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Can otolith elemental signatures record the capture site of Patagonian toothfish (*Dissostichus eleginoides*), a fully marine fish in the Southern Ocean?

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Abstract: Otolith chemistry has been successfully used to reconstruct the environmental history experienced by estuarine-dependent teleost fish, including movement between estuaries and coastal areas. However, application has been more limited in species exposed exclusively to oceanic waters, where gradients in physical and chemical properties are less extreme. To test whether otolith elemental signatures record spatial information in an oceanic species, we sampled otoliths from Patagonian toothfish (*Dissostichus eleginoides*) and used an inductively coupled plasma mass spectrometer (ICP-MS) coupled to a laser ablation system to target the outer otolith edges corresponding to the period immediately before capture. Using multivariate analysis of variance and multivariate discriminant analysis, we found that edge signatures discriminated toothfish by geographic region with near complete success: only 5% of fish caught off South America and in the Antarctic were misclassified to sampling areas in the other region. Moreover, edge signatures showed strong differences between sampling areas within each region: fish captured off South America classified to sampling areas therein with 79%–84% success, and Antarctic fish classified to sampling areas therein with 50%–67% success. These results compare favourably with rates of classification for estuarine-dependent fish, demonstrating that otolith elemental signatures can discriminate the geographic provenance of oceanic and estuarine-dependent fish.

Résumé : La chimie des otolithes permet de retracer avec succès le passé environnemental des poissons téléostéens qui dépendent des estuaires et, en particulier, leurs déplacements entre les estuaires et les milieux côtiers. La méthode est cependant plus rarement utilisée chez les espèces qui sont exposées exclusivement aux eaux de l'océan, car les gradients des propriétés physiques et chimiques y sont moins marqués. Afin de vérifier si les signatures des éléments dans les otolithes enregistrent des renseignements de nature spatiale chez une espèce océanique, nous avons prélevé des otolithes de la légine australe (*Dissostichus eleginoides*) et nous avons utilisé un spectromètre de masse à plasma inductif (ICP-MS) couplé à un système d'ablation laser pour cibler les couches externes des otolithes qui correspondent à la période qui précède immédiatement la capture. Une analyse de variance multidimensionnelle et une analyse discriminante multidimensionnelle démontrent que les signatures sur les couches externes permettent de discriminer les otolithes d'après la région géographique avec un succès presque complet; seuls 5 % des poissons capturés au large de l'Amérique du Sud et dans l'Antarctique sont mal classifiés dans des zones d'échantillonnage de l'autre région. De plus, les signatures des couches externes indiquent de fortes différences entre les zones d'échantillonnage de chacune des régions : les poissons récoltés au large de l'Amérique du Sud sont placés à 79–84 % dans des zones d'échantillonnage de la région et ceux de l'Antarctique à 50–67 % dans des zones d'échantillonnage de cette région. Ces résultats se comparent avantageusement aux taux de classification obtenus chez les poissons associés aux estuaires, ce qui démontre que les signatures des éléments dans les otolithes permettent de reconnaître l'origine géographique des poissons océaniques aussi bien que celle des poissons estuariens.

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Introduction

Recent advances in ecology have highlighted the importance of spatial considerations in population dynamics. For instance, critical habitats may contribute disproportionately to recruitment (Beck et al. 2003), and linkage between geographic areas through migration can fundamentally affect population dynamics (e.g., Pulliam 1988; Polacheck 1990; Hanski and Simberloff 1997). Where mortality exceeds recruitment, changes in immigration and emigration may determine local persistence or extinction.

To measure fish movement and link spawning adults to earlier habitat, researchers have historically used genetic markers and artificial tags. However, genetic markers cannot link fish to geographic area specifically, but rather to a population, limiting their use for identifying the critical habitats used by fish that survive to reproduce. Moreover, genetic techniques rely on identifying unique fixed markers when populations are known to be separate, usually during spawning. On the other hand, artificial tags can only link fish to the area where they were marked or recaptured, and the technique depends on several assumptions that can be difficult to meet. Moreover, tagging the number of fish necessary to generate sufficient recaptures is costly and logistically challenging.

In the last decade, however, new technology has led to renewed interest in trace and minor element analysis of otoliths. Because they have similar properties, divalent elements substitute for Ca in the aragonite lattice of the otolith. Consistent concentrations of Sr, Ba, and Mn recorded for fishes between the ambient water, blood plasma, and otolith suggest that seawater concentrations influence the rate at which the elements are incorporated into the otolith (Campana 1999). Moreover, validating studies have estimated this relationship experimentally by manipulating ambient Sr/Ca and Ba/Ca and measuring their resulting otolith concentrations (Bath et al. 2000). The preferred instrumentation available to make these measurements is inductively coupled plasma mass spectrometry (ICP-MS) because of its sensitivity and mass resolution (Campana 1999). When coupled with lasers, ICP-MS combines these advantages with the capacity to sample material at fine spatial scales (Jones and Chen 2003).

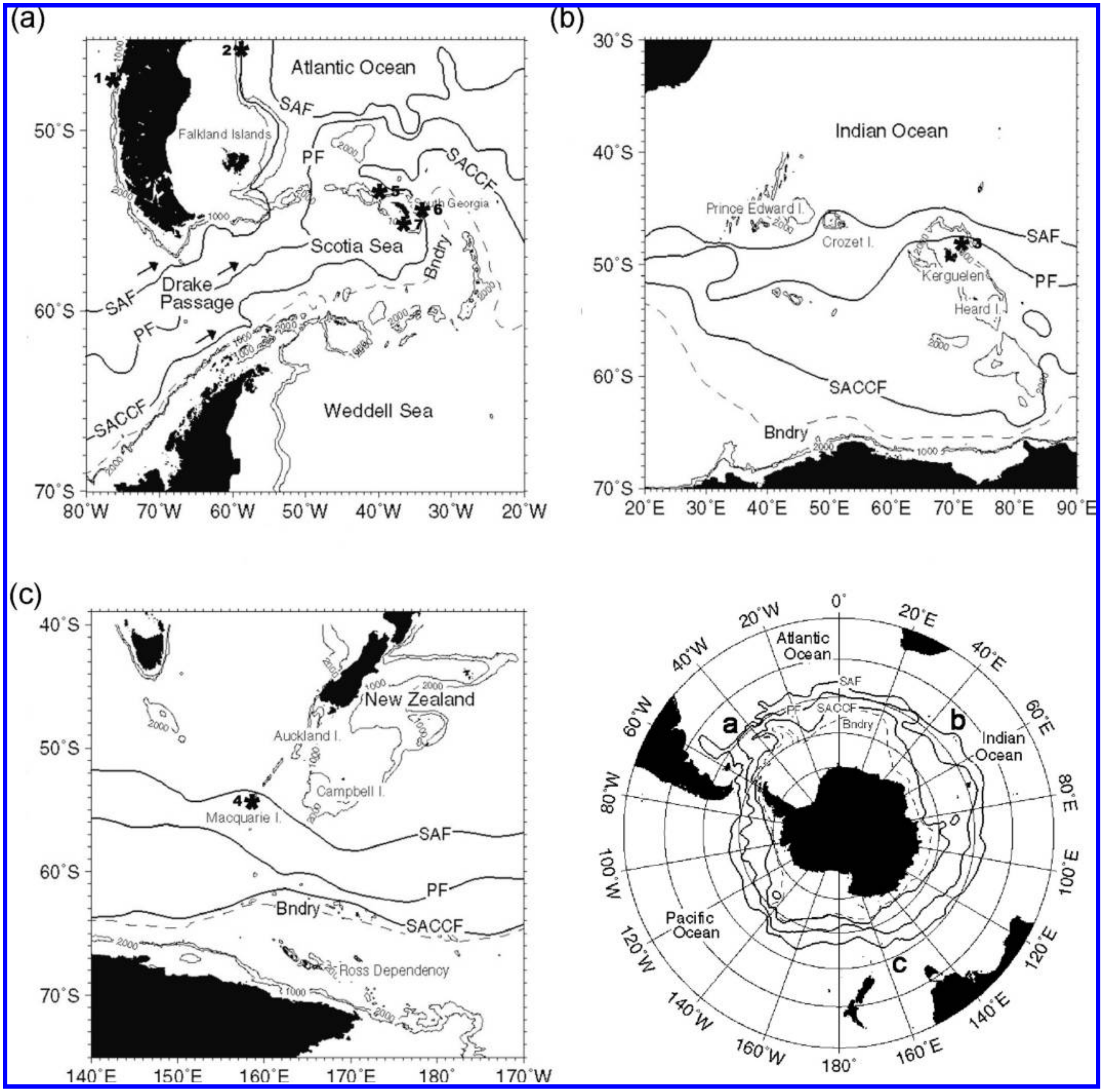
As a result, researchers have successfully used natural elemental markers in otolith growth increments to reconstruct the environmental history experienced by estuarine-dependent fish, including movement between oceanic and estuarine environments (see Thresher (1999) for review). However, application of laser ICP-MS to exclusively marine species has been more limited because of the perception that otolith chemistry is less effective as a natural marker for discriminating between environments offshore, where gradients in physical and chemical properties are much less pronounced. Yet earlier studies discriminated between fish from different capture areas (Thresher 1999), even when they used dissolved whole otoliths, which integrated the otolith chemistry over the entire life history prior to capture (Edmonds et al. 1989, 1991). Furthermore, the environment was sufficiently heterogeneous between five spawning grounds for elemental markers deposited in otolith nuclei to discriminate between Atlantic cod (*Gadus morhua*) returning as adults (Campana et al. 1994).

In the Southern Ocean, strong zonal wind stress acting on surface waters drives the Antarctic Circumpolar Current (ACC) eastward, and thermohaline variation generates a complex pelagic environment that is stratified in a polar–temperate direction and characterized by broad quiescent zones interspersed with fronts (Hofmann 1985; Orsi et al. 1995). The Subantarctic Front (SAF) and Polar Front (PF) penetrate the entire water column at the Drake Passage (Nowlin and Clifford 1982). They persist around the Antarctic continent, appearing stable where they flow over large bathymetric features (Hofmann 1985; Orsi et al. 1995; Rintoul and Sokolov 2001) (Fig. 1). Vertical stratification also varies spatially: Circumpolar Deep Water is more shallow towards the pole, whereas after sinking in the Polar Frontal Zone, Antarctic Intermediate Water is found at depth off South America. Differential exposure to trace elements from wind-driven, riverine, glacial, and geothermal sources, combined with biological processes and physical mixing downstream of sources, leads to environmental gradients in trace elements (e.g., Nolting et al. 1991; Westerlund and Öhman 1991).

Fish moving within this biogeochemical array may incorporate elements as spatially specific signatures in their otoliths that would provide a record of movement-at-age when combined with otolith chronology. Patagonian toothfish (*Dissostichus eleginoides*) are distributed on the continental shelves and shelf breaks of banks and island groups in the ACC and along western and eastern South America. They are bentho-pelagic tertiary predators (Eastman 1993) that attain a maximum total length of more than 2 m and live over 50 years (Ashford 2001; Horn 2002). They are managed as spatially discrete stocks within zones broadly corresponding to island groups under the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR) and the Exclusive Economic Zones (EEZ) of neighboring national authorities. Consistent with spatial separation, genetic studies discounted panmixia and showed heterogeneity between management areas in different ocean basins (Smith and McVeagh 2000; Appleyard et al. 2002) and between South Georgia and the Falkland Islands (Shaw et al. 2004). However, rather than resulting from separation, this spatial heterogeneity may be the result of movement and mixing of populations in different proportions. This is difficult to discount using genetic approaches, however, because toothfish spawning areas are unknown and population-specific markers remain unidentified. On the other hand, management zones across the western Indian Ocean showed genetic homogeneity (Appleyard et al. 2004), suggesting a single population moving between seamounts and islands. But instead, this may reflect several populations, as movement of only a few fish between spatially discrete populations can counteract divergence through natural selection or genetic drift.

The evidence from tagging studies indicates that most recaptures were caught near the area of marking (e.g., Williams et al. 2002), consistent with spatial separation. However, tagging effort concentrated on sizes corresponding to immature fish, which are negatively buoyant in *D. mawsoni*, the congener of Patagonian toothfish (Eastman 1993; Near et al. 2003). It did not address early stages, which are pelagic (North 2002), or older stages in which *D. mawsoni* become neutrally buoyant at maturity, thereby considerably reducing

Fig. 1. Map of the Southern Ocean showing mean position of major fronts: SAF, Subantarctic Front; PF, Polar Front; SACCF, southern Antarctic Circumpolar Current Front; southern boundary (Bndry) of the ACC is shown as a broken line (Orsi et al. 1995). Detailed maps of the (*a*) Atlantic, (*b*) Indian, and (*c*) Pacific sectors of the Southern Ocean. Arrows show current direction. Sampling areas: 1, Chile, 1996; 2, Falkland Islands, 1997; 3, Kerguelen, 1996; 4, Macquarie, 1996; 5, South Georgia, 1996; 6, South Georgia, 1997; 7, South Georgia, 1998.



the energy needed to move using currents in the ACC (Ashford et al. 2003). Yet some tagged fish moved considerable distances (Williams et al. 2002), and similar size-at-age distributions between Kerguelen and South Georgia, despite differences in diet (Duhamel 1981, Goldsworthy et al. 2002), suggested that spatial differences due to growth may be homogenized by movement along the ACC (Ashford et al. 2003).

If it occurs, movement may be only of nonbreeding vagrants (Sinclair 1988), mixing in their new locality with resident fish that are reproductively active. Even so, a large rate of vagrancy will reduce the number of spawners in the source population, and arriving vagrants may inflate the apparent number of spawners at their destination area, carrying the risk of recruitment overfishing. Indeed, restriction of the breeding population may help explain strong differentiation in mtDNA where differences in nuclear DNA were not detected (Appleyard et al. 2002). Alternatively, if migrating fish return to breed in their natal population, variation in their mortality or movement would directly affect spawning biomass. It is unlikely that this effect would be spatially uniform: some areas or habitats may be more critical to future recruitment than others.

To assess these questions using otolith chemistry, researchers need to reliably link signatures to geographic area. Once they are able to do so, they can begin to identify the provenance of captured oceanic fish from otolith material laid down earlier in the life history and hence study movement and use of critical habitat. The important first step, however, is to demonstrate that otoliths do, in fact, record spatially specific signatures. To test this, we compared otoliths taken from toothfish caught from five fishing management zones within two geographic regions around southern South America and in the Antarctic. In contrast to the analyses of whole otoliths and nucleus signatures undertaken previously for marine fish using ICP-MS, we used lasers to target the outer edges of otoliths, which specifically correspond to the environment occupied in the period immediately prior to capture, to validate the use of otolith chemistry in differentiating known marine habitats.

Materials and methods

Fishery observers collected otoliths between March and April 1996 from commercial catches of Patagonian toothfish taken in the Antarctic off the northern continental slope of South Georgia (United Nations Food and Agriculture Organization Statistical Subarea 48.3) and off the eastern continental slope of Kerguelen (Division 58.5.1), both situated on the Antarctic continental side of the PF in the southern Atlantic and Indian oceans, respectively (Fig. 1). Otoliths were also collected from Antarctic fish caught off the west coast of the Macquarie Island Exclusive Economic Zone (EEZ) located between the SAF and PF in the southern Pacific. For the region around South America, observers sampled fish from artisanal fishermen returning to Chiloe Island in the Chilean EEZ, north of the SAF. As fish could not be collected off the Falkland Islands in the same year, observers sampled again between April and May 1997 off the South American continental slope north of the Falkland Islands Conservation Zone (FCZ) and the eastern slope of South Georgia. Otoliths were also selected randomly from observer sample sets taken from the slope south of South Georgia in 1998. At each collection, female toothfish between 90 and 110 cm total length were sampled to minimize effects resulting from sex and life stage. Off Chile, otoliths could not be collected before removal of gonads by fishermen, and sex data were not recorded. All fish were taken from depths greater than 1000 m, except for those taken at Macquarie Island, where the depths were ~400–500 m.

Otoliths were dried and stored in envelopes and returned to the laboratory at Old Dominion University. One otolith was selected randomly from each pair, rinsed in milli-Q water, sonicated for 2 min, and rinsed again to remove any surface contamination. After drying, otoliths were ground from the anterior and posterior sides using a Hillquist Thin Section Machine (Hillquist Inc., Denver, Colorado) to produce thick transverse sections. Final processing was done in a class-100 clean room. We rinsed all remaining sections in milli-Q water under a laminar flow hood and lapped each manually using clean plastic clamps and Mark V Laboratory polishing film (Mark V Laboratory, East Granby, Connecticut). Each otolith was lapped successively on three pieces of clean 3 μm film and finished on 0.3 μm film. One otolith from each treatment was randomly selected and mounted in random order on a clean petrographic slide using silicon glue. Two otoliths were damaged during preparation and were not used in the analysis. The mounted sections were rinsed, sonicated for 5 min, then rinsed again twice, all in milli-Q water, and left to dry under a positive flow hood.

We used a Finnegan Mat Element 2 double-focusing sector-field ICP-MS located at the Laboratory for Isotope and Trace Element Research (LITER) at Old Dominion University (Norfolk, Virginia) to examine minor and trace element signatures. Instrument details are given in Jones and Chen (2003). Samples were introduced in automated sequence (Chen et al. 2000) using a New Wave Research EO LUV 266 laser ablation system and a PFA microflow nebulizer. Ablated otolith material from the sample cell was mixed in the spray chamber with an aerosol of 1% HNO_3 introduced by the nebulizer, and the mixture was then carried to the ICP torch. Laboratory calibration standards consisted of known-concentration multi-element solutions synthesized from stock single element standards and were similarly introduced to the spray chamber by the nebulizer as an aerosol before being carried to the ICP torch. Blanks of 1% HNO_3 aerosol also were introduced to the chamber by the nebulizer. For quality control, we used dissolved otolith reference material obtained from the National Research Council of Canada. To control for operational variability in the laser ICP-MS, a randomized blocks design was used with each petrographic slide as the blocking factor, considered randomly drawn, with each sampling area considered a fixed treatment. Blank and standard readings of count rate ($counts \cdot s^{-1}$) were obtained before and after random presentation of the otolith sections in each block. Readings of reference material were obtained before sample presentation. To calculate element-to-Ca ratios (Me/Ca), background counts were subtracted from otolith counts by interpolating between readings taken before and after each block of otoliths, and the corrected otolith counts were converted to Me/Ca concentrations using the standards. To sample the edge, we used a line raster type with a laser beam of diameter 20 μm, frequency at 10 Hz, and power at 60%, travelling ~900 μm along the proximo-dorsal edge of the otolith section at 6 $\mu m \cdot s^{-1}$ and giving a predicted mean crater width of 17 μm and crater depth of 105.4 μm (Jones and Chen 2003). Dwell time was 15.0 ms.

Response variables were concentrations of Mg/Ca, Mn/Ca, Sr/Ca, and Ba/Ca. Overall, Ba/Ca and Sr/Ca showed a strong relationship implying some redundancy, but because the relationship was weak for southern Chile, both variables were included. We applied multivariate analysis of variance (MANOVA) and discriminant analysis (MDA) and used separate univariate analyses to examine the behaviour of each element. Multivariate outliers were identified by plotting robust squared Mahalanobis distances of the residuals (D_i^2) against the corresponding quantiles (Q–Q plot) of the chi-square distribution. We checked the assumption of multivariate normality analytically using tests ($\alpha = 0.05$) based on Mardia's multivariate skewness and kurtosis measures (Khattree and Naik 1999) and graphically using Q–Q plots of squared Mahalanobis distances (d_i^2). As data were not normally distributed, we used a reciprocal transformation to fulfill the assumption of multivariate normality; univariate normality followed as a property of multivariate normality (Khattree and Naik 1999).

For the MANOVA, as variance–covariance matrices were not equal according to Bartlett's modification ($\chi^2 = 140.0$, df = 60, $\alpha = 0.10$), transformed data were normalized using the square root of the sample dispersion matrix for each area, allowing the sample mean vectors to float. We tested six contrasts for departures from null hypotheses of no differences in elemental concentrations, using an experimentwise $\alpha = 0.0083$. To test between fish caught in the region around South America versus the Antarctic region, we used the following contrasts: (*i*) 1996, Chile vs. south of SAF (South Georgia, Kerguelen, Macquarie); (*ii*) 1997, Falkland Islands vs. South Georgia. To test between the subset of sampling areas in the Antarctic, we used contrasts to test between treatments from 1996: (*iii*) South Georgia vs. Kerguelen; (*iv*) South Georgia vs. Macquarie; (*v*) Kerguelen vs. Macquarie. We also tested (*vi*) the northern slope (1996) vs. the eastern slope (1997) of South Georgia.

As the MANOVA indicated populations were distinguishable from each other, we proceeded to apply MDA. However, because variance–covariance matrices were not equal and pooled variance–covariance matrices were therefore inappropriate, we used individual variance–covariance matrices instead and applied quadratic MDA (Khattree and Naik 2000). The error rates were estimated by cross-validation, using equal prior probabilities. Finally, we examined the data using univariate analysis of variance (ANOVA) ($\alpha = 0.05$) on transformed data to detect the behaviour of individual elements, using pairwise comparisons with SNK tests. The assumption of homogeneity of variance was fulfilled for all concentrations except Mn/Ca.

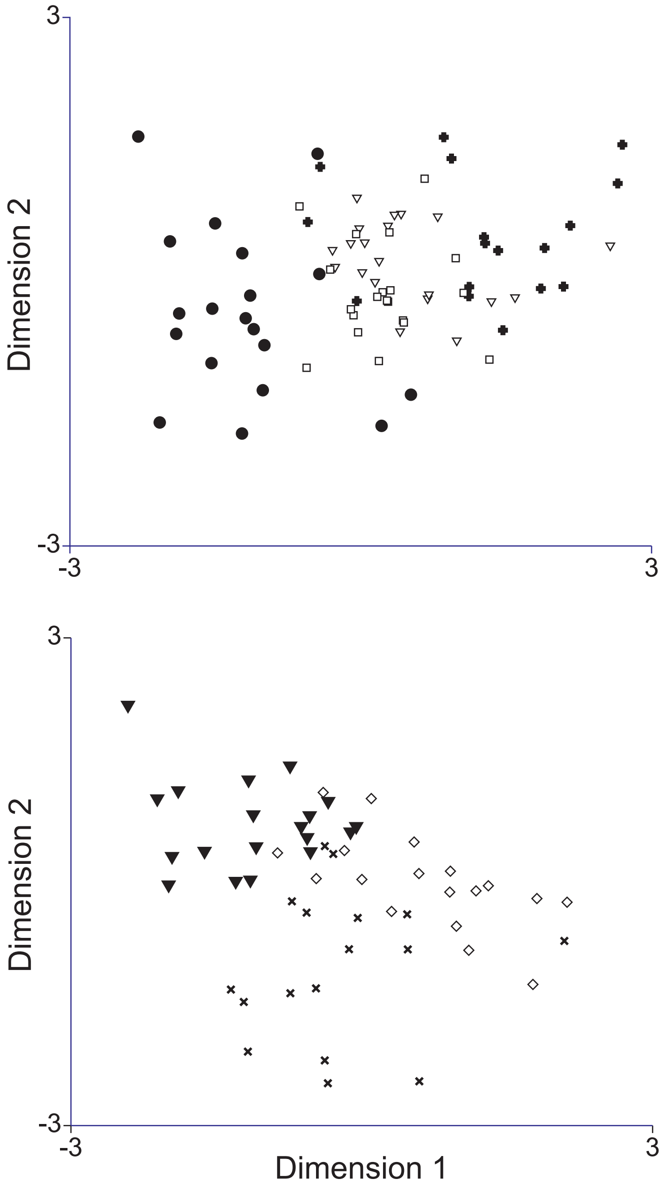
In addition to parametric statistics, we examined the data graphically using nonmetric multidimensional scaling (nMDS) (Kruskal and Wish 1978; Schiffman et al. 1981). Because the variables had different absolute magnitudes and ranges, they were standardized to the same scale. We constructed a dissimilarity matrix based on Euclidean distances from which we created a two-dimensional projection of distance between individual fish using a convergence criterion of Stress < 0.01.

Results

Otolith chemistry was significantly different between all comparisons. Multidimensional scaling showed strong separation between fish captured off South America and those captured in the Antarctic, plotted by year for clarity (Fig. 2). Fish taken off Kerguelen in 1996 showed considerable overlap with those from Macquarie Island, but fish from both sampling areas separated from those taken in the same year from South Georgia. South Georgia fish taken from the southern continental slope in 1998 clearly differentiated from those taken from the eastern slope in 1997.

The MANOVA showed significant differences between sampling areas (Pillai's Trace = 2.95, *F* = 57.9, *P* < 0.0001). Elemental signatures from toothfish caught off South America were significantly different from those of Antarctic fish (Tables 1 and 2). We also detected significant differences between fish taken off South Georgia, Kerguelen, and Macquarie in 1996 and significant differences between fish taken off the northern and eastern continental slopes of South Georgia.

Fig. 2. Relationships between samples of Patagonian toothfish (*Dissostichus eleginoides*) taken from sampling areas in the Southern Ocean between 1996 and 1998, using nonmetric multidimensional scaling (nMDS) based on Euclidean distances. For clarity of illustration, samples are separated by year. (*a*) Samples taken in 1996: Chile, ●; Kerguelen, ▽; Macquarie, □. (*b*) Samples taken in 1997–1998: Falkland Islands, 1997, ▼; South Georgia, 1997, ◇; South Georgia, 1998, ×.



The univariate analysis showed significantly higher Sr/Ca values for fish from the Antarctic region compared with those off South America. Concentrations of Ba/Ca were significantly lower for the Falkland Islands than elsewhere, but significantly higher at South Georgia in 1996 than at other areas sampled that year. Fish from Chile and the Falkland Is-

Table 1. Concentrations of trace elements ratioed to Ca ($\mu mol \cdot mol^{-1}$), sampled from the outer edge of the otoliths from Patagonian toothfish (*Dissostichus eleginoides*) taken in commercial fisheries at five different sites in the Southern Ocean.

Year	Site	*n*	Mean	CV
(*i*) Mg/Ca				
1996	Chile	19	75.1	0.61
	Macquarie	20	105.6	0.38
	South Georgia	17	44.1	0.51
	Kerguelen	20	67.4	0.47
1997	Falklands	19	54.6	0.31
	South Georgia	18	63.4	0.39
1998	South Georgia	17	290.9	0.73
(*ii*) Mn/Ca				
1996	Chile	19	2.79	2.06
	Macquarie	20	0.19	1.68
	South Georgia	17	0.15	0.56
	Kerguelen	20	0.13	0.73
1997	Falklands	19	0.79	0.69
	South Georgia	18	0.27	0.68
1998	South Georgia	17	1.59	1.09
(*iii*) Sr/Ca				
1996	Chile	19	3790	0.24
	Macquarie	20	5233	0.15
	South Georgia	17	6574	0.24
	Kerguelen	20	5520	0.25
1997	Falklands	19	3827	0.14
	South Georgia	18	6052	0.29
1998	South Georgia	17	5467	0.36
(*iv*) Ba/Ca				
1996	Chile	19	2.61	0.42
	Macquarie	20	2.65	0.38
	South Georgia	17	4.51	0.45
	Kerguelen	20	2.99	0.64
1997	Falklands	19	1.53	0.25
	South Georgia	18	3.84	0.59
1998	South Georgia	17	3.11	0.57

Note: CV, coefficient of variation.

Table 2. Contrasts from multivariate analysis of variance of Patagonian toothfish *(Dissostichus eleginoides)*: (*i* and *ii*) samples taken off South America vs. those taken in the Antarctic in 1996 and 1997; (*iii–v*) between samples taken in the Antarctic in 1996; and (*vi*) samples taken from the northern vs. the eastern continental slopes of South Georgia in 1996 and 1997.

Comparison	Pillai's trace	*F*	Pr > *F*
(*i*) CH96 vs. SG, K, M96	0.926	373	<0.0001
(*ii*) FI97 vs. SG97	0.908	295	<0.0001
(*iii*) SG96 vs. K96	0.757	93	<0.0001
(*iv*) SG96 vs. M96	0.829	146	<0.0001
(*v*) K96 vs. M96	0.881	223	<0.0001
(*vi*) SG96 vs. SG97	0.853	175	<0.0001

Note: Dependent variables: Mg/Ca, Mn/Ca, Sr/Ca, Ba/Ca. $\alpha = 0.0083$. Sites: CH, Chile; FI, Falkland Islands; K, Kerguelen; M, Macquarie; SG, South Georgia. "96" and "97" after the site codes refer to the years 1996 and 1997, respectively.

Table 3. Classification rates (%) from quadratic multivariate discriminant analysis undertaken for Patagonian toothfish (*Dissostichus eleginoides*) taken from sampling areas in the Southern Ocean, using four response variables (Mg/Ca, Mn/Ca, Sr/Ca, Ba/Ca).

	Area classified to						
Area sampled	CH	FI	K	M	SG96	SG97	SG98
CH	**84**	16	0	0	0	0	0
FI	5	**79**	11	0	0	5	0
K	0	5	**65**	10	10	10	0
M	0	0	25	**50**	5	10	10
SG96	0	6	12	6	**53**	23	0
SG97	0	5	11	5	11	**67**	0
SG978	6	0	6	23	6	6	**53**

Note: Sampling areas: CH, southern Chile, 1996 ($n = 19$); FI, Falkland Islands, 1997 ($n = 19$); K, Kerguelen, 1996 ($n = 20$); M, Macquarie, 1996 ($n = 20$); SG96, South Georgia, 1996 ($n = 17$); SG97, South Georgia, 1997 ($n = 18$); SG98, South Georgia, 1998 ($n = 17$). Bold values indicate rates of correct classification to sampling area.

lands had significantly higher concentrations of Mn/Ca than Antarctic fish in 1996–1997 but were not significantly different from fish taken in 1998 from the southern slope of South Georgia. Southern slope fish showed significantly higher Mg/Ca concentrations than all other samples, whereas fish caught off Macquarie Island showed significantly higher concentrations of Mg/Ca than all other 1996–1997 samples.

The quadratic MDA successfully classified fish to the region where they were captured: only four Antarctic fish were incorrectly classified to South American sampling areas and three South American fish were incorrectly allocated to Antarctic sampling areas. Within region, fish caught off South America were classified most successfully, with 79% of Falkland Island fish and 84% of fish from southern Chile correctly allocated (Table 3). Moreover, all misclassifications for Chilean fish were allocated to the Falkland Islands. Classification success for Antarctic fish was between 50% and 67%, but of the fish taken off the northern slope of South Georgia in 1996, half of the misclassifications were allocated to the nearest sampling area, on the eastern South Georgia slope in 1997. Similarly, half the misclassifications of Macquarie fish were to Kerguelen. Nevertheless, 67% of South Georgia eastern slope fish and 65% of Kerguelen fish were allocated correctly.

Discussion

Although previous researchers examined whole otoliths (Edmonds et al. 1989, 1991) and otolith nuclei (Campana et al. 1994) of marine fish, the present study uniquely used laser ICP-MS to place the otolith elemental signatures in their geographic context by examining material at the edge of the sectioned otolith, deposited in the interval immediately prior to capture. Edge signatures discriminated Patagonian toothfish caught off South America from those caught in the Antarctic with near-complete success, direct evidence that otolith elemental signatures of exclusively marine fish can record specific spatial locations. By extension, because otolith material once deposited is not reworked (Campana and Neilsen 1985), otolith growth increments can record toothfish provenance at regional scales over the life history prior to capture, providing a means to measure movement directly and test hypotheses of population structure. In contrast, genetic markers can link fish only indirectly to geographic area at the time of spawning, and artificial tags are costly to use, are applied only to a proportion of the targeted population, and can link fish to geographic area only at the time of marking and recapture.

Moreover, strong differences between sampling areas within each region indicated edge signatures could record provenance at finer scales. With only four elements, 79%–84% of South American fish were correctly allocated to sampling area, success rates that compared favourably with the range reported for estuarine-dependent fish on the east coast of North America. Thus, juvenile weakfish (*Cynoscion regalis*) classified to their natal estuaries with 63% success using trace element data (Thorrold et al. 1998*a*), whereas American shad (*Alosa sapidissima*) classified to natal river with 90% success (Thorrold et al. 1998*b*). For Patagonian toothfish caught in the Antarctic, success rates were still comparable for Kerguelen fish and those caught off the eastern slope of South Georgia in 1997. Lower rates for fish from Macquarie and the northern and southern slopes of South Georgia were due largely to misclassification to a single other area, respectively, Kerguelen, the eastern South Georgia slope, and Macquarie, which may be resolved by the use of other chemical markers (Thorrold et al. 1998*a*). Even with only the markers used in this study, few South Georgia eastern slope fish were classified to the northern or southern slopes, and few of those caught along the southern slope were allocated to other South Georgia areas, suggesting that otolith chemistry may be capable of recording differences at spatial scales comparable to those achieved for spotted sea trout (*Cynoscion nebulosus*) within the Chesapeake Bay (Dorval et al. 2005).

However, the fact that Macquarie fish classified to Kerguelen at similar rates as northern slope to eastern slope fish at South Georgia argues that the discriminatory power of the signatures is not simply related to spatial distance, but instead reflects environmental gradients in trace and minor element concentrations. Thus, Mn is a scavenged element (Whitfield and Turner 1987), decreasing with depth in contrast to recycled elements like Ba. Deep-water flow patterns on a global scale mean that enrichment of recycled elements and uptake of scavenged elements along the direction of deep-water flow leads to fractionation between ocean basins (Donat and Bruland 1995). These larger trends are overlaid by finer-scale spatial differences between and within islands and seamounts.

For Ba, Dehairs et al. (1992) observed that barite accumulation in the open ocean of the ACC was generally associated with new production fueled by nitrate. Transport to depth occurred through surface uptake of dissolved Ba as barite particles ($BaSO_4$) in association with aggregates of biogenic debris. On sinking, the aggregates break down through bacterial activity, freeing enclosed barite crystals to accumulate at 200–700 m (Stroobants et al. 1991; Dehairs et al. 1997), where they settle slowly and dissolve at depth, consistent with the high mean otolith Ba/Ca found in the South Georgia toothfish in the present study. In contrast, Dehairs et al. (1992) found that continental shelf areas were associated with regenerated production dominated by ammonium uptake and little accumulated barite at depth, consistent with the low mean otolith Ba/Ca concentrations found in the Falkland Island fish.

For Mn, the thermodynamically stable form in oxidising seawater is insoluble Mn(IV), but Sunda and Huntsman (1988) suggested that photoreduction of Mn oxides and photoinhibition of Mn-oxidising microorganisms may maintain the dissolved Mn^{2+} maximum observed at the surface. Similarly, high dissolved Mn near the benthic layer where toothfish are caught is associated with effluxes from anoxic sediments. Upwelling has also been demonstrated in the wake of oceanic islands (e.g., Gordon et al. 1998); similarly, Bucciarelli et al. (2001) found the continental shelf zone to the northeast of Kerguelen to be influenced by enrichment from lithogenic origin and the resuspension of sediments. Off the eastern shelf slope, however, where Kerguelen fish were taken for the present study, they found that concentrations of dissolved Mn decreased abruptly offshore of the shelf front, below the surface layer and in association with Antarctic water. On the other hand, authigenic Mn activity recorded on the eastern side of South America (Anonymous 1989) is consistent with the higher concentrations of Mn/Ca in the otoliths of Falkland Island fish.

In contrast to estuaries, where Sr/Ca and Ba/Ca are inversely related because of the exchange of cations in seawater for Ba adsorbed on river-borne clays (Guay and Falkner 1998), Sr is quasiconservative in the oceanic environment: concentrations vary slightly, probably as a result of biogenic carbonate cycling (De Villiers 1999; Müller and de Deckker 2002). If this process were linked to barite formation (Bernstein et al. 1992), a common transport pathway to depth would help explain the relationship we found in toothfish between otolith Ba/Ca and Sr/Ca compared with the inverse relationship found in estuarine-dependent fish. It would also help explain the higher otolith Sr/Ca concentrations at Antarctic sampling areas compared with South American ones.

However, variation in ambient concentrations of oceanic Sr/Ca are at the limits at which otolith chemistry has so far been able to resolve experimentally (Bath et al. 2000). An alternative explanation is that Sr/Ca in toothfish otoliths is linked to physical variables such as salinity and temperature, but even though these drive the complex pelagic environment in the Southern Ocean, their variation at depth is small compared with shallower and more temperate regions. Temperature-dependent fractionation may behave differently at the lower temperatures encountered in the Southern Ocean (Townsend et al. 1992), but nevertheless, these ranges approach the demonstrated limits of resolution for otolith chemistry. For instance, the difference in temperature between warm Upper Circumpolar Deep Water and colder Bransfield Strait Water is in the order of 2 °C (Smith et al. 1999) compared with differences almost an order of magnitude greater between surface water types off the east coast of North America. Similarly, the difference between salinities found in Antarctic Winter Water and the salinity maximum in Lower Circumpolar Deep Water vary between only ~0.5 and 1.0 ppt. Instead, another explanation is that variation in the rate of matrix protein formation linked to somatic growth may control otolith crystallization (Campana 1999), accounting for variation in otolith Sr/Ca concentrations. In consequence, divergent growth rates (Ashford 2001) could contribute to the differences in otolith Sr/Ca concentrations between the toothfish sampled in the present study off South America and that sampled in the Antarctic.

Thus, the spatial variation in toothfish otolith chemistry appears consistent with environmental structure in the Southern Ocean, mediated by physiological processes (Kalish 1989, 1991) such as growth that themselves vary spatially in rate. As a result, by empirically linking otolith elemental markers to geographic areas within a species' distribution, researchers can identify the provenance of captured oceanic fish over their entire life history and hence study their prior movement and use of critical habitat. Because otoliths also provide a chronology recorded in growth increments, these life history processes can be related to age and to calendar year when the capture date is known. Where elemental signatures can demonstrably discriminate areas of interest, these important advantages make otolith chemistry a valuable addition to the suite of tools available to study oceanic fish and a useful complement to older approaches employing genetic and artificial markers.

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