Otolith elemental evidence for spatial structuring in a temperate reef fish population

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ABSTRACT: Elemental fingerprints of calcified structures can provide useful insight into dispersal, structure and connectivity of fish and invertebrate populations, but an assumption must be made that fingerprints reflect local physico-chemical conditions at the time of accretion. Physiological control of elemental uptake and temporal variability in environmental conditions can confound interpretation of spatial patterns. Multiple lines of evidence suggest that a commercially important temperate reef fish *Parapercis colias* (Forster 1801) (Pinguipedidae) in the New Zealand fjords forms discrete subpopulations at the mesoscale (10 to 20 km). We investigate otolith microchemistry as a tool to elucidate population structure and connectivity in the southern fjords. Concentrations of 12 elements (Li, B, Mg, P, S, Ca, Mn, Cu, Zn, Sr, Ba and Pb) were measured at the otolith edge (the most recently accreted region) using laser ablation inductively coupled plasma mass spectrometry (ICP-MS). Demographic and temporal variability were accounted for before spatial patterns were investigated. P:Ca, Sr:Ca and Ba:Ca ratios were positively correlated while Li:Ca was negatively correlated with age. Inter-annual variability in signatures within a single site was widespread. Independent of demography, fingerprints differed between inner and outer fjord subpopulations in Bradshaw–Thompson, Breaksea and Dusky Sounds but not in Doubtful Sound or Long Sound–Preservation Inlet. Solution and laser ablation ICP-MS were similarly able to discriminate between subpopulations of fish in a subset for which one sagitta was analysed by each technique. The assumption that fingerprints represent environmental conditions at the time of accretion is supported by a relocation study between habitats in Doubtful Sound. These results have important implications for temperate reef fish management and support the adoption of spatially explicit measures such as those in place in the New Zealand fjords.

KEY WORDS: Population connectivity · Otolith microchemistry · ICP-MS · Trace element analysis · Fiordland · Blue cod · *Parapercis colias*

INTRODUCTION

The microchemical composition of calcified structures in marine vertebrates and invertebrates can provide useful insights into stock structure (Thresher 1999), larval dispersal (Thorrold et al. 2002) and connectivity between habitats associated with ontogenetic (Secor 1992, Gillanders et al. 2003) and adult movements (Campana et al. 2007). Trace, minor and major elemental concentrations have been recorded in teleost otoliths, scales, fin spines and eye lenses

(Gillanders 2001), but due to their biochronological properties, otoliths have proved the most useful structures for detecting geographic origin or change (Campana 1999). Determining population structure from otolith elemental fingerprints assumes that material is metabolically inert (Campana & Neilson 1985) and that elements are incorporated in such a way as to reflect the physico-chemical environment at the time of accretion (Fowler et al. 1995, Gallahar & Kingsford 1996), albeit with a degree of physiological regulation (Kalish 1989, Campana & Thorrold

2001). Ions are incorporated into the aragonitic oto-lith matrix or bound to the protein phase from the surrounding endolymph, and must pass across a branchial or intestinal cellular boundary (Campana 1999). In continuously drinking marine fishes, ions are sourced primarily from the water, with smaller branchial and dietary inputs (Campana 1999, Milton & Chenery 2001). Whilst it is not always necessary to understand the physiological mechanisms underly-ing variability in otolith elemental fingerprints, con-sideration should be given to ionic sources and uptake pathways to prevent the age and size struc-ture of the sampled population from confounding spatial patterns (Campana 1999). Physiologically regulated elements (e.g. Mg, P, S, Cu, Zn and Pb) may be maintained at constant levels in otoliths despite considerable ambient variability, but can still act as useful markers provided concentrations vary among populations (Campana et al. 2000). Other ele-ments such as Li, Sr and Ba are likely to reflect physico-chemical conditions (Elsdon & Gillanders 2003, Hicks et al. 2010), although the Sr:Ca ratio may also be related to growth rate (Sadovy & Severin 1992, 1994). Mn is consistently elevated in otolith cores (Brophy et al. 2004, Ruttenberg et al. 2005), suggesting that in the natal and larval life history stages the Mn:Ca ratio is not related to environmen-tal conditions or ambient concentrations (Bath et al. 2000, Elsdon & Gillanders 2003, Miller 2009) but may be influenced by diet (Pentreath 1976, Mugiya et al. 1991, Sanchez-Jerez et al. 2002). However, in juve-nile otoliths, Mn:Ca ratios may be affected by sedi-ment type (Fodrie & Herzka 2008) and by tempera-ture in adults (S. E. Swearer unpubl. data).

Inter-annual differences in temperature, salinity and water chemistry mean that a population's ele-mental fingerprint may be temporally variable (Gillanders & Kingsford 2000, Elsdon et al. 2008). This can complicate investigations of spatial structure or movement patterns. Otolith elemental concentrations are commonly measured using solution-based or laser ablation (LA) inductively coupled plasma mass spectrometry (ICP-MS). Solution-based ICP-MS mea-sures bulk element concentrations in the whole otolith, integrated over the entire life history of an or-ganism, and thereby avoids concerns over within-otolith heterogeneity (Sanborn & Telmer 2003, Ludsin et al. 2006). LA ICP-MS on the other hand can target discrete portions of otolith growth; combined with the optical characteristics of the otolith, this ability to record chronologically explicit microchemical infor-mation allows a detailed record of a fish's environ-mental and physiological history to be reconstructed.

Temperate reef fish typically form spatially struc-tured metapopulations at the mesoscale (10s to 100s m), with limited larval dispersal and adult mix-ing among subpopulations (Gunderson & Vetter 2006). In the New Zealand fjords, the commercially and recreationally important blue cod *Parapercis col-ias* (Forster 1801) (Pinguipedidae) forms discrete sub-populations at the scale of 10 to 20 km, with a subsidy of outer fjord and open coast adults into the highly resident inner fjord populations evident from mark–recapture (Carbines & McKenzie 2004) and stable isotopic studies (Rodgers & Wing 2008). Decoupling of vital rates between inner and outer fjord popula-tions is linked to habitat quality and may render pop-ulations vulnerable to localized depletion, particu-larly in the less productive inner fjords (N. Beer & S. Wing unpubl.). The spatially explicit management measures introduced under the Fiordland Marine Management Act (2005) exclude commercial fishing from inner fjord waters and limit recreational catches, in addition to providing a network of refugia in the form of 10 no-take marine reserves. To ade-quately protect Fiordland blue cod populations, how-ever, these management measures must act at an ecologically meaningful spatial scale.

Here, we employ an orthogonal sampling design to investigate spatial structuring between blue cod pop-ulations at paired inner and outer fjord sites in the 5 southern fjords. Size, age and sex effects on elemen-tal uptake and the inter-annual stability of finger-prints are quantified before spatial structuring among sampled subpopulations is examined on the basis of otolith microchemical differences. The rela-tive discriminatory power of solution and LA ICP-MS is tested using a subset of individuals for which one otolith of the sagittal pair was analyzed using each technique. In addition, we use otoliths from blue cod relocated from the outer fjord to the inner fjord (Rodgers & Wing 2008) to test the assumption that otolith elemental fingerprints of a wild population reflect local physico-chemical conditions.

MATERIALS AND METHODS

Sample collection

Blue cod were sampled using artificial lures at paired inner and outer fjord sites throughout the southern fjords in November 2004 (n = 95) and April 2009 (n = 95) (Fig. 1). For the relocation study, 46 adult blue cod were caught in outer Doubtful Sound from May to June 2004, tagged with individually
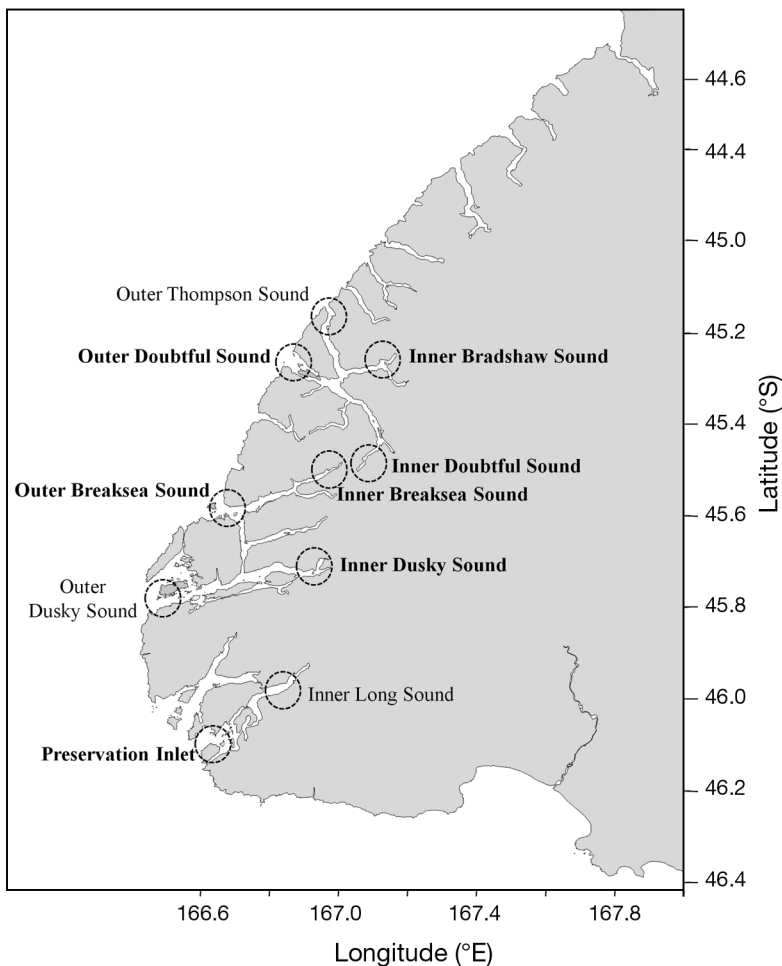
Fig. 1. Study sites in southern Fiordland, New Zealand. Not all sites were sampled in 2004; sites sampled in both 2004 and 2009 are **in bold**

otolith pair was embedded in K142 epoxy resin (NUPLEX Construction Products). It is not expected that left and right otoliths of a pair would differ in their elemental composition (Campana et al. 1994), but for consistency and ease of sectioning, the right otolith was selected whenever possible. Transverse sections (~0.7 mm thick) were cut through the primordium using a Buehler Isomet low-speed diamond-tipped saw and the sections mounted on glass slides using a small amount of resin. Age was estimated from sectioned otoliths prior to ICP-MS analysis.

LA ICP-MS

LA ICP-MS was carried out at the University of Melbourne's School of Earth Sciences using a Varian high sensitivity quadrupole ICP-MS fitted with a HelEx laser ablation system (Laurin Technic and Australian National University) constructed around a Compex 110 (Lambda Physik) Excimer laser. Preliminary analyses were conducted to determine the suite of elements that were consistently above detection limits and were not unduly subject to interference by polyatomic species (Table 2). The laser was operated at 60 mJ with a firing frequency of 5 Hz. A 206 μm diameter spot in the most recently deposited otolith material was preablated to remove surface contamination. Triplicate 55 μm diameter spots were then positioned within this preablated zone and, after a 40 s blank acquisition, were ablated for 30 s each to record a 'capture site signature'. This spot size was a compro-

numbered dart tags and released at Deep Cove in inner Doubtful Sound (Rodgers & Wing 2008). Between June and November 2004, 6 tagged fish were recaptured in the vicinity of the release site after 15 to 184 d at liberty (Table 1). All fish were measured (±1 mm total length [TL]) and sexed by macroscopic examination of the gonads. Although blue cod are protogynous hermaphrodites, sex inversion is a rapid process and terminal sex can be accurately determined macroscopically (Carbines 2004a,b). Sagittal otoliths were removed by cranial dissection, cleaned of adhering cerebral tissue in deionized water, transferred to sterile centrifuge tubes and left to air dry for several days. Otoliths were handled with Teflon-coated forceps only. Once fully dry, one of each

Table 1. Release and recapture date (dd.mm.yyyy), days at liberty, total length (TL), sex and age of tagged fish relocated from outer to inner Doubtful Sound

Fish	Released	Recaptured	Days at liberty	TL (mm)	Sex	Age (yr)
1	25.05.2004	09.06.2004	15	339	M	7
2	26.05.2004	23.08.2004	89	439	F	8
3	22.06.2004	10.11.2004	170	395	M	8
4	22.06.2004	11.11.2004	171	386	M	6
5	25.05.2004	24.11.2004	184	354	M	8
6	25.05.2004	24.11.2004	184	375	M	8

Table 2. Detection limits (DL) and instrument precision estimates (RSD) for suite of elements quantified using laser ablation (LA) inductively coupled plasma mass spectrometry (ICP-MS) in 2008 and 2009

Element	Isotope	Year	DL (µmol mol^{-1})	RSD (%)
Li	^{7}Li	2008	0.879	4
		2009	4.614	4
B	^{11}B	2008	2.200	6
		2009	2.623	5
Mg	^{24}Mg	2008	2.442	5
		2009	1.316	3
P	^{31}P	2008	16.201	5
		2009	19.470	4
S	^{34}S	2008	43.859	6
		2009	54.272	7
Mn	^{55}Mn	2008	0.473	3
		2009	0.809	3
Cu	^{63}Cu	2008	0.139	4
		2009	0.436	4
Zn	^{66}Zn	2008	0.204	5
		2009	0.400	5
Sr	^{88}Sr	2008	0.068	5
		2009	0.302	4
Ba	^{138}Ba	2008	0.002	4
		2009	0.007	4
Pb	^{208}Pb	2008	0.005	5
		2009	0.008	4

mise between temporal sensitivity and analytical detection power and represents ~6 to 12 mo of otolith growth. As elemental incorporation may be heterogeneous within an otolith (Hamer & Jenkins 2007), all spots were positioned at the dorsal tip of the transverse section. Standards (NIST 610, NIST 612 and NIST 614) were analysed before and after blocks of 10 samples. Samples were analysed in a random order to avoid any potential instrument drift confounding examination of spatial variability in otolith elemental concentrations. Data were low-pass filtered off-line to remove transient spikes before being smoothed using a moving average of 3 cells, blank-subtracted and drift-corrected. The low-pass filter compared the cell value to the median of the target and 2 bracketing cells. For high background elements (all but Ba and Pb), if the value was greater than twice the median, the cell value was replaced with the median value; for Ba, the cell value was replaced if greater than $100 + 2 \times$ Median, and for Pb, if greater than $150 + 2 \times$ Median. These threshold values were necessary to prevent filtering out high variation at low count rates during blank acquisition for low background elements. Elemental concentrations were then calculated from single-point calibrations using the NIST standard that most closely

matched the concentration in the sample, and expressed as ratios relative to ^{43}Ca to correct for variable ablation yield. Detection limits were calculated for each element (3 SD above the mean blank for all sample and standard acquisitions) and expressed in molar ratios using the average ^{43}Ca count of the samples. External precision (relative standard deviation [RSD]) was based on replicate analyses (1 per block of 10 samples) of either NIST 612 (for elements calibrated using NIST 610) or NIST 614 (for elements calibrated using NIST 612) treated as an unknown.

Although individual scans sometimes fell below the selected instrumental detection limits, we chose to include these data as they can be useful in detecting spatial separation between sampled groups (Hicks et al. 2010, Fairclough et al. 2011), and we agree with Ben-Tzvi et al. (2007) that potentially interesting data may be unnecessarily discarded when they fall below arbitrarily determined thresholds. An average capture site signature was calculated from up to triplicate spots per sample following close examination of the raw data for each element; only 3 of these averaged replicates fell below the instrumental detection limits for the element (0.14% of elemental averages). As the concentrations of certain metals (Cu, Zn and Pb) are particularly prone to surface or edge contamination, replicates (individual spots) were excluded from the individual average calculation if the concentration was >3 orders of magnitude greater than the mean. Photomicrographs of the ablated otolith sections were also examined to assess the quality of these sections; anomalously high concentrations were predominantly recorded in spots that were positioned too close to the edge of the otolith (i.e. touching the resin) or straddled a visible flaw in the section.

Solution ICP-MS

The remaining otolith from a subset of fish sampled in 2004 (n = 55) was prepared for solution ICP-MS. Otoliths were washed for 8 to 10 s in 1% HNO$_3$ (65%, Merck Suprapure®), then rinsed 3 times in Milli-Q water, placed in labelled, acid-washed 10 ml polypropylene centrifuge tubes and dried in a Class-100 laminar flow cabinet. Once dry, otoliths were weighed to the nearest 0.01 mg, dissolved in 1.5 ml of 20% HNO$_3$ and made up to a final volume of 8 ml with Milli-Q water. A subsample of this solution (0.5 or 1 ml, depending on otolith weight) was made up to 10 ml with 1% HNO$_3$. A further 100× dilution was required to measure Sr. Procedural blanks were pre-

pared in the same manner, but contained no otoliths. Solution ICP-MS was conducted at James Cook University's Advanced Analytical Centre using a Varian UltraMass 700 ICP-MS, calibrated using certified reference material NIES 22 (Fish Otolith Certified Reference Materials, Japan National Institute for Environmental Studies) and matrix-matched calibration solutions made of matrix otolith and a multi-element standard solution supplied by Choice Analytical. Concentrations were blank-subtracted and converted into ratios to Ca, based on otolith dry wt and dilution factor. Detection limits (3 SD of the blanks; Gillanders & Kingsford 2000) and instrument precision estimates (RSD, based on replicate measurements of a spiked otolith sample) are given in Table 3.

Statistical analysis

Unless otherwise stated, statistical analyses were performed using PERMANOVA+ for PRIMER v6 (PRIMER-E), which calculates p-values under permutation and therefore avoids the assumptions of normality of distribution and homogeneity of variance inherent in traditional ANOVA tests. The effect of terminal sex (fixed effect), age and size (random effects) on 2009 capture site signatures was tested using permutational multivariate analysis of variance (MANOVA); where significant effects were detected, a permutation-based least squares linear model framework (DistLM) was used to correlate demographic factors with individual elemental concentrations. For those elements that were significantly correlated with demographic factors (p < 0.05), subsequent spatio-temporal comparisons used analysis of covariance (ANCOVA) with interactions included in the model to allow for heterogeneity of slopes (Quinn & Keough 2002); non-significant interactions

were pooled with residuals to increase model power. Capture site elemental signatures of blue cod sampled in 2009 were compared between fjords (fixed effect, 5 levels), habitats (fixed effect, 2 levels) and sites (fixed effect, 10 levels) using permutational MANOVA based on Euclidean distance between samples. Data were first normalized (mean = 0, SD = 1) to account for inter-elemental differences in concentration ranges. Canonical analysis of principal coordinates (CAP) was used to measure reclassification-to-site success rates. Spatial trends in individual elements were examined using permutational ANOVA.

Capture site elemental signatures were compared between sampling years (random effect, 2 levels) using permutational MANOVA. As samples were analysed on 2 separate occasions (November 2004 samples were analysed in September 2008; April 2009 samples were analysed in October 2009), detection limits and machine precision estimates were compared between sampling events to ensure that analytical artefacts did not confound identification of temporal trends. Boron was excluded due to instrument calibration errors in 2008. CAP was used to compare reclassification success rates to year (pooled across sites) and to site (pooled across years). A separate CAP analysis used 2004 samples as a training dataset with which to assign 2009 samples to site.

Data from LA and solution ICP-MS analyses were compared using the subset of 2004 samples for which one otolith of the sagittal pair was analysed using each technique. The suite of elements was restricted to those measured by both techniques (Li, Mg, Mn, Cu, Sr, Ba). Euclidean distance matrices of normalized data were compared using a Mantel test with 9999 permutations of the data in R v2.11.1 (The R Foundation for Statistical Computing), after Gillanders (2001).

Capture site signatures of relocated individuals were compared to signatures of fish of a similar age (6 to 8 yr) sampled from inner (n = 15) and outer Doubtful Sound (n = 9) during November 2004. Group was treated as a fixed effect (3 levels). Distances of relocated signatures to multivariate inner and outer fjord group centroids were used to test the hypothesis that newly deposited otolith material will incorporate elements in concentrations reflecting local physico-chemical conditions and that consequently, over time, a relocated individual's otolith elemental signature will become progressively more similar to that of the local population and less similar to that of the population in its former habitat.

Table 3. Detection limits (DL) and instrument precision estimates (RSD) for suite of elements quantified using solution inductively coupled plasma mass spectrometry (ICP-MS)

Element	Isotope	DL (μmol mol^{-1})	RSD (%)
Li	^{7}Li	<0.001	18
Mg	^{24}Mg	0.018	7
Mn	^{55}Mn	0.005	–
Cu	^{63}Cu	0.002	10
Sr	^{88}Sr	0.011	3
Ba	^{138}Ba	<0.001	5

RESULTS

Demographic variability in otolith elemental fingerprints

Blue cod sampled in 2009 ranged in age from 4 to 18 yr and in size from 215 to 555 mm. Elemental fingerprints did not vary with fish size or sex (nested within site, p > 0.05) but did vary with age at sampling (nested within site, pseudo-$F_{47,94}$ = 1.2985, p = 0.0399). Age distributions differed between sites (pseudo-$F_{9,94}$ = 9.4251, p = 0.0001) and between all paired inner and outer fjord sites. Age was positively correlated with P:Ca, Sr:Ca and Ba:Ca ratios and negatively correlated with Li:Ca (Table 4).

Spatial variability in otolith elemental fingerprints

In 2009, spatial variability in multivariate fingerprints was evident at the fjord (pseudo-$F_{4,94}$ = 2.4564, p = 0.0001), habitat (pseudo-$F_{1,94}$ = 2.1148, p = 0.0449) and site level (pseudo-$F_{9,94}$ = 2.7412, p = 0.0001). Fingerprints differed among blue cod sampled in the 5 inner fjord basins (pseudo-$F_{4,45}$ = 3.4632, p = 0.0001) and among samples from the 5 outer fjord sites (pseudo-$F_{4,48}$ = 2.1589, p = 0.0005). Blue cod from inner and outer fjord habitats differed in their elemental fingerprints in Bradshaw–Thompson Sound (pseudo-$F_{1,19}$ = 2.2672, p = 0.0234), Breaksea Sound (pseudo-$F_{1,18}$ = 3.7258, p = 0.0014) and Dusky Sound (pseudo-$F_{1,20}$ = 4.9650, p = 0.0001) but not in Doubtful Sound (pseudo-$F_{1,14}$ = 1.8621, p = 0.0866) or Long Sound–Preservation Inlet (pseudo-$F_{1,19}$ = 0.7480, p = 0.5894). Reclassification-to-site success was 41% overall; 5.3 to 11.6% would be expected by

chance according to sample size at each site. Within each fjord, reclassification to habitat was more successful with >80% of samples correctly reclassified (chance: 33.3 to 66.7%). Reclassification to fjord was more successful among inner (63%) than outer (49%) fjord samples. Spatial variability in individual elements was most evident at the site level (Table 5, Figs. 2 & 3). B, Mg and Mn did not vary at any of the spatial scales modelled. Sr and Ba showed spatial variability independent of age distributions but Li and P did not.

Table 4. Correlations between elemental concentrations in recently deposited otolith material and age-at-sampling. Significant correlations (p < 0.05) are **in bold**

Element	Equation	R^2	p
Li	Li = 9.331 − 0.323(Age)	0.06	**0.0192**
B	B = 0.155 − 0.007(Age)	<0.01	0.7801
Mg	Mg = 0.116 − 0.002(Age)	0.01	0.3194
P	P = 0.241 + 0.008(Age)	0.07	**0.0108**
S	S = 0.823 − 0.0004(Age)	<0.01	0.8890
Mn	Mn = 1.242 − 0.047(Age)	0.04	0.0567
Cu	Cu = 0.662 + 0.035(Age)	<0.01	0.5894
Zn	Zn = 1.806 − 0.004(Age)	0.02	0.1299
Sr	Sr = 2.590 + 0.082(Age)	0.19	**0.0001**
Ba	Ba = 1.491 + 0.146(Age)	0.08	**0.0062**
Pb	Pb = 0.037 − 0.0002(Age)	0.02	0.1963

Table 5. Permutational multivariate analysis of variance (PERMANOVA) tests comparing elemental concentrations between fjords, habitats and sites including test statistic pseudo-F, significance level p calculated under permutation, number of unique values obtained from 9999 permutations and df (factor, error). Age was included as a co-variable in Li, P, Sr and Ba models. Significant results (p < 0.05) are **in bold**

| Element	Test	Pseudo-F	p	Unique perms.	df
Li	Fjord	2.0081	0.0867	9944	4,94
	Habitat	2.4581	0.1198	9847	1,94
	Site	1.8479	0.0624	9954	9,94
B	Fjord	0.6211	0.6520	9956	4,94
	Habitat	0.2036	0.6478	9928	1,94
	Site	1.5014	0.1505	9949	9,94
Mg	Fjord	0.7792	0.5428	9944	4,94
	Habitat	0.0802	0.7690	9859	1,94
	Site	0.8957	0.5326	9957	9,94
P	Fjord	1.2623	0.1627	9961	4,94
	Habitat	1.5002	0.7672	9862	1,94
	Site	1.2484	0.3823	9948	9,94
S	Fjord	9.9721	**0.0001**	9948	4,94
	Habitat	0.7178	0.4027	9835	1,94
	Site	5.2984	**0.0002**	9962	9,94
Mn	Fjord	1.1897	0.3303	9947	4,94
	Habitat	0.4930	0.4900	9822	1,94
	Site	1.4707	0.1695	9939	9,94
Cu	Fjord	2.2916	0.0639	9950	4,94
	Habitat	0.5496	0.4694	9849	1,94
	Site	2.6401	**0.0092**	9950	9,94
Zn	Fjord	1.0354	0.2717	9942	4,94
	Habitat	2.0598	**0.0466**	9818	1,94
	Site	4.1547	**0.0003**	9948	9,94
Sr	Fjord	1.5967	0.1767	9946	4,94
	Habitat	18.397	**0.0003**	9842	1,94
	Site	4.1306	**0.0002**	9950	9,94
Ba	Fjord	8.6474	**0.0001**	9949	4,94
	Habitat	0.0580	0.0857	9836	1,94
	Site	5.1582	**0.0001**	9945	9,94
Pb	Fjord	2.9963	**0.0176**	9956	4,94
	Habitat	3.8983	**0.0446**	9856	1,94
	Site	5.3122	**0.0001**	9948	9,94
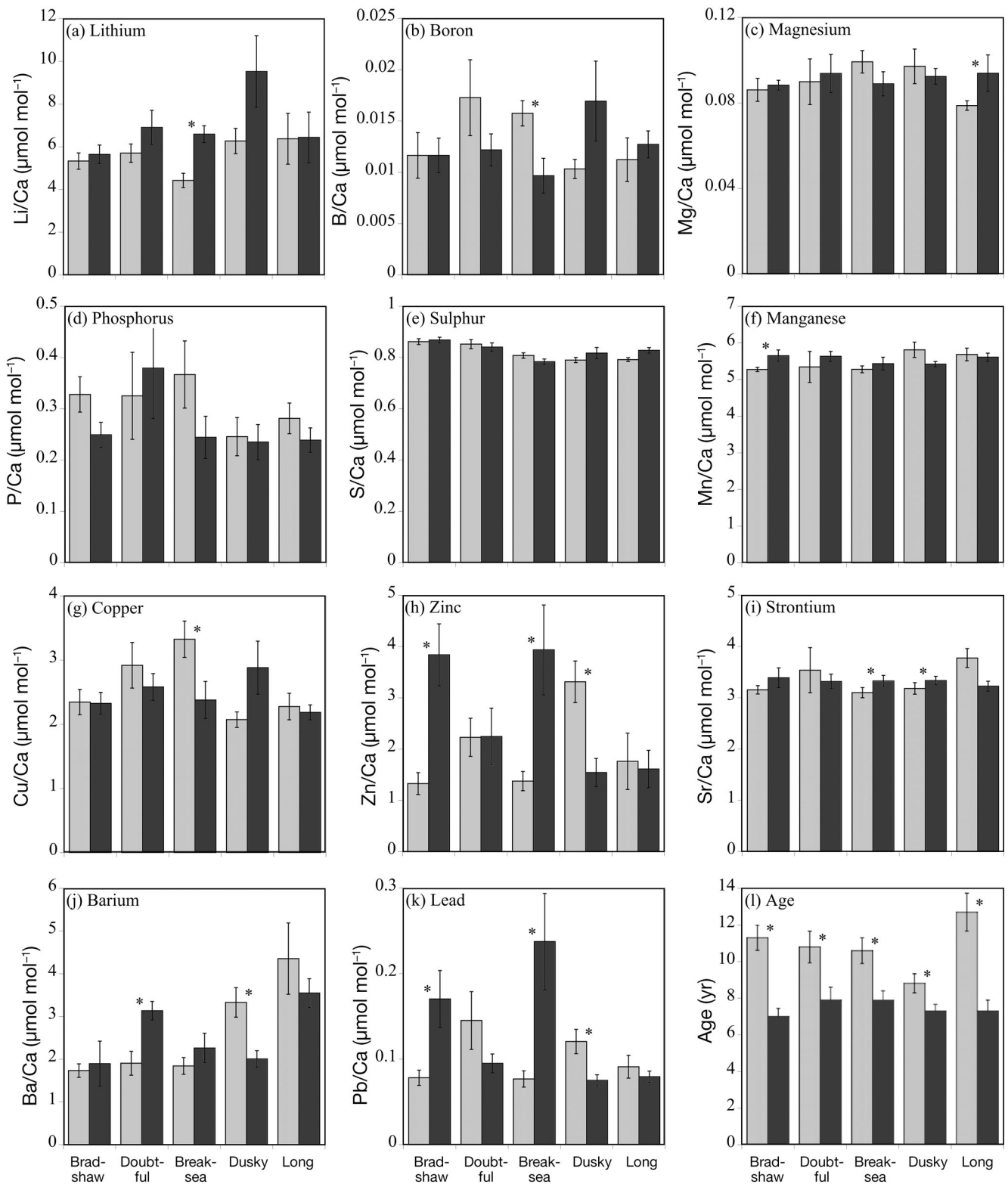
Fig. 2. Mean (±SE) concentrations of (a) Li (b) B (c) Mg (d) P (e) S (f) Mn (g) Cu (h) Zn (i) Sr (j) Ba (k) Pb and (l) mean age at inner (grey bars) and outer (dark grey bars) fjord sites in each fjord in 2009. *Significant difference ($p < 0.05$) between paired inner and outer fjord sites
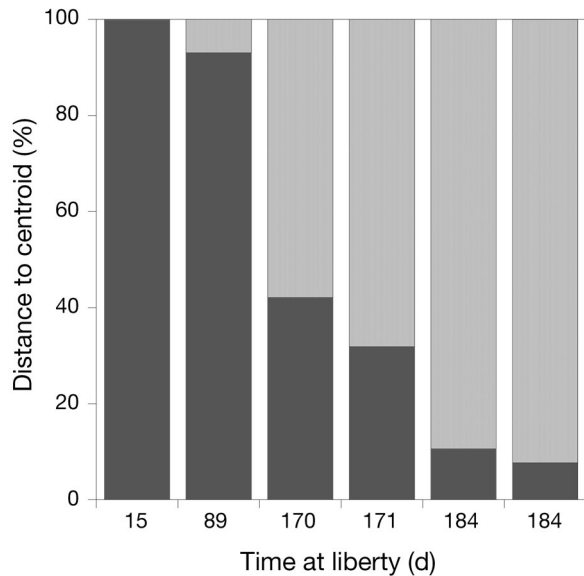
Fig. 3. Relative similarity of multivariate otolith elemental signatures of relocated fish to inner (light grey) and outer (dark grey) Doubtful Sound signatures after 15 to 184 d at liberty in the inner fjord

Temporal variability in otolith elemental fingerprints

Multivariate elemental fingerprints differed between blue cod sampled in 2004 and 2009 (nested within site: pseudo-$F_{6,139}$ = 4.5142, p = 0.0001), and at each site >76% of samples could be correctly reclassified to sampling year. Temporal effects were detected in inner Bradshaw Sound (pseudo-$F_{1,19}$ = 2.0454, p = 0.0464), inner Doubtful Sound (pseudo-$F_{1,25}$ = 2.2670, p = 0.0401), outer Breaksea Sound (pseudo-$F_{1,19}$ = 5.1595, p = 0.0001), inner Dusky Sound (pseudo-$F_{1,15}$ = 5.5212, p = 0.0003) and Preservation Inlet (pseudo-$F_{1,27}$ = 6.2896, p = 0.0001) but not outer Doubtful Sound (pseudo-$F_{1,29}$ = 1.7796, p = 0.0824). Of samples, 44% could be successfully reclassified to site and year overall (3.6 to 15.0% would be expected by chance alone). Reclassification to sampling year (pooled across sites: 79%) was more successful than reclassification to sampling site (pooled across years: 48%); however, 39.2 to 60.7% of samples could have been correctly reclassified to year by chance alone while just 11.4 to 21.4% could have been reclassified to site by chance. Using 2004 multivariate data as a training dataset by which to classify 2009 samples to site resulted in low success rates (16.4% overall). Concentrations of Mg, S, Cu, Zn, Sr, Ba and Pb differed between sampling years, nested within site (p < 0.05), but Li, P and Mn did not (p > 0.05).

Comparison of LA and solution ICP-MS

Successful reclassification to capture site included 47% of LA samples and 44% of solution samples (chance: 10.9 to 29.1%). Under both techniques, 19 individuals (34.5%) were misclassified, and 8 (14.5%) of these were wrongly assigned to the same site. Sampling did not include all possible groups in the region so the direction of misclassifications is not discussed. Normalized Euclidean distance matrices for the 2 techniques were poorly correlated (r = −0.0048. p = 0.4912). Examination of the raw data revealed considerable variability in the relative concentrations of Mg, Mn and Cu recorded at each site by both ICP-MS techniques. Age distributions were similar between sites and multivariate signatures did not vary with age for either technique (p > 0.05), so it is unlikely that the difference in spatial patterns is related to the temporal sensitivity of each technique in terms of ontogeny. Relative concentrations of Li, Sr and Ba at each site were broadly similar between techniques; including just these 3 elements, normalized Euclidean distance matrices for the 2 techniques were significantly correlated (r = 0.1932, p = 0.0292).

Acclimatization to local physico-chemical conditions

Otolith elemental fingerprints of blue cod relocated from outer to inner Doubtful Sound differed from those of the local inner fjord population (pseudo-t = 1.6752, p = 0.0271, df = 18) but not from those of their native outer fjord population (pseudo-t = 0.9486, p = 0.4635, df = 12). However, fingerprints of relocated fish after 170 to 184 d at liberty in the inner fjord could be distinguished from those of both inner fjord (pseudo-t = 2.4297, p = 0.0002, df = 16) and outer fjord fish (pseudo-t = 1.6225, p = 0.0258, df = 10). Over time, the elemental fingerprint of relocated fish became progressively more similar to an inner fjord signature and less similar to an outer fjord signature (Fig. 3). After just 15 d at liberty in the inner fjord, otolith microchemistry was typical of the fish's native outer fjord subpopulation; after 89 d, an influence of the new inner fjord habitat was visible, and between 89 and 170 d this influence increased appreciably, but complete naturalization had not yet occurred after 184 d.

DISCUSSION

Management of spatially structured populations must operate at an ecologically appropriate spatial

scale in order to be effective. Consistent differences in otolith elemental fingerprints of Fiordland blue cod imply limited mixing between inner and outer fjord subpopulations over the scale of weeks to months, consistent with the results of mark–recapture and stable isotopic studies (Carbines & McKenzie 2004, Rodgers & Wing 2008). As reported for sea perch *Helicolenus percoides* in the southern fjords (Lawton et al. 2010), blue cod elemental fingerprints were more discrete among inner fjord subpopulations, either as a result of isolation or distinct physico-chemical conditions in each fjord. Lower reclassification success rates among outer fjord subpopulations could reflect population mixing or the more dynamic environment in this exposed coastal habitat. The homogeneous fingerprints of blue cod sampled from inner and outer fjord habitats in Long Sound–Preservation Inlet is likely to reflect physico-chemical conditions in the relatively sheltered outer fjord environment, with its atypically estuarine ecosystem (Wing et al. 2003, Wing & Jack 2010, N. Beer & S. Wing unpubl.). Extensive mixing between inner and outer fjord subpopulations in this fjord is unlikely due to the strong spatial separation in population structure and growth rates (N. Beer & S. Wing unpubl.).

An ontogenetic decrease in otolith Li:Ca ratio and an increase in P:Ca ratio has been observed in other species (Kalish et al. 1996, Begg et al. 1998, Stransky et al. 2005) but no mechanism has been suggested for either phenomenon. A positive correlation between otolith Sr:Ca ratio and age and has been attributed to changes in relative protein growth following maturation (Kalish 1989, Fuiman & Hoff 1995, Friedland et al. 1998). We are unaware of any previous reports of a positive correlation between otolith Ba:Ca ratio and age for adult marine teleosts. The increase in blue cod Sr:Ca and Ba:Ca ratios with age may reflect an ontogenetic shift in habitat use (i.e. an environmental effect) or may be a physiological effect related to the kinetics of Sr^{2+} and Ba^{2+} incorporation into otolith material.

Interannual variability in blue cod otolith elemental fingerprints was considerable within a sampling location. As trophic ecology of subpopulations is consistent over a period of several years (N. Beer & S. Wing unpubl.), the temporal variability in otolith microchemistry is unlikely to be driven by diet. Rather, it is likely to be due to complex variability in water chemistry at a range of temporal scales (Elsdon & Gillanders 2006).

Spatial trends in Li:Ca, Sr:Ca and Ba:Ca ratios were well-correlated between solution and LA ICP-MS analyses; previous comparative studies have reported similar results for Sr:Ca and Ba:Ca ratios (Thorrold et al. 1997, Ludsin et al. 2006), but no study to date has compared results for Li:Ca ratios between ICP-MS techniques. The similar discriminatory power of time-averaged and time-resolved techniques, despite the observed inter-annual variability in elemental signatures, likely reflects the highly resident nature of blue cod subpopulations in enclosed waterways (Cole et al. 2000, Carbines & McKenzie 2004).

The results of our relocation study support the assumption that spatial variability in otolith microchemistry reflects the physico-chemical environment at the time of accretion. While we cannot determine the mechanism underlying the shift in elemental fingerprints following relocation, there is evidence to support both a dietary and a physico-chemical source. The same experimental fish displayed a transition in stable isotopic signatures after relocation, reflecting differences in the basal food web between habitats (Rodgers & Wing 2008). Physico-chemical conditions in inner and outer Doubtful Sound are markedly different (Gibbs et al. 2000, Gibbs 2001) and mussels transplanted to cages in the inner, mid and outer fjord acquired discrete elemental fingerprints (Beer et al. 2011).

These results have important implications for the management of blue cod and other temperate reef fish populations that are spatially structured at the mesoscale. The tendency to form discrete subpopulations necessitates the adoption of management measures that act at a smaller, more ecologically meaningful spatial scale than is traditionally the case in fisheries management. Whilst it has long been recognised that invertebrate populations can be highly variable in their vital rates and productivity (Orensanz & Jamieson 1998), there is evidence for a similar phenomenon among Fiordland blue cod subpopulations that is apparently related to differences in the food web between inner and outer fjord habitats (N. Beer & S. Wing unpubl.). The persistence of discrete otolith elemental fingerprints over a period of several months and the spatial asynchrony in vital rates renders subpopulations vulnerable to localized depletion and necessitates spatially explicit management measures that are precautionary enough to allow for differential yields among adjacent populations.

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