia & Jeannin 1984; Fahrbach et al. 1992;

Ashford et al. 2005) may also occur. Advection of larvae from the Southern Indian Ocean into the Amundsen Sea would be less likely given the Antarctic Peninsula partially impedes the Antarctic Coastal Currents flow (Fahrbach et al. 1992). It should be noted that overlapping mixtures observed here may have resulted from some oceanic basins having similar water mass compositions during early growth making the detection of spatial differences in otolith nucleus chemistry insignificant (Campana 1999; Elsdon et al. 2008). However, as the spatial disparity of edge compositions among the same fish (Chapter 2) differed to natal signatures, mixing would not be amenable to homogenous geochemical conditions in otoliths (Campana 1999).

Moreover, the prevalence of larval exchange likely explains, the inconsistency of genetic markers to clearly distinguish Antarctic toothfish populations (Smith et al. 2001; Parker et al. 2002; Smith & Gaffney 2005; Kuhn & Gaffney 2008; Mugue & Petrov 2014), and further illustrates the utility of using multifaceted genetic and otolith microchemistry approaches in population studies of fully marine fish species (Campana 2005; Elsdon et al. 2008; Taillebois et al. 2017). This indicates that some larvae utilised different spawning areas to where they were originally captured, suggesting that among some oceanic basins the effective habitat range of larval recruits is much broader than the management area in which adults were captured.

The present study demonstrated that the otolith nucleus chemistry of age 10-year-old and 14-year-old Antarctic toothfish varied sufficiently to distinguish two distinct geochemical spawning areas within the Ross Sea and Southern Atlantic. However, neighbouring regions to the RS and SAO showed considerable overlap in natal chemistry consistent with downstream mixing through the ACC generating

a mixture of natal types similar to Patagonian toothfish (Ashford et al. 2012; 2007). The Ross Sea and Southern Atlantic Ocean may therefore contribute disproportionately to recruitment in neighbouring areas of the Southern Indian Ocean and Amundsen Sea facilitated by large scale ocean circulations. This would indicate that fisheries management of the Ross Sea and Southern Atlantic Antarctic toothfish populations should be considered as separate stocks.

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4. Preliminary examination of otolith microchemistry to determine stock structure in Antarctic toothfish (Dissostichus mawsoni) between SSRU 88.1C and 88.2

4.1. Abstract

Life history characteristics of Antarctic toothfish (*Dissostichus mawsoni*) were evaluated using otolith microchemistry techniques. The aims of this study were to (i) determine whether adult (age 17) Antarctic toothfish from Small Scale Research Units (SSRUs) 88.1C (Northern Ross Sea) and 88.2H (North-eastern Ross Sea) of the Ross Sea fishery could be differentiated by the trace elemental concentrations in the otolith edge and then to (ii) assess the potential of natal signatures to distinguish stocks among these regions. For otolith edge elemental compositions (Li, Mg, Al and Zn), spatial heterogeneity between regions was not evident resulting in low discriminatory power, and classification success (53%) of fish to their original capture sites. This suggests the water mass between areas was similar or that there was possibly mixing between them through regional scale circulation currents. However, in contrast to edge chemistry, otolith nucleus compositions of the same age class (age 17) revealed stronger patterns of separation. Univariate ANOVA analyses showed significant differences in Li, Al and Zn among toothfish being significantly higher in fish from 88.1H (ANOVA, $P < 0.01$) compared to 88.1C.

Visualisation of the same elements in the otolith nucleus using a 2-dimensional ordination plot and Ward's AHC cluster solutions showed reasonable separation between toothfish from 88.1C and 88.2H suggesting some toothfish may have

utilised different spawning grounds. Moreover, patterns of overlap were also observed indicating both areas may contribute recruits to the Ross Sea fishery disproportionately. However, given these analyses are only preliminary and included a small number of adults (age 17), further investigations using juvenile and adult age classes collected from shelf and slope regions within SSRU 88.1 and 88.2 would provide stronger evidence of structuring between these regions.

4.2. Introduction

Life history and stock structure information around Antarctic toothfish (*Dissostichus mawsoni*) has been particularly elusive. However, the range of approaches that have expanded our knowledge of these aspects has steadily increased to include genetic evidence (Parker et al. 2002; Smith & Gaffney 2005; Kuhn & Gaffney 2008; Mugue & Petrov 2014) parasitology and more recently otolith microchemistry techniques (Ashford et al. 2012). A range of aspects on the stock structure of Antarctic toothfish within the Ross Sea region have also been outlined (Hanchet & Rickard 2008; Hanchet et al. 2015), along with assessments of life history and larval dispersal (Hanchet & Rickard 2008; Ashford et al. 2012). Collectively, these lines of evidence support the existence of a single stock within the Ross Sea.

However, there is less information characterising the stock structure of Antarctic toothfish within the Amundsen Sea region (SSRU 88.2C-H), or whether the two regions are connected biologically (Parker et al. 2014). Mark-recapture data indicate the movement of Antarctic toothfish rarely exceed 50–100 km from their release site yet some limited connectivity between the Ross Sea shelf/slope SSRUs 88.2 (1748-2193 km) has been documented by the movement of four tagged fish between areas (Parker et al. 2014). However, considering the large number of tagged fish in these areas and the small number of recaptures, current evidence on movements of Antarctic toothfish between these areas is weak. In the present study, we used otolith microchemistry techniques with the aims of (i) determining whether adult fish from these regions could be differentiated based on the trace elemental concentrations in the otolith edge and (ii) assessing the potential of natal

signatures to delineate stocks and determine whether these regions are biologically connected.

4.3. Materials and methods

4.3.1 Sample collection

Fish collections were carried out from four New Zealand long-line vessels operating in CCAMLR managed SSRUs 88.1C (Ross Sea) and 88.2H (Amundsen Sea) during the 2011–2012 austral summer (Fig. 4.1).

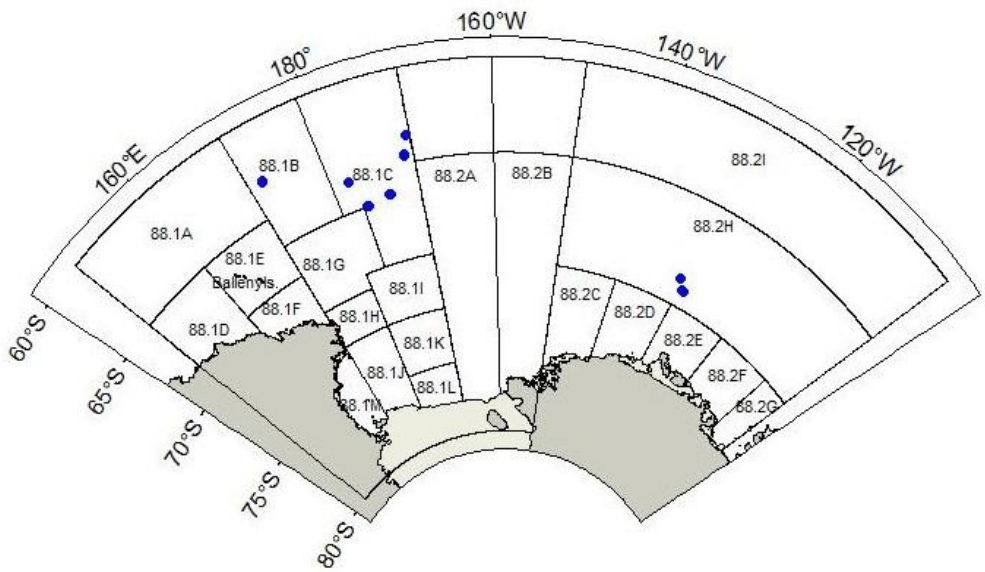

Figure 4.1 Small scale research units (SSRUs) in CCAMLR Subareas 88.1 and 88.2. Antarctic toothfish were collected in SSRUs 88.1C and 88.2H during the austral summer of 2011–2012. Points represent relative sample locations for fish in each sample region.

Sagittal otolith pairs from adult Antarctic toothfish (*Dissostichus mawsoni*) were extracted by scientific observers. Otoliths were stored in paper envelopes and labelled with catch information for each fish including total length, weight, sex, maturity stage, trip code and species code. Analysis of 36 adults collected from the northern Ross Sea in SSRU 88.1C and 26 adults from the Amundsen Sea in SSRU

88.2H were carried out for otolith edge chemistry (Fig. 4.1; Table 4.1). Otolith growth adjacent to the edge reflects elemental growth profiles consistent with the time when fish were captured regardless of age (Elsdon et al 2008). However, for the otolith nucleus, only a subset of age 17-year-old Antarctic toothfish (N = 30) hatched in the same year were used for analysis as fish hatched in different years may constitute differences in water chemistry that could be misconstrued as population differences (Elsdon et al 2008).

Table 4.1 Summary information of Antarctic toothfish sample collections including mean (±SD), total length, sex, sample size and sample collection location in NERS and NRS.

			Length (cm)	Sex	
Location	N	SSRU	Mean ± SD	F	M
NERS	26	88.2H	146.8 ± 12.70	18	8
NRS	36	88.1C	148.1 ± 11.63	14	22
Total	62	--	147.5 ± 12.02	32	30

4.3.2 Otolith preparation

Otolith cleaning protocols and preparation for laser ablation inductively coupled mass spectrometry (ICP-MS) were conducted with modifications to the methods of Secor et al. (1992). All handling and storage utensils were acid washed in a 10% HNO_3 bath for 24 h, air dried in a laminar flow cabinet and stored in sealed containers until use. Sagittal otolith pairs were sonicated in ultrapure Milli-Q water in batches of 28 for 3 min then drained and left to soak in a 3% solution of H_2O_2 for ~3 min to remove connective tissue. The solution was then drained and rinsed in Milli-Q water and sonicated a further 3 min. Otoliths were then drained and soaked in 2% HNO_3 solution for 15 s before being thoroughly rinsed and sonicated

for 2 min (Secor et al. 2001). Otoliths were dried in their vials in a laminar flow cabinet over 24 h prior to mounting, then embedded in silicon moulds using Nuplex ® K36 epoxy resin. Selections for embedding were made randomly to account for bias due to operational effects associated with laser instrument drift within and between days that might mask the signatures of capture location. Transverse sections of the nucleus were made using a low-speed Isomet diamond cutting saw (Buehler-USA), fitted with two blades spaced 250–300 µm apart. Sections were polished to expose the otolith nucleus using a sequence of SIC-2400 wetted carbide paper (Struers) followed by finer grit 4000 µm. Polishing was carried out within a tray of Milli-Q water that was replaced with each new sample. Accuracy of polishing was determined using a Leica MZ12 microscope at x 40 magnification (Sutton & Horn 2010). Surface otolith material was gradually removed by polishing to expose the nucleus just under the otolith surface. The section was then glued to a 50 x 25 mm petrographic slide using Crystalbond^{TM} 509 thermo-setting adhesive and stored in covered slide holders ready for laser ablation.

4.3.3 Otolith elemental analysis

Trace elements in the otolith edge (recent growth), and nucleus (natal growth), of Antarctic toothfish were assayed using laser ablation inductively coupled mass spectrometry (LA-ICP-MS). The system comprised of a Perkin Elmer DRCII ELAN 6000 ICP-MS (Waltham, MA) coupled to a New Wave Research UP-213 nm NdYAG laser ablation system (Fremont, CA), and was operated at the University of Waikato Mass Spectrometry Facility in New Zealand. Laser optimisation and sensitivity parameters and running conditions were operated in accordance with settings from Chapter Two (section 2.3.3). Transport and detection of elements was optimised using helium (He) and argon (Ar) carrier gas

configurations, with elemental interferences associated with oxygen accounted for by measuring ThO^+/Th^+ ratios during standard analyses until ratios ~1% were achieved (Lichte et al. 1987). Sensitivity checks were carried out by monitoring Th^+/U^+ ratios and adjusting nebuliser gas flow rates until counts were > 20,000 (0.7 L min^{-1}), (Jackson 2007). A total of 12 isotopes were analysed in otoliths and in National Institute of Standards and Technologies (NIST612) standard reference material (SRM), for which the concentration values of 50 elements are known (Pearce et al. 1997). However, only seven isotopes were above minimum detection limits (MDL) and the remainder were therefore excluded.

A single day's run included processing one slide with 28 samples and a wafer of NIST612 SRM. The laser was operated in Q-switched time resolved mode with the average energy reading of 0.07 mJ, scan speed 15 µm s^{-1} and repetition rate of 20 Hz. A beam diameter of 25 µm was used with the laser fired at 60% power, while travelling in raster mode configuration along the otolith edge at a distance of ~700 µm. Although the properties of otolith crater depth were not measured in this study, a beam diameter of 25 µm, laser pulse setting of 20 Hz and power output of 65% would likely give a predicted crater depth of around 110 µm as per experimental laser ablation properties reported by Jones and Chen (2003). The nucleus was ablated as a continuous grid (150 µm x 150 µm), centred within the first annual increment at the same raster distance as edge analyses (~700 µm). It has been shown that processes associated with embryological development may produce elevated manganese concentrations in the otolith nucleus that could confound the environmental signal associated with larval rearing habitats (Brophy et al. 2004). As a result, Mn concentrations were excluded from analyses associated with nucleus compositions. Before each sample ablation, background intensity readings

of residual material were recorded. Similarly, NIST612 reference material was analysed twice before sample analysis, once every seven samples and twice at the end of the run. Final output data from individual ablations were acquired for each isotope as an average reading of the peak laser signal and given as elemental concentrations, with calibration and instrument drift corrections carried out using GLITTER data reduction software (Macquarie University Australia SKU: version 4.0). For each s, mean minimum detection limits (MDL) at 99% confidence were calculated for individual elements with GLITTER using background readings and Poisson counting statistics (Griffin et al. 2008). Similarly, GLITTER was used to review and select time-resolved peak laser signals for each element enabling 100% readings for all elements used in this study.

4.3.4 Data analysis

To meet parametric assumptions of normality element-Ca concentrations in the otolith edge and nucleus were transformed using lambda-based power transformations. Data were then evaluated for normality using Shapiro-Wilks ($\alpha = 0.05$), homogeneity of variance was evaluated using Levene's test and covariance matrices for moderate to small sample sizes by Box's M tests. To evaluate spatial differences in the otolith edge chemistry of fish collected from SSRUs 88.1C and 88.2H, mean differences in elemental concentrations were assessed using univariate (ANOVA) and elemental effects that may be confounded by size and sex between capture locations were evaluated using multivariate ANCOVA analyses.

Quadratic discriminant function analyses (QDFA) were used to assess classification success between capture locations as it does not assume homogeneity of variance covariance (Everitt et al. 1991) with standardised coefficients from the QDFA used

to assess which elements contributed most to capture site separation analyses. Classification accuracy of fish to their known capture location was determined using cross-validated 'leave-one-out' jack-knife classification. For otolith nucleus analyses, the same adult age classes were examined using univariate ANOVA. To visualise spatial relationships of toothfish during early growth (otolith nucleus), non-metric multidimensional scaling (nMDS) ordinations (measuring dissimilarity between data points) were carried out using distances (Kruskal & Wish 1978). How well ordinations represented the original dissimilarities was determined by the lowest stress coefficient (Kruskal stress < 0.1). Ordinations were constructed in two dimensions using standardised (mean = 0, SD = 1) element-Ca composition and Zn) to account for any scale differences, while the selection of elements was based on those that showed significant spatial variability among sample regions. The extent to which one or more populations might exist was investigated using Ward's minimum variance hierarchical clustering approach using Euclidean distances (Khattree & Naik 2000). The quality of clustering was evaluated using the within cluster variance with cluster truncation determined using Shannon's entropy (Shannon 1948) whereby the number of clusters were identified by the largest decrease between nodes. visualisations All elemental values are reported as transformed data standardised to calcium (i.e., Sr:Ca) with analyses performed using STATISTICA 13 (StatSoft Inc, Tulsa, USA).

4.4. Results

4.4.1 Spatial variability in otolith edge chemistry

Significant effect differences between element-Ca compositions and fish length, sex or between capture locations were not evident from multivariate ANCOVA

analyses of Antarctic toothfish otolith edge compositions. This enables their use as environmental proxies (Table 4.2). However, univariate ANOVA analyses revealed no patterns of spatial heterogeneity between NRS and NERS among element-Ca compositions suggesting the water mass between these areas may have been similar (Table 4.3, Fig 4.2). Of some notable mention, were compositions of Al (Table 4.3) which was slightly higher in otoliths of toothfish from the NERS compared to NRS (Fig 4.2).

Table 4.2 Univariate ANCOVA results testing for differences in otolith edge chemistry using elemental compositions of Li, Mg, Al, and Zn (transformed data) of age 17 year old Antarctic toothfish according to capture location, length, and sex

		ANCOVA				
Effect	Element	df	SS	MS	F	P
Location*Sex	Li:	3	0.48	0.16	0.23	0.87
Location*Length		1	0.36	0.36	0.52	0.48
Sex*Length		1	0.09	0.09	0.13	0.73
Location*Sex*Length		1	0.02	0.02	0.02	0.88
Error		22	15.18	0.69		
Total		29	16.70			
Location*Sex	Mg	3	2.97	0.99	2.16	0.12
Location*Length		1	0.10	0.10	0.21	0.65
Sex*Length		1	1.03	1.03	2.24	0.15
Location*Sex*Length		1	1.32	1.32	2.88	0.10
Error		22	10.11	0.46		
Total		29	16.85			
Location*Sex	Al	3	1.65	0.55	0.28	0.84
Location*Length		1	0.11	0.11	0.06	0.81
Sex*Length		1	0.92	0.92	0.47	0.50
Location*Sex*Length		1	0.60	0.60	0.31	0.59
Error		22	43.40	1.97		
Total		29	58.90			
Location*Sex	Zn	3	1.01	0.34	0.67	0.58
Location*Length		1	0.59	0.59	1.17	0.29
Sex*Length		1	0.20	0.20	0.41	0.53
Location*Sex*Length		1	0.19	0.19	0.38	0.54
Error		22	11.04	0.50		
Total		29	12.67			

Table 4.3 Univariate ANOVA results testing for differences in otolith edge element-Ca compositions between the NRS and NERS of age 17 year old Antarctic toothfish.

			ANOVA			
Effect	Element	df	SS	MS	F	*P*
Location	Li	1	0.70	0.70	1.22	0.28
Error		28	16.00	0.57		
Total		29	16.70			
Location	Mg	1	0.71	0.71	1.24	0.27
Error		28	16.14	0.58		
Total		29	16.85			
Location	Al	1	7.61	7.61	4.16	0.05
Error		28	51.29	1.83		
Total		29	58.90			
Location	Zn	1	0.39	0.39	0.89	0.35
Error		28	12.28	0.44		
Total		29	12.67			

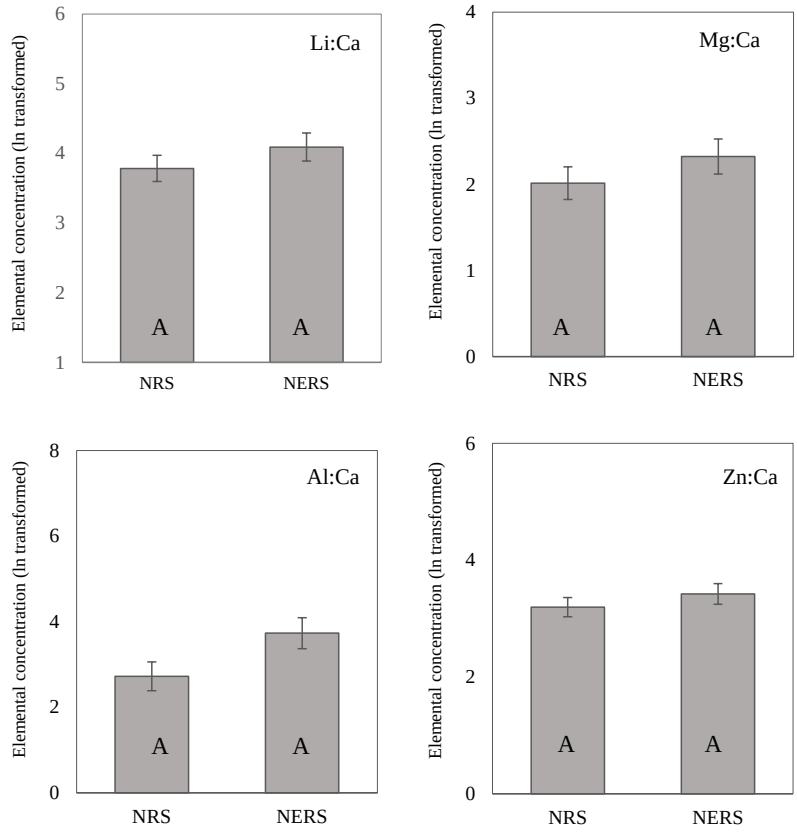

Figure 4.2 Mean (± SE) element-Ca compositions of ln transformed Li, Mg, Al and Zn in the otolith edge of age 17 year old Antarctic toothfish collected from SSRUs 88.2H and 88.1C. Letters that differ between locations represent significant differences (Tukey's P <0.05).

4.4.2 Distinguishing capture location using otolith elemental signatures

The utility of elemental concentrations in the otolith edge in assigning adult Antarctic toothfish to their known capture location was examined using QDFA analyses and three predictor elements (Mg, Zn and Al), (Table 4.3). Evaluation of the discriminant model showed considerable overlap in elemental ratios between capture locations with jack-knife classification success of 53% overall (Table 4.4).

Classification success was much the same for fish in SSRU 88.2H 57% and in 88.1C 50% (Table 4.4). However, the number of misclassifications was greatest

for fish from SSRU 88.1C (8) assigned to 88.2H and slightly fewer fish (6) being misclassified from 88.1C to 88.2H (Table 4.4).

Table 4.3. Canonical discriminant functions (CDF) and standardised canonical discriminant functions (SCDF) of otolith edge elements (Mg, Al and Sr) and nucleus (Al and Zn) predictors used in the QDFA analyses alongside sample means for elements among capture locations.

	Element	CDF	SCDF	88.2H	88.1C
Edge	Mg:Ca	0.5	0.34	2.23	1.91
	Al:Ca	0.69	0.87	3.62	2.55
	Sr:Ca	-0.46	-0.22	1.24	1.62
Nucleus	Zn:Ca	1.71	0.83	3.13	2.63
	Al:Ca	0.66	0.45	2.07	1.63

Table 4.4 Cross validated (jack-knife) classification matrix of QDFA analyses using otolith edge signatures Mg, Al, and Zn to assign age 17 year old Antarctic toothfish to capture locations within SSRUs 88.1C and 88.2H. Rows represent original capture region, columns represent predicted capture location. Analyses were weighted to group size.

	Predicted location			
Actual location	NERS	NRS	Total	% Correct
NERS	8	6	14	57.14%
NRS	8	8	16	50.00%
Total	16	14	30	53.33%

4.4.3 Differences in natal structuring of adults between 88.1C and 88.2H

In contrast to otolith edge chemistry, the spatial variability in the otolith nucleus varied significantly between locations (Table 4.5, Fig 4.3). For example, Tukey's HSD tests showed element-Ca compositions of Li were significantly higher in toothfish from the NERS than in fish from the NRS. This was also the case for element-Ca compositions of Mg and Zn which were also significantly higher in

fish from the NERS (Table 4.5, Fig 4.3). Differences between regions based on Tukey's pairwise (HSD) comparisons showed significantly higher concentrations of Mg in fish from SSRU 88.2H than were present in fish from 88.1C (Fig 4.3). Elemental concentrations were also significantly higher in the nucleus of fish taken from 88.2H for Al and Zn (Table 4.5).

Table 4.5 Univariate ANOVA results testing for differences in otolith nucleus element-Ca compositions between the NRS and NERS of adult (age 17) toothfish.

			ANOVA			
Effect	Element	df	SS	MS	F	*P*
Location	Li	1	1.09	1.09	7.74	*0.010*
Error		28	3.94	0.14		
Total		29	5.03			
Location	Mg	1	1.66	1.66	8.76	*0.006*
Error		28	5.29	0.19		
Total		29	6.95			
Location	Al	1	0.94	0.94	2.29	0.142
Error		28	11.50	0.41		
Total		29	12.44			
Location	Zn	1	1.69	1.69	6.4	*0.02*
Error		28	7.42	0.27		
Total		29	9.11			

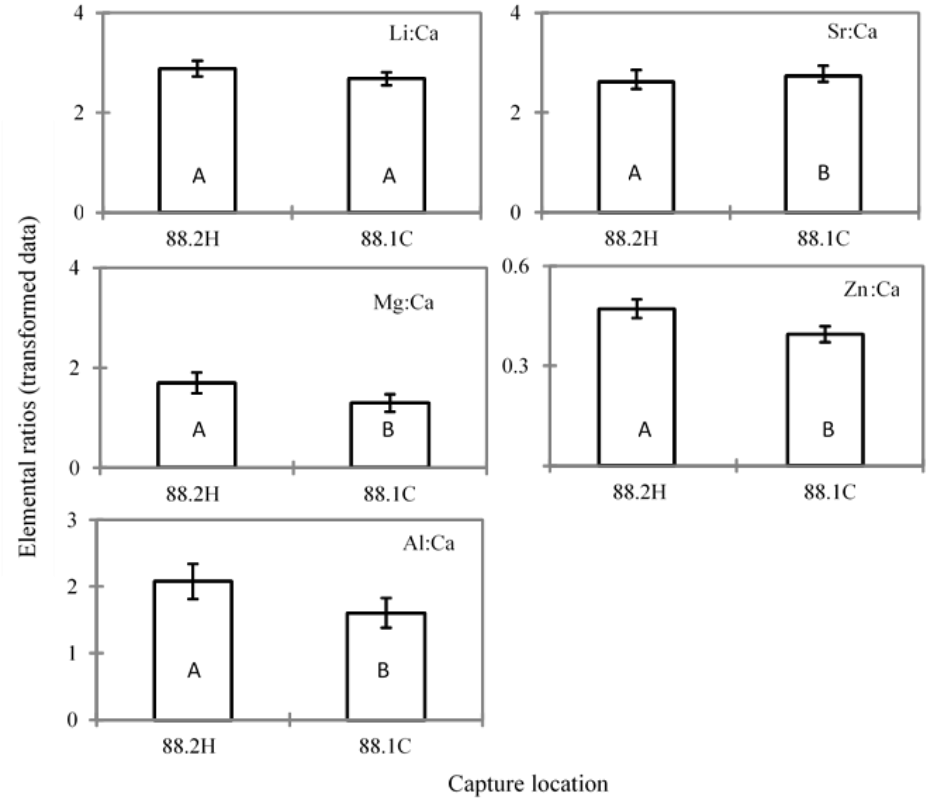

Figure 4.3 Mean (± SE) elemental ratios (transformed data), Li, Mg, Al, and Zn in the otolith nucleus of age 17 year old Antarctic toothfish collected from NERS (n = 14) and NRS (n = 16). Letters that differ between locations represent significant differences (Tukey's $P < 0.05$).

To visualise whether otolith nucleus signatures show spatial separation between NERS and NRS fishing grounds, element-Ca compositions were plotted as an nMDS ordination (Fig 4.4). All significant elements were included in the model with the best fitting ordination represented by the lowest stress coefficient (Kruskall Stress < 0.1), (Fig 4.4). Notable patterns of separation among adult toothfish from the NERS and NRS was evident, although overlapping cases were also shown indicating some toothfish may have utilised different spawning areas (Fig 4.4). These patterns were also supported by the Wards AHC cluster solution using Euclidean distances and by the composition of the two natal groupings.

Group one consisted of 50% of NERS and 50% of NRS toothfish whereas for group two, 40% of toothfish were from NERS and 60% were from the NRS (Fig 4.4).

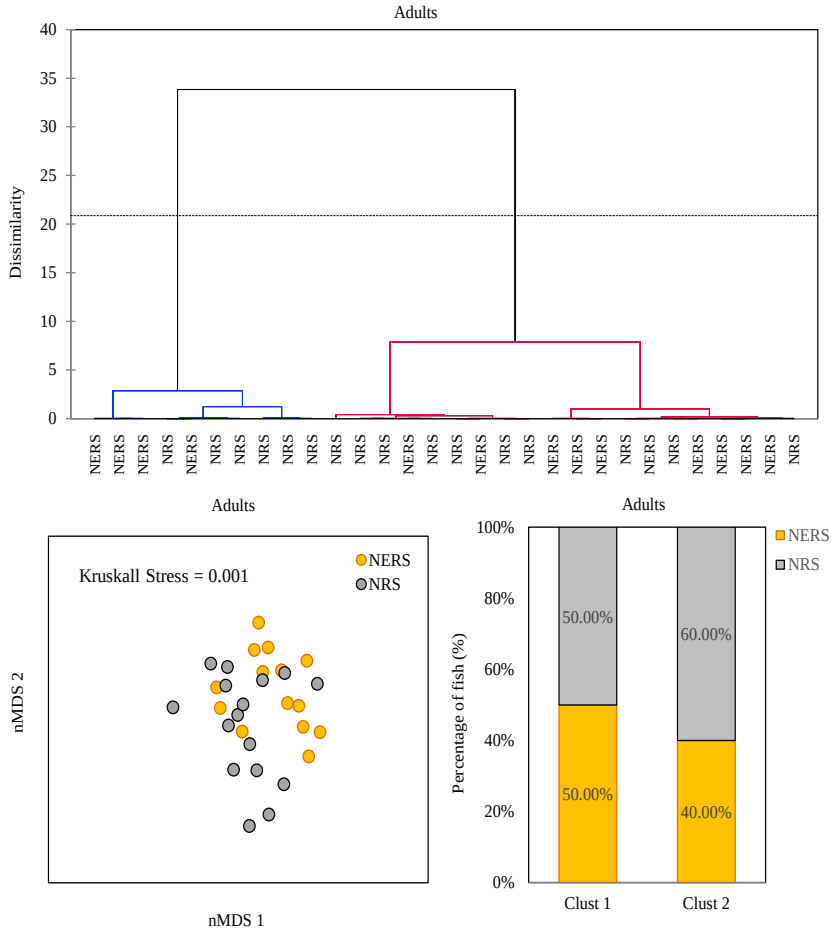

Figure 4.4 Wards AHC cluster solution (top) and nMDS ordination plot (bottom left) showing group structuring between the NERS (n = 14) and NRS (n = 16) alongside cluster assignment (%) cases using element-Ca compositions of Al, Zn and Mg extracted from the otolith nucleus of age 17 year old Antarctic toothfish.

4.5. Discussion

To distinguish fish between locations, environmental conditions must leave characteristic site-specific elemental markers in the portion of the otolith that corresponds to that specific time (Brown 2006; Campana 1999). In this study, analysis of trace elements in the otolith edge showed no significant spatial

heterogeneity between fishing areas which corresponded with low discriminatory classification success (53%), (Table 4.4). This could arise due to the lack of strong trace elements present in the water at these two locations, such that differences in otolith chemistry were not detectable in otoliths or that fish may have only recently moved into these areas from elsewhere and have not yet had time to incorporate site specific signatures into their otoliths. Alternatively, the influence of the regional scale Ross Sea gyre which is known to mix between slope and open water areas of the Ross Sea could also facilitate homogeneous water chemistries (Locarnini 1994; Budillon et al. 2003; Rickard et al. 2010).

In contrast to otolith edge chemistry, univariate ANOVA analyses of the same element-Ca signatures in the otolith nucleus of 17-year-old age classes showed stronger spatial separation between populations. Similarly, that adult toothfish were of the same age (17yr) between areas and likely subject to the same environmental conditions during early growth, reduced the influence of indifferent chemistries associated with fish that may have been spawned in different years which could lead to misleading inferences about the structure of populations (Elsdon et al 2008).

Significantly higher Li, Mg and Al compositions were evident in fish from 88.2H compared to fish from 88.1C indicating environmental conditions during larval growth differed among toothfish from these areas. However, while corresponding nMDS and Wards AHC cluster analysis results showed reasonable separation between fish from 88.2H and 88.1C suggesting two likely spawning populations, there were also clear patterns of overlap between these same populations. This suggests two possible spawning populations exist which may contribute

disproportionately to the Ross Sea fishery. The first group consisted of an equal mixture of 88.2H (50%) and 88.1C (50%) Antarctic toothfish while the second grouping mainly consisted of 88.2H (60%) and to a lesser extent 88.1C (40%) populations.

Unlike otolith edge chemistry where capture location is utilised as an elemental fingerprint corresponding to environmental conditions of the water mass prior to capture (Campana 1999) otolith nucleus chemistries may reflect early growth phases post spawning or reflect longer periods of drift as larvae disperse from spawning grounds in ocean currents (Campana 1999, Standish et al 2008).

In the Ross Sea (88.1C) and Amundsen Sea (88.2H) where toothfish were captured, spawning location is not precisely known (Hanchet et al 2008). Instead, otolith nucleus compositions of adult toothfish reflect larval growth histories well within the first annual increment (~6 months growth). This suggests the two possible spawning populations may contribute disproportionately to the Ross Sea fishery. The first group reflecting an equal mixture of 88.2H (50%) and 88.1C (50%) Antarctic toothfish while the second grouping mainly consisted of 88.2H (60%) and to a lesser extent 88.1C (40%) populations. Reproductive evidence of Antarctic toothfish from the Ross Sea (Yukhov 1971; Fenaughty 2006; Hanchet & Rickard 2008; Parker & Grimes 2010b) suggests spawning may occur over banks and ridges along the PAR north of 88.1C (Hanchet et al. 2015).

The presence of two Ross Sea Gyres that rotate clockwise and anticlockwise not only facilitate mixing between deep open ocean water masses with slope and shelf regions (Locarnini 1994; Budillon et al. 2003) but also connects larval recruits from spawning areas over the PAR (Fenaughty et al. 2008; Hanchet & Rickard 2008) to

shallower continental shelf nursery grounds adjacent to NERS and NRS (Hanchet & Rickard 2008).. The relative closeness of the natal groupings shows reasonable uncertainty, and this study was only conducted as a preliminary analysis. Subsequently, analysis of adult and juvenile age classes collected from shelf and slope regions within the Ross Sea and the Amundsen Sea would be essential to determining whether observed patterns are consistent with separate populations between these regions.

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5. Spatial and temporal variation in the otolith chemistry of Antarctic toothfish (*Dissostichus mawsoni*) from the Ross Sea

5.1 Abstract

Individual life history chronologies of Antarctic toothfish were derived from fish otoliths collected during consecutive longline operations (2012–2013) within the Ross Sea. Chronologies were acquired with laser ablation ICP-MS to determine if otolith edge chemistry could differentiate capture locations of fish across different seasons. The stability of elemental compositions in the otolith nuclei across successive years was also evaluated to determine if any population differences were evident. Significant spatial separation between the PAR and continental Slope for adults (age 14) in consecutive seasons (2012–2013) was not evident based on otolith edge compositions of Mg, Al, Sr and Ba. This suggested environmental conditions prior to when adults were captured were much the same between locations and seasons possibly because of the close proximity of these locations and the influence of regional scale circulations mixing between these areas. However, significant spatial differences in Ba and Mg were observed among sub-adult (age 9) toothfish between Shelf and Slope areas among 2012 samples. Differences in Ba were associated with upwelling events along the continental Slope and Mg constituted physiological regulation. Temporal patterns of variation among element-Ca compositions in Antarctic toothfish suggest caution must be given to the use of otolith chemistry to evaluate movements of toothfish over consecutive years.

ANOVA analysis of the otolith nucleus chemistry of sub-adult (age 9) and adult (age 14) Antarctic toothfish showed no significant spatial variability among

elements (Li, Mg, Al and Sr) in either year, consistent with nMDS ordinations indicating that the majority of Antarctic toothfish from the Ross Sea fishery used a common spawning ground. However, a very small proportion of adults in both years reflected elemental compositions that ranged outside of the 95% confidence ellipse of nMDS ordinations indicating these fish may have utilised different spawning areas. These findings provide further support to the primary existence of a single spawning population within the Ross Sea that appears to be consistent over both years.

5.2 Introduction

Antarctic toothfish (*Dissostichus mawsoni*) are a large bentho-pelagic fish found in sub-zero (-2°C) waters from the Antarctic Convergence at about 60°S southwards towards the Antarctic continent (Gon & Heemstra 1990). These fish can be found at depths ranging from 70–2000 m (Eastman & DeVries 1982) and have been aged as old as 38 years (Brooks et al. 2010). Antarctic toothfish have been the focus of a longline fishery operating out of the Ross Sea where it was first initiated in 1996–1997 (Hanchet & Rickard 2008). The fishery is managed as an "exploratory" fishery, as the emphasis is around obtaining data and carrying out research that is implemented through biennial long-line operations during the austral summer (December– March) across discrete small-scale research units and statistical management areas. While aspects of the biology and ecology of Antarctic toothfish has been reasonably well described and much is known about its life history (Hanchet et al. 2015), information around the structure of populations across is less certain (Mugue & Petrov 2014; Hanchet et al. 2015). Several studies have used genetic markers to address population uncertainties with

varying degrees of success (Parker et al. 2002; Smith & Gaffney 2005; Kuhn & Gaffney 2008; Mugue & Petrov 2014). For example, using a combination of mitochondrial DNA (mtDNA) analyses and nuclear DNA introns (Smith & Gaffney 2005) found no population differences between Antarctic toothfish from the South Shetland Islands, Ross Sea Dependency and Australian Antarctic Territory. However, using mtDNA and nuclear single nucleotide polymorphism (SNPs) approaches, Kuhn and Gaffney (2008) reported evidence of genetically distinct differences between the same regions as Smith and Gaffney (2005) that were not apparent in a following study (Mugue & Petrov 2014). These authors found methodological inconsistencies in the assessment of polymorphisms at a number of loci reported by Kuhn and Gaffney (2008), such that conclusions about genetic isolation of toothfish in the Ross Sea should be considered with caution (Mugue et al. 2014).

In an alternative stock assessment approach using fish otoliths, Ashford et al. (2012) found that the otolith edge chemistry of Antarctic toothfish aligned with spatial differences within the Ross Sea. Moreover, in the same study, the otolith nucleus corresponding to larval growth further supported the existence of a single spawning population within the Ross Sea (Ashford et al. 2012) consistent with genetic evidence (Kuhn & Gaffney 2008). Notably, significant differences in the nucleus chemistry between juvenile fish caught in the Ross Sea and off the South Shetland Islands agreed with broader findings of Kuhn and Gaffney (2008) and findings of Chapter 2 where spatial heterogeneity between fish from the Ross Sea and Southern Atlantic Ocean were also shown in otolith nucleus chemistry, further expanding information around the population structure of this species. The aim of this study was to examine patterns of variation in the otolith chemistry of Antarctic toothfish

from collections obtained over two consecutive seasons (2012–2013) within the Ross Sea to determine if otolith edge chemistry, corresponding to periods of elemental uptake prior to capture, could distinguish capture locations, and where evidence of spatial differences was shown, to ascertain whether the same spatial differences in otolith chemistry were evident in fish over time. Finally, the existence of one or more spawning populations within the Ross Sea was evaluated based on the assessment of differences in natal chemistry between seasons.

5.3 Materials and methods

5.3.1 Fish otolith collections

Antarctic toothfish (*Dissostichus mawsoni*) collections were obtained by observers on board benthic longline fishing vessels operating in the Ross Sea over two consecutive years (2011–2012 and 2012–2013). Fishing operations occurred during the austral summer (December–February) in small scale research unit (SSRU) management areas within the Convention for Conservation of Antarctic Marine Living Resources (CCAMLR) Subareas 88.1 in the Ross Sea (Fig. 5.1). Length at age relationships for Antarctic toothfish have been previously established within the Ross Sea fishery using catch data (Eastman & DeVries 2000; Horn 2002; Hanchet & Rickard 2008; Brooks et al. 2010; Parker & Grimes 2010b). Following length-at-age relationships, specimens used in this study are attributed as juveniles (55–65 cm), sub-adults (66–100 cm), and adults (> 100 cm) and were collected during a pre–recruit survey in open-water polynyas on the continental Shelf, over the Ross and Hayes Banks and near the Iselin Bank south of the continental Slope by a single New Zealand longline vessel (San Aotea II) during 2011–2012. As outlined in Hanchet et al. (2015) the Ross Sea region can be divided into the 'Shelf'

(the Ross Sea Shelf out to the Shelf break at about 750 m), the 'Slope' (the Ross Sea Slope from 750 to 2000 m depth), and the 'North' (the area of seamounts, and the Pacific–Antarctic Ridge to the north of the Ross Sea). Hereafter, the Ross Sea Shelf and Ross Sea Slope will be referred to as the "Shelf and Slope". Adults in the same season were collected northeast of the Slope on banks, ridges and seamounts along the Pacific–Antarctic Ridge (PAR) by observers on board three additional New Zealand longline vessels (Fig. 5.1). Sample sizes of Antarctic toothfish collected during the 2012–2013 season were smaller than in the previous years and only included sub-adult (84–100 cm) and adults (107–174 cm) with no juveniles collected. All otolith extractions were carried out onboard fishing vessels by observers. Otoliths were stored in paper envelopes with catch data comprising total length measurements (TL cm), sex, maturity stage, trip code and fish identification. Envelopes were shipped to New Zealand, then delivered to the University of Waikato.

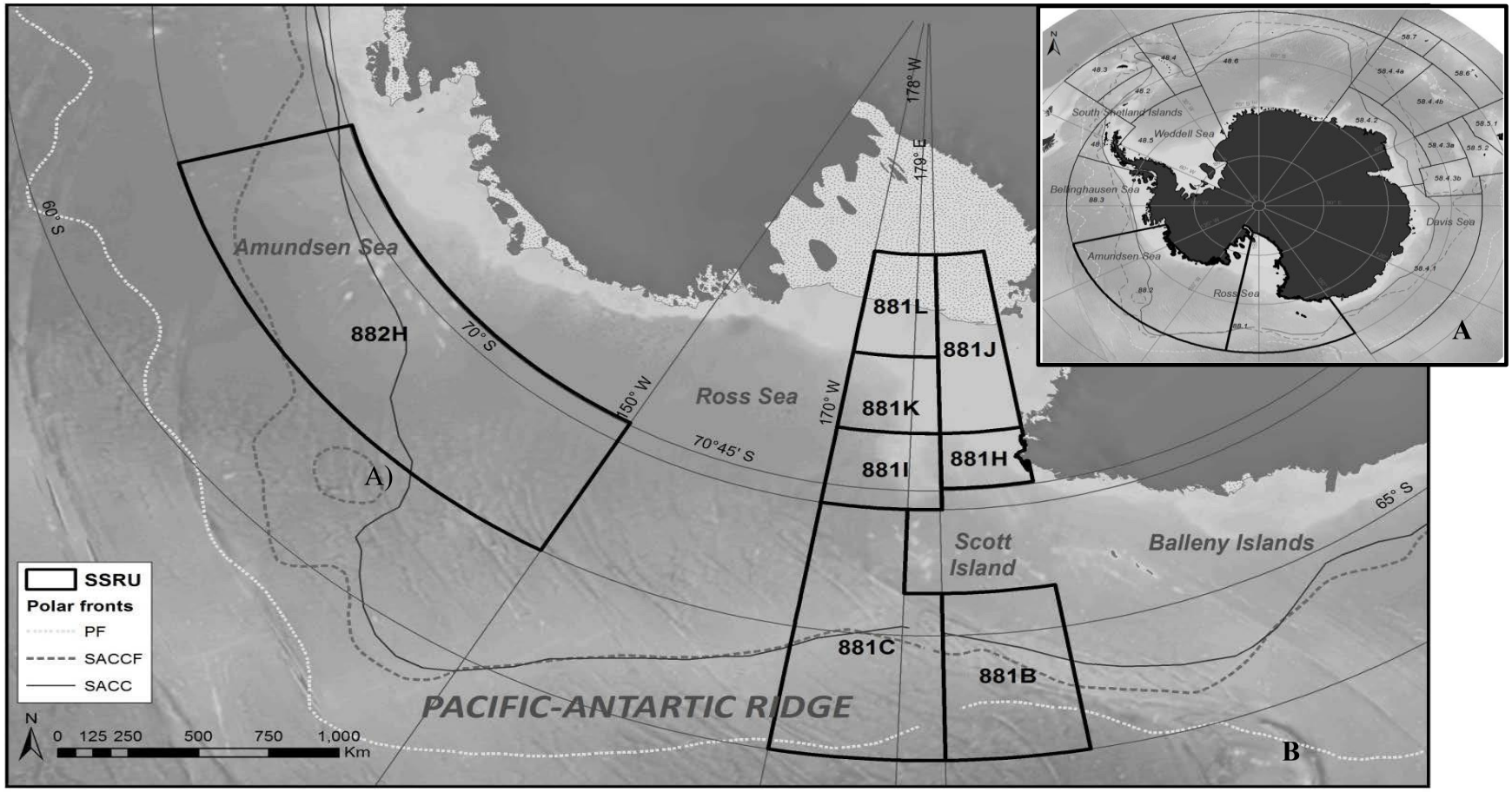

Figure 5.1. Location of CCAMLR SSRUs, A) where juveniles, sub-adult and adult Antarctic toothfish were collected in consecutive years (2012–2013), during the austral summer (December–February). Shaded and dotted lines represent hydrographic currents (Polar Front), which flow eastwards across the Ross Sea and Amundsen Sea and around the Antarctic continent B) SSRUs (88.1I, L, K J and H) on the Shelf and Slope, SSRUs (88.1C, B) along the Pacific–Antarctic Ridge (PAR) within Subareas 88.1, where fish collections were carried out. Pale grey shaded areas near the continent represent shallow continental Shelf areas (< 1000 m depth).

5.3.2 Otolith cleaning and preparation

Otolith handling and preparation was carried out using acid-washed plastic utensils soaked in a 10% HNO_3 for 24 h. Although otolith extractions were performed with metal tweezers, the effect of metal contamination after stringent cleaning protocols would be negligible (Secor 2002). Sagittal otolith pairs were cleaned in 19 mm glass scintillation vials topped up with ultrapure Milli-Q water and run through an ultrasonic bath for 4 min. Vials were drained and a 3% solution of H_2O_2 was added and left to sit for ~3 min to remove connective tissue, rinsed in Milli-Q water and sonicated for 3 min, then drained and rinsed with clean Milli-Q water. Otoliths were dried in a laminar flow cabinet over 24 h prior to mounting. Only the left sagittal otoliths were used for chemical analyses except where only one otolith was available which was then used. Otoliths were embedded in silicon moulds using Nuplex® K36 epoxy resin with the distal surface facing up. Resin blocks were air dried for 24 h prior to sectioning. Transverse sections were made across the primordial using a low-speed Isomet diamond cutting saw (Buehler, USA) fitted with two blades spaced 250–300 µm apart.

Each thin section was submerged within Milli-Q water and polished, using a sequence of SIC-2400 µm followed by finer 4000 µm grit carbide paper (Struers). Accuracy of polishing was determined using a Nikon SMZ645 with stereo microscope (C-W 10 x A/22) so that ring structures radiating from the outer growth zones exposed the nucleus just short of the otolith surface. Sectioned otoliths were glued to a small 50 x 25 mm petrographic slide, rinsed in Milli-Q water, then sonicated for 4 min, drained and left to dry in a laminar flow cabinet before storage in a cover slide container ready for laser ablation.

5.3.3 Laser ablation ICP-MS optimisation

The acquisition of trace elements in the otolith edge (recent growth), and nucleus (growth during hatching), of Antarctic toothfish were assayed using laser ablation inductively coupled mass spectrometry (LA-ICP-MS). The system consisted of a Perkin Elmer DRCII ELAN 6000 ICP-MS (Waltham, MA) coupled to a New Wave Research UP-213 nm Nd-YAG laser ablation system (Fremont, CA), operated at the University of Waikato, New Zealand. Prior to sample analyses, transport and detection of elemental concentrations, the ICP-MS was optimised using a helium (He) and argon (Ar) carrier gas configuration. A suite of 12 isotopes were initially analysed both in otoliths and National Institute of Standards and Technologies (NIST612) standard reference material (SRM), the concentration of which 50 elements have been reported (Pearce et al. 1997).

The selection of elements was based on the lack of matrix-matched standards, which interact differently with the laser beam (Mokgalaka & Gardea-Torresdey 2006), resulting in laser induced elemental fractionation (Longerich et al. 1996). As a result, the lithophile elements and their isotopes ^{7}Li, ^{27}Al, ^{25}Mg, ^{42}Ca, ^{43}Ca, ^{55}Mn, ^{66}Zn^{66}, ^{88}Sr, and ^{137}Ba, which have the lowest fractionation potential (Fryer et al. 1995; Jackson et al. 2004), were analysed. To account for elemental interferences produced by the presence of oxygen in the ICP-MS, carrier gas transport lines or on samples, optimisation involved monitoring oxide production in the ICP-MS by measuring ThO^{+}/Th^{+} ratios during analyses of NIST612 standards. The standard was analysed with a single slide of 20–30 samples using a continuous line scan set at a repetition rate of 20 Hz, output power of 60% and a spot size of 60 µm for ~4 min. As Th+ has a high affinity for oxygen, the ICP-MS was tuned by manually

adjusting the nebuliser gas flow to give a ThO+/Th+ ratio of ~1%. Once this was achieved it was assumed that all other oxide interferences were negligible (Lichte et al. 1987) and otolith sample analysis could begin. Additionally, sensitivity checks were carried out by monitoring Th+/U+ ratios closely until counts were > 20,000 cps. Pearce et al. (1997) demonstrated that the concentration of 50 trace elements in NIST612 glass standards were homogeneous.

Concurrently, elemental concentrations (ppm) from NIST 612 reference material were standardised to internal ICP-MS machine standards using ^{42}Ca by way of GLITTER data reduction software (Version 4.4.1, Macquarie Research Limited© 1991–2000). A single day's run included processing one slide with 20–28 samples and a wafer NIST612 SRM. The laser was operated in Q-switched time resolved mode with an average energy reading of 0.0734 mJ, a scan speed of 15 µm s^{-1} to enable analysis of all elements closely in time and a repetition rate of 20 Hz. Beam diameter was set at 25 µm with the laser fired at 60% power, while travelling in rastar mode immediately adjacent to the otolith edge at a distance of ~700 µm. Material from the same otolith at the nucleus was ablated as a continuous rastar line grid under the same ablation settings, while travelling an equivalent distance (~700 µm) in a raster mode grid formation (150 µm x 150 µm) centred well within the first annual increment. Prior to each sample ablation background intensity readings of residual material settled within the sample chamber, transport lines, and ICP-MS were recorded and subtracted online using data reduction software GEMOC Laser ICP-MS total trace element reduction (GLITTER). Variability in laser signal intensities over prolonged periods of use for elements were accounted for by analysing NIST612 reference material twice before sample analysis, once every seven samples and twice at the end of the run. Replicate standard analyses were

interpolated across samples enabling correction for within and between day variations in instrument sensitivity (drift) across the atomic mass range. Agreement of elements in each standard run were monitored in real time against reported NIST612 values and where readings were not in good agreement the NIST612 was run again. Relative standard deviations (RSD %) of elements from repeat NIST612 standard analyses (N = 42), within and between days were 4.5% (^{7}Li), 3.9% (^{25}Mg), 1.7% (^{27}Al), 1.3% (^{43}Ca), 1.9% (^{55}Mn), 4.5% (^{66}Zn), 1.2% (^{88}Sr) and 1.8% (^{137}Ba). Similarly, energy output readings (J cm^{2}) for each otolith and standard ablation were recorded and assessed against real time trace plots to determine if fluctuations in power might correspond to changes in concentration yields or via artefacts of the laser, with any major fluctuations resulting in re-runs of the analysis. For each days analyses, mean minimum detection limits (MDL) at 99% confidence (ppm) were calculated for individual elements with GLITTER using background readings and Poisson counting statistics (Griffin et al. 2008). Similarly, GLITTER was used to review and select time-resolved peak laser signals for each element enabling 100% readings for all elements used in this study. Final output data from individual ablations were acquired for each isotope as an average reading of the peak laser signal (elemental concentrations in ppm) all elements were normalised to ^{43}Ca as molar ratios (mmol mol^{-1}).

5.3.4 Age estimation

Age estimates were conducted according to protocols outlined in Chapter 2 and are discussed in more detail in Section 2.3.2. This was in accordance with standardised protocols developed by Sutton and Horn (2010) for Antarctic toothfish as part of the Scientific Committee CCAMLR work plan for 2010 (see SC-CAMLR-FSA

2009, paragraphs 9.4–9.8). The manual also forms the basis of protocols used by the National Institute of Water and Atmospheric Research Limited (NIWA) for estimating age of Antarctic toothfish collected from the Ross Sea (Sutton & Horn 2010), (Section 2.3.2).

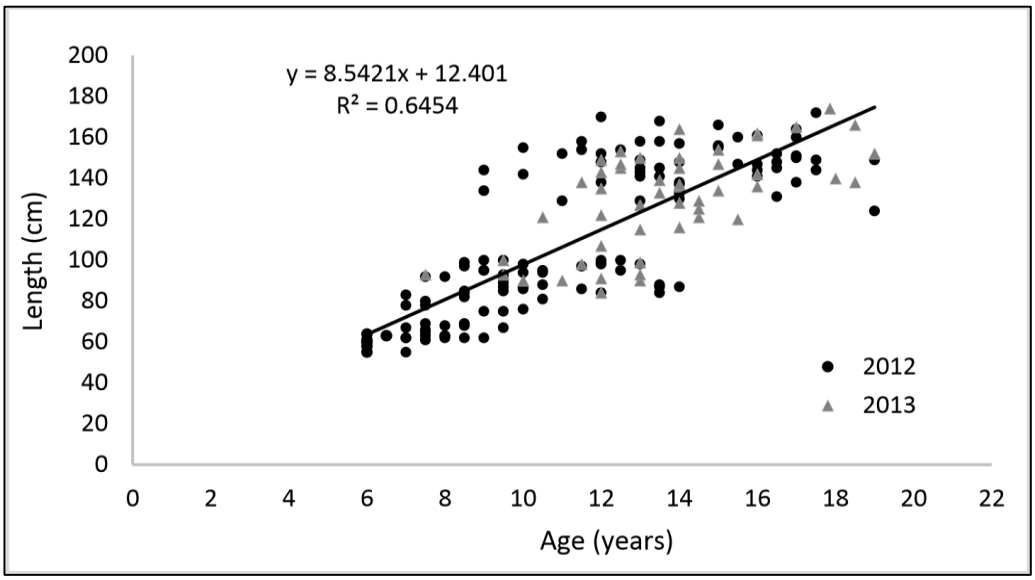

Figure 5.1 Age-length relationship of Antarctic toothfish collected within the Ross Sea (N = 170) in consecutive seasons during the austral summer (December–January) of 2012–2013.

5.3.5 Data analysis

Antarctic toothfish otolith edge and nucleus element-Ca compositions of Mg, Al, Sr and Ba were analysed using multivariate and univariate ANOVA approaches after natural log transformations (ln) ensured normality assumptions were met (Shapiro Wilks $P > 0.05$) across seasonal datasets (2012–2013). For consistency among spatial comparisons between years and capture locations, element-Ca compositions of age 9 sub adult (80-100 cm) and age 14 adult (100-120 cm) year classes were used in analyses (Hanchet et al 2008). Similarly, for otolith nucleus analyses using fish of the same age would limit temporal differences associated

with individuals hatching at different times (Elsdon et al 2008). Similarly, factorial ANOVA analyses were carried out on otolith nucleus signatures to determine if effect differences between sex and location were evident. Spatial differences in element-Ca compositions between capture locations were assessed using univariate ANOVA analyses with homogeneity of variance assumptions examined using Levene's test. When significant differences were identified, Tukey's (HSD) tests were used to identify where *a posteriori* difference may lie. Quadratic discriminant function analysis (QDFA) was used to explore the utility of otolith edge compositions to correctly classify toothfish to their capture locations as class variance/covariance matrices were not equal. Elements in the model that contributed most to the discrimination were based on standardised canonical coefficients and significant univariate (ANOVA) results among locations. Final classifications were determined using cross validated 'leave-one-out' jack-knife classification.

5.3.6 Spatial and temporal analysis of natal signatures

To visualise whether discrete groups exist among Antarctic toothfish collections from the continental Shelf and Slope and along the PAR, otolith nucleus compositions (representing larval growth) were examined in separate seasons using nonmetric multidimensional scaling (nMDS) (Kruskal & Wish 1978). Prior to using nMDS, a dissimilarity matrix was constructed using Euclidean distances after elemental compositions of Mg, Al, and Sr, and had been standardised (x – mean/standard deviation). The quality of nMDS configurations were determined using Kruskal's Stress convergence criterion, a measure of how much the ranked distances in the ordination deviate from the original ranked distances allowing for

a common natal signature among groups to be assessed in each year. It should be noted that testing for significant temporal differences in otolith edge and nucleus chemistry between years was not ideally suited in this study as otolith extractions and LA-ICP-MS analyses were performed in the year collections were made. Similarly, fishing in the same locations in each year was not entirely possible despite the same SSRUs being fished, the actual sites within them could have limited fishing vessel access by ice cover or range distances several 100s of km apart. As a result, analyses are run as standalone separate years.

5.4 Results

5.4.1 Spatial and temporal variability in otolith edge chemistry

Variability in otolith edge element-Ca compositions were only examined among the same sub-adult (age 9 and age 14 year classes taken during longline operations in the Ross Sea 2012–2013. These ages were examined for purposes of consistency and because the selected age classes were the most common among the entire dataset with reasonably sufficient sample sizes across groups (Table 5.1). Similarly, for otolith nucleus analyses, using the same age classes would provide some assurances that all individuals of the same age class would have hatched around the same time and this would allow for elemental comparisons during larval growth to be made (Elsdon et al. 2008).

Table 5.1 Mean length (TL cm) of size and age classes for sub-adult (age 9) and adult (age 14) Antarctic toothfish taken over the continental Shelf, Slope and PAR of the Ross Sea during the austral summers of 2012–2013. -- = missing values.

	Coordinates			2012			2013		
Size class	Lat	Long	Location	N	Mean Length (cm)	Age	N	Mean Length (cm)	Age
Sub adults	174.09E	-73.98S	Shelf	9	84.3	9	9	91.4	9
	-71.57S	-177.04E	Slope	9	86.4	9	9	88.5	9
			Total	18	85.3	--	18	90.0	--
Adults	174.16E	-62.80S	PAR	10	134.9	14	10	128.6	14
	-71.73S	-174.04W	Slope	10	138.5	14	10	128.3	14
			Total	20	112.4	--	20	109.0	--

Effect differences (interactions) between the sex of toothfish and capture locations were not evident for adult (age 14) or subadult (age 9) age classes between seasons (Table 5.2) suggesting this factor did not confound site specific signatures in otolith edge chemistry. Resulting univariate ANOVA analyses showed significant spatial heterogeneity among element-Ca concentrations between some locations for 2012 collections but not in 2013 (Table 5.2). For adults (age 14) no spatial variability in element-Ca compositions of Mg, Al, Sr or Ba were shown between the PAR or Slope in either season where adults have been regularly taken in the fishery indicating the water mass may be similar between years in these areas (Table 5.3, Fig 5.3).

Among sub-adults (age 9), spatial heterogeneity was only evident among 2012 toothfish collections where Mg and Ba compositions were significantly different between capture locations (Table 5.3, Fig 5.4). Tukey's pairwise comparisons indicated that spatial variability in Mg was significantly higher in toothfish captured on the Shelf compared to fish taken over the continental Slope (Fig. 5.4). Similarly,

Ba compositions were also marginally higher in sub-adult (age 9) toothfish taken from the shelf compared to those captured over the continental slope (Fig 5.4). In contrast, no spatial differences in element-Ca compositions were shown among sub-adult collections taken in 2013 indicating the composition of these elements in the water mass may be reasonably variable between years and between locations (Table 5.3, Fig 5.4).

Table 5. 2 Details of factorial ANOVA analyses testing for effect differences among (ln) transformed otolith edge compositions (Mg, Al, Sr and Ba) of sub adult (age 9) and adult (age 14) toothfish between capture locations and sex for each season (2012–-2013).

			Adults (age 14+)									Subadults (age 9+)									
			Factorial ANOVA 2012				Factorial ANOVA 2013						Factorial ANOVA 2012				Factorial ANOVA 2013				
Effect	Element	df	SS	MS	F	*P*	df	SS	MS	F	*P*	Effect	Element	df	SS	MS	F	*P*	df	SS	MS	F	*P*
Location	Mg:Ca	1	0.001	0.001	0.002	0.97	2	1.68	0.84	2.32	0.13	Location	Mg:Ca	1	2.84	2.84	6.53	*0.02*	1	0.37	0.37	1.94	0.18
Sex		1	0.52	0.52	1.01	0.33	1	0.14	0.14	0.40	0.54	Sex		1	0.08	0.08	0.18	0.68	1	0.01	0.01	0.06	0.81
Location*Sex		1	0.26	0.26	0.51	0.49	2	2.40	1.20	3.31	0.06	Location*Sex		1	0.30	0.30	0.68	0.42	1	0.60	0.60	3.14	0.10
Error		17	8.79	0.52			15	5.43	0.36			Error		15	6.53	0.44			15	2.87	0.19		
Total		20	9.54				20	8.90				Total		18	9.60				18	3.78			
Location	Al:Ca	1	2.89	2.89	3.39	0.08	2	2.19	1.10	2.96	0.08	Location	Al:Ca	1	0.25	0.25	0.35	0.56	1	4.66	4.66	3.04	0.10
Sex		1	0.12	0.12	0.14	0.71	1	0.13	0.13	0.34	0.57	Sex		1	0.02	0.02	0.03	0.86	1	0.36	0.36	0.23	0.64
Location*Sex		1	6.34	6.34	7.44	0.14	2	0.25	0.13	0.34	0.72	Location*Sex		1	0.28	0.28	0.39	0.54	1	0.27	0.27	0.18	0.68
Error		17	14.49	0.85			15	5.55	0.37			Error		15	10.80	0.72			15	23.00	1.53		
Total		20	23.52				20	7.84				Total		18	11.31				18	28.18			
Location	Sr:Ca	1	0.03	0.03	0.54	0.47	2	0.16	0.08	0.67	0.53	Location	Sr:Ca	1	0.001	0.001	0.01	0.92	1	0.03	0.03	0.40	0.54
Sex		1	0.004	0.004	0.07	0.79	1	0.28	0.28	2.26	0.15	Sex		1	0.05	0.05	0.47	0.50	1	0.01	0.01	0.09	0.77
Location*Sex		1	0.08	0.08	1.38	0.26	2	0.07	0.04	0.30	0.75	Location*Sex		1	0.02	0.02	0.24	0.63	1	0.003	0.003	0.04	0.84
Error		17	0.98	0.06			15	1.85	0.12			Error		15	1.52	0.10			15	0.98	0.07		
Total		20	1.09				20	2.53				Total		18	1.59				18	1.03			
Location	Ba:Ca	1	0.19	0.19	0.53	0.48	2	0.67	0.34	1.14	0.35	Location	Ba:Ca	1	4.33	4.33	6.12	*0.03*	1	0.02	0.02	0.10	0.75
Sex		1	2.89	2.89	8.18	0.10	1	0.05	0.05	0.17	0.69	Sex		1	2.15	2.15	3.04	0.10	1	0.24	0.24	1.62	0.22
Location*Sex		1	0.08	0.08	0.21	0.65	2	0.63	0.32	1.07	0.37	Location*Sex		1	0.13	0.13	0.18	0.68	1	0.15	0.15	0.86	0.37
Error		17	6.00	0.35			15	4.45	0.30			Error		15	10.61	0.71			15	2.62	0.17		
Total		20	9.18				20	5.98				Total		18	16.78				18	3.09			

Table 5. 3 Details of univariate ANOVA tests using (ln) transformed otolith edge compositions (Mg, Al, Sr and Ba) to determine spatial differences among sub adult (age 9) and adult (age 14) toothfish sampled in different seasons (2012–2013).

		Adults (age 14+)										Subadults (age 9+)									
		ANOVA 2012					ANOVA 2013					ANOVA 2012					ANOVA 2013				
Effect	Element	df	SS	MS	F	*P*	df	SS	MS	F	*P*	Effect	Element	df	SS	MS	F	*P*	df	SS	MS	F	*P*
Location	Mg:Ca	1	0.0004	0.0004	0.001	0.98	2	0.93	0.47	1.05	0.37	Location	Mg:Ca	1	2.71	2.71	6.69	*0.02*	1	0.30	0.30	1.48	0.24
Error		19	9.54	0.50			18	7.97	0.44			Error		17	6.89	0.41			17	3.47	0.20		
Total		20	9.54				20	8.90				Total		18	9.60				18	3.78			
Location	Al:Ca	1	2.47	2.47	2.23	0.15	2	1.98	0.99	3.04	0.07	Location	Al:Ca	1	0.22	0.22	0.34	0.57	1	4.49	4.49	3.22	0.09
Error		19	21.05	1.11			18	5.86	0.33			Error		17	11.09	0.65			17	23.70	1.39		
Total		20	23.52				20	7.84				Total		18	11.31				18	28.18			
Location	Sr:Ca	1	0.03	0.03	0.50	0.49	2	0.21	0.11	0.83	0.45	Location	Sr:Ca	1	0.0002	0.0002	0.002	0.97	1	0.04	0.04	0.64	0.43
Error		19	1.06	0.06			18	2.32	0.13			Error		17	1.585	0.093			17	0.99	0.06		
Total		20	1.09				20	2.53				Total		18	1.586				18	1.03			
Location	Ba:Ca	1	0.25	0.25	0.53	0.48	2	0.85	0.42	1.49	0.25	Location	Ba:Ca	1	3.94	3.94	5.22	*0.04*	1	0.02	0.02	0.06	0.80
Error		19	8.93	0.47			18	5.13	0.29			Error		17	12.84	0.76			17	2.55	0.28		
Total		20	9.18				20	5.98				Total		18	16.78				18	2.56			

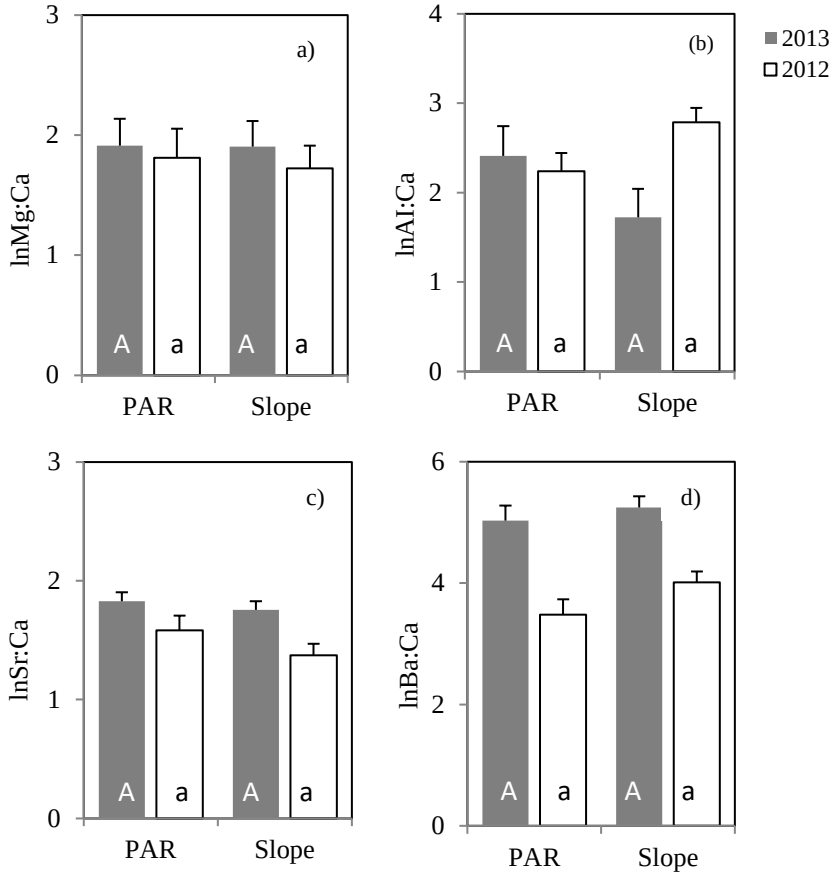

Figure 5.2 Mean (± SE) otolith edge element-Ca compositions of ln transformed (a) Mg (b) Al (c) Sr and (d) Ba from adult (age 14) toothfish collections from the Ross Sea, Slope and Shelf. Letters that differ between locations represent significant differences (Tukey's HSD).

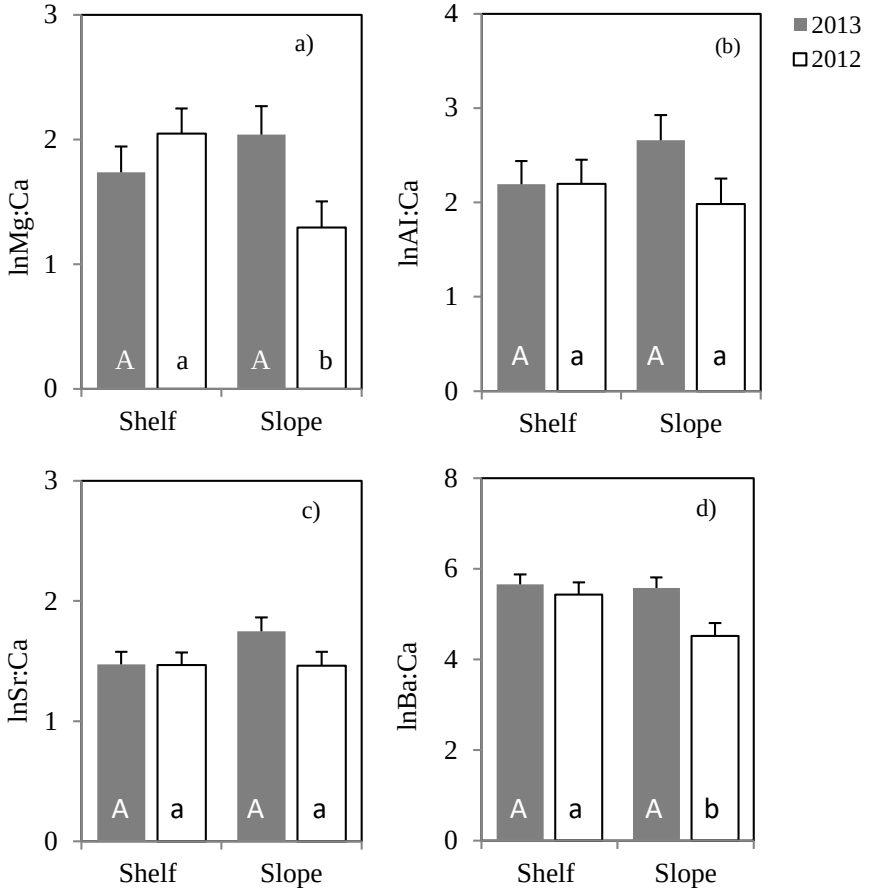

Figure 5.3 Mean (± SE) otolith edge element-Ca compositions of ln transformed (a) Mg (b) Al (c) Sr and (d) Ba from sub-adult (age 9) toothfish collections from the Ross Sea, Slope and Shelf. Letters that differ between locations represent significant differences (Tukey's HSD).

Spatial differences in otolith edge signatures for toothfish age classes from 2012 and 2013 were obtained using QDFA models. All univariate ANOVA elements were included in the discrimination model (Table 5.3; Fig. 5.4). The low degree of separation of Antarctic toothfish between capture locations was reflected in cross-validated "leave-one-out" results with overall classification success for 2012 adult (age 14) collections only 43% (Table 5.4). Of these, the highest classification success was observed between fish from the Slope (55%), with fish from the PAR (30%) revealing poor classification overall. As both locations are relatively bound (compared to the shelf), misclassifications between the PAR and Slope consisted of

several toothfish misclassified to the Slope (7) from the PAR and from the PAR (5) fish were misclassified to the Slope (Table 5.4). Classification of adult toothfish from 2013 discriminant analyses revealed improved classification success overall (67%) (Table 5.4).

Table 5.4 Jack-knife classification matrix showing predicted vs actual classification success of adult (age 14) and sub-adult (age 9) toothfish to capture locations using otolith edge element-Ca compositions of Mg, Al, Sr and Ba in the QDFA model. Columns represent actual capture locations and rows represent predicted locations.

Adults			Predicted location		
Actual location		N	PAR	Slope	% Correct
2012	PAR	10	3	7	30%
	Slope	11	5	6	55%
	Total	21	8	13	43%
2013	PAR	8	4	4	50%
	Slope	13	3	10	77%
	Total	21	7	14	67%

Sub adults			Predicted location		
Actual location		N	Slope	Shelf	% Correct
2012	Slope	9	3	6	33%
	Shelf	10	4	6	60%
	Total	19	7	12	47%
2013	Slope	5	1	4	20%
	Shelf	6	1	5	83%
	Total	11	2	9	55%

Similarly, adults from the Slope had the highest classification success (77%), with fish from the PAR (50%) being less distinguishable. Misclassifications were similarly attributed to fish from the PAR (4) misclassified to the Slope and fish from the Slope (3) misclassifying to the PAR (Table 5.4). For 2012, sub-adults (age 9) toothfish, classification success overall (47%) was much the same as adults from 2012 (Table 5.4). The highest classification success was among sub-adults (age 9) from the Shelf (60%) with poor classification of toothfish from the Slope (33%). Discrimination of 2013 sub-adults showed largely poor classification overall (55%) although fish from the Shelf (83%) had the highest classification success among all years followed by poor classification of sub-adults from the Slope (20%) (Table 5.4). Discrimination of sub-adults from the Slope (6) in 2012 were misclassified to the adjacent Shelf, while sub-adults from the Shelf (4) were misclassified northwards to the Slope. Similarly, for 2013 the classification of sub-adults from the Slope (4) were misclassified to the Shelf, whereas one fish from the Shelf was misclassified to the Slope (Table 5.4). The variability in classification success between locations and seasons indicate environmental conditions within the Ross Sea were quite variable between seasons and locations.

5.4.2 Spatial and temporal variability in otolith nucleus chemistry

Univariate ANOVA analyses of adult (age 14) and sub-adult (age 9) otolith nucleus compositions indicated no significant spatial differences between locations and within years suggesting the environmental conditions after hatching may have been similar for adult and sub-adult toothfish from the Ross Sea (Table 5.5, Fig 5.5).

Table 5. 6 Univariate ANOVA tests using (ln) transformed otolith nucleus compositions (Li, Mg, Al and Sr) to determine spatial differences among sub adult (age 9) and adult (age 14) toothfish sampled across different seasons (2012–2013).

		Adult (age 14+)										Subadult (age 9+)									
		ANOVA 2012					ANOVA 2013					ANOVA 2012					ANOVA 2013				
Effect	Element	df	SS	MS	F	P	df	SS	MS	F	*P*	Effect	Element	df	SS	MS	F	P	df	SS	MS	F	*P*
Location	Li	1	0.29	0.29	0.28	0.60	1	1.58	1.58	1.36	0.26	Location	Li	1	0.64	0.64	0.56	0.46	1	0.13	0.13	0.27	0.61
Error		19	19.65	1.03			19	22.13	1.16			Error		17	19.35	1.14			17	8.15	0.48		
Total		20	19.93				20	23.71				Total		18	19.99				18	8.28			
Location	Mg	1	0.82	0.82	1.10	0.31	1	0.003	0.003	0.01	0.92	Location	Mg	1	0.04	0.04	0.19	0.67	1	0.11	0.11	0.48	0.50
Error		19	14.19	0.75			19	5.01	0.26			Error		17	4.04	0.24			17	3.97	0.23		
Total		20	15.02				20	5.02				Total		18	4.08				18	4.08			
Location	Al	1	0.55	0.55	1.05	0.32	1	0.01	0.01	0.01	0.92	Location	Al	1	0.78	0.78	0.89	0.36	1	0.09	0.09	0.45	0.51
Error		19	9.92	0.52			19	10.24	0.54			Error		17	15.01	0.88			17	3.40	0.20		
Total		20	10.47				20	10.25				Total		18	15.80				18	3.49			
Location	Sr	1	0.002	0.002	0.04	0.84	1	0.19	0.19	1.14	0.30	Location	Sr	1	0.006	0.006	0.22	0.64	1	0.003	0.003	0.06	0.81
Error		19	0.69	0.04			19	3.14	0.17			Error		17	0.47	0.03			17	0.91	0.05		
Total		20	0.69				20	3.33				Total		18	0.47				18	0.91			

This contrasted with otolith edge compositions, at least among sub-adults from 2012, where environmental exposures where clearly different between continental slope and shelf regions (Table 5.3, Fig 5.4).

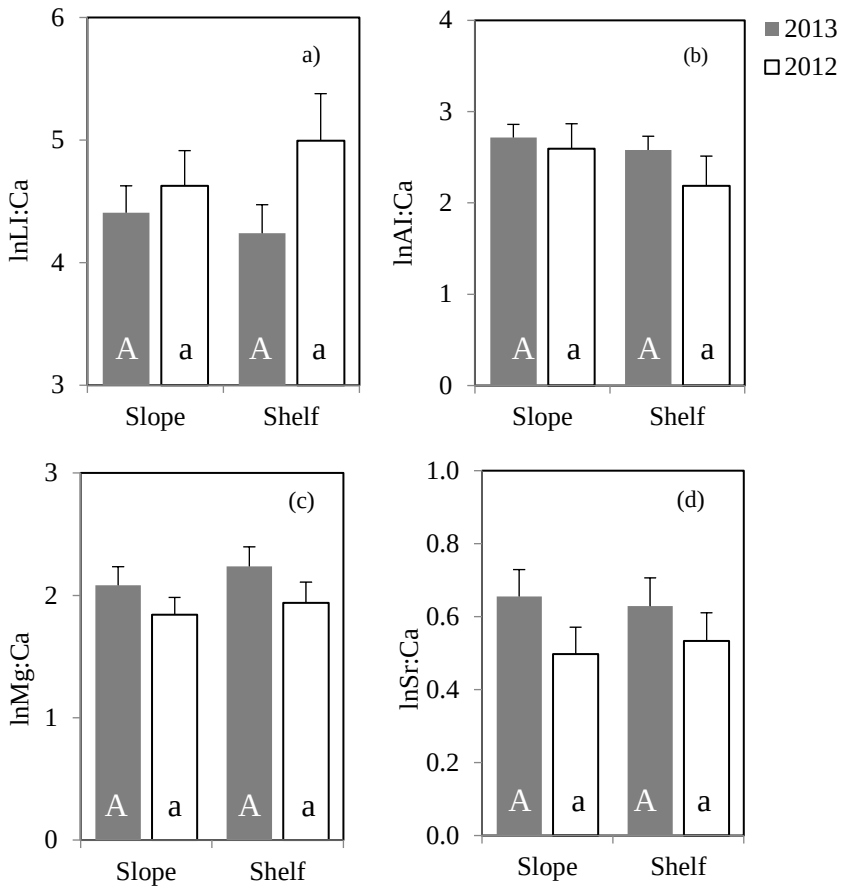

Figure 5.4 Mean (± SE) elemental compositions of (ln) transformed (a) Li (b) Al (c) Mg and (d) Sr extracted from the otolith nucleus of sub-adult (age 9) Antarctic toothfish from the Slope and Shelf in 2012 and 2013. Letters that differ between locations represent significant differences (Tukey's HSD).

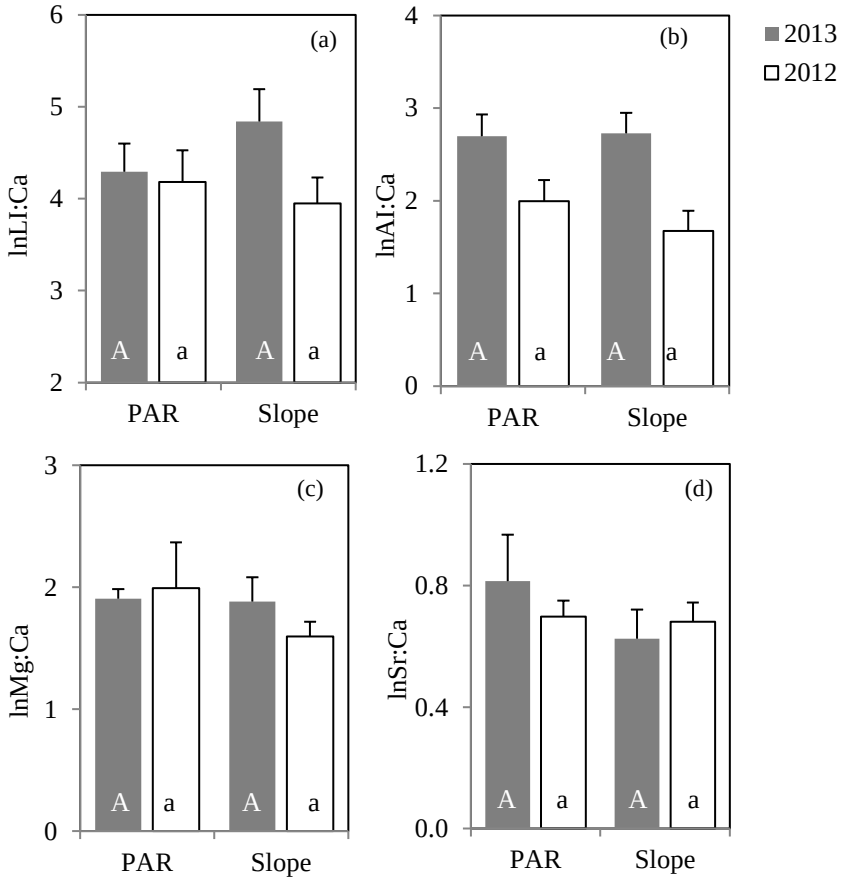

Figure 5.5 Mean (± SE) elemental compositions of (ln) transformed (a) Li (b) Al (c) Mg and (d) Sr extracted from the otolith nucleus of adult (age 14) toothfish from the PAR and Slope in 2012 and 2013. Letters that differ between locations represent significant differences (Tukey's HSD). 5.4.3 Natal structuring within the Ross Sea across seasons

Structuring of otolith nucleus compositions based on nMDS two dimensional visualisations with 95% confidence intervals showed no clear separation between sub-adult (age 9) or adult (age 14) age classes in either season suggesting toothfish may have utilised the same spawning areas (Fig. 5). Similarly, nMDS Kruskal stress values corresponded with good overall fit (Stress ≤ 0.08) indicating the data were well represented in 2 dimensions (Fig. 5.7). However, one adult from the continental Slope in 2012 had element-Ca compositions that ranged outside the 95% confidence ellipse suggesting this fish may have utilised a different spawning habitat not characterised in the current sample collections (Fig 5.7).

Similarly, two adults from the PAR in 2013 also had element-Ca compositions that ranged outside the 95% confidence ellipse indicating they may have utilised different spawning areas.

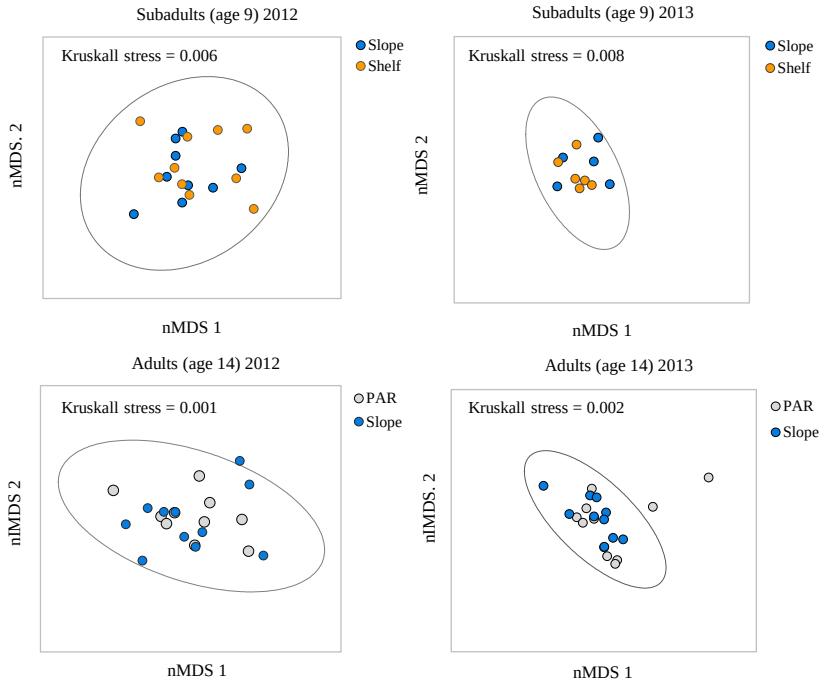

Figure 5.6 Nonmetric MDS ordination plots with distance measures of best fit (Kruskall Stress < 0.01) and Fishers 95% confidence ellipses using element-Ca compositions of Mg. Al, and Sr in the otolith nucleus of Antarctic toothfish sub-adults (age 9) from the Slope and Shelf and adult (age 14) from the PAR and Slope during 2012 and 2013 seasons.

5.5 Discussion

5.5.1. Spatial and temporal variability of otolith edges

Examination of the same otolith edge compositions (Mg, Al, Sr and Ba) in consecutive years highlighted that the environmental conditions within the Ross Sea were largely variable between age classes and seasons. Among adult (age 14) toothfish, where no spatial differences in otolith edge chemistry between the PAR and Slope were evident in either year, possibly reflects their close proximity within the Ross Sea limiting any distinction between water masses. It is also possible, that

regional scale gyres mixing the water mass would diminish elemental differences or that smaller sample sizes among 2013 collections may constitute a lack of statistical or discriminatory power. Nevertheless, despite a lack of spatial heterogeneity among adult otolith edge compositions, significant spatial differences were strongly evident at least among the same aged sub-adult collections from 2012. This underscores the variability in environmental conditions between years and age classes for toothfish in the Ross Sea. Such a finding may confound the ability to identify the contribution of juvenile spawning grounds in one year from adults of unknown provenance in another year (Gillanders 2002) promoting data misinterpretation (Reis-Santos et al. 2012).

Similar patterns of temporal variability in otolith chemistry have been observed in European anchovy otoliths (Guidetti et al. 2013) indicating the need for a long-term study to determine the stability of elemental signatures over time (Campana 1999). In many cases, characterisation of the temporal variability of otolith chemical compositions in marine fish has seldom been addressed among different coastal environments (Gillanders 2002; Hamer et al. 2003; Swearer et al. 2003; Clarke et al. 2009; Mateo et al. 2012). In this study evidence of temporal variability in otolith edge chemistries of Antarctic toothfish within the Ross Sea suggest further examinations of otolith elemental contingents over time if they're corresponding otolith nucleus chemistries are to be applied as stock delineators (Elsdon et al. 2008).

5.5.2. Natal structuring of Antarctic toothfish within the Ross Sea

The otolith nuclei of adult and sub-adult Antarctic toothfish across consecutive seasons revealed no significant spatial differences in element-Ca compositions (Li,

Mg, Al and Sr). Structuring of otolith nucleus chemistries contrasted with significant spatial heterogeneity observed in otolith edges (at least for sub-adults 2012) suggesting that most adults and sub-adults of the same age from the Ross Sea shared similar larval growth histories. Similarly, that only the natal chemistries of the same age classes (9-year-old and 14-year-old) were examined, meaning fish were subject to the same environmental conditions during early growth, reduced the influence of indifferent chemistries associated with fish that may have spawned in different years (Elsdon et al 2008). Among nMDS visualisations, no clear patterns of separation in the otolith nuclei of Antarctic toothfish were shown adding further credence to the existence of a single spawning population within the Ross Sea (Hanchet & Rickard 2008).

However, the otolith nucleus chemistry of a few adults appeared to have different larval growth histories between seasons suggesting they possibly utilised different spawning grounds or that the water mass across spawning grounds may be variable overtime. These patterns were similar to otolith nucleus analyses of adult toothfish from the NRS (88.1C) and NERS (88.2H), where 2D ordinations and cluster analyses indicated two possible spawning groups within the Ross Sea with overlapping populations (Chapter 4). Large scale ocean circulations have already been associated with mixing between adjoining basins such the Antarctic Peninsula in the Southern Atlantic and Macquarie Island in the Southern Indian Ocean (Ashford et al. 2005; Ashford et al. 2007) or across broader spatial scales between the Ross Sea, Amundsen Sea, Southern Atlantic and Southern Indian Ocean (Chapter 3).

The same processes may explain the small portion of uncharacterised mixtures identified in the Ross Sea through this study. Overall, these results appear consistent with genetic evidence (Smith & Gaffney 2005; Kuhn & Gaffney 2008) and corroborate homogeneous otolith nucleus chemistries reported for Antarctic toothfish by Ashford et al. (2012). Hanchet and Rickard (2008) hypothesised a life cycle for Antarctic toothfish in which spawning occurs during winter through to spring (May–November) on ridges and banks of the PAR in the northern Ross Sea where adults were obtained in this study. Depending on the precise location of spawning, eggs and larvae released over the PAR may be advected west by the western Ross Sea gyre settling around the Balleny Islands and adjoining continental Shelf. Alternatively, larvae could also be exported south onto the continental Shelf, or eastwards via the eastern arm of the Ross Sea gyre settling out along the continental Slope and Shelf east of the Ross Sea (Hanchet & Rickard 2008; Hanchet et al. 2015).

As juveniles mature, they move west towards the Ross Sea shelf and then towards the slope region where sub-adults reflected homogenous natal chemistries in this study. As juveniles mature they feed on the slope and move to deeper water where they gain condition before moving north onto the PAR to start the cycle again (Hanchet and Rickard 2008). A life history along these lines is consistent with tagging data where large fish (100– 130 cm) followed a northward direction between the Shelf and Slope and from the Slope, North, consistent with ontogenetic movements into deeper water and spawning (Yukhov 1971; Fenaughty 2006; Hanchet & Rickard 2008; Parker & Grimes 2010b).

Connectivity of Antarctic toothfish from the PAR with Shelf areas is necessary to complete the life cycle of Antarctic toothfish (Ashford et al 2012; Hanchet & Rickard 2008). Correspondingly, the findings of this study provide supporting evidence of connectivity among sub-adults from nursery grounds on the Shelf and spawning areas over the PAR for adults year classes suggesting the existence of a single spawning population of Antarctic toothfish within the Ross Sea (Smith & Gaffney 2005; Hanchet & Rickard 2008; Kuhn & Gaffney 2008; Ashford et al. 2012).

However, a small proportion of individuals had natal chemistries that suggest they utilised different spawning areas or seamounts indicating the population structure of Antarctic toothfish within the Ross Sea is more spatially complex. Moreover, patterns of temporal variability in the otolith chemistry of Antarctic toothfish over consecutive seasons has important implications if future use of otolith chemistry is applied across years and year classes as stock delineators (Elsdon et al. 2008; Régnier et al. 2017). However, temporal uncertainties were likely attributed to smaller sample sizes and further analyses of trace element stability in Antarctic toothfish otoliths is recommended.

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6. Research summary

The aim of this research was to expand current understanding of Antarctic toothfish population structuring across different spatial and temporal scales of fisheries management convention areas and determine the extent at which these populations may be connected. Also critical is identification of the primary factors that limit interpretation of otolith microchemistry as indicators of life history.

6.1 Distinguishing broad-scale spatial areas around Antarctica

In the first investigation (Chapter Two), trace element compositions in the otolith edge were used to test their efficacy at distinguishing the capture locations of Antarctic toothfish across spatially discrete fishery management areas around Antarctica. Several studies have used otolith chemistry profiles as a valuable tool in understanding the spatial ecology of marine species (Wells et al. 2012; Rooker et al. 2014; Ashford et al. 2007; MacDonald et al. 2013).

The use of otolith elemental tags as natural markers is contingent on their continuous growth characteristics and ability to record chronological records of the environment fish occupied (Campana 1999). However, physiological processes (e.g., growth, metabolism, and reproductive stage) can also influence otolith composition (Elsdon and Gillanders 2003; Gaetani and Cohen 2006; Walther et al. 2010; Sturrock et al. 2014; Stanley et al. 2015; Mazloumi et al. 2017; Walsh and Gillanders 2018). Therefore, knowledge of how exogenous and endogenous factors affect otolith chemistry is essential to reconstruct life history patterns and migration among fish species (Elsdon et al. 2008; Reis-Santos et al. 2013). In accounting for these effects, many studies have shown that differentiation of fish from

geographically separated areas is possible (Edmonds et al. 1992; Patterson et al. 1999; Rooker et al. 2001), or that habitat utilisation and early life history movements can be traced (Ashford & Duhamel 2005; Elsdon & Gillanders 2005; Ruttenberg et al. 2008; Longmore et al. 2014).

Based on compositions of Sr, Al, Mg and Ba, the otolith edge chemistry of Antarctic toothfish showed strong patterns of separation between the Ross Sea and Southern Atlantic highlighting the potential of this approach as a valuable tool in understanding the spatial ecology of marine species (Wells et al. 2012; Rooker et al. 2014; Ashford et al. 2007; MacDonald et al. 2013). However, not all locations were spatially resolved with areas immediately adjacent to primary source regions having overlapping trace element signatures that resulted in poor separation and classification.

These discrepancies were reasonably consistent with their nearest neighbour and were not directly attributable to recent movement of fish between areas as large-scale movements of toothfish (> 50 km) based on tag data is limited (Williams & Tuck 2002; Marlow & Agnew 2003; Hanchet & Rickard 2008; Parker et al. 2014). Instead, elemental similarities were most likely due to large scale hydrographic processes (oceanic gyres) and the Antarctic Circumpolar Current (ACC) mixing within and across adjoining areas.

These findings demonstrated that the otolith edge chemistry of Antarctic toothfish can be an effective habitat marker in distinguishing toothfish between capture locations and provides a strong basis in future studies aimed at discerning the structure of stocks or the extent to which populations in these areas may be connected.

6.2 Connections among toothfish spawning areas

The second investigation (Chapter Three) was aimed at identifying the spatial structure of toothfish populations among fisheries management areas around Antarctica and the extent at which they may be connected. In this study, element-Ca compositions were extracted from the otolith nucleus of age 10-year-old and age 14-year-old toothfish, where trace element profiles correspond to environmental conditions experienced during larval growth (Campana 1999). Univariate ANOVA analyses of trace elements showed significant spatial separation in Al, Mg and Zn compositions between toothfish from the Ross Sea and Southern Atlantic a finding further evidenced by Ward's AHC cluster analyses and nMDS visualisations.

This indicated that for the majority of toothfish, the primary structure of spawning populations was mostly affiliated with the Ross Sea and Southern Atlantic giving credence to these areas sustaining separate spawning populations (Kuhn and Gafney 2008). This finding, appears to counter genetic evidence that proposed a single population around Antarctica (Mugue & Petrov 2014) suggesting Antarctic toothfish from Ross Sea and Southern Atlantic have reasonably close affinities to their original capture locations. Moreover, that the same age classes were examined across regions precludes any interferences from interannual noise that might occur from fish that were spawned in different years (Elsdon et al 2008).

Both regions would therefore constitute an important source area for Antarctic toothfish recruits. However, while most fish from the Ross Sea and Southern Atlantic showed close affinities to their respective capture areas, some individuals primarily from adjoining areas of the RS (AMS) and SAO (SIO) showed patterns

of connectivity consistent with downstream transport of larvae into these areas through the ACC. This would diminish any unique elemental signatures between areas similar to patterns observed in the otolith edge chemistry of the same fish and suggest the population structure of Antarctic toothfish is more spatially complex and the spatial sampling resolution of my study may limit conclusions about spawning areas at this point.

Nevertheless, the Antarctic Circumpolar Current (ACC) is a likely mechanism of ocean scale transport of toothfish larvae downstream of their respective spawning grounds into neighbouring basins (Eastman 1988; Ashford et al. 2005; Ashford et al. 2007; Ashford et al. 2008; Hanchet & Rickard 2008; Kuhn & Gaffney 2008; Ashford et al. 2012). By similar means, finer scale cyclonic oceanic gyres can retain larvae within a region based on the proximity of these features to spawning grounds along open ocean ridges and banks to shallow maturing habitat.

Genetic evidence reported by (Kuhn & Gaffney 2008) suggest limited gene flow may occur among Antarctic toothfish populations within the Australian Antarctic Territory, Ross Dependency and the South Shetland Islands. To support this finding, Kuhn and Gaffney (2008) proposed that two major cyclonic clockwise circulations in the Southern Ocean, the Weddell Sea gyre and the Ross Sea gyre likely play an important role in the isolation of toothfish populations between these regions (Kuhn and Gaffney 2008; Orsi et al. 1995). This might also be facilitated by homing tendencies among Antarctic toothfish which would further promote population subdivision between these regions (Parker et al. 2002). Fisheries management of Antarctic toothfish stocks between these areas would need to consider these populations as separate management units to ensure the fishery is sustainable.

6.3 Structure of the Ross Sea and Amundsen Sea stocks

The third investigation (Chapter 4) focussed primarily on finer scale evaluations of stock structure between two adjoining fishing areas in the northern Ross Sea (SSRU 88.1C) and the north-eastern Ross Sea (SSRU 88.2). Until the present investigation, no such analyses had been carried out on fish from the north-eastern Ross Sea. As a result, preliminary assessments of adult (age 17) otolith chemistry profiles were examined. Univariate ANOVA analysis of trace elements in the otolith edge showed no significant spatial heterogeneity between fishing areas which corresponded with low discriminatory classification success (53%) (Table 4.4). Low classification success may have been due to there being no differences in trace elements present in the water at these two locations, or that fish may have only recently moved into these areas from elsewhere having not yet had sufficient time to incorporate site specific signatures into their otoliths.

Compared to otolith edge chemistries, ANOVA analyses of otolith nucleus chemistries showed stronger spatial separation between adult (age 17) toothfish. Significantly higher Li, Mg and Al concentrations were evident among fish from the Ross Sea (88.2H) compared to fish from the Amundsen Sea (88.1C), indicating environmental conditions during larval growth of toothfish differed between these areas. However, while corresponding nMDS and Wards AHC cluster analysis showed reasonable patterns of separation, suggesting separate populations, there were also clear patterns of overlap between the same populations, suggesting two possible spawning populations exist, which may contribute more to the Ross Sea fishery than to the Amundsen Sea. The relative closeness of the natal groupings shows reasonable uncertainty, and this study was only conducted as a preliminary analysis. Subsequently, analysis of a larger sample base of representative adults and

juveniles age classes collected from shelf and slope regions within Small Scale Research Units (SSRUs) 88.1 and 88.2 would be essential to determining whether observed patterns are consistent and whether the movement of individuals from juveniles to adult habitats can be tracked.

6.4 Spatial and temporal stability of otolith microchemistry in the Ross Sea

In the final investigation (Chapter 5), spatial and temporal stability of otolith microchemistry signatures and patterns of structuring among Antarctic toothfish within the Ross Sea were evaluated. This was because successful retrospective determinations of natal sources depend on the principle that chemical signatures are sufficiently stable across time to allow for their accurate discrimination (Campana 1999; Elsdon et al. 2008). Many studies have examined the issue of temporal stability among otolith elemental compositions, which suggest signatures may vary among years (Campana et al. 2000). In turn, this can limit their reliability as useful markers of population structure (Elsdon et al. 2008). In this study, the otolith edge chemistry of adults Antarctic toothfish from the Pacific–Antarctic Ridge (PAR), and continental Slope revealed no significant spatial or temporal heterogeneity between seasons.

This may be due to the relatively close proximity of the PAR and continental Slope areas within the Ross Sea and regional scale gyres mixing the water mass between them which would diminish elemental differences. However, univariate ANOVA analyses revealed significant spatial differences at least among subadult collections from 2012, which underscores the variability in environmental conditions between years and age classes for toothfish. Such a finding may confound the ability to identify the contribution of juvenile spawning grounds in one year from adults of

unknown provenance in another year (Gillanders 2002) promoting data misinterpretation (Reis-Santos et al. 2012). Similar patterns of temporal variability in otolith nucleus chemistry have been observed in European anchovy otoliths (Guidetti et al. 2013) indicating the need for a long-term study to determine the stability of elemental signatures over time (Campana 1999).

The otolith nucleus chemistry of Antarctic toothfish within the Ross Sea showed no significant spatial variability among elements (Li, Mg, Al and Sr) in either year. Similarly, nMDS visualisations of the otolith nuclei showed no clear separation among individuals of the same 9-year-old and 14-year-old year classes which would be negligible to interannual noise (Elsdon et al 2008). This suggests Antarctic toothfish from the Ross Sea fishery utilised a common spawning ground. These findings suggest that a single spawning population of Antarctic toothfish exists within the Ross Sea and is reasonably consistent over both years. However, a small proportion of individuals had natal chemistries that suggest they utilised different spawning areas or seamounts within the Ross Sea or possibly outside of the Ross Sea indicating the population structure of Antarctic toothfish within the Ross Sea may also receive larval contributions from outside of the region.

6.2 Future research directions

Juvenile and subadult age classes are under-represented in all Antarctic otolith analyses and addressing this deficiency would be essential to removing uncertainties around population structure of Antarctic toothfish. This is because the collection of representative age classes, in particular juveniles across the convention area is difficult without targeted pre-recruitment surveys and because juveniles are difficult to collect because of the size-biased fishing. Across broad

spatial scales, the indication that two distinct spawning populations of Antarctic toothfish exist within the Ross Sea and Southern Atlantic Ocean sector highlights two areas of interest where further research using otolith microchemistry could be directed. For example, a spatial and temporal study of otoliths from subadults and adult Antarctic toothfish from the Southern Atlantic sector would provide a stronger basis of understanding the structure of populations within this region and determine the extent to which elements may change over time. If the elemental signatures of the Ross Sea and Southern Atlantic sector can be fully characterised by a finer scale spatial and temporal study, then it may be possible to look at the contributions fish from these areas may have on areas outside them.

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