Ministry for Primary Industries
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Exploratory microchemical analysis of blue cod otoliths from potting surveys in Dusky Sound, Fiordland 2002 and 2008

New Zealand Fisheries Assessment Report 2012/21

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ISSN 1175-1584 (print)
ISSN 1179-5352 (online)
ISBN 978-0-478-38851-0 (online)

May 2012

New Zealand Government

Growing and Protecting New Zealand

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Beer, N.A.; Carbines, G.D. (2012). Exploratory microchemical analysis of blue cod otoliths from potting surveys in Dusky Sound, Fiordland 2002 and 2008.

***New Zealand Fisheries Assessment Report 2012/21.* 32 p.**

This report describes the results of microchemical analysis of blue cod (*Parapercis colias*) otoliths collected during two potting surveys of Dusky Sound in Fiordland in 2002 and 2008. We also combine otolith microchemical data with tagging data (from fish released in 2001) to assess the ability of elemental composition to record known movements of fish. A larger scale comparison with additional blue cod otoliths taken from a 2008 potting survey of Banks Peninsula is also included.

Laser ablation inductively coupled plasma mass spectrometry (LA ICP-MS) was used to record concentrations of a suite of elements, which were converted to molar ratios relative to ^{43}Ca. Data were normalised to account for inter-elemental differences in concentration ranges and multivariate data analysis was used to explore spatial and temporal variability. In addition to standard hypothesis testing, an information-theoretic approach was used to rank multivariate models in terms of their statistical power to describe variability in trace element signatures. Some evidence was found to support the use of otolith trace element composition as a natural tag to detect blue cod movement. However, capture site signatures varied between sampling years highlighting the need to control for temporal effects when investigating spatial variability. Within both sampling years, trace element signatures revealed three regions along the fiord axis that were distinct in their environmental signals.

Capture site signatures
Multivariate trace element signatures of the most recently deposited otolith material did not vary with individual age, sex or total length, but did differ between sampling years. In 2002 capture site signatures varied between the five Dusky Sound strata overall with significant differences between some individual strata. Reclassification to stratum was more successful (44% of individuals) than expected by chance alone. In 2008 capture site signatures were less distinct, but there remained significant differences between some individual strata, and 68% of individuals were successfully reclassified to capture stratum. When strata were re-grouped into three larger regions, 54% of 2002 samples and 73% of 2008 samples could be correctly reassigned to their capture region.

Temporal variability in multivariate signatures was driven by Li, B, S and Ba while spatial differences between strata were driven by B, P and Mn. As P and S also varied with age, age was included as a covariable in these models. Model selection revealed that stratum, sampling year, age, total length and sex individually explained small but significant portions of the variability in multivariate capture site signatures. However, overall only 23% of the total variability in trace element signatures was explained by the factors included in the models. The variance explained by spatial factors was greatly increased by including region rather than stratum in the model.

A supplementary microchemical analysis of blue cod otoliths from a 2008 Banks Peninsula potting survey showed that populations from the two regions (inshore/offshore) differed in their multivariate trace element signatures. Comparing between 2008 survey areas, trace element signatures of blue cod sampled off Banks Peninsula differed from Dusky Sound blue cod. However, the inshore Banks Peninsula region did not differ significantly from any strata in Dusky Sound, whereas the offshore Banks region differed significantly from all Dusky Sound strata except the open coast.

0+ signatures
Similar spatial patterns were observed in individuals from the same cohort sampled at different times (2002 and 2008) and multivariate signatures did not differ between strata overall or in either year. Adjacent strata were generally indistinguishable but more distant strata could sometimes be differentiated, implying a gradient in trace element incorporation along the fiord axis. No evidence

was found of discrete larval source pools and the gradient in post-recruitment signatures along the fiord axis suggests that larvae from one pool disperse along the fiord and acquire a trace element fingerprint that reflects physico-chemical conditions in the environment in which they settle.

Tagged individuals
The two individuals that had moved between strata during their year at liberty were reclassified to their release stratum rather than their recapture stratum. It was possible to differentiate between recapture strata more powerfully than release strata and 45% of fish were successfully reclassified to their recapture stratum. The individual that moved the greatest distance was misclassified to an intermediate stratum through which it had traversed.

In 2002, results reflect spatial variability observed in capture site signatures and strata were consequently pooled up to a regional scale as before. At this broader spatial scale environmental histories could be more successfully differentiated.

Management
The results of this study support the use of otolith microchemical analysis to identify discrete subpopulations of blue cod in Fiordland and to determine stock movements between regions. The results provide no evidence to reject the findings of previous studies that suggest a highly resident inner fiord blue cod population supplemented by inward migration from the outer fiord and open coast. As the current management regime excludes fishing from the inner waters of the fiords but not the outer fiords and open coast, it is possible that the source population remains unprotected.

1. INTRODUCTION

Blue cod (*Parapercis colias*) is heavily targeted and the species most frequently landed by recreational fishers in the South Island (Ministry of Fisheries 2008). Conventional external tagging studies reveal that most blue cod have a restricted home range (Carbines 2004a; Carbines & McKenzie 2004; Mace & Johnston 1983; Mutch 1983; Rapson 1956) and stocks of this species are likely to consist of many largely independent subpopulations within current Fisheries Management Areas (FMAs). Blue cod are therefore susceptible to localised depletion within an FMA, and in response to local changes in fishing pressure, bag limits vary among and within South Island FMAs (BCO 3, BCO 5 and BCO 7).

In Fiordland, fishing is one of the most popular recreational activities and is most intensively focused on Dusky and Doubtful Sounds, and off Preservation Inlet (Davey & Hartill 2008). The Fiordland Marine Area was developed by the Guardians of Fiordland (Guardians of Fiordland 2003) and established by the Fiordland Marine Management Act in 2005 as an integrated management strategy for Fiordland. The Fiordland Marine Area includes several marine reserves, and its establishment brought a halt to daily bag limit accumulation and a reduced daily bag limit of 20 blue cod for recreational fishers in the outer fiords. Commercial fishing was removed from the inner half of most fiords and the daily bag limit for recreational fishers was further reduced to only three blue cod in these areas. Milford and Doubtful Sounds have also been closed to all blue cod fishing since 2005. Estimates of dispersal distance and the number of individuals that disperse over short or long distances are needed to determine the most appropriate scale for localised management areas.

Movement studies

Over four thousand blue cod were tagged with T-bar tags throughout Dusky Sound in 2001 and 6.8% were recovered after 17 months (Carbines & McKenzie 2004). The largest distance moved was 30 km, but the median was only 570 m, with 65% of fish moving less than 1 km. Using standardised catch data, a variation of the Peterson mark–recapture model was used to calculate proportional population mixing rates. The two outermost strata of Dusky Sound drained an annual point estimate of 7.4% and 9.2% of their respective populations into the inner half of the fiord, which acted as a collecting sink with 100% residency (Carbines & McKenzie 2004).

Stable isotope analysis has also been used to investigate likely migration patterns among subpopulations of blue cod in Fiordland; in Doubtful Sound consistent differences in $\delta^{13}C$ and $\delta^{15}N$ of blood and muscle collected from open coast (n=34) and inner sound (n=35) blue cod reflected long-term (more than one year) residency, due to an apparent difference in the available diet at these locations (Rodgers & Wing 2008). This study concluded that populations of blue cod within Doubtful Sound are made up of long-term residents with some subsidy from the open coast and corroborates the conclusions of Carbines and McKenzie (2004). It is not known however whether inner fiord populations are capable of self-recruitment or are reliant on immigration from the outer fiords and open coast.

Potting surveys of blue cod

Recreational blue cod stocks are monitored by the Ministry of Fisheries (now the Ministry for Primary Industries) using standardised potting surveys. There are currently nine time series of relative abundance indices located in areas with important recreational fisheries throughout the South Island, repeated approximately every three to four years. The aim of these surveys is to provide localised abundance indices, and to monitor the size and population age structure of geographically separate blue cod populations. During these surveys a sample of otoliths are removed and used to develop survey specific age-length keys to describe the age frequency distribution of the catch and hence to estimate total mortality rates (e.g. Carbines & Beentjes 2011). Two potting surveys have been done in Dusky Sound, an initial survey in 2002 (Carbines & Beentjes 2003) and a repeat survey in 2008 (Carbines & Beentjes 2011).

Otolith microchemistry

Currently blue cod otoliths collected from potting surveys are used only for estimating age (See Carbines 2004b). However, the microchemical composition of otoliths is increasingly being used overseas to identify natal origin, larval dispersal and migratory patterns and to aid in stock identification (Campana et al. 2000; Thorrold et al. 2007; Thresher 1999). During deposition, certain trace elements accumulate within otoliths in proportion to the concentrations in the seawater, although proportions can also vary with temperature and salinity (Campana 1999). Otolith elemental composition can therefore reflect ambient water conditions at the time of deposition and studies on otolith microchemistry have been able to document levels of isolation among populations that were not evident in genetic studies (Thorrold et al. 2001). The presence of a microchemical difference does not however imply a genetic difference and provides no information regarding population identity (Campana et al. 1995). By corollary, the absence of a chemical difference does not necessarily imply a common origin.

Because otoliths are metabolically inert, concentrations can be retrospectively measured for previous life-history stages. This technique is of particular interest in the study of blue cod, which are extremely difficult to sample as juveniles. High spatial resolution sampling techniques such as laser ablation inductively coupled plasma-mass spectrometry (LA ICP-MS) also allow continuous recording of elemental profiles along a growth axis; in conjunction with the optical properties of the otolith this facilitates reconstruction of an environmental history referenced to ontogenetic development (Elsdon et al. 2008). One limitation in the use of otolith microchemical composition as a natural tag is temporal variation in water chemistry (Dorval et al. 2005; Elsdon & Gillanders 2006). While inter-annual variability has been documented in blue cod otolith elemental signals in Fiordland, considerable spatial variability also exists, including between inner and outer Dusky Sound (Beer 2011). This study makes use of the fine sampling scale of the standard potting surveys and combines otolith microchemical data with tagging data to assess the ability of elemental composition to record known movements of adults. In addition, retrospective sampling of post-settlement signatures from a single cohort is used to test the hypothesis that the inner fiord population is sustained by export from the outer fiord and open coast.

Overall objective

1. To investigate otolith trace element signatures in blue cod (*Parapercis colias*) otoliths collected in Dusky Sound during potting surveys in 2002 and 2008.

Specific objectives

1. To undertake a selection and preparation of otoliths available from the two Dusky Sound potting surveys.

2. To analyse samples.

3. To report on the feasibility of using otolith microchemistry to determine stock movements of blue cod populations in Dusky Sound and potentially other areas of Fiordland.

2. METHODS

2.1 Timing

Surveys were conducted from the 15th to 26th October 2002 (Carbines & Beentjes 2003) and the 16th to 29th October 2008 (Carbines & Beentjes 2011). Otolith analysis was undertaken at the University of Melbourne's School of Earth Sciences from 30th September to 2nd October 2009.

2.2 Survey areas

The area for the 2002 Dusky Sound potting survey was divided arbitrarily into five strata traversing the length of Dusky Sound from the open coast stratum at the mouth to the inner stratum at the head of the fiord (Figure 1). To allow comparisons with the previous survey the same five strata were used in 2008, but no biological samples were taken from the new Taumoana (Five Fingers Peninsula) Marine Reserve (Figure 2).

2.3 Survey design

The sampling methods used in the 2002 and 2008 potting surveys remained consistent and standardised with all surveys in the Ministry of Fisheries blue cod potting survey time series (Carbines & Beentjes 2003, 2011). For all blue cod the total length (rounded down to the nearest centimetre), sex and gonad maturity were recorded, and the sagittal otoliths removed from a representative size range.

2.4 Sample selection

To investigate whether trace element signatures can be used to identify place of origin, a sample was taken of the youngest blue cod available from the otoliths collected throughout Dusky Sound in 2002 (BCO2002/01). Only 35 otoliths of individuals less than four years old (five 2-year-olds and thirty-one 3-year-olds) were available throughout all five strata in the 2002 potting survey (Table 1). These individuals represent the 1999 (n=5) and 2000 (n=31) year classes available from the 2002 otolith collection. To examine potential patterns of residency in trace element signatures, otoliths from the 1999 and 2000 year classes were selected from the available otoliths taken throughout Dusky Sound six years later (as 8- and 9-year-olds) in the 2008 potting survey (Table 2).

To investigate the use of otolith microchemistry as a tool for tracing movement of individual fish, otoliths from tagged and recaptured blue cod were analysed from both the 2002 (Table 3) and 2008 (Table 4) potting surveys. As an additional spatial comparison, 20 otoliths were selected from a Banks Peninsula blue cod potting survey done in 2008 (See Appendix A).

2.5 Otolith preparation

During each potting survey, otoliths were rinsed with water, air-dried, and stored in paper envelopes. One otolith from each fish sampled was then sectioned and aged as part of previous studies (Carbines et al. 2008; Carbines & Beentjes 2011). A sub-sample of remaining otoliths was selected for trace element analysis by LA ICP-MS (See Section 1.6). Only those otoliths whose core region was deemed sufficiently intact to allow a transverse section to be taken were selected. These otoliths were carefully cleaned of any adhering cerebral tissue using Teflon coated forceps before being embedded in K142 epoxy resin (NUPLEX Construction Products, Auckland). Transverse sections (approximately 0.7 mm thick) were cut through the core region using a Buehler Isomet diamond-tipped low speed saw and the

sections mounted on slides using a small amount of resin. Table 5 gives details of the final samples selected from each survey collection.

2.6 Trace element analysis

LA ICP-MS was carried out using a Varian high sensitivity quadrupole ICP-MS (Varian, Australia) fitted with a HelEx (Laurin Technic and Australian National University) laser ablation system constructed around a Compex 110 (Lambda Physik) Excimer laser. The laser was tuned at an energy level of 40 mJ and samples were ablated at 60 mJ; frequency of firing was constant at 5 Hz. Energy levels, firing frequency, spot diameters and scan speeds were designed to optimise the amount of material ablated and maintain concentrations above detection limits, and were based on extensive preliminary analyses (N.A. Beer, unpublished data). The suite of elements measured (Li, B, P, S, Mg, Mn, Cu, Zn, Sr, Ba, Pb) comprised those which were consistently above detection limits and which were not subject to interference by polyatomic species (S. Swearer, pers. comm.).

206 μm diameter zones were preablated for 40 seconds to remove surface contamination from the most recent otolith growth (the 'capture site' growth) and the 0+ newly settled recruit growth (identified as the first translucent band outside the core region, see Carbines 2004b). Within each of these regions, three 55 μm diameter spot samples were ablated for 30 s (Figure 3). Transects (93 μm diameter; 30 μm s^{-1} scan speed) were preablated from one side of the core to the tip of the opposite lobe, bisecting the annual bands along the longest growth axis (Figure 3a). The same path was ablated at a speed of 10 μm s^{-1} using a 55 μm diameter laser spot to record elemental profiles. Multiple transects sampled on the same otolith were also used to investigate heterogeneity in trace element incorporation within an individual otolith. As a basic qualitative check, a sub-set of samples (n=3) was used to compare growth axes (Figure 3a) and another sub-set (n=2) was used to compare parallel transects along the same axis (Figure 3b).

Before and after each block of about ten samples, three National Institute of Standards and Technology (NIST) standards (614, 612 and 610) were sampled to calibrate the system. Samples were analysed in a random order to avoid machine drift confounding examination of spatial variability in otolith trace element concentrations.

Successive standard runs were used to calculate machine precision estimates for each element (relative standard deviation, RSD). Detection limits were calculated for each element using standard acquisitions. Constants were added to certain elements in order to raise all concentrations above zero, but maintain the variance structure of the data. Details of the final suite of elements selected for analysis of Dusky Sound otoliths, including detection limits, machine precision estimates and constants added are given in Table 6.

2.7 Data processing

Data were low pass filtered to remove transient spikes, cut-outs were removed and data were smoothed using a moving average of three counts to reduce the influence of high frequency noise. Element counts were blank-subtracted and drift-corrected using the three NIST standards which bracketed each block of samples before being converted to molar ratios relative to ^{43}Ca.

The agreement between replicate capture site or 0+ spots was examined before an overall average for each region of growth was calculated for individual fish. If a replicate spot sample differed greatly from the others for a particular fish, or there was any concern over the accuracy of any element concentrations (particularly common contaminants such as Cu, Zn and Pb), it was excluded from the final average.

2.8 Data analysis

All analyses were carried out using PERMANOVA+ for PRIMER v6 (PRIMER-E Ltd, Plymouth, UK). Data were normalised to account for inter-elemental differences in concentration ranges. Euclidean distances were calculated between each pair of samples. Permutational MANOVA, PERMANOVA (Anderson 2001) was used to explore spatial and temporal variability. Adopting a permutational approach avoids the assumptions of normality of distribution and homogeneity of variance inherent in traditional tests. Discriminant function analysis and leave-one-out reclassification was performed using CAP (canonical analysis of principal coordinates); this maximises the differences between *a priori* groups and calculates (under permutation) the probability associated with the groups in the form of a misclassification error (Anderson & Willis 2003). Spatiotemporal variability in capture site signatures was investigated using all samples while assessment of the variability in 0+ signatures was limited to individuals of the same cohort (1999–2000).

In addition to standard hypothesis testing, an information-theoretic approach was used to rank multivariate models in terms of their statistical power to describe variability in trace element signatures. Akaike's Information Criterion, AIC (Akaike 1974) is an extension of Kullback-Liebler information and likelihood theory and enables the combination of estimation (e.g. maximum likelihood or least squares) and model selection under an optimisation theoretical framework. It is particularly applicable to observational type studies and avoids the pitfalls of data dredging, over-fitted models and subsequent problems with inference associated with traditional hypothesis-testing methods (Burnham & Anderson 1998). AIC values are calculated for each model, with lower values indicating a better approximation of the truth for a given data set:

$$AIC = -2\ln(L) + 2K$$

AIC is the index of model power to describe the "true" situation for a given data set, *L* is the value of the likelihood and *K* is the number of parameters included in a model.

For small sample sizes the equation is modified by a bias correction term. If the ratio of *n/K* is sufficiently large AIC and AIC_c will be similar and tend to select the same model.

$$AIC_c = -2\ln(L) + 2K + \frac{2K(K+1)}{n-K-1}$$

As it is the relative power of a particular model compared to all other proposed models that is of interest, results are scaled to the minimum value of AIC achieved:

$$\Delta_i = AIC_i - AIC_{\min}$$

AIC_i is the value of AIC_c for the *i*th model and AIC_{min} is the minimum value of AIC_c achieved. Akaike weights were then calculated from normalised likelihoods:

$$W_i = \frac{\exp\left(-\Delta_i/2\right)}{\sum_{r=1}^{R}\exp\left(-\Delta_i/2\right)}$$

These weights represent the weight of evidence in favour of model *i* from the set of models *R*. Models were constructed and compared using the DistLM routine. When Euclidean distance is used as the basis of the analysis, this equates to traditional multivariate multiple regression, or redundancy analysis, but *p*-values are calculated under permutation thereby avoiding the common assumptions of normality etc.

Time series (i.e. the number of point measures recorded along a transect) varied between samples depending on the size of the otolith and consisted of a large number of data points for 11 elements per sample. Each time series was therefore decomposed to a suite of 13 statistical descriptors or "extracted features" which aimed to capture patterns of variation and covariation among elemental ratio time series (Shima & Swearer 2009; Wang et al. 2006). The suite of descriptors consisted of a serial correlation function, a nonlinear function, a skewness function, a kurtosis function, a hurst function, a lyapunov function, a frequency function, a trend function, a seasonal function, a trend and seasonally adjusted (TSA) serial correlation function, a TSA non-linear function, a TSA skewness function and a TSA kurtosis function (Wang et al. 2006). Functions were estimated using R v2.11.1 (The R Foundation for Statistical Computing). Elements prone to contamination and spikes (Cu, Zn and Pb; S. Swearer, pers. comm.) were excluded from time series analyses. Descriptors were normalised before Euclidean distance was calculated between samples. PERMANOVA was used to compare multivariate environmental histories between strata and CAP was used to identify misclassified individuals. Results were interpreted in conjunction with spatial information from the tag-return data.

3. RESULTS

3.1 Capture site signatures

Multivariate trace element signatures of the most recently deposited otolith material (the 'capture site signature') did not vary with individual age (PERMANOVA, pseudo-$F_{34,98}$=1.3965; p=0.0766), sex (pseudo-$F_{5,98}$=1.1068; p=0.3072) or total length (pseudo-$F_{77,98}$=0.7774; p=0.8163) nested within stratum. However, capture site signatures did vary between sampling years nested within stratum (pseudo-$F_{5,98}$=1.6417; p=0.0341) so spatial variability was examined within 2002 and 2008 separately.

In 2002 capture site signatures varied between strata overall (pseudo-$F_{4,58}$=1.7338; p=0.0157) with post-hoc tests detecting significant differences between adjacent inner and Mid strata (pseudo-t=1.5436, p=0.0325, df=26) and Outer and EO (extreme outer) strata (pseudo-t=1.4866, p=0.0478, df=19) but not Mid and Outer (pseudo-t=1.2962, p=0.1132, df=17) or EO and OC (open coast) strata (pseudo-t=0.9181, p=0.5580, df=17). Details of all post-hoc tests comparing capture site signatures between strata in 2002 are given in Table 7.

CAP successfully reclassified 44.1% of individuals to capture stratum overall; this is considerably higher than the reclassification success rate expected by chance alone. Reclassification success rate was 52.4% in the inner stratum (chance: 35.6% based on the relative sample size in this stratum), 57.1% in the Mid stratum (chance: 11.9%), 50.0% in the Outer stratum (chance: 20.3%), 22.2% in the EO stratum (chance: 15.3%) and 30.0% on the OC stratum (chance: 16.9%).

In 2008 capture site signatures did not differ significantly between strata overall (pseudo-$F_{4,39}$=1.6444; p=0.0650) although the p-value was not much higher than the 0.05 significance level and post-hoc tests still detected significant differences between Mid and Outer strata (pseudo-t=1.7064, p=0.0109, df=16) and Outer and EO strata (pseudo-t=1.3451, p=0.0330, df=18). Details of all post-hoc tests comparing capture site signatures between strata in 2008 are given in Table 8.

CAP successfully reclassified 67.5% of individuals to capture stratum overall and was again more successful than expected by chance alone. Reclassification success rate was 72.7% in the inner stratum (chance: 27.5% based on the relative sample size in this stratum), 75.0% in the Mid stratum (chance: 20.0%), 70.0% in the Outer stratum (chance: 25.0%), 50.0% in the EO stratum (chance: 25.0%) and 100.0% on the OC stratum (chance: 2.5%).

Based on these results and understanding of predominant water chemistry and mixing patterns within the fiords (Beer 2011), strata were grouped into three regions: "Inner fiord", equivalent to the inner

stratum; "Mid fiord", encompassing Mid and Outer strata and "Outer fiord", incorporating EO and OC strata (See Figure 1). At this broader spatial scale, sample sizes were more even between groups and the power to detect spatial variability was increased (2002: pseudo-$F_{2,58}$=2.7110, p=0.0014; 2008: pseudo-$F_{2,37}$=2.0597, p=0.0094). Post-hoc tests showed that all regions could be distinguished in both 2002 and 2008 (See Table 9).

At this larger spatial scale 54.2% of 2002 samples and 72.5% of 2008 samples could be correctly reassigned to their capture region (chance: 33.3%). In 2002 reclassification success rate was 66.7% in the inner fiord region (chance: 35.6%), 57.9% in the mid fiord (chance: 32.2%) and 36.8% in the outer fiord (chance: 32.2%). In 2008 reclassification success rate was 72.7% in the inner fiord (chance: 27.5%), 77.8% in the mid fiord (chance: 45.0%) and 63.6% in the outer fiord (chance: 27.5%).

Univariate tests for individual elements revealed age effects for P (pseudo-$F_{34,98}$=2.6213; p=0.0009), S (pseudo-$F_{34,98}$=2.7022; p=0.0005) and Sr (pseudo-$F_{34,98}$=2.2381; p=0.0244) pooled across years. Linear regressions are shown in Figure 4 and Table 10. Within individual sampling years, significant effects were found in 2002 but not 2008; this is likely to be related to the larger age range sampled in 2002 (2–24 years) compared to 2008 (8–22 years, but only two samples were older than 9 years), and means that the overall age effects detected are not likely to be artefacts of temporal variability between sampling years. Similar effects of age on otolith Sr concentrations have been reported in other marine fish species (Fowler et al. 2004; Kalish 1989; Proctor et al. 1995) and for blue cod throughout Fiordland (Beer 2011). Age-related increases in P and S have previously been reported in scombrids (Begg et al. 1998; Proctor et al. 1995). Patterns are likely to be related to ontogenetic changes in growth and maturation.

Temporal variability in multivariate signatures was driven by Li (pseudo-$F_{5,98}$=3.0860; p=0.0292), B (pseudo-$F_{5,98}$=4.6657; p=0.0099), S (pseudo-$F_{5,98}$=3.7531; p=0.0040) and Ba (pseudo-$F_{5,98}$= 2.5644; p=0.0391) while spatial differences between strata were driven by B (pseudo-$F_{4,98}$=2.5587; p=0.0402), P (pseudo-$F_{4,98}$=3.2535; p=0.0145) and Mn (pseudo-$F_{4,98}$=3.5591; p=0.0058). However, as P and S also varied with age, apparent spatiotemporal differences may be an artefact of skewed age distributions between strata and years. Mean P concentration was highest in the inner stratum (Figure 5), where the mean age of fish was higher than in other strata. Analysis of covariance (ANCOVA) using age as a covariate confirmed this hypothesis (pseudo-$F_{4,98}$=1.8269; p=0.1268). Mean S concentration and mean age were both higher in 2002 than 2008, however, ANCOVA showed that temporal variability in S was independent of age distributions (pseudo-$F_{5,98}$=3.1737; p=0.0104). Concentrations of individual elements are shown by stratum in Figure 5.

Model selection revealed that stratum, sampling year, age, total length and sex individually explained small (R^2<0.1) but significant (p<0.05) portions of the variability in multivariate capture site signatures (Table 11). Spatiotemporal effects were evident on top of the variability explained by demographic factors. However, overall only 22.5% of the total variability in trace element signatures was explained by the factors included in the models (Table 12). Age was the most important factor in terms of model weight, followed by total length, sampling year and sex; stratum was the least heavily weighted factor (Table 13). Including the larger scale of region in the models rather than stratum revealed a much stronger influence of spatial variability (Table 14) with region as the most important factor in terms of model weight (Table 15).

3.2 0+ signatures

Newly settled recruit (0+) signatures did not vary with terminal sex (pseudo-$F_{4,56}$=1.2850; p=0.1930) or size (pseudo-$F_{43,56}$=1.3969; p=0.1851) nested within stratum. An effect of both individual age (pseudo-$F_{4,56}$=1.5858; p=0.0293) and sampling year (pseudo-$F_{5,56}$=2.6027; p=0.0006) nested within stratum was detected. Within each sampling year, however, no effect of age was found (2002: pseudo-$F_{2,20}$=1.6142, p=0.1144; 2008: pseudo-$F_{4,35}$=0.6317, p=0.8978).

Multivariate signatures did not differ between strata overall (pseudo-$F_{4,56}$=1.0146; p=0.4320) in 2002 (pseudo-$F_{4,20}$=1.4227; p=0.0882) or 2008 (pseudo-$F_{4,35}$=1.4367; p=0.1257). It should be noted that within an individual year, available samples were unevenly distributed between strata. Post-hoc tests showed that adjacent strata were generally indistinguishable but more distant strata could sometimes be differentiated, implying a gradient in trace element incorporation along the fiord axis. CAP successfully reclassified 47.6% of 2002 samples and 47.2% of 2008 samples to their original capture stratum.

Univariate tests revealed that the temporal variability observed in multivariate 0+ signatures was predominantly driven by Mg (pseudo-$F_{5,56}$=3.3174; p=0.0107), S (pseudo-$F_{5,56}$=11.342; p=0.0001), Sr (pseudo-$F_{5,56}$=4.1162; p=0.0025) and Pb (pseudo-$F_{5,56}$=4.2923; p=0.0184). Mean concentrations of Mg, Mn and Ba, the suite of elements commonly used to identify the core region of otoliths (Ruttenberg et al. 2005), were higher in 2002 than 2008 for all strata. Meanwhile, mean concentrations of P, S, and Sr were higher in 2008 than 2002 for all strata. An ontogenetic increase in capture site concentrations of P, S and Sr has been observed in Dusky Sound (see section 2.1). These results suggest that although the same criteria were used to identify the "0+" region of otolith material for all samples, for 2002 samples the material ablated was apparently from an earlier stage in development than 2008 samples.

In 2002 Mg concentrations varied significantly between strata (pseudo-$F_{4,20}$=3.8555; p=0.0225) while in 2008 significant spatial variability was detected for S (pseudo-$F_{4,35}$=5.6268; p=0.0052) and Mn (pseudo-$F_{4,35}$=3.1226; p=0.0290). Overall, however, no clear spatial trends are apparent on an individual element basis (Figure 5).

3.3 Within otolith heterogeneity

The profiles of individual elements along different transects within an otolith (Figure 3) were compared visually to qualitatively assess the degree of within-otolith heterogeneity in trace element incorporation. Example profiles are shown in Figures 6 and 7. Generally, while the absolute concentrations varied between growth axes, relative patterns along transects were similar although for some elements (e.g. B, P) there is some evidence for within-otolith heterogeneity. Parallel transects along the same growth axis tended to yield more similar trace element profiles than transects along different growth axes.

Transect sampling of tagged fish followed approximately the same path on every otolith (i.e. the ventral lobe of the transverse section was always sampled and transects were aligned to be as perpendicular to growth increments as possible). Consequently, within-otolith heterogeneity is not expected to be a significant issue when comparing profiles between otoliths (Hamer & Jenkins 2007). Spot samples were also positioned within the same region of each otolith to minimise potential effects of heterogeneous elemental incorporation between different lobes.

3.4 Tagged individuals

Age was included as a covariate in models to account for variability in seasonality between individuals of different ages. In 2002, multivariate environmental histories did not vary between strata overall (pseudo-$F_{4,33}$=1.2075; p=0.0612) although the result was close to the 0.05 significance level and post-hoc tests detected a significant difference between Inner and Mid strata (pseudo-t=1.2140; p=0.0282; df=12). A marginal difference was detected between Outer and EO (extreme outer) strata (pseudo-t=1.2118; p=0.0667; df=8) but no difference was found between Mid and Outer strata (pseudo-t=1.0314; p=0.3980; df=8) or EO and OC (open coast) strata (pseudo-t=1.0009; p=0.4639; df=8). These results reflect the spatial variability observed in capture site signatures and strata were

consequently pooled up to a regional scale as before (three levels: inner fiord, mid fiord and outer fiord; see Section 2.1). At this broader spatial scale, environmental histories could be more successfully differentiated (pseudo-$F_{2,33}$=1.3805; p=0.0155) and 70.6% of individuals could be correctly reassigned to their region (32.4–35.3% could be achieved by chance alone) compared to the stratum level reclassification success rate of just 50% (chance: 8.8–38.2%). The two individuals that had moved between strata during their year at liberty were reclassified to their release stratum rather than their recapture stratum (Table 16), implying that environmental histories at the time of sampling did not reflect their recent movement.

It was hypothesised that as only a small proportion of total otolith material would have been accreted between tagging and recapture, capture site signatures may be a more powerful tool to detect migrants in the current data set. PERMANOVA was able to differentiate between individuals based on their recapture stratum (pseudo-$F_{4,37}$=1.7489; p=0.0178) more powerfully than release stratum (pseudo-$F_{4,37}$=1.5736; p=0.0446). CAP analysis successfully reclassified 44.7% of fish to their recapture stratum (chance: 7.9–39.5%). Misclassified individuals included several fish that had moved hundreds to thousands of metres but also fish that had not moved appreciably in a year at liberty. The capture site signature of the individual that moved the greatest distance, which integrated over several weeks of recently accreted otolith material, was classified as typical of the Mid stratum, intermediate between the release (Outer) and recapture (Inner) strata.

The three tagged individuals recaptured in 2008 after approximately seven years at liberty had moved less than 1 km. No statistical comparison was possible due to the lack of replication.

4. DISCUSSION

4.1 Spatiotemporal variability in capture site signatures

Capture site signatures varied between sampling years, highlighting the need to control for temporal effects when investigating spatial variability. Within both sampling years, trace element signatures revealed three regions along the fiord axis that were distinct in their environmental signals. Relative to the reclassification success expected by chance alone, in both 2002 and 2008 subpopulations were most easily distinguished in the inner fiord and least distinguishable in the extreme outer fiord/open coast region. Otolith microchemical results therefore support those of a previous tagging study that found residency to be highest in the inner fiord and lowest in the extreme outer fiord and open coast (Carbines & McKenzie 2004).

In each sampling year, capture site trace element signatures differed between some but not all of the five strata. As strata were arbitrarily assigned, two pots (from different stations) set in adjacent strata could be closer together than two pots from different stations within the same stratum. It is possible that the clustering of pots near stratum boundaries may be responsible for some of the apparent homogeneity in otolith microchemistry between blue cod from adjacent strata (for example, Inner and Mid strata and EO (extreme outer) and OC (open coast)). To investigate this, pot location information was examined for the eight individuals misclassified to an adjacent stratum in 2002. Of these misclassifications, five could potentially have resulted from proximity to a stratum boundary (less than one kilometre). Finer-scale spatial variability may exist along the axis of Dusky Sound than was detected by the current sampling design; a fully nested sampling design incorporating a range of spatial scales from metres to kilometres would be needed to investigate this and was beyond the scope of the present study.

In a supplemental pilot microchemical analysis of blue cod otoliths from a 2008 Banks Peninsula potting survey, trace element signatures of inshore and offshore regions (pooled from survey strata) were also distinct in their environmental signals (See Appendix 1). As in Dusky Sound, spatial

separation between Banks Peninsula multivariate trace element signatures was driven mainly by B and Mn, both of which were higher in the inshore region of Banks Peninsula (Appendix 1, Figures B and C) and the extreme outer (EO) stratum of Dusky Sound (Figure 5).

When a comparison was made between both survey areas (2008, west versus east coast), trace element signatures of blue cod sampled off Banks Peninsula were significantly different from Dusky Sound (see Appendix 1) with the variability between the two survey areas driven by Mg, Mn, S, Cu and Sr. However, the trace element signatures of inshore Banks Peninsula were not distinguishable from any strata in Dusky Sound, while the offshore Banks region was significantly different from all Dusky Sound strata except the open coast (Appendix 1, Figure D). While this is an interesting observation, the mechanisms behind spatial patterns in otolith concentrations of individual elements cannot be ascertained in the absence of ambient water chemistry data for each site. This warrants further investigation as establishing a link between local water chemistry and blue cod otolith microchemistry would enable a library of site-specific elemental signatures to be compiled. This would facilitate stock discrimination in the absence of genetic heterogeneity.

4.2 Spatial variability in 0+ signatures

Sampling of 0+ material was apparently more accurate in otoliths sampled earlier in the lifetime of the studied cohort (i.e. those sampled in 2002 at 2–3 years of age). However, although the material sampled was deposited at a slightly later stage in development, similar spatial patterns were observed in individuals from the same cohort sampled at age 8–9. Due to the difficulty in accurately sectioning blue cod otoliths to expose the core region, and the variability observed in the structure of annual banding between individuals, it was not possible to sample a sufficient quantity of otolith material from the earliest life history stages to support an accurate analysis at such a fine temporal scale. Despite the demonstrated spatial variability in trace element incorporation into adult otolith growth, no evidence was found of discrete larval source pools within the sampled population. Based on the current data set, no conclusions can be drawn regarding the geographic origin of larvae, although the gradient in post-recruitment signatures along the fiord axis seems to suggest that larvae from one pool disperse along the fiord and acquire a trace element fingerprint that reflects physico-chemical conditions in the environment in which they settle. Although the natal origin of dispersing larvae cannot be determined based on these data, no evidence was found to contradict the current understanding of population dynamics and movement/mixing patterns of blue cod in Dusky Sound which assumes that the source population is centred in the outer fiord/open coast region (Carbines & McKenzie 2004; Rodgers & Wing 2008). However, it is important to bear in mind that the inability to detect discrete source pools does not mean that they do not exist, as environmental conditions at the time of settlement may have been unsuitable for the induction of significantly different elemental fingerprints (Thorrold et al. 1997).

4.3 Using otolith microchemistry to determine movement patterns

Some evidence was found to support the use of otolith trace element composition as a natural tag to detect movement. However, as tagged fish recaptured in 2002 had only been at liberty for one year and ranged in age from 7–24 years at the time of sampling, otolith material accreted between tagging and recapture represented only a small proportion of total transect length. Trace element signatures of fish relocated from outer to inner Doubtful Sound had not fully acclimatised to their new environment after 6 months (Beer 2011). The broad range of ages that were represented meant that transects were highly variable in length and seasonality etc; this technique has been more successfully used to compare environmental histories of juveniles of other species (e.g., Shima & Swearer 2009).

With mark–recapture data such as these, an assumption is made that between tagging and recapture, fish have remained in the immediate vicinity (in the case of individuals which were recaptured close to

their release location). For those individuals that moved a considerable distance between tagging and recapture, assumptions are made regarding the timing and number of movements during their time at liberty. When examining environmental histories it is also important to consider the possibility that apparent differences may be due to the environment altering around a stationary fish rather than reflecting a move between habitats (Elsdon et al. 2008); such inter-annual environmental variability has been observed in Fiordland (Beer 2011), making this a possibility. The effects of stress experienced during tagging on behaviour and otolith elemental composition are also unquantified. These assumptions must be considered when interpreting spatial patterns, particularly as the time at liberty was not much greater than the temporal sensitivity of the capture site sampling technique. This sensitivity could be increased through the use of a narrow slit sample rather than a spot sample of the otolith, but this would lower detection limits and preclude measurement of certain elements.

4.4 Management implications

Fiordland-wide microchemical analysis of blue cod otoliths was able to distinguish between outer fiord populations along the coast from Preservation to outer Thompson Sound and between inner fiord populations (Beer 2011, Beer et al. 2011). However, these studies only compared inner with outer fiord populations, not populations along an entire fiord axis as in the current study. Beer (2011) detected significant differences between fish in Bradshaw/Thompson, Breaksea and Dusky Sounds, but not between Long/Preservation or Doubtful Sounds.

At a finer scale, the results of the current study support the use of otolith microchemical analysis to determine stock movements of blue cod among regions within a single fiord and potentially other geographically distinct areas such as inshore and offshore Banks Peninsula. However, to be used effectively at this scale it would be necessary to establish a link between local water chemistry and blue cod otolith microchemistry (Beer 2011), and to develop a library of site-specific elemental signatures. The scale of temporal variability in otolith elemental signatures (e.g. seasonal, annual) would also need to be quantified and may necessitate cataloguing site-specific signatures at regular intervals. Unfortunately, these requirements may make the use of otolith microchemical analysis difficult for determining fine scale movement patterns of blue cod as part of routine stock assessment potting surveys.

The results of this study do not refute the findings of previous tagging and stable isotope studies that have detected a subsidy of isolated, highly resident inner fiord populations by inward migration from the outer fiord and open coast (Carbines & McKenzie 2004; Rodgers & Wing 2008). As the current management regime excludes fishing from the inner waters of the fiords but not the outer fiords and open coast, it is possible that the source population remains unprotected. Despite mounting evidence to suggest a source–sink population structure in the fiords, it remains to be tested whether the reproductive outputs of inner and outer fiord populations reflect this (i.e. whether reproductive excess in the outer fiord and open coast populations sustains population growth in the inner fiord, where reproductive output may be insufficient to maintain the local population).

5. ACKNOWLEDGMENTS

This research was carried out by Saltwater Science Ltd under contract to the Ministry of Fisheries (MFish Project SAP2009/03) and a research initiative grant from Saltwater Science Ltd (Appendix 1). We thank Stephen Swearer and the University of Melbourne's School of Earth Sciences for providing advice and laboratory facilities.

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Table 1: Numbers of blue cod 2 and 3 year-old otoliths sampled in the five strata of Dusky Sound in 2002 (BCO2000/01), strata are defined in Figure 1.

Age	Inner	Mid	Out	EO	OC	Total
2	2	-	2	-	1	5
3	6	8	4	9	4	31

Table 2: Numbers of blue cod 8 and 9 year-old otoliths sampled in the five strata of Dusky Sound in 2008 (BCO2008/02), strata are defined in Figure 1.

Age	Inner	Mid	Out	EO	OC	Total
8	4	-	4	13	-	21
9	7	7	7	24	1	46

Table 3: Numbers of otoliths sampled from tagged (2001) blue cod recaptured in Dusky Sound in 2002 (BCO2002/01), strata are defined in Figure 1.

Stratum	Inner	Mid	Out	EO	OC	Total
Total	15	3	7	5	8	38

Table 4: Numbers of otoliths sampled from tagged (2001) blue cod recaptured in Dusky Sound in 2008 (BCO2008/02), strata are defined in Figure 1.

Stratum	Inner	Mid	Out	EO	OC	Total
Total	1	2	-	-	-	3

Table 5: Numbers of otoliths selected for trace element analysis, strata are defined in Figure 1.

Group	Inner	Mid	Out	EO	OC	Total
2002 2–3 year olds	6	4	5	4	2	21
2008 8–9 year olds	10	6	10	10	1	37
2002 tag recaptures	15	3	7	5	8	38
2008 tag recaptures	1	2	-	-	-	3

Table 6: The suite of elements analysed by LA ICP-MS. Details of isotopes, detection limits (DL), machine precision (RSD) and constants added are given.

Element	Isotope	DL (μmol/mol)	RSD (%)	Constant
Lithium	^{7}Li	4.614	4	7.9495
Boron	^{11}B	2.623	6	-
Magnesium	^{24}Mg	1.316	5	-
Phosphorous	^{31}P	19.470	5	-
Sulphur	^{34}S	54.272	6	-
Manganese	^{55}Mn	0.809	3	4.6714
Copper	^{63}Cu	0.436	4	1.2860
Zinc	^{66}Zn	0.400	5	0.6447
Strontium	^{88}Sr	0.302	5	-
Barium	^{138}Ba	0.007	4	-
Lead	^{208}Pb	0.008	5	0.0539

Table 7: Details of all post-hoc tests comparing capture site signatures between strata in 2002. Significant results (p<0.05) are shown in bold, strata are defined in Figure 1.

	Inner	Mid	Out	EO
Mid	**pseudo-*t*=1.5436; *p*=0.0325; df=26**			
Out	**pseudo-*t*=1.6272; *p*=0.0163; df=31**	pseudo-*t*=1.2962; *p*=0.1132; df=17		
EO	pseudo-*t*=0.9346; *p*=0.5205; df=28	pseudo-*t*=1.2335; *p*=0.1658; df=14	**pseudo-*t*=1.4866; *p*=0.0478; df=19**	
OC	pseudo-*t*=1.2564; *p*=0.1287; df=29	**pseudo-*t*=1.4352; *p*=0.0471; df=15**	pseudo-*t*=1.1782; *p*=0.2045; df=20	pseudo-*t*=0.9181; *p*=0.5580; df=17

Table 8: Details of all post-hoc tests comparing capture site signatures between strata in 2008. Significant results (p<0.05) are shown in bold, strata are defined in Figure 1.

	Inner	Mid	Out	EO
Mid	pseudo-*t*=1.3400; *p*=0.0745; df=17			
Out	pseudo-*t*=1.3319; *p*=0.0539; df=19	**pseudo-*t*=1.7064; *p*=0.0109; df=16**		
EO	**pseudo-*t*=1.5081; *p*=0.0151; df=19**	pseudo-*t*=1.2588; *p*=0.1087; df=16	**pseudo-*t*=1.3451; *p*=0.0330; df=18**	
OC	pseudo-*t*=0.8964; *p*=0.5119; df=10	pseudo-*t*=1.2418; *p*=0.2322; df=7	pseudo-*t*=1.2360; *p*=0.2141; df=9	pseudo-*t*=0.6174; *p*=0.8004; df=9

Table 9: Details of all post-hoc tests comparing capture site signatures between regions in 2002 and 2008. Significant results (p<0.05) are shown in bold, strata are defined in Figure 1.

	2002		2008	
	Inner	Mid	Inner	Mid
Mid	**pseudo-*t*=2.0284; *p*=0.0013; df=38**		**pseudo-*t*=1.2429; *p*=0.0310; df=27**	
Out	**pseudo-*t*=1.2934; *p*=0.0321; df=38**	**pseudo-*t*=1.5733; *p*=0.0221; df=36**	**pseudo-*t*=1.4778; *p*=0.0224; df=20**	**pseudo-*t*=1.5010; *p*=0.0148; df=27**

Table 10: Linear regressions of capture site P, S and Sr concentrations against fish age.

Element	Linear regression	R^2	*P*
P	P = 0.128061 + 0.111621(Age)	0.205	0.0009
S	S = 0.7937177 + 0.0046345(Age)	0.096	0.0005
Sr	Sr = 2.438097 + 0.0953974(Age)	0.368	0.0244

Table 11: Marginal and sequential tests of the relationship between predictor factors and multivariate capture site signatures, including the test statistic pseudo-*F*, the significance level p calculated under permutation and the variance explained (R^2). Marginal tests fit each explanatory variable individually; sequential tests fit the first variable and test if the second variable explains a significant portion of the total variance given that explained by the first etc. Significant results (*p*<0.05) are shown in bold.

	Marginal			Sequential		
Factor	Pseudo-*F*	*p*	R^2	Pseudo-*F*	*p*	R^2
Age	10.600	**0.0001**	0.099	10.600	**0.0001**	0.099
Total length (mm)	2.7659	**0.0038**	0.028	2.9928	**0.0015**	0.126
Sex	9.6499	**0.0001**	0.091	1.3204	0.2279	0.138
Sampling year	2.0330	**0.0361**	0.021	1.6986	**0.0026**	0.153
Capture stratum	2.0077	**0.0014**	0.079	2.0807	**0.0015**	0.225

Table 12: Details of log-likelihood, number of parameters (K), AIC_c values, relative power (Δ_i), Akaike weights (w_i) and variance explained (R^2) for models where Δ_i<5.

Capture site signature =	Log (L)	K	AIC_c	Δ_i	w_i	R^2
Age + year	2.97	2	229.33	0.00	0.080	0.126
Age + sex + year	2.97	3	229.59	0.26	0.071	0.142
Age + TL + year	2.97	3	230.14	0.81	0.054	0.138
TL + year	2.98	2	230.22	0.89	0.052	0.118
Age	2.99	1	230.24	0.91	0.051	0.099
Age + year + stratum	2.94	3	230.28	0.95	0.050	0.194
Age + sex	2.98	2	230.34	1.01	0.049	0.117
TL + sex + year	2.97	3	230.41	1.08	0.047	0.135
Age + sex + year + stratum	2.93	4	230.46	1.13	0.046	0.212
TL + year + stratum	2.94	3	230.51	1.18	0.045	0.192
Age + TL + sex + year	2.96	4	230.59	1.26	0.043	0.153
Age + TL + year + stratum	2.93	4	230.64	1.31	0.042	0.210
Age + TL	2.98	2	230.78	1.45	0.039	0.113
Age + TL + sex	2.97	3	230.82	1.49	0.038	0.132
TL + sex + year + stratum	2.93	4	231.05	1.72	0.034	0.207
TL + sex	2.98	2	231.07	1.74	0.034	0.110
TL	2.99	1	231.12	1.79	0.033	0.090
Age + TL + sex + year + stratum	2.92	5	231.21	1.88	0.031	0.225
Age + sex + stratum	2.94	3	231.32	1.99	0.030	0.185
Age + stratum	2.95	2	231.41	2.08	0.028	0.165
Age + TL + sex + stratum	2.93	4	231.49	2.16	0.027	0.203
Age + TL + stratum	2.94	3	231.50	2.17	0.027	0.184
TL + stratum	2.96	2	231.61	2.28	0.026	0.164
TL + sex + stratum	2.95	3	231.67	2.34	0.025	0.182

Table 13: Summed weights for each factor calculated as the sum of the Akaike weights for each model in Table 12 that includes a given factor.

Factor	Summed weight
Age	0.706
TL	0.596
Year	0.593
Sex	0.474
Stratum	0.411

Table 14: Details of log-likelihood, number of parameters (K), AIC_c values, relative power (Δ_i), Akaike weights (w_i) and variance explained (R^2) for models where Δ_i<5.

Capture site signature =	Log (L)	K	AIC_c	Δ_i	w_i	R^2
Age + sex + year + region	2.94	4	228.01	0.00	0.080	0.193
Age + year + region	2.95	3	228.26	0.25	0.071	0.173
Age + TL + year + region	2.94	4	228.59	0.58	0.060	0.189
Age + sex + region	2.95	3	228.61	0.60	0.059	0.170
TL + year + region	2.95	3	228.62	0.61	0.059	0.170
TL + sex + year + region	2.94	4	228.72	0.10	0.076	0.188
Age + TL + sex + year + region	2.93	5	228.73	0.72	0.056	0.206
Age + TL + sex + region	2.94	4	228.75	0.74	0.055	0.187
TL + sex + region	2.95	3	229.04	1.03	0.048	0.166
Age + region	2.96	2	229.08	1.07	0.047	0.147
Age + TL + region	2.95	3	229.11	1.10	0.046	0.166
Age + year	2.97	2	229.33	1.32	0.041	0.126
TL + region	2.96	2	229.35	1.34	0.041	0.145
Age + sex + year	2.97	3	229.59	1.58	0.036	0.142
Age + TL + year	2.97	3	230.14	2.13	0.028	0.138
TL + year	2.98	2	230.22	2.21	0.026	0.118
Age	2.99	1	230.24	2.23	0.026	0.099
Age + sex	2.98	2	230.34	2.33	0.025	0.117
TL + sex + year	2.97	3	230.41	2.40	0.024	0.135
Age + TL + sex + year	2.96	4	230.59	2.58	0.022	0.153
Age + TL	2.98	2	230.78	2.77	0.020	0.113
Age + TL + sex	2.97	3	230.82	2.81	0.020	0.132
TL + sex	2.98	2	231.07	3.06	0.017	0.110
TL	2.99	1	231.12	3.11	0.017	0.090

Table 15: Summed weights for each factor calculated as the sum of the Akaike weights for each model in Table 14 that includes a given factor.

Factor	Summed weight
Region	0.697
Age	0.692
TL	0.615
Year	0.579
Sex	0.518

Table 16: Details of movements undertaken by individuals tagged in 2001 and recaptured in 2002 (BCO2002/01) and 2008 (BCO2008/02).

Otolith	Tag	Distance (km)	Release		Recapture		Days @ liberty	Stratum	
			Long	Lat	Long	Lat		Release	Recapture
Glen1	3374	17.59	166.57	45.78	166.78	45.73	365	Out	Inner
5d	0007	6.39	166.58	45.80	166.51	45.79	373	Out	EO
107e	2289	1.89	166.94	45.71	166.96	45.70	375	Inner	Inner
112c	0956	1.48	166.60	45.80	166.61	45.80	376	Mid	Mid
Glen3	1176	1.39	166.44	45.74	166.44	45.75	369	OC	OC
118c	0074	1.06	166.44	45.81	166.45	45.81	373	OC	OC
118e	0221	0.76	166.45	45.81	166.44	45.81	373	OC	OC
74b	1941	0.76	166.53	45.69	166.53	45.69	361	Out	Out
22a	3494	0.59	166.44	45.81	166.44	45.81	361	OC	OC
96b	2511	0.56	166.50	45.71	166.50	45.71	367	Out	Out
110e	3227	0.52	166.81	45.75	166.80	45.75	366	Inner	Inner
123e	1983	0.50	166.51	45.70	166.52	45.70	377	Out	Out
106b	2217	0.48	166.81	45.71	166.81	45.71	376	Inner	Inner
123a	1875	0.46	166.53	45.69	166.53	45.69	370	Out	Out
44c	3505	0.43	166.44	45.81	166.44	45.81	361	OC	OC
92a	3593	0.41	166.44	45.81	166.44	45.81	360	OC	OC
105d	2207	0.33	166.79	45.72	166.80	45.72	375	Inner	Inner
83b	2208	0.33	166.79	45.72	166.80	45.72	375	Inner	Inner
111d	3195	0.28	166.81	45.75	166.80	45.75	366	Inner	Inner
86e	3202	0.28	166.81	45.75	166.80	45.75	366	Inner	Inner
15c	3041	0.26	166.79	45.74	166.79	45.73	366	Inner	Inner
100c	0414	0.24	166.49	45.80	166.49	45.80	379	EO	EO
107c	2241	0.19	166.83	45.70	166.83	45.70	375	Inner	Inner
49d	1344	0.19	166.49	45.78	166.49	45.78	369	EO	EO
80b	1350	0.19	166.49	45.78	166.49	45.78	369	EO	EO
123b	1760	0.15	166.53	45.69	166.54	45.69	370	Out	Out
27d	3535	0.15	166.44	45.81	166.44	45.81	361	OC	OC
63d	1343	0.13	166.49	45.78	166.49	45.78	369	EO	EO
111b	3186	0.11	166.81	45.75	166.81	45.75	366	Inner	Inner
48a	3750	0.11	166.55	45.78	166.55	45.78	360	Out	Out
53e	3179	0.11	166.81	45.75	166.81	45.75	365	Inner	Inner
68b	3185	0.11	166.81	45.75	166.81	45.75	365	Inner	Inner
86c	3181	0.11	166.81	45.75	166.81	45.75	365	Inner	Inner
107a	2248	0.07	166.83	45.70	166.83	45.70	375	Inner	Inner
113e	2150	0.06	166.63	45.77	166.63	45.77	370	Mid	Mid
88b	2155	0.04	166.63	45.77	166.63	45.77	371	Mid	Mid
121a	3591	0.00	166.44	45.81	166.44	45.81	361	OC	OC
94b	1821	0.00	166.53	45.69	166.53	45.69	370	Out	Out
343	3213	0.78	166.81	45.75	166.82	45.75	2565	Inner	Inner
333	2148	0.19	166.63	45.77	166.63	45.77	2567	Mid	Mid
338	2664	0.13	166.68	45.75	166.68	45.76	2567	Mid	Mid

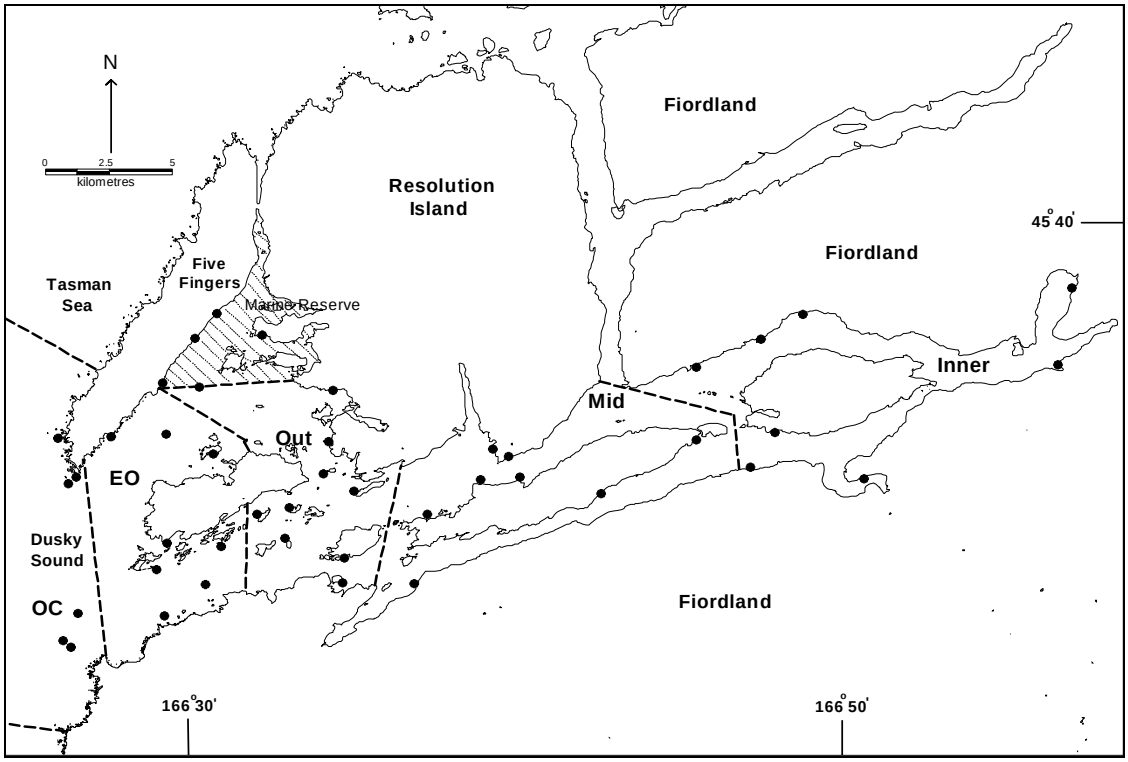

Figure 1: Map of the Dusky Sound area showing the strata and stations of the 2002 blue cod potting survey. Strata are OC (open coast), EO (extreme outer), Out (outer), Mid and Inner. Note the Marine Reserve was not established until 2005, formerly part of stratum Out. Detailed results of this survey were given by Carbines & Beentjes (2003). Figure from Carbines & Beentjes (2011).

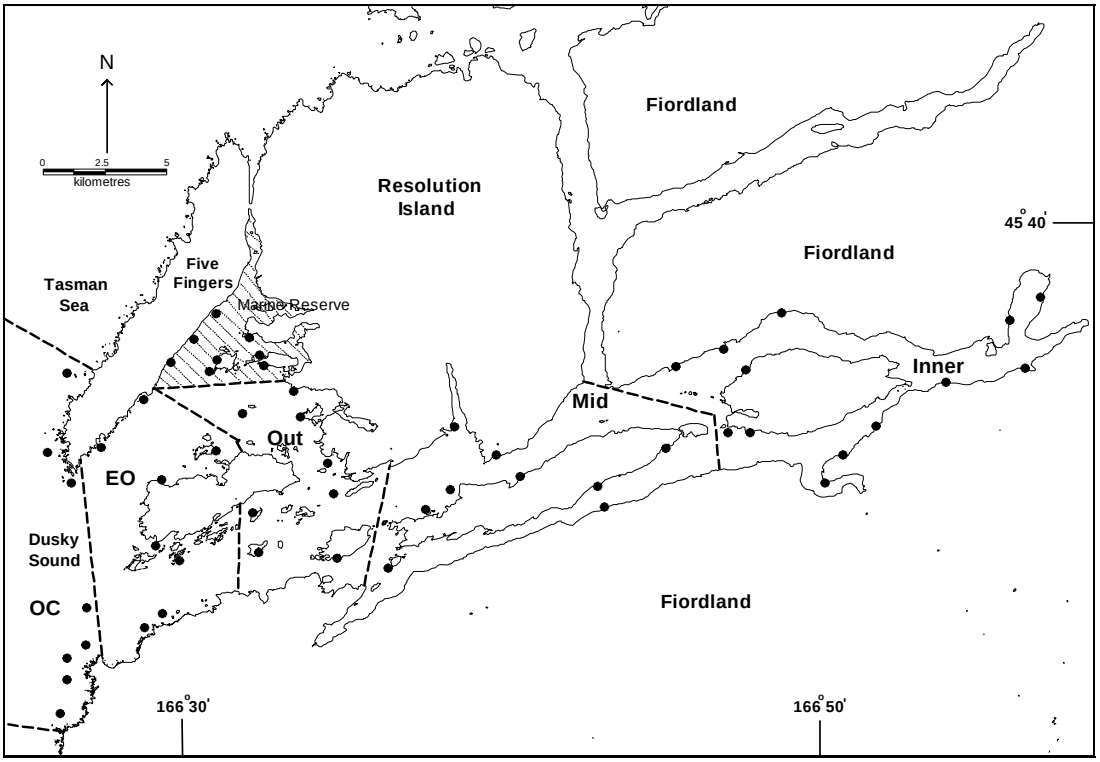

Figure 2: Map of the Dusky Sound area showing the strata and stations of the 2008 blue cod potting survey. Detailed results of this survey were given by Carbines & Beentjes (2011). Figure from Carbines & Beentjes (2011).

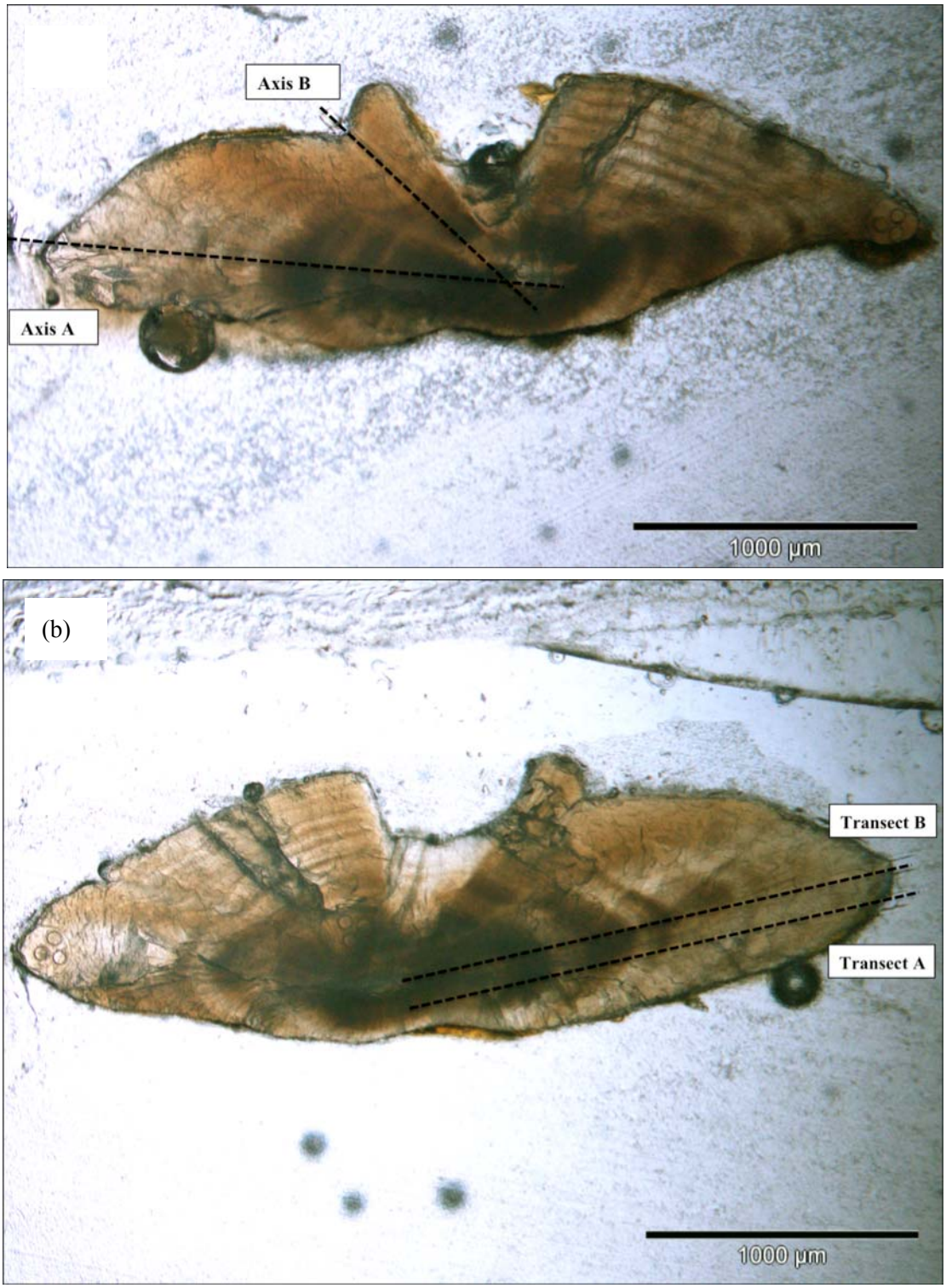

Figure 3: (a) Transects scanned along two different growth axes and (b) parallel transects scanned along the same growth axis within a single otolith. Three spot samples can be seen on the right tip of (a) and the left tip of (b).

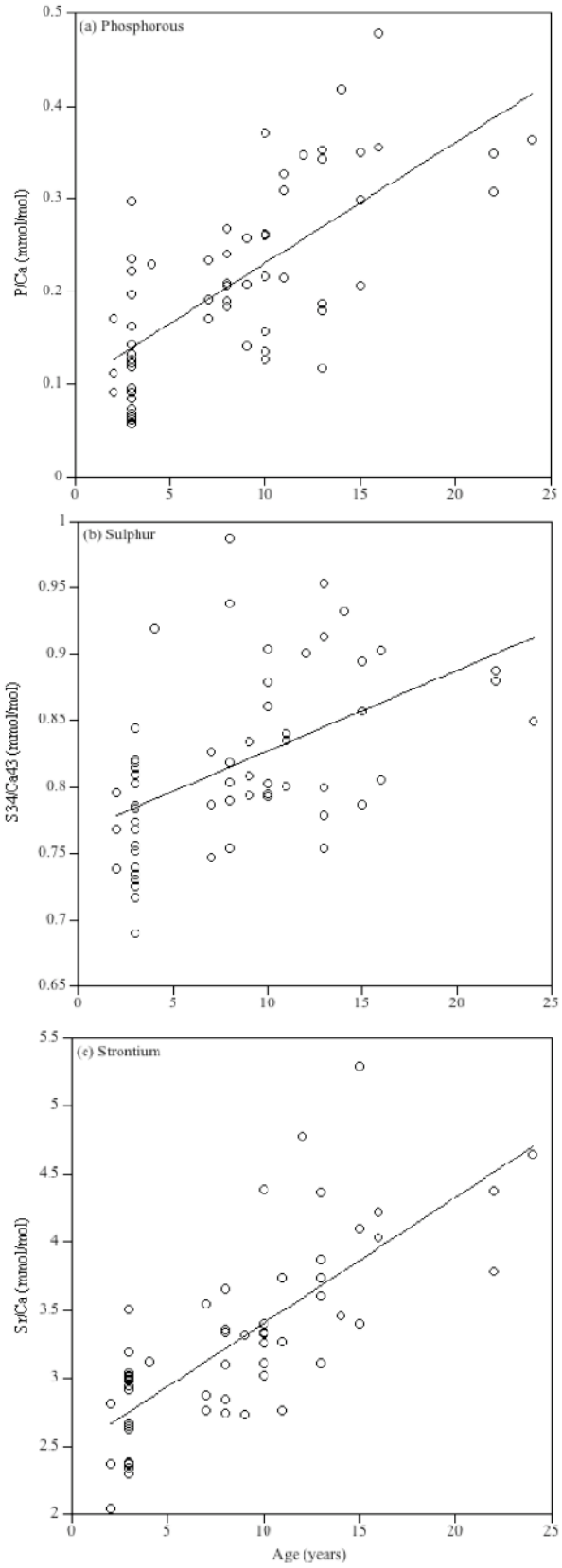

Figure 4: Linear regressions of capture site concentrations of (a) P/Ca (b) S/Ca and (c) Sr/Ca versus age for all fish sampled in 2002 and 2008 (n=124). Equations and R^2 for regression lines are given in Table 10.

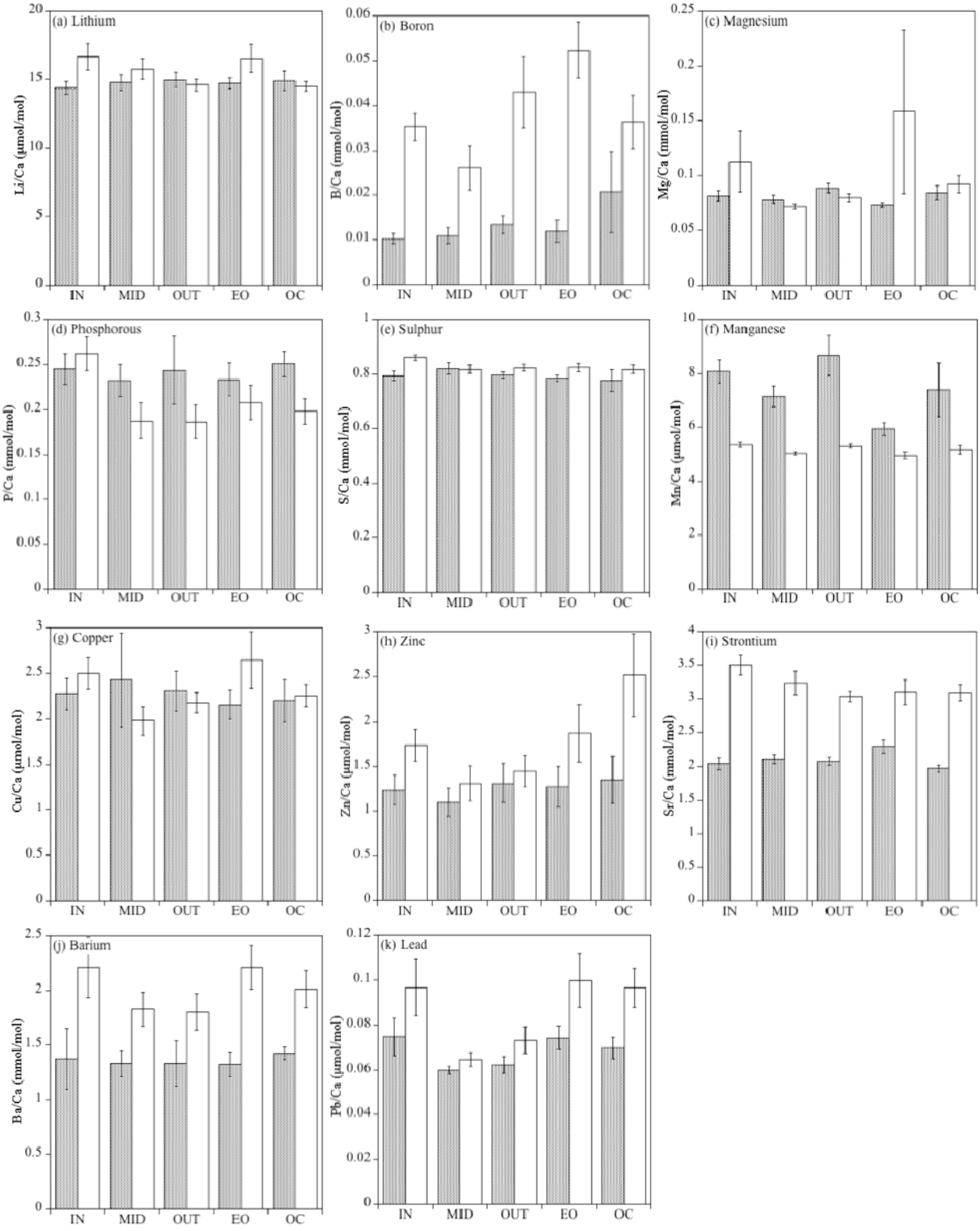

Figure 5: Mean concentrations of individual elements in 0+ (filled bars) and capture site (open bars) otolith material in each stratum, pooled across years. Standard error bars are shown.

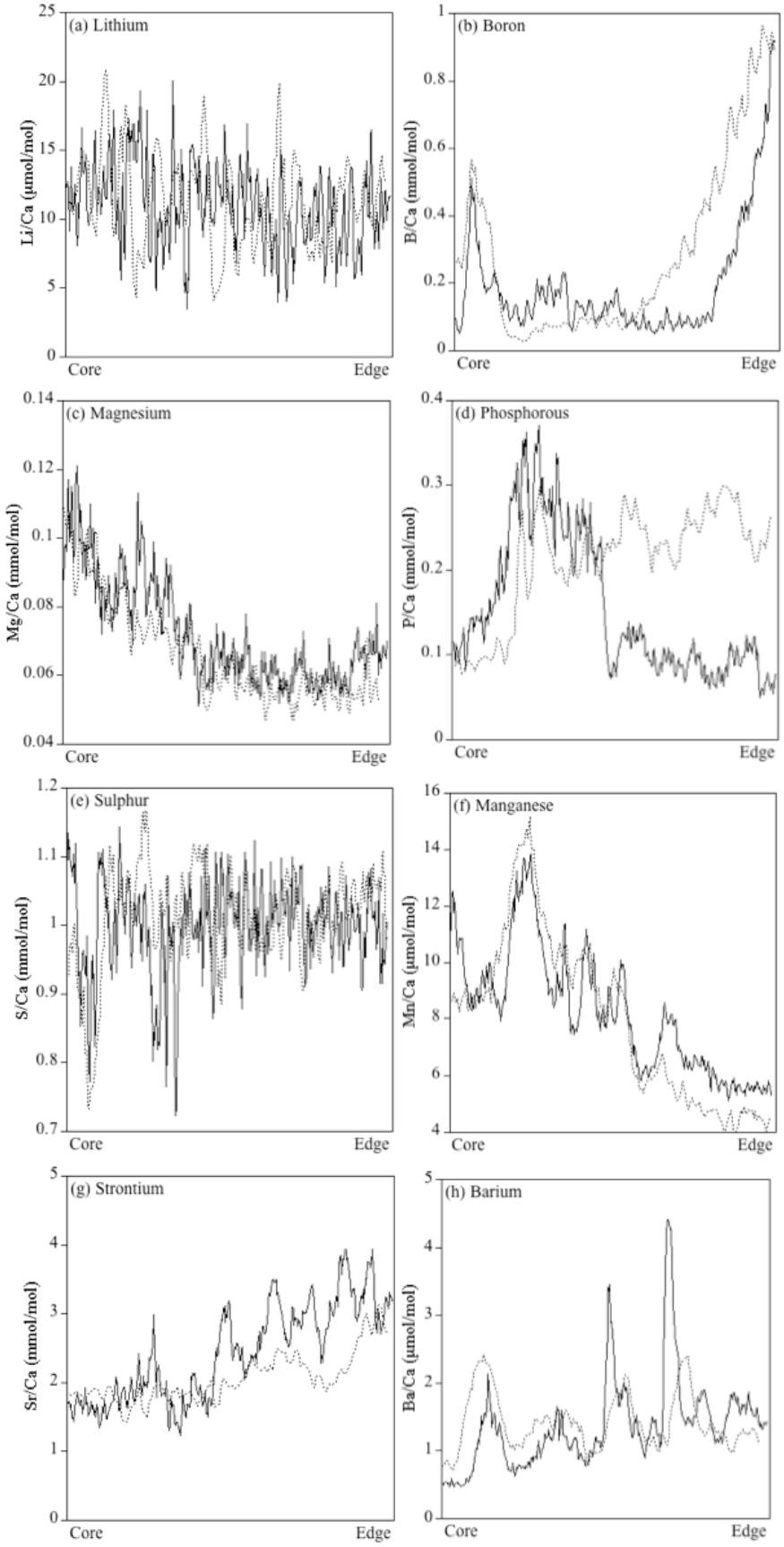

Figure 6: Individual element profiles from core to edge scanned along growth axis A (solid line) and growth axis B (dashed line). Axes A and B are shown in Figure 3a.

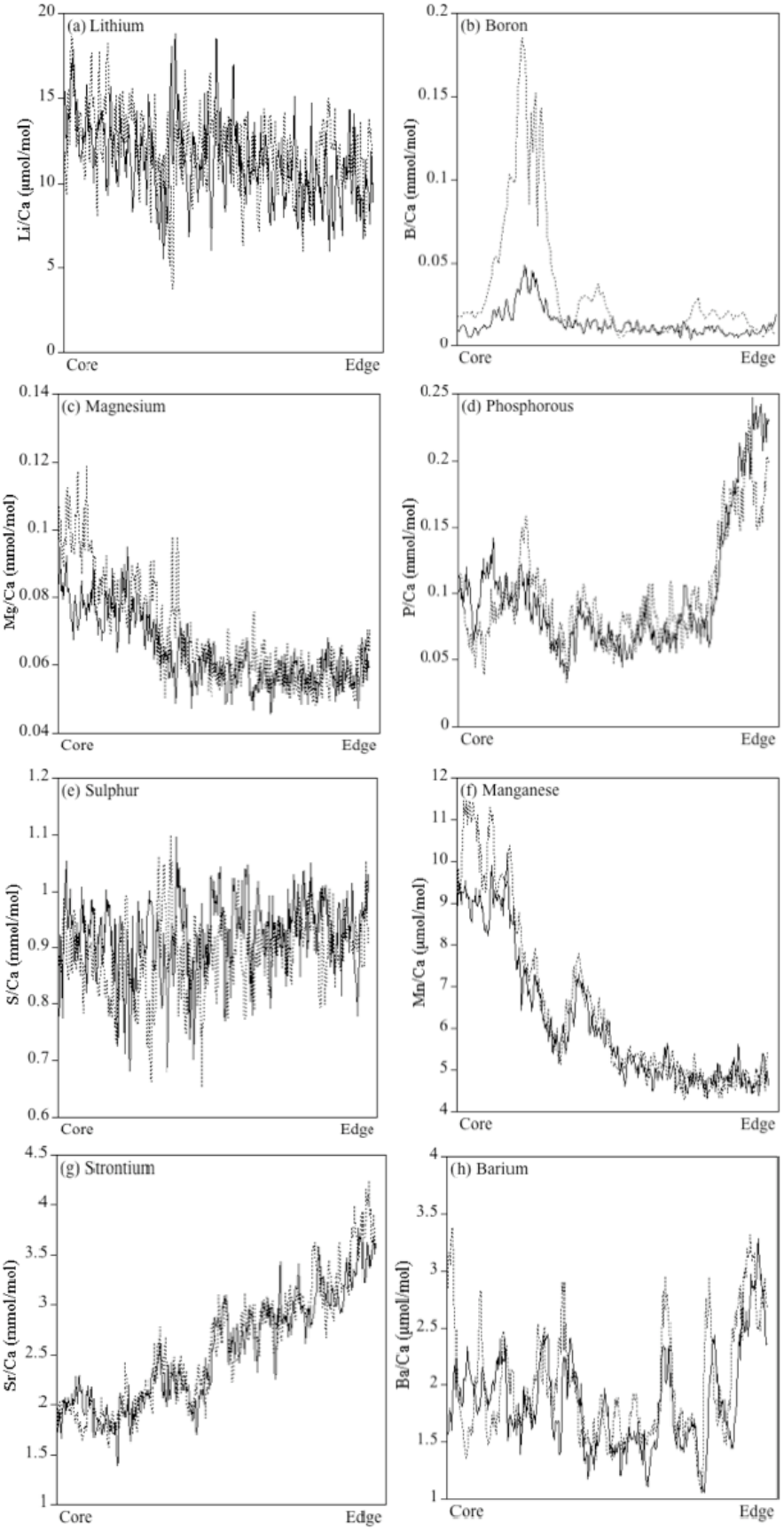

Figure 7: Individual element profiles from core to edge scanned along parallel axes A (solid line) and B (dashed line). Axes A and B are shown in Figure 3b.

Appendix 1: Trace element analysis of Banks Peninsula blue cod

Using the available otoliths collected from the April 2008 Banks Peninsula blue cod potting survey (Beentjes & Carbines 2009), a sub-sample of otoliths was taken from inshore (n=10) and offshore (n=10) regions (Table A & Figure A). Otolith microchemical analysis was carried out in September 2008 at the University of Melbourne's School of Earth Sciences using the same methods as described in Section 2.6.

Multivariate trace element signatures varied with total length (pseudo-$F_{11,19}$=2.0095; p=0.0401) and sex (pseudo-$F_{2,19}$=4.6220; p=0.0001) nested within stratum. Age did not have a significant effect on signatures but the result was close to the 0.05 significance level (pseudo-$F_{9,19}$=1.8609; p=0.0516). The similar result for all three measures stems from the broad range of ages/sizes in the offshore sample and the marked age/size difference between males and females. For this reason, only size effects were considered in subsequent tests as controlling for size (using ANCOVA) should control for age and sex too. Size effects within strata were driven by B (pseudo-$F_{11,19}$=3.1262; p=0.0367), Mg (pseudo-$F_{11,19}$=6.8142; p=0.0127), Sr and Pb (pseudo-$F_{11,19}$=5.7777; p=0.0269).

Regions (inshore/offshore) differed significantly in multivariate trace element signatures (pseudo-$F_{1,19}$=2.2164; p=0.0318) independent of size distributions. CAP successfully reclassified 75% of samples to strata group; reclassification success rates were 60% in the inshore stratum and 90% in the offshore stratum. For both strata groups, 50% would be expected by chance alone. The first two PCO axes explained 58.9% of the variability in trace element signatures (Figure B). Spatial effects were driven by B (pseudo-$F_{1,19}$=4.5844; p=0.0454) and Mn (pseudo-$F_{1,19}$=20.026; p=0.0003), both of which were higher in the inshore stratum (Figure C).

Trace element fingerprints of blue cod sampled off Banks Peninsula differed significantly from Dusky Sound blue cod sampled in 2008 overall (pseudo-$F_{1,55}$=5.0451; p=0.0002). However, the inshore Banks Peninsula strata group did not differ significantly from any strata in Dusky Sound. The offshore Banks strata group differed significantly from all Dusky Sound strata except the open coast. TL was included as a covariable in the models; B/Ca was excluded due to a machine calibration issue in 2008. The first two PCO axes explained 46.5% of the variability in trace element signatures (Figure D). CAP correctly assigned 90% of Banks Peninsula samples and 100% of Dusky Sound samples to capture region (i.e. Banks Peninsula versus Dusky Sound). Sixty-three percent of samples could be correctly reassigned to site (=region + stratum). By chance, 1.9–18.5% would be expected. Only one Dusky Sound sample was misclassified as a Banks Peninsula sample (caught in inner Dusky Sound, reassigned to Banks offshore). One of the three incorrectly reclassified inshore Banks Peninsula samples was assigned to outer Dusky Sound. Three of the four misclassified offshore Banks Peninsula samples were more similar to Dusky Sound signatures (inner and open coast strata). Variability between the two regions was driven by Mg, Mn, S, Cu and Sr.

Table A: Blue cod otoliths sampled in the seven strata of Banks Peninsula in 2008 (BCO2007/01).

				Inshore Strata Group		Offshore Strata Group		Total
Age\Strata	1	2	3	4	5	6	7	
2	2	-	2	-	-	1	2	7
3	4	-	1	1	-	-	-	6
5	-	-	-	-	-	1	-	1
6	-	-	-	-	-	-	1	1
13	-	-	-	-	-	1	-	1
17	-	-	-	-	-	1	-	1
18	-	-	-	-	-	1	-	1
20	-	-	-	-	-	1	-	1
25	-	-	-	-	-	1	-	1
Total	6	0	3	1	0	7	3	20

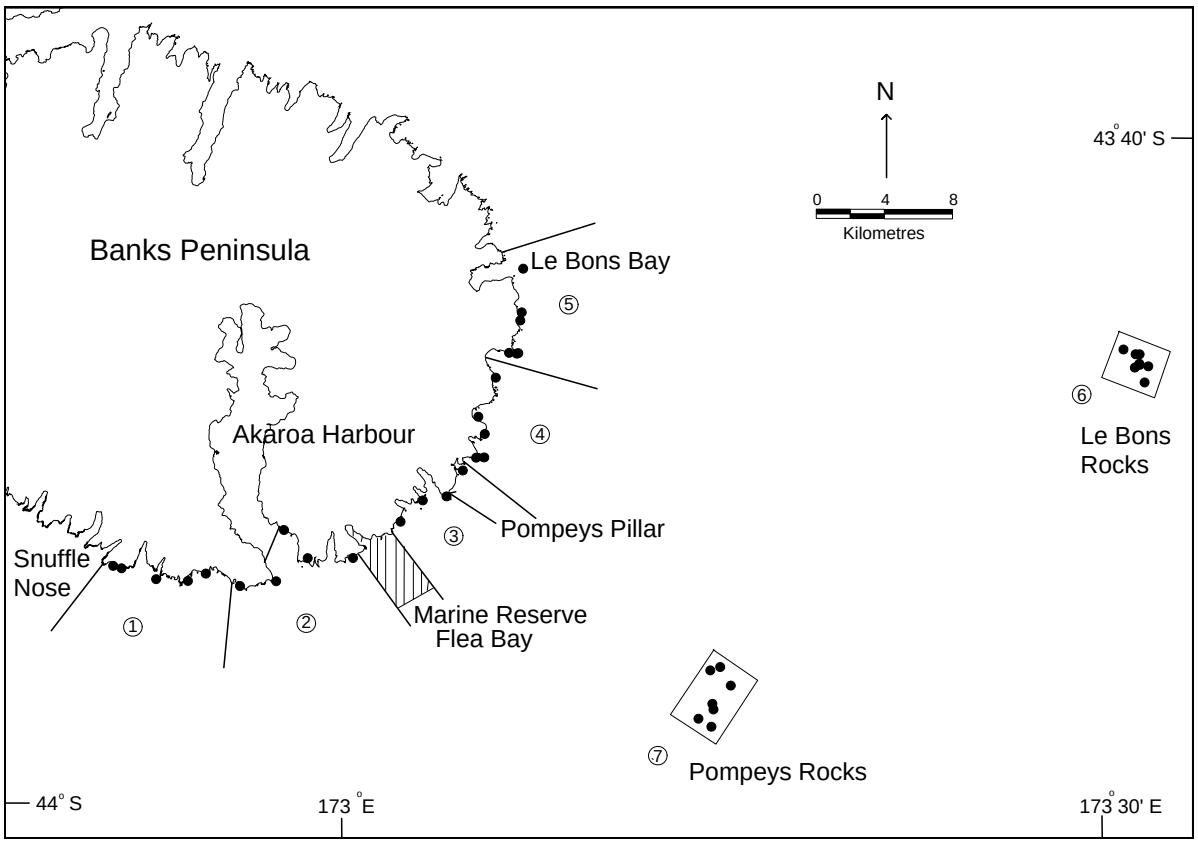

Figure A: Map of Banks Peninsula showing onshore (1–5) and offshore (6–7) strata, stations surveyed in 2008 are shown as black dots. Figure from Beentjes & Carbines 2009.

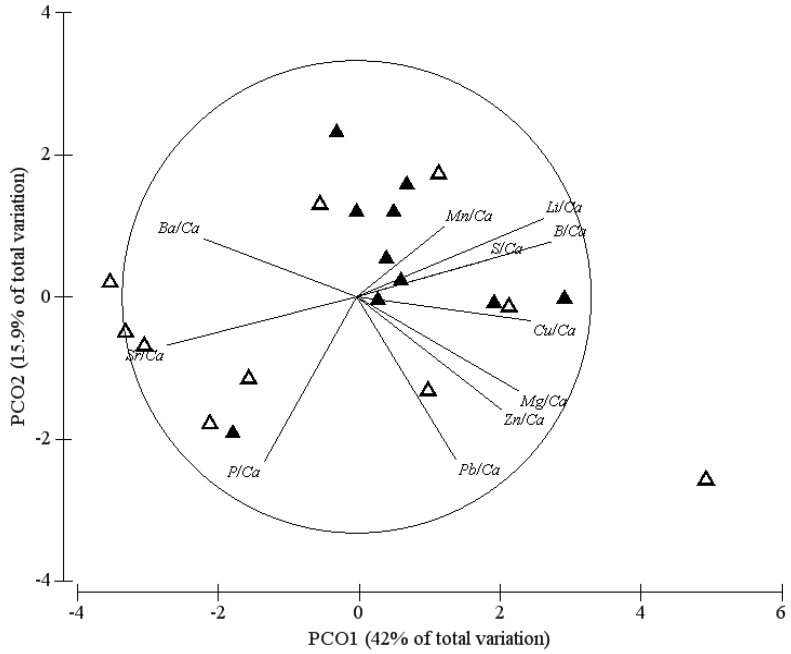

Figure B: PCO ordination of the Euclidean distance between normalised multivariate trace element signatures of fish sampled from inshore (filled triangles) and offshore Banks Peninsula (open triangles) in 2008. Pearson correlation vectors are included.

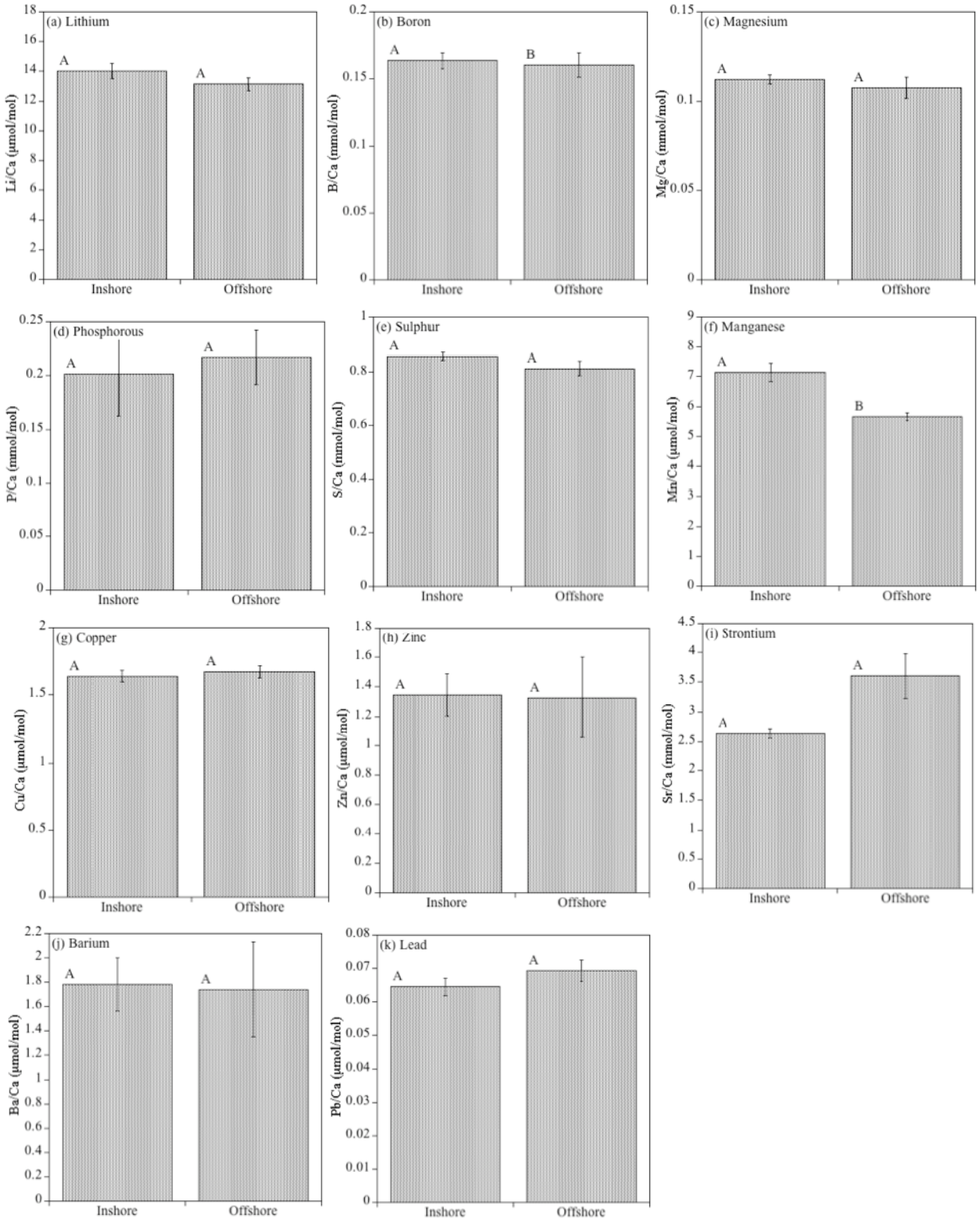

Figure C: Mean capture site concentrations of individual elements in inshore and offshore strata group of Banks Peninsula in 2008. Standard error bars are shown.

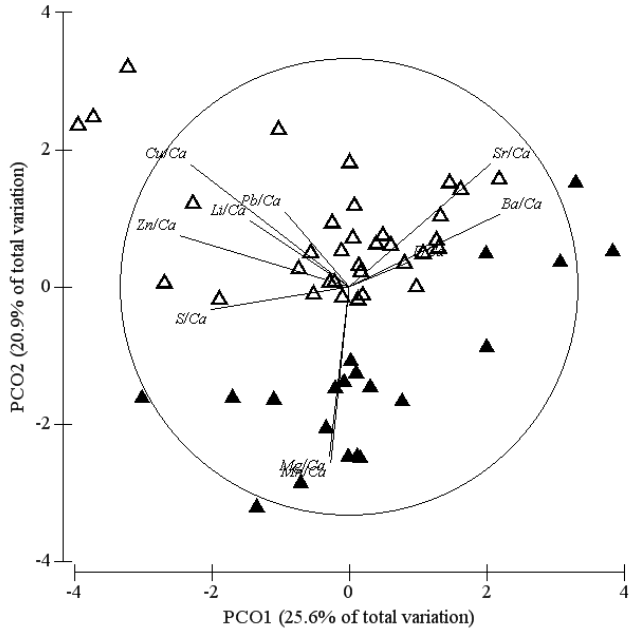

Figure D: PCO ordination of the Euclidean distance between normalised multivariate trace element signatures of fish sampled from Banks Peninsula (filled triangles) and Dusky Sound (open triangles) in 2008. Pearson correlation vectors are included.