Available online at www.sciencedirect.com
ScienceDirect
Geochimica et Cosmochimica Acta 71 (2007) 87–94
Geochimica
www.elsevier.com/locate/gca

Oxygen and carbon stable isotopes in otoliths record spatial isolation of Patagonian toothfish (*Dissostichus eleginoides*)

Julian Ashford *, Cynthia Jones

Center for Quantitative Fisheries Ecology, Old Dominion University, Norfolk, Virginia, VA 23529, USA

Received 25 April 2006; accepted in revised form 23 August 2006

Abstract

Strong contrasts in ambient isotope ratios and in diet suggest stable isotopes in the otoliths of oceanic fish can resolve water masses and geographic areas, promising a powerful multivariate approach for examining population structure and provenance. To test this, whole otoliths were taken from Patagonian toothfish (*Dissostichus eleginoides*) sampled off the Patagonian Shelf and South Georgia, on either side of a population boundary, and otolith $\delta^{18}O$ and $\delta^{13}C$ values were measured to see if they could distinguish South American-caught fish from those taken in the Antarctic. Values of otolith $\delta^{18}O$ and $\delta^{13}C$ predicted capture area with 100% success, validating their use for distinguishing provenance and corroborating the prior evidence of population isolation. Values of $\delta^{18}O$ in the otoliths reflected ambient values as well as seawater temperature: low values in Patagonian Shelf fish were consistent with exposure to Antarctic Intermediate Water (AAIW), and high values in South Georgia fish were consistent with exposure to Circumpolar Deep Water (CDW). In contrast, differences in otolith $\delta^{13}C$ appeared to reflect diet: relative depletion of otolith $\delta^{13}C$ at South Georgia compared to the Patagonian Shelf were most likely linked to differences in sources of metabolic carbon, as well as $\delta^{13}C$ in dissolved inorganic carbon (DIC) of seawater. These contrasting properties strongly suggest that stable isotopes can resolve the provenance of toothfish from Antarctic sampling areas that hitherto have been difficult to separate. These results show that, by using the chemistry recorded in otoliths, researchers can exploit biogeochemical variation in fully marine environments to examine the spatial ecology of oceanic fish.

© 2006 Elsevier Inc. All rights reserved.

1. Introduction

1.1. Otolith chemistry and stable isotopes

Otolith chemistry shows considerable promise as a technique for studying population structure in oceanic fishes: the chemistry of otolith nuclei, laid down during early life, can record early spatial separation resulting from segregation during spawning, and hence population differences (e.g. Campana et al., 1994). The technique takes advantage of biogeochemical variation in the ocean environment. As a result of the spatial array this generates, minor and trace elements incorporated in the otoliths show spatial properties which link fish to specific environments (e.g. Thresher, 1999). Concentrations of strontium, for instance, characterize marine exposure and are related to water temperature (Bath et al., 2000); the trace element barium, on the other hand, is indicative of freshwater exposure (e.g. Dorval et al., 2005). In estuarine-dependent species, fisheries biologists often use these properties to detect fish movement between marine and estuarine environments (e.g. Kalish, 1990; Limburg, 1995).

Like trace and minor elements, stable isotopes incorporated in otoliths display characteristic properties: $\delta^{18}O$, the ratio of stable isotopes of oxygen reported relative to a standard (Vienna Pee Dee belemnite, or VPDB), is deposited in fish otoliths close to equilibrium with ambient water, and fractionation is predictably related to temperature (Kalish, 1991; Thorrold et al., 1997; Weidman and Millner, 2000). However, in focusing on the effect of temperature, researchers have paid less attention to the fact that ambient $\delta^{18}O$ also varies between water types. Evaporation increas-

---

* Corresponding author. Fax: +1 757 683 5293.
E-mail addresses: jashford@odu.edu (J. Ashford), cjones@odu.edu (C. Jones).

0016-7037/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.gca.2006.08.030

es ocean surface $\delta^{18}O$, whereas precipitation reduces it, because the $H_2{}^{18}O$ molecule is heavier. A consequence is that ambient values vary with latitude: the $\delta^{18}O$ in precipitation decreases poleward as water vapour moves from temperate source regions and the heavier $H_2{}^{18}O$ is depleted. As a result, polar glacial ice is isotopically very light and glacial meltwater in polar oceans, as well as precipitation, influences the local $\delta^{18}O$ balance (Meredith et al., 1999a,b). Moreover, once away from the surface, $\delta^{18}O$ in oceanic waters is a conservative tracer, changing only by mixing between water masses. These properties make otolith $\delta^{18}O$ a good candidate for linking fish to oceanic water types that show characteristic temperatures and come from sources at different latitudes.

Values of $\delta^{13}C$, the ratio of stable isotopes of carbon reported relative to the VPDB standard, found in dissolved inorganic carbon (DIC) also vary between water types (Kroopnick, 1980). Moreover, $\delta^{13}C$ deposited in otoliths showed evidence of a relationship with temperature, but also showed depleted values that were inconsistent with an equilibrium reaction for carbonates (Kalish, 1991; Thorrold et al., 1997). As metabolic carbon is severely depleted in $\delta^{13}C$, the internal DIC pool from which biogenic carbonate is formed is thought to be replenished from both seawater and metabolic carbon derived from the fish diet (Kalish, 1991; Schwarcz et al., 1998; Høie et al., 2003). Hence otolith $\delta^{13}C$ values are linked to temperature by metabolic rate which is controlled by ambient temperature in most marine fish. These properties indicate the potential of otolith $\delta^{13}C$ to link marine fish to environments where fish access different sources of metabolic carbon, and water types are characterized by distinct temperatures and DIC $\delta^{13}C$ concentrations.

1.2. Circulation and water masses in the Southern Ocean

In the Southern Ocean, the physical environment is structured by the Antarctic Circumpolar Current (ACC) which transports water eastward around the polar continent. Within it, fronts of fast-flowing water (Hofmann, 1985; Orsi et al., 1995) penetrate to depth (Nowlin and Clifford, 1982). South of the Polar Front (PF), surface waters are cold and fresh reflecting significant heat loss to the atmosphere in winter and dilution by high-latitude precipitation and ice-melt. The Southwest Atlantic is a key site for the formation of Antarctic Intermediate Water

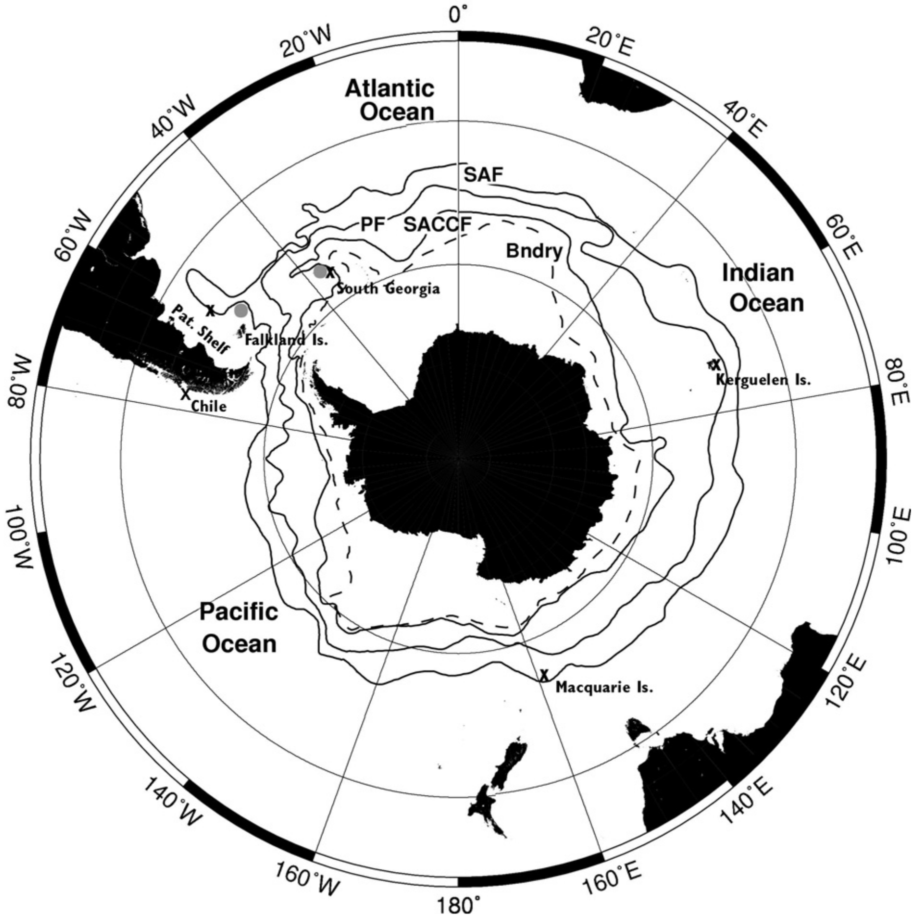

Fig. 1. Map of the Southern Ocean showing mean position of fronts in the Antarctic Circumpolar Current (ACC) in relation to areas (●) sampled for Patagonian toothfish in 1999 and areas (X) sampled in 1996 by Ashford et al. (2005). Positions of fronts are taken from Orsi et al. (1995). Pat. Shelf, Patagonian Shelf; SAF, Subantarctic Front; PF, Polar Front; SACCF, Southern ACC Front; Bndry, southern boundary of the ACC.

(AAIW), which includes subducted Antarctic waters from south of the PF, that is then advected along the loop of the ACC off the Patagonian Shelf (Meredith et al., 1999a) (Fig. 1). There, AAIW has a temperature above 3 °C (Arhan et al., 2002), $\delta^{18}O$ values of approximately −0.2‰ (Meredith et al., 1999a), and $\delta^{13}C$ values of approximately 0.5‰ (Kroopnick, 1980). In contrast, North Atlantic Deep Water (NADW), transported southwards from source regions in the northern Atlantic, is the parent of Circumpolar Deep Water (CDW) which circulates in the ACC, becoming shallower towards the polar continent. CDW, because of its provenance from outside the Antarctic, is generally characterized by temperatures between 1.5 and 2.0 °C (e.g. Orsi et al., 1995; Brandon et al., 1999), and high $\delta^{18}O$ values of 0.00–0.05‰ in the western South Atlantic (Meredith et al., 1999a). Values of $\delta^{13}C$ in CDW are low, down to a minimum of 0.2‰ (Kroopnick, 1980).

1.3. Otolith chemistry and Patagonian toothfish

Distributed along the ACC and around southern South America, Patagonian toothfish (*Dissostichus eleginoides*) are opportunistic, bentho-pelagic predators which show considerable spatial variation in diet (e.g. Duhamel, 1981; Goldsworthy et al., 2002). They grow to more than 2 m, live up to 50 years (Ashford, 2001; Horn, 2002), and are commercially fished using benthic longlines, mostly along the continental slopes. Their otolith chemistry has already been shown capable of distinguishing provenance: when sampled using lasers, trace and minor elements laid down along the otolith edges during the period immediately prior to capture, successfully distinguished fish caught in the Antarctic from those taken off South America, and also separated fish caught off the western and eastern South American shelf-slopes (Ashford et al., 2005). However, they were less successful in classifying fish to capture areas around the Antarctic, off South Georgia in the Atlantic Ocean, Kerguelen Island in the Indian, and Macquarie Island in the western Pacific Ocean (Fig. 1).

Differences in temperature, $\delta^{18}O$ and $\delta^{13}C$ between water types, as well as diet, indicate otolith $\delta^{18}O$ and $\delta^{13}C$ have potential to resolve these Antarctic sampling areas. Before they can be used to study population questions, however, validation studies are needed to confirm that these markers will in fact distinguish known environmental differences between areas. Researchers outside the Southern Ocean have successfully used the otolith chemistry of dissolved whole otoliths to distinguish fish between geographic areas (Edmonds et al., 1989; Edmonds et al., 1991). Whole-otolith analyses, though, risk homogenizing spatial differences through fish movement: if fish move, the chemistry laid down corresponding to the area of capture can be obscured by otolith material accumulated while in other areas. To avoid this, stable isotopes can be validated by testing whole otoliths for spatial differences between areas where fish movement is constrained. One way to do this is to test between spatially discrete populations either side of a population boundary.

In toothfish, microsatellite data showed evidence of population differentiation around the Southern Ocean (Smith and McVeagh, 2000). Although differences between sampling areas in the Indian and Pacific Ocean sectors were not consistent across loci and samples, fish showed strong, consistent differences from those caught off the Patagonian Shelf. Moreover, although fish caught off South Georgia in the South Atlantic and Kerguelen in the Indian Ocean showed similar size-at-age distributions, distributions in fish off the Patagonian Shelf were significantly different (Ashford, 2001) suggesting that there was little mixing between South America and the Antarctic. More recent genetic evidence confirmed a sharp population division in the vicinity of the Polar Front between toothfish caught along the Patagonian Shelf, and others caught around South Georgia (Shaw et al., 2004). Moreover, the trace element chemistry of the otolith nuclei from the very same fish resolved the population structure, indicating substantial differences in the environments to which the two populations were exposed during their early life histories (Ashford et al., 2006).

Accordingly, in this study we sampled fish taken from areas on either side of the population boundary, on the Patagonian Shelf and off South Georgia, and used whole otoliths to test whether otolith $\delta^{18}O$ and $\delta^{13}C$ values could distinguish the South American-caught toothfish from those taken in the Antarctic. Furthermore, we examined whether expected values of otolith $\delta^{18}O$ and $\delta^{13}C$ calculated using relationships estimated by Kalish (1991) and Thorrold et al. (1997) were consistent with the values observed in our study.

2. Materials and methods

Otoliths of toothfish from the Patagonian Shelf were sampled in 1999 from commercial catches taken by benthic longline at a fishing depth of 950 m within the Falkland Island Outer Conservation Zone (Fig. 1); otoliths were also sampled the same year from commercial longline catches taken around South Georgia. Observers recorded total length, weight, sex, and maturity stages for each fish; collected the otoliths; and stored them in paper envelopes. We randomly selected one otolith from each of four fish from each sampling area for a preliminary assay. Based on these results, the main assay was undertaken using larger sample sizes. Because data values were relative to a common standard, results from preliminary and main assay were pooled. The pooled sample for the Patagonian Shelf included all fish between 100 and 120 cm total length (TL) taken at two consecutive stations at the end of August; for South Georgia, the pooled sample included all fish between 100 and 120 cm TL sampled by one observer at the end of April, and included two fish of 97 cm TL to supplement sample size.

To measure values of $\delta^{18}O$ and $\delta^{13}C$, a single otolith was randomly selected from each pair, cleaned by soaking in 20% hydrogen peroxide, and rinsed and sonicated in milli-Q water. Otoliths were individually crushed using an agate pestle and mortar cleaned with 10% nitric acid, and rinsed with milli-Q water. The crushed powder was transferred to acid-cleaned vials. The preliminary assay was processed using the Isoprime dual inlet gas source mass spectrometer, with Multiprep carbonate reaction devices, at the Geochemistry Laboratories of the Department of Geology at the University of Maryland. The reaction of the carbonate powder with anhydrous phosphoric acid (density ~1.90 g/cc) was for 10 min at 90 °C. Values were normalized to house standards in turn calibrated against the international standard NBS-19 (National Bureau of Standards), and reported relative to PDB. The precision of analyses based on the measurement of multiple standard aliquots during the run of samples is generally better than 0.05‰ of both carbon and oxygen isotopes. The same procedures were followed for the main assay, and samples were processed using the instrument at the Department of Geological Sciences at Rutgers University, normalized to the standard NBS-19.

Sampling area was used as a fixed treatment factor. When tested using Mardia's multivariate skewness and kurtosis measures (Khattree and Naik, 1999), residuals conformed to multivariate normality; variance–covariance matrices were not significantly different according to Bartlett's modification ($\chi^2 = 6.00$, df = 3, $\alpha = 0.10$). We used multivariate analysis of variance (MANOVA) to test whether sampling areas were distinguishable from each other with respect to their population means. Because the means were significantly different, we applied multivariate discriminant analysis (MDA), using a pooled variance–covariance matrix (Khattree and Naik, 2000). We used equal prior probabilities of 50%, and estimated the error-rate by cross-validation. Finally, we examined the data using univariate ANOVA ($\alpha = 0.05$) to detect the behaviour of δ^{18}O and δ^{13}C individually. Residuals fulfilled the assumptions of univariate normality and homogeneity of variance for both δ^{18}O and δ^{13}C for both treatments.

Finally, we examined whether observed otolith stable isotope values were consistent with those from previous studies. Kalish (1991) estimated the relationship between mean values of otolith δ^{18}O for a species ($\delta^{18}\mathrm{O}_\mathrm{m}$) and water temperature (T) for an assemblage of wild-caught fish, including some from the Southern Ocean:

$$\delta^{18}\mathrm{O}_m = 3.58 - 0.196T\ ^\circ\mathrm{C} \tag{1}$$

whereas Thorrold et al. (1997) used Atlantic croaker (*Micropogonias undulatus*) raised at temperatures between 18 and 25 °C in experimental tanks to estimate the same relationship but incorporated a term correcting for ambient δ^{18}O (δ_w):

$$\delta_c - \delta_w = 4.64 - 0.21T\ ^\circ\mathrm{C} \tag{2}$$

where δ_c is the value of otolith δ^{18}O. Thorrold et al. (1997) also estimated a similar relationship for δ^{13}C:

$$\Delta^{13}\mathrm{C} = -1.78 - 0.18T\ ^\circ\mathrm{C} \tag{3}$$

where Δ^{13}C represents values of δ^{13}C corrected for variations in δ^{13}C in DIC. We substituted values of seawater temperature and, respectively, ambient δ^{18}O and δ^{13}C in seawater DIC, obtained from the literature for depths at which the fish were sampled. To convert between δ^{18}O values standardized to VPDB and SMOW, we used the conversion factor of Friedman and O'Neil (1977):

$$\delta_w = 0.99978(\delta^{18}\mathrm{O}_\mathrm{SMOW}) - 0.22 \tag{4}$$

Using t-tests, we then tested for differences between the observed and expected values of otolith δ^{18}O and δ^{13}C.

3. Results

Values for δ^{18}O and δ^{13}C are presented in the electronic annex. The data showed complete separation between Patagonian Shelf and South Georgia fish (Fig. 2), with little evidence of any effect due to size (Fig. 3). Fish taken off South America showed lower δ^{18}O values ($\bar{x}_\mathrm{O,PS} = 2.840$, $s = 0.159$, $n = 22$) and higher δ^{13}C values ($\bar{x}_\mathrm{C,PS} = -0.790$, $s = 0.37$) than South Georgia fish ($\bar{x}_\mathrm{O,SG} = 3.442$, $s = 0.148$, $n = 21$; $\bar{x}_\mathrm{C,SG} = -1.739$, $s = 0.36$). There was some evidence of a small offset in the δ^{18}O values from the preliminary test (Fig. 2) even after correcting against the NBS-19 standard, but since this affected only a small number of samples, they were left in the analysis. Even without removing this source of variation, the data fulfilled multivariate parametric assumptions and the MANOVA confirmed that the differences were highly significant (Pillai's Trace = 0.861; $F = 123.8$; $p < 0.0001$). The univariate results showed that differences between sampling areas were highly significant for both elements (Table 1). For the MDA, 100% of the observations correctly classified to sampling area (error-rate = 0%).

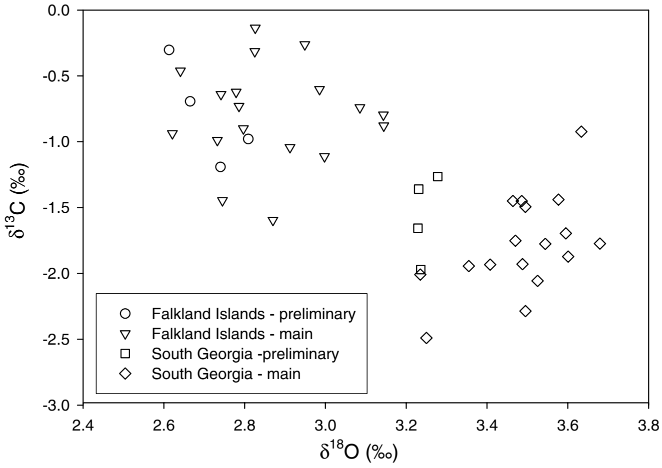

Fig. 2. Stable isotope values found in whole otoliths of Patagonian toothfish captured off the Patagonian Shelf and South Georgia ($n_\mathrm{PS} = 22$, $n_\mathrm{SG} = 21$).

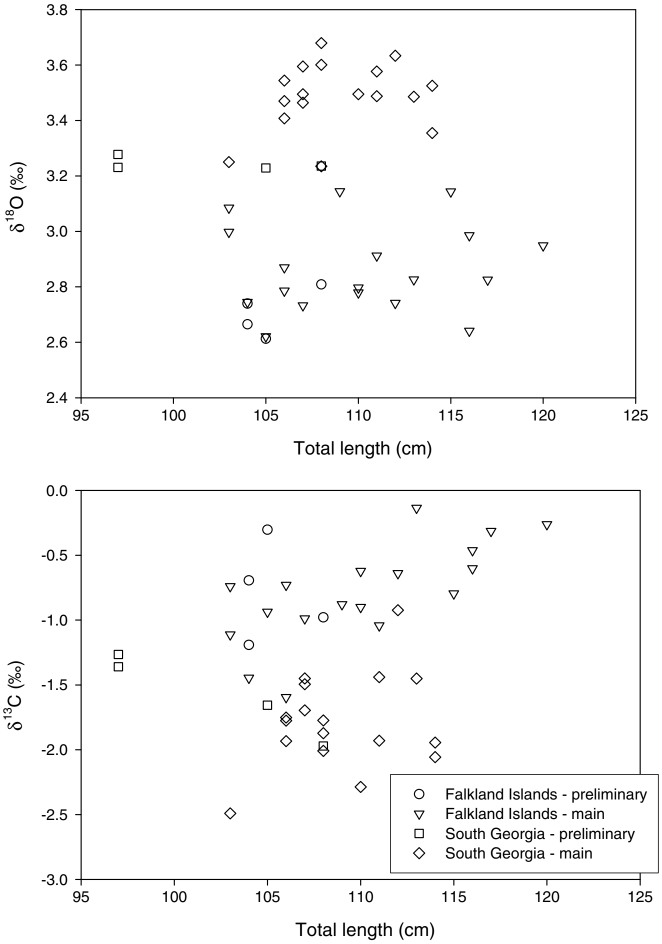

Fig. 3. Stable isotope values found in whole otoliths of Patagonian toothfish captured off the Patagonian Shelf and South Georgia plotted against size, demonstrating little evidence of a relationship.

Table 1
Results from ANOVA of $\delta^{18}O$ and $\delta^{13}C$ values measured in whole otoliths of Patagonian toothfish caught off the Patagonian Shelf and South Georgia ($\alpha = 0.05$)

	df	SS	MS	*F*	*p*
(a) $\delta^{18}O$					
Area	1	3.93	3.93	167.11	<0.0001
Error	41	0.97	0.02		
Total	42	4.90			
(b) $\delta^{13}C$					
Area	1	9.69	9.69	72.35	<0.0001
Error	41	5.49	0.134		
Total	42	15.18			

For $\delta^{18}O$, the value expected from Eq. (1) for Patagonian Shelf-caught fish was $\delta_{\mathrm{cPS}} = 2.99$‰, using $T = 3$ °C corresponding to AAIW at the fishing depth of 950 m (Arhan et al., 2002); and that for South Georgia-caught fish was $\delta_{\mathrm{cSG}} = 3.19$‰, using $T = 2$ °C corresponding to CDW at depths >250 m (Brandon et al., 1999). These were not significantly different from the observed values (*t*-tests, $p > 0.05$), but considerably under-estimated the observed difference between fish from the two sampling areas. On the other hand, the expected value for Patagonian Shelf fish from Eq. (2) was $\delta_{\mathrm{cPS}} = 3.59$‰, whereas the expected value for South Georgia fish was $\delta_{\mathrm{cSG}} = 4.00$‰, using the same temperatures and $\delta_{\mathrm{w}} = -0.2$‰ for AAIW and $\delta_{\mathrm{w}} = 0.0$‰ for CDW (Meredith et al., 1999a). Both expected values significantly over-estimated those observed (*t*-tests, $p < 0.05$) and under-estimated the observed difference between fish from the two sampling areas, but less than the values from Eq. (1).

In the case of $\delta^{13}C$, Eq. (3) gave very similar predicted values for the Patagonian Shelf ($\delta^{13}C_{\mathrm{c}} = -1.82$‰) and South Georgia ($\delta^{13}C_{\mathrm{c}} = -1.94$‰), using values for $\delta^{13}C = 0.5$‰ and 0.2‰ in DIC for AAIW and CDW, respectively (Kroopnick, 1980). Both values departed significantly from the observed values of −0.79‰ for the Patagonian Shelf (*t*-test, $p < 0.05$), and −1.74‰ for South Georgia (*t*-test, $p < 0.05$).

Nevertheless, despite the discrepancies with predicted values, observed values for $\delta^{18}O$ and $\delta^{13}C$ discriminated between sampling areas with complete success: observed values of $\delta^{18}O$ diverged between sampling areas by 0.60‰, the equivalent of 3.75 standard deviations, whereas values of $\delta^{13}C$ diverged by 0.95‰, representing over 2.5 standard deviations. These observed differences between areas were much larger than those expected from Eqs. (1)–(3): in the case of $\delta^{18}O$, the observed difference was at least 50% larger, whereas for $\delta^{13}C$, the observed difference was nearly an order of magnitude larger.

4. Discussion

Previous studies of wild-caught marine fish examined otoliths obtained from a range of species (Kalish, 1991), or used stable isotopes to validate age estimates (Weidman and Millner, 2000). In contrast, we successfully used stable isotope chemistry from the whole otoliths of an oceanic species to predict capture area, validating the use of $\delta^{18}O$ and $\delta^{13}C$ for distinguishing toothfish provenance in the Southern Ocean, and supporting prior evidence of population isolation between toothfish found off the Patagonian Shelf and South Georgia. Unlike trace and minor element data from the same species, the stable isotope data were normally distributed and fulfilled all multivariate and univariate parametric assumptions. We did not need to transform data, and were able to use pooled variance–covariance matrices. As a result, the isotope data were amenable to powerful parametric multivariate approaches that either could not be applied when using trace and minor element data, or could be applied only after transformation (Ashford et al., 2005; Ashford et al., 2006).

For the univariate analyses, the $\delta^{18}O$ data separated Patagonian Shelf and South Georgia fish without overlap, whereas $\delta^{13}C$ separated the two areas only slightly less strongly. Eq. (1) successfully predicted concentrations of otolith $\delta^{18}O$ from seawater temperature alone, but under-estimated the difference between sampling areas. On the other hand, Eq. (2), which incorporated a term for $\delta^{18}O$ in seawater, significantly over-estimated the observed values but estimated the difference between sampling areas considerably better. Considering that whole otoliths integrate measurements over the life history prior to capture, these results were promising and confirmed the importance of ambient $\delta^{18}O$ in predicting spatial variability in otolith stable oxygen values. Even so, the greater than expected disparity between our observed values suggested that the slope term may be different for toothfish, despite the fact that the intercept terms explain the difference between Eqs. (1) and (2), and the slope terms are similar (Thorrold et al., 1997). However, Eq. (2) would explain this disparity if the temperature differential between our two sampling areas was 1 °C larger. Given that observed values reflect chemistry integrated over the whole otolith, this is not unreasonable: toothfish tend to move from shallower water with age (Agnew et al., 1999), and shallower water off the Patagonian Shelf has a higher temperature (Arhan et al., 2002) whereas the converse is true off South Georgia (Brandon et al., 1999; Meredith et al., 2005). When the temperatures are adjusted accordingly, the predicted values from Eq. (2) still over-estimate the observed, suggesting that the intercept for toothfish has an intermediate value between those estimated by Thorrold et al. (1997) and Kalish (1991).

In the case of $\delta^{13}C$, Eq. (3) gave expected values that were very similar for the Patagonian Shelf ($\delta^{13}C_c = -1.82‰$) and South Georgia ($\delta^{13}C_c = -1.94‰$). These departed significantly from the observed values (−0.79‰ for the Patagonian Shelf, and −1.74‰ for South Georgia) and indicated smaller differences than were in fact measured. Increasing the temperature differential in the same way as for $\delta^{18}O$ could not account for these discrepancies. Instead, since otolith carbon is considered to be a function of carbon from seawater and metabolically derived carbon (Høie et al., 2003), ambient $\delta^{13}C$ in DIC and diet may explain the differential ($\delta^{13}C_{cPS} - \delta^{13}C_{cSG} = 0.95‰$) we observed between otoliths of toothfish from the Patagonian Shelf and South Georgia. Thus, the corresponding difference in $\delta^{13}C_{DIC}$ between areas estimated from the literature was 0.3‰, which would account for a part of the differential in the otoliths, but toothfish also display considerable spatial variation in diet which has been extensively documented (Duhamel, 1981; Duhamel and Pletikosic, 1983; Zhivov and Krivoruchko, 1990; Garcia de la Rosa et al., 1997; Goldsworthy et al., 2002): over the Patagonian shelf/slope, diet consists almost entirely of fish; in contrast, toothfish on the South Georgia shelf feed more on *Euphausia* and, on the shelf slope, on other crustaceans and cephalopods as well.

Although $\delta^{13}C$ values for these prey groups are not available for the Southern Ocean, each trophic level is generally enriched by 1‰ compared to its prey (McConnaughey and McRoy, 1979; Peterson and Fry, 1987). Off Georges Bank in the North Atlantic, non-planktivore fish show $\delta^{13}C$ values of between −16.1‰ and −17.6‰, whereas euphausiids show $\delta^{13}C$ values of $-19.8 \pm 0.4‰$ (Fry, 1988). If differences are similar in the Southern Ocean, toothfish that feed on fish exclusively, like those off the Patagonian Sheif would be expected to have enriched otolith $\delta^{13}C$ compared to fish like those at South Georgia, that include a large proportion of krill in their diet. Researchers have found depletion in otolith $\delta^{13}C$ is also related to elevated metabolism (Høie et al., 2003), but this does not appear to explain the differential for toothfish: although generally characterized by low rates of metabolism (Eastman, 1993), toothfish off South Georgia grow significantly more slowly (Ashford, 2001) yet are depleted in otolith $\delta^{13}C$ relative to the Patagonian Shelf. As a result, differences in metabolically derived carbon and values of $\delta^{13}C$ in seawater DIC are the most likely explanation of the observed differential in otolith $\delta^{13}C$.

In other sampling areas in the Antarctic, toothfish show evidence of accessing a range of different sources of metabolic carbon. Unlike on the South Georgia shelf, toothfish at Macquarie Island have not been caught with euphausiids in their stomachs; fish, though present, were of different species. Moreover, diet proportions by mass were reasonably consistent between years at the two fishing grounds examined (Goldsworthy et al., 2002); and cephalopods and crustaceans were more prevalent than at Kerguelen Island where diet consisted mostly of fish. Similarly, $\delta^{13}C$ in seawater DIC varies around the Southern Ocean: at 1000 m, values were enriched in the southern Indian Ocean compared to South Georgia, and more so in the south-western part of the Pacific Ocean (Kroopnick, 1985). Given the evidence presented here, these spatial differences argue for the potential of otolith $\delta^{13}C$ to link fish to environments around the Southern Ocean.

Values of ambient $\delta^{18}O$ also vary. Although a conservative tracer in oceanic waters, it is modified by mixing processes. Away from the source region in the western South Atlantic, values of $\delta^{18}O$ increase in AAIW eastward, consistent with core erosion (Meredith et al., 1999a). Around South Africa, Red Sea Water (RSW) enters the South Atlantic via eddy formation associated with the Agulhas Retroflection, leading to substantial changes in intermediate waters. As a result, Indian Ocean AAIW is more saline and isotopically heavier than in the South Atlantic, increasing up to −0.07‰ at the depths sampled in this study off the Patagonian Shelf. As δ_w is a constant, Eq. (2) indicates that mean $\delta^{18}O$ deposited in toothfish otoliths will change by the corresponding amount of 0.13‰, or nearly a full standard deviation. Even further from its source, the AAIW core minimum continues to erode into the southern Pacific Ocean (Meredith et al., 1999b), and mixing processes modify AAIW off South America as it moves north (Pierre et al., 1991). Similarly, transfer of NADW in the western Georgia Basin in the South Atlantic modifies CDW in the ACC (Arhan et al., 2002).

Such changes in metabolic carbon sources and ambient values of stable isotopes, recorded in the otolith chemistry, may improve the detection of provenance from Antarctic sampling areas. Trace and minor elements from otolith edges distinguished South American-caught toothfish from those caught in the Antarctic with 95% success, and classified 79–84% of South American fish correctly to their capture area. But toothfish caught in the Antarctic classified to sampling area at lower rates, between 50% and 67% (Ashford et al., 2005). Mostly, this was due to misclassification to a single other sampling area: for example, half of the misclassified fish sampled at Macquarie Island were allocated to Kerguelen, whereas half of the fish from the western slope of South Georgia classified to Macquarie. Oceanographic structure and toothfish diet vary sharply between Macquarie and both Kerguelen and South Georgia: as a result, otolith δ^{18}O and δ^{13}C may resolve these sampling areas more powerfully than was possible with elemental markers alone.

Use of δ^{18}O and δ^{13}C substantially increased success of classifying estuarine-dependent weakfish to their natal estuaries in North America, improving error rates from 37% to 10% (Thorrold et al., 1998). Similarly, the two markers substantially improved the success of classifying juvenile spotted sea trout to seagrass habitats within the Chesapeake Bay (Dorval et al., 2005). Our results show that the two markers by themselves achieved complete separation between toothfish taken from the Patagonian Shelf and South Georgia. By using the contrasting spatial properties of δ^{18}O and δ^{13}C, researchers can target water masses containing different mixing proportions of the water types AAIW and NADW within the Antarctic, and systems with different sources of metabolic carbon, in effect using the markers to triangulate on a given geographic area. Lasers already allow researchers to sample otolith minor and trace elements at scales corresponding to a year in adult toothfish; sampling stable isotopes at similar scales would allow researchers to use the chronology laid down in otoliths. By doing so, they can fully exploit the variation in environmental biogeochemistry recorded by otoliths to answer ecological questions involving the population structure and provenance of oceanic fish.

Acknowledgments

We thank Dr. Alexander Arkhipkin of the Falkland Island Fisheries Department, and Dr. Mark Belchier of the British Antarctic Survey, for providing otoliths. Our thanks also go to Dr. Arkhipkin for his comments on the draft manuscript; Karen Underkoffler of the Center for Quantitative Fisheries Ecology at Old Dominion University, who prepared the samples; Dr. A.J. Kaufman of the Department of Geology for running the sample assays at the University of Maryland and arranging the sample assays at Rutgers University; and three anonymous reviewers. We also thank Dr. John Klinck of the Center for Coastal Physical Oceanography at Old Dominion University for providing the map with fronts in the ACC, used in Fig. 1. Funding was provided by the National Science Foundation (Project No. NSF OPP-0338294).

Associate editor: Kelly Falkner

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.gca.2006.08.030.

References

Agnew, D.J., Heaps, L., Jones, C., Watson, A., Berkieta, K., Pearce, J., 1999. Depth distribution and spawning pattern of *Dissostichus eleginoides* at South Georgia. *CCAMLR Sci.* **6**, 19–36.

Arhan, M., Naveira Garabato, A.C., Heywood, K.J., Stevens, D.P., 2002. The Antarctic Circumpolar Current between the Falkland Islands and South Georgia. *J. Physical Oceanography* **32**, 1914–1931.

Ashford, J.R., 2001. In support of a rationally managed fishery: age and growth in Patagonian toothfish (*Dissostichus eleginoides*). Ph.D. Dissertation, Old Dominion University, Virginia, United States.

Ashford, J.R., Jones, C.M., Hofmann, E.E., Everson, I., Duhamel, G., Moreno, C., Williams, R., 2005. Can otolith elemental signatures record the capture site of a fully marine fish, *Dissostichus eleginoides*, in the Southern Ocean?. *Can. J. Fish Aquat. Sci.* **62** (12) 2832–2840.

Ashford, J.R., Arkhipkin, A.I., Jones, C.M., 2006. Can the chemistry of otolith nuclei determine population structure of Patagonian toothfish (*Dissostichus eleginoides*)? *J. Fish Biol.* **69**, 708–721.

Bath, G.E., Thorrold, S.R., Jones, C.M., Campana, S.E., McLaren, J.W., Lam, J.W.H., 2000. Sr and Ba uptake in otoliths of marine fish. *Geochim. Cosmochim. Acta* **64**, 1705–1714.

Brandon, M.A., Murphy, E.J., Whitehouse, M.J., Trathan, P.N., Murray, A.W., Bone, D.G., Priddle, J., 1999. The shelf break front to the east of the sub-Antarctic island of South Georgia. *Cont. Shelf Res.* **19**, 799–819.

Campana, S.E., Fowler, A.J., Jones, C.M., 1994. Otolith elemental fingerprinting for stock identification of Atlantic cod (*Gadus morhua*) using laser ablation ICPMS. *Can. J. Fish Aquat. Sci.* **51** (9), 1942–1950.

Dorval, E., Jones, C.M., Hannigan, R., van Montfrans, J., 2005. Can otolith chemistry be used for identifying essential seagrass habitats for juvenile spotted seatrout, *Cynoscion nebulosus*, in Chesapeake Bay? *Mar. Freshwat. Res.* **56**, 645–653.

Duhamel, G., 1981. Caracteristiques biologiques des principales espèces de poissons du plateau continental des Iles Kerguelen. *Cybium 3e Sér.* **5**, 19–32.

Duhamel, G., Pletikosic, M., 1983. Donnés biologiques sur les Nototheniidae des Iles Crozet. *Cybium 3e Sér.* **7**, 43–57.

Eastman, J.T., 1993. *Antarctic Fish Biology: Evolution in a Unique Environment*. Academic Press, San Diego.

Edmonds, J.S., Moran, M.J., Caputi, N., 1989. Trace element analysis of fish sagittae as an aid to stock identification: pink snapper (*Chrysophrys auratus*) in Western Australian waters. *Can. J. Fish Aquat. Sci.* **46**, 50–54.

Edmonds, J.S., Caputi, N., Morita, M., 1991. Stock discrimination by trace-element analysis of otoliths of Orange Roughy (*Hoplostethus atlanticus*), a deep-water marine teleost. *Aust. J. Mar. Freshwat. Res.* **42**, 383–389.

Friedman, I., O'Neil, J.R., 1977. Compilation of stable isotope fractionation factors of geochemical interest. In: Fleisher, M. (Ed.), *Data of Geochemistry, 6th ed.* US Geol. Survey Prof. Pap., vol 440. pp. 1–12.

Fry, B., 1988. Food web structure on Georges Bank from stable C, N and S isotopic composition. *Limnol. Oceanogr.* **33**, 1182–1189.

Garcia de la Rosa, S.B., Sanchez, F., Figueroa, D., 1997. Comparative feeding ecology of Patagonian toothfish (*Dissostichus eleginoides*) in the southwestern Atlantic. *CCAMLR Sci.* **4**, 105–124.

Goldsworthy, S.D., Lewis, M., Williams, R., He, X., Young, J.W., van den Hoff, J., 2002. Diet of Patagonian toothfish (*Dissostichus *eleginoides*) around Macquarie Island, South Pacific Ocean. *Ocean Mar. Freshwater Res.* **53**, 49–57.

Hofmann, E.E., 1985. The large-scale structure of the Antarctic Circumpolar Current from FGGE drifters. *J. Geophys. Res.* **90** (C4), 7087–7097.

Høie, H., Folkvord, A., Otterlei, E., 2003. Effect of somatic and otolith growth rate on stable isotopic composition of early juvenile cod (*Gadus morhua L*) otoliths. *J. Exp. Mar. Bio. Ecol.* **289**, 41–58.

Horn, P.L., 2002. Age and growth of the Patagonian toothfish (*Dissostichus eleginoides*) and Antarctic toothfish (*D. mawsoni*) in waters from the New Zealand subantarctic to the Ross Sea, Antarctica. *Fish Res.* **56** (3), 275–287.

Kalish, J.M., 1990. Use of otolith microchemistry to distinguish the progeny of sympatric anadromous and non-anadromous salmonids. *Fish Bull.* **88**, 657–666.

Kalish, J.M., 1991. ^{13}C and ^{18}O disequilibria in fish otoliths : metabolic and kinetic effects. *Mar. Ecol. Prog. Ser.* **75**, 191–203.

Khattree, R., Naik, D.N., 1999. *Applied multivariate statistics with SAS® software*, second ed. SAS Institute, Cary, North Carolina.

Khattree, R., Naik, D.N., 2000. *Multivariate data reduction and discrimination with SAS® software*. SAS Institute, Cary, North Carolina.

Kroopnick, P.M., 1980. The distribution of ^{13}C in the Atlantic Ocean. *Earth Planet. Sci. Lett.* **49**, 469–484.

Kroopnick, P.M., 1985. The distribution of ^{13}C of CO_2 in the world oceans. *Deep-Sea Res.* **32** (1A), 57–84.

Limburg, K.E., 1995. Otolith strontium traces environmental history of subyearling American shad *Alosa sapidissima. Mar. Ecol. Prog. Ser.* **119**, 25–35.

McConnaughey, T., McRoy, C.P., 1979. Food-web structure and fractionation of carbon isotopes in the Bering Sea. *Mar. Biol.* **53**, 257–262.

Meredith, M.P., Grose, K.E., McDonagh, E.L., Heywood, K.J., Frew, R.D., Dennis, P.F., 1999a. Distribution of oxygen isotopes in the water masses of Drake Passage and the South Atlantic. *J. Geophys. Res.* **104** (C9), 20949–20962.

Meredith, M.P., Heywood, K.J., Frew, R.D., Dennis, P.F., 1999b. Formation and circulation of the water masses between the southern Indian Ocean and Antarctica: results from δ^{18}O. *J. Mar. Res.* **57**, 449–470.

Meredith, M.P., Brandon, M.A., Murphy, E.J., Trathan, P.N., Thorpe, S.E., Bone, D.G., Chernyshkov, P.P., Sushin, V.A., 2005. Variability in hydrographic conditions to the east and northwest of South Georgia, 1996–2001. *J. Mar. Systems* **53** (1–4), 143–167.

Nowlin Jr., W.D., Clifford, M., 1982. The kinematic and thermohaline zonation of the Antarctic Circumpolar Current at Drake Passage. *J. Mar. Res.* **40**, 481–507.

Orsi, A.H., Whitworth, T., Nowlin, W.D., 1995. On the meridional extent and fronts of the Antarctic Circumpolar Current. *Deep-Sea Res. Part I Oceanogr. Res. Pap.* **42** (5), 641–673.

Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. *Ann. Rev. Ecol. Syst.* **18**, 293–320.

Pierre, C., Vergnaud-Grazzini, C., Faugeres, J.C., 1991. Oxygen and carbon stable isotope tracers of the water masses in the Central Brazil Basin. *Deep-Sea Res.* **38** (5), 597–606.

Schwarcz, H.P., Gao, Y., Campana, S., Browne, D., Knyf, M., Brand, U., 1998. Stable carbon isotope variations in otoliths of Atlantic cod (*Gadus morhua*). *Can. J. Fish. Aquat. Sci.* **55**, 1798–1806.

Shaw, P.W., Arkhipkin, A.I., Al-Khairulla, H., 2004. Genetic structuring of Patagonian toothfish populations in the Southwest Atlantic Ocean: the effect of the Antarctic Polar Front and deep water troughs as barriers to genetic exchange. *Mol. Ecol.* **13** (11), 3293–3303.

Smith, P., McVeagh, M., 2000. Allozyme and microsatellite DNA markers of toothfish population structure in the Southern Ocean. *J. Fish Biol.* **57** (Suppl. A), 72–83.

Thresher, R.E., 1999. Elemental composition of otoliths as a stock delineator in fishes. *Fish Res.* **43**, 165–204.

Thorrold, S.R., Campana, S.E., Jones, C.M., Swart, P.E., 1997. Factors determining δ^{13}C and δ^{18}O fractionation in aragonite otoliths of marine fish. *Geochim. Cosmochim. Acta* **61** (14), 2909–2919.

Thorrold, S.R., Jones, C.M., Swart, P.K., Targett, T.E., 1998. Accurate classification of juvenile weakfish (*Cynoscion regalis*) to estuarine nursery areas based on chemical signatures in otoliths. *Mar. Ecol. Prog. Ser.* **173**, 253.

Weidman, C.R., Millner, R., 2000. High-resolution isotope records from North Atlantic cod. *Fish Res.* **46**, 327–342.

Zhivov, V.V., Krivoruchko, V.M., 1990. On the biology of the Patagonian Toothfish, *Dissostichus eleginoides*, of the Antarctic part of the Atlantic. *Vopr. Ikhtiol.* **30** (5), 861–864.