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# High-resolution otolith elemental signatures in eteline snappers from valuable deepwater tropical fisheries

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## Abstract

Marine resources are often shared among countries, with some fish stocks straddling multiple Exclusive Economic Zones, therefore understanding the structure of populations is important for the effective management of fish stocks. Otolith chemical analyses could discriminate among populations based on differences in the chemical composition of otoliths. We used otoliths from two deepwater snappers (flame snapper *Etelis coruscans* and ruby snapper *Etelis boweni*) to examine the evidence for population structure across six Pacific Island countries using solution-based inductively coupled plasma mass spectrometry (ICP-MS) for otolith core and whole otolith samples and laser ablation ICP-MS (LA-ICP-MS) for core and edge areas of a cross-sectioned otolith. The inter-species comparison of these methods is important as the two species are often managed under the same regulations. For both species, the two methods demonstrated separation among the locations sampled with high classification accuracy. Smaller laser ablation spot size gave greater temporal resolution over the life-history transect. Comparing the early life-history section of the otoliths (*i.e.*, the core), one interpretation is that young fish experienced more uniform environments in the open ocean as larvae than adults, as the elemental fingerprints had greater overlap among multiple locations. LA-ICP-MS methods had some advantages over solution-based ICP-MS and generally better discrimination for the trace elements investigated. There were substantial differences between species, but both methods suggested nonmixing populations at the regional scale. Otolith chemistry can be an effective tool in discriminating variation for deepwater marine species in multispecies fisheries, and edge measurements from LA-ICP-MS provided the greatest resolution. Although caution should be taken in interpreting the results from relatively small samples sizes, otolith chemical analyses could be useful at these spatial scales to investigate population structure. This information on separate or overlapping populations could be used in future regional fishery management plans.

**KEYWORDS**
deepwater fisheries, Lutjanidae, otolith chemistry, Pacific islands, stock structure, trace element ICP-MS

## 1 | INTRODUCTION

The management of global fish catch is of critical importance for human societies. Various conventions and policies define the rights and obligations of nations and societies to extract marine resources. One important mandate, the United Nations Convention on the Law of the Seas (UNCLOS), allows nations to have jurisdiction over a 200-nautical mile Exclusive Economic Zone (EEZ), which includes all fishing rights in these territorial waters. Pacific island EEZs are allocated according to the UNCLOS agreement, but closely neighbouring countries likely have overlapping fish stocks and unequal areas of productive fishing grounds. Regional organizations such as the Pacific Community (SPC, New Caledonia) and the Western Pacific Fisheries Management Council (WPFMC) can provide countries in the Pacific region with information on which to base fisheries management decisions. However, fisheries research in this region is often limited by funding and resources (Newman *et al*., 2015; Williams *et al*., 2015). In practice, fisheries management often defines stock management units and the spatial separation of stocks based on units of convenience (*i.e*., EEZs) rather than ecological evidence on the spatial structure of stocks (Begg *et al*., 1999).

Greater fishing effort has been directed toward deepwater fisheries in recent decades (Morato *et al*., 2006), placing greater urgency on determining stock structure so that accurate assessments of stocks can be made (Newman *et al*., 2016). Some Pacific countries, including Tonga and Vanuatu, have established deepwater fisheries, with eteline snappers among the most economically valuable and potentially vulnerable fishes (Newman *et al*., 2015; Williams *et al*., 2013). Although knowledge of deepwater fish spatial ecology is limited (Gomez *et al*., 2015; Kobayashi, 2008; Weng, 2013), there is growing evidence for spatial variation in demography (Williams *et al*., 2017), suggesting the existence of nonmixing populations and/or separate fish stocks. Previous genetic studies have revealed panmictic populations of some deepwater snapper species in the Indo-Pacific, suggesting widespread stock-mixing and highly connected populations (Andrews *et al*., 2014, 2016, 2020; Gaither *et al*., 2011; Goldstein *et al*., 2016), although there is some genetic evidence for population structure at spatial scales of hundreds of kilometres (Gaither *et al*., 2011; Ovenden *et al*., 2002, 2004). However, only low levels of gene flow are needed to maintain population connectivity (Andrews *et al*., 2016), and there likely is population structure at scales more relevant to fisheries management.

Analysis of the chemical composition of otoliths provides an alternative method for discriminating among populations and subpopulations for the purposes of identifying management units (Cadrin & Secor, 2009; Campana, 2005; Hammer & Zimmermann, 2005). Concentric layers of calcium-based materials are layered as the fish ages, providing a chronological record of the environmental history of the fish (Campana, 1999). Otolith chemical composition includes metals in trace amounts that, when measured against an internal standard such as calcium, can discriminate between environments or locations where the fish has been (Campana *et al*., 2000). Otolith chemistry has the potential to provide evidence on the connectivity among populations from multiple locations (Jones *et al*., 2016). Differences in water chemistry or diet may result in differences in the trace elemental composition of the otolith, which can delineate ecological subpopulations or manageable stock units (Campana, 2005; Walther *et al*., 2017). Otolith microchemistry can also give insight into possible movements or ontogenetic shifts through comparisons of otolith composition from point of origin (core) versus catch-location (edge) chemistries (Elsdon *et al*., 2008). Defining stock structure, as it applies to fisheries management, is the process of spatially delineating parts of a fishery into biological units of low connectivity that can be fished with little or no immediate consequences for sustainable yield from subpopulations within the metapopulation on ecologically relevant temporal scales (*i.e*., 5–10 years; Thresher & Proctor, 2007).

Chemical analyses of fish otoliths have been useful as natural tags of the environments fish have been exposed to over their lifespan (Campana *et al*., 2000). These methods complement information from other methods such as morphometrics (*e.g*., Haddon & Willis, 1995), parasite markers (*e.g*., Lester & Moore, 2015), genetic analyses (*e.g*., Smith & Campana, 2010) and catch record comparisons to provide insights on which fisheries managers can base decisions. Where there may be gaps or uncertainty in data collection, the combination of multiple techniques has been especially useful where decisions need to be made based on incomplete assessments (Brodziak *et al*., 2011; Welch *et al*., 2015) and may provide a more holistic view of the fishery (Begg *et al*., 1999; Begg & Waldman, 1999), yet advanced techniques have not been used to look at region-wide stock discrimination for deepwater species.

There are multiple techniques that could help to delineate stocks based on trace element otolith chemistry. The primary techniques are solution-based inductively coupled plasma mass spectrometry (ICP-MS) and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Both techniques measure trace element concentrations, but they have different resolution capabilities, and each technique has strengths and weaknesses. Given the challenges of researching deepwater fisheries, methods are needed that maximize the information on the structure of deepwater fish populations for the region. The delineation of stocks using otolith chemistry relies on the assumptions that otolith material, once deposited, is metabolically inert (Campana, 1999), elements taken into the otolith reflect the ambient environment experienced by the fish (Bath *et al*., 2000; Campana *et al*., 2000) and there is sufficient geographic variation in water or other factors to influence the chemistry of the otolith (Campana, 2005; Elsdon *et al*., 2008). Solution-based ICP-MS is relatively faster in terms of time and efficiency for laboratory protocols. This technique is faster (Kingsford *et al*., 2009) because there is less post-processing of data, but may be limited in questions that can be addressed because the whole otolith is dissolved in solution. This results in a 'whole-structure fingerprint' (Kerr & Campana, 2014) that integrates the entire lifetime of the fish and can only distinguish among groups of fish that have experienced different environments across their life history (Campana, 1999; Thorrold *et al*., 1998). However, there can be some resolution of life-history stages, for instance, by isolating the core (*e.g*., Dove *et al*., 1996) it is possible to infer nursery origin for groups

of fish (Burns *et al.*, 2020; Campana, 2005; Gillanders & Kingsford, 2000). LA-ICP-MS has greater fine-scale spatial resolution, as specific areas of the otolith are selected for comparison. Selecting a 'life-history transect' from the core to the edge of the otolith can be useful to investigate how the elemental signatures change over the lifespan of an individual fish. This allows the discrimination of groups within a specific time-frame when matched with specific portions of the otolith or specific annuli in the otoliths. This method may be useful for species whose ecology is less well known and where variations in distributions with growth may potentially be inferred from environmental information.

Both otolith analyses have been used successfully to delineate stocks of shallow-water demersal species (*e.g.*, LA-ICP-MS of Western Australian dhufish, *Glaucosoma hebraicum*, and snapper, *Chrysophyrs auratus*, ~1000 km, Fairclough *et al.*, 2013; solution-based ICP-MS of snapper, ~400 km, Gillanders, 2002) and even deepwater species (*e.g.*, solution-based ICP-MS and electron probe microanalysis of orange roughy, *Hoplostethus atlanticus*, ~1300 km and ~5000 km, Edmonds *et al.*, 1991; Thresher & Proctor, 2007) over varying spatial scales. However, it is not known if the environmental variation is sufficiently different among locations (hundreds to thousands of kilometres apart) to discriminate stocks of deepwater fish, which are further from coastal influences, in a deepwater environment with limited biological, physical and chemical information over this spatial scale. There is some evidence that these species are highly site-attached with limited adult mobility (Weng, 2013), and therefore otolith chemical analyses have the potential to successfully discriminate between nonmixing stocks. There are some studies that have compared trace elemental composition across similarly broad regions on more mobile species (*e.g.*, pelagic tuna populations; Proctor *et al.*, 1995; Rooker *et al.*, 2016), but there are few studies that have examined otolith trace elemental composition for more site-attached reef species at large spatial scales. The few otolith chemical analyses of deepwater (>200 m) species indicate that fish have high site fidelity, especially where seamount habitats are limited and geographically separated (*e.g.*, orange roughy, *Hoplostethus atlanticus*, Edmonds *et al.*, 1991; roundnose grenadier, *Coryphaenoides rupestris*, Longmore *et al.*, 2010; Régnier *et al.*, 2017).

Fisheries management relies on accurate species-specific information, and previous otolith chemical studies indicate there are greater similarities between closely related species and species with similar ecology (Reis-Santos *et al.*, 2008; Swearer *et al.*, 2003), including strong taxonomic signals in fishes from the same region (Chang & Geffen, 2013). It may be possible to use the otolith chemistry of one species as a proxy for a related species (Nelson & Powers, 2019; Prichard *et al.*, 2018; Reis-Santos *et al.*, 2008). However, other studies indicate significant differences among species from the same family collected at multiple estuaries (Gillanders & Kingsford 2003). More interspecies comparisons of otolith chemical signatures, over varying spatial scales, are warranted.

The objective of this study was to evaluate the utility of solution-based ICP-MS and LA-ICP-MS for discriminating among populations of two closely related species of deepwater snapper (flame snapper *Etelis coruscans* Valenciennes 1862 and ruby snapper *Etelis boweni*; Andrews et al., 2021) from multiple locations in the Pacific island region. In the previous literature, *E. boweni* has been referenced as the pygmy ruby snapper *Etelis carbunculus* Cuvier 1828 in some locations. In the South Pacific, this species often co-occurs with *E. carbunculus*, which is a cryptic sister species (Andrews *et al.*, 2016; Andrews *et al.*, 2021; Loeun *et al.*, 2014; Smith, 1992; Wakefield *et al.*, 2014). Both species are fully marine fishes, demonstrating high site-attachment as adults (Weng *et al.*, 2013). Both species generally inhabit depths of 250 m or more, which makes telemetry studies and mark-recapture studies more difficult (Kobayashi, 2008). Deepwater snappers live in heterogeneous seascapes and species may use habitat differently (Sih *et al.*, 2017, 2019).

Our specific aims were (1) to determine which elements and which technique yielded greatest separation of elemental fingerprints for inferring stock structure, (2) to elucidate the likelihood of detecting spatial differences based on the part of the otolith that represented early and late life history by comparing the resolution of dissolved core and whole otoliths (solution-based ICP-MS) and (3) to investigate the differences between representative core and edge ablation spots from LA-ICP-MS transect measurements. This study provides a useful prerequisite for broader application of elemental chemistry to potentially discriminate among tropical deepwater fish stocks.

## 2 | MATERIALS AND METHODS

### 2.1 | Sampling design

Otoliths for this study were collected from 2012 to 2015 during scientific surveys on commercial vessels and from artisanal landings using vertical multihook droplines from depths ranging between ~100 and 400 m. Samples were collected from fish collected from Fiji, New Caledonia, Papua New Guinea, Tonga, Vanuatu, and Wallis and Futuna. The EEZs for these Pacific countries span over 4500 km (Table 1 and Figure 1).

Ethical approval was not required for this study, as all fish were collected as part of routine fishing procedures. No samples were collected by the authors. All samples in this study originated from commercial or artisanal fisheries in Tonga, Vanuatu, Fiji, New Caledonia, Papua New Guinea, and Wallis and Futuna, and were already dead when provided to the sampler. Fish were sacrificed by the commercial or artisanal fisher at sea using standard fisheries practices (most fish were dead when landed). Permission was granted from the fishers who donated these samples.

### 2.2 | Solution-based ICP-MS protocol

Elemental signatures were obtained for juvenile (otolith core) and whole-life integrated (whole otolith) with solution-based ICP-MS. Sixty-six otoliths from the two species from multiple EEZs were selected for solution-based analyses. Otolith cores were isolated using a hand-held rotating diamond-blade saw (similar to Dove *et al.*, 1996).

**TABLE 1** Geographic locations of otolith samples used for solution-based ICP-MS and LA-ICP-MS

| | Species | *Etelis coruscans* | | | | *Etelis boweni* | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Method** | **Exclusive Economic Zone** | **Latitude (°S)** | **Longitude (°E)** | ***n*** | **Mean age (years)** | **Latitude (°S)** | **Longitude (°E)** | ***n*** | **Mean age (years)** |
| Solution-based ICP-MS | Papua New Guinea | 2.35–2.57 | 150.40–150.80 | Three otolith cores | 15.7 | 2.35–2.50 | 150.40–150.60 | Three otolith cores | 12.7 |
| | | | | Three whole otoliths | 14.7 | | | Three whole otoliths | 13.7 |
| | Vanuatu | 15.55 | 167.33 | Three otolith cores | 12.7 | 15.55 | 167.33 | Three otolith cores | 13 |
| | | | | Three whole otoliths | 10.3 | | | Three whole otoliths | 13.3 |
| | New Caledonia | 20.94 | 165.59 | Three otolith cores | 12.3 | 20.54–21.13 | 164.99–165.76 | Three otolith cores | 13.3 |
| | | | | Three whole otoliths | 12 | | | Three whole otoliths | 12 |
| | Fiji | 22.36 | 181.03 | Three otolith cores | 9.7 | | | | |
| | | | | Three whole otoliths | 9.7 | | | | |
| | Wallis and Futuna | 13.42–13.59 | 180.77 | Three otolith cores | 15.3 | 13.42 | 180.77 | Three otolith cores | 17 |
| | | | | Three whole otoliths | 15.3 | | | Three whole otoliths | 20.3 |
| | Tonga | 22.98–23.52 | 183.75–184 | Three otolith cores | 9.3 | 18.35–19.78 | 185.25–185.70 | Three otolith cores | 11.7 |
| | | | | Three whole otoliths | 6.7 | | | Three whole otoliths | 11 |
| Laser-ablation ICP-MS | Papua New Guinea | 2.35–2.57 | 150.40–150.80 | 3 | 13.7 | 2.35–2.50 | 150.40–150.60 | 3 | 10 |
| | Vanuatu | 15.55 | 167.33 | 3 | 9.7 | 15.55 | 167.33 | 3 | 13 |
| | New Caledonia | 20.94 | 165.59 | 3 | 10.3 | 20.61–21.12 | 164.99–165.76 | 3 | 14.7 |
| | Fiji | 22.36 | 181.03–181.04 | 3 | 13.3 | | | | |
| | Wallis and Futuna | 13.42 | 180.77 | 3 | 15.3 | 13.40–13.59 | 180.75–180.77 | 3 | 19.3 |
| | Tonga | 22.98–23.52 | 183.78–184 | 3 | 11 | 19.05–22.98 | 184–185.70 | 3 | 11.7 |

*Note*: ICP-MS, inductively coupled plasma mass spectrometry; LA-ICP-MS, laser ablation inductively coupled plasma mass spectrometry. Otoliths were collected in multiple Exclusive Economic Zones for two species, *Etelis coruscans* and *Etelis boweni*. Latitude and longitude are expressed in decimal degrees.

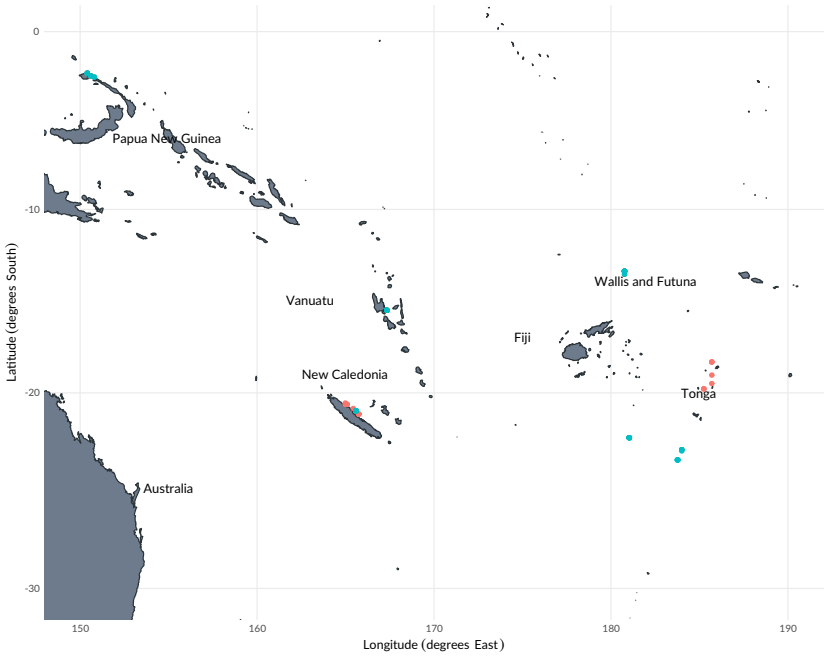


**FIGURE 1** Map of sampling locations for two species of deepwater snapper, *Etelis boweni* and *Etelis coruscans*. Ninety-nine otoliths were collected from six locations representing the Exclusive Economic Zones of multiple Pacific Island nations. ● *Etelis boweni*; ● *Etelis coruscans*

Prior to dissolution, otolith cores and whole otoliths were weighed to the nearest 0.001 g, washed three times in Milli-Q Ultra-Pure (Type 1) water, placed in an ultrasonic bath for 2 min and then rinsed three times in Milli-Q water. Otoliths were placed in acid-washed vials and dried for 48 h in a laminar-flow hood. For solution-based samples, 33 cores and 33 whole otoliths (18 *E. coruscans* and 15 *E. boweni*, respectively) were dissolved in 20% $HNO_3$ solution based on otolith weight, then diluted to a solution of 2% acidity and concentration of 1 g/l of otolith material. Elements $^{138}$Ba, $^{88}$Sr, $^{44}$Ca, $^{24}$Mg, $^{55}$Mn, $^{65}$Cu, $^{66}$Zn and $^{57}$Fe were measured against blank solutions and certified reference material (CRM) #22 from *Lutjanus sebae* otoliths from Western Australia (National Institute for Environmental Studies, Japan) and each line was tested five times. CRM is used as a quality control for ICP-MS analyses, and a *L. sebae* CRM calibration standard was representative of the Lutjanidae family (Yoshinaga *et al*., 2000). Elemental concentrations were measured in ppm and expressed as a ratio to calcium concentrations (metal:calcium, abbreviated as Me:Ca).

## 2.3 | LA-ICP-MS protocol

Spatial and temporal resolution elemental fingerprints were obtained from the time fish hatched (core) to the time of collection (edge). Furthermore, the results were compared for two different ablation spot sizes that would integrate different amounts of the otolith chronology elemental deposition. Thirty-three otoliths from two species were selected for laser-based analyses. Otoliths were transverse-sectioned, then embedded in CrystalBond 509 Amber resin to maintain an even ablation surface, using a combination of 600, 1200 and 3000-grit grinding wheels and 3 μm lapping film and Milli-Q water for polishing. For all LA-ICP-MS measurements, the area was pre-ablated to remove potential contamination using a larger ablation spot-size. Each LA-ICP-MS transect consisted of a 20 second background scan followed by a continuous ablation scan of 10 Hz pulses with a 193 nm Geolas Pro Excimer laser paired with a Varian 820-MS mass spectrometer. The elements measured with LA-ICP-MS included $^{7}$Li, $^{24}$Mg, $^{43}$Ca, $^{44}$Ca, $^{55}$Mn, $^{57}$Fe, $^{60}$Ni, $^{65}$Cu, $^{66}$Zn, $^{88}$Sr and $^{138}$Ba. For each otolith, LA-ICP-MS samples were taken in the following areas of each otolith: (a) a 'core-to-edge' transect with a 24 μm ablation mask; (b) an adjacent 'core-to-edge' transect with a 32 μm ablation mask and (c) an edge measurement from the sulcus acusticus along the proximal surface-edge (approximately 200 μm long, using a 24 μm ablation mask). NIST610 and NIST612 readings were taken at the start, midpoint and end of each sample chamber (16–18 otoliths). NIST readings are considered reliable for determining the accuracy of measurements for a calcium carbonate matrix (Craig *et al*., 2000). LA-ICP-MS spectral data was analysed using IGOR PRO 6.37 software with Iolite v.2.2 interface with a mean and three standard deviation outlier rejection scheme. Calcium readings were checked for consistency across the otolith and elements were expressed as a ratio to calcium as an internal standard (Me:Ca).

If calcium varied across the otolith, this could confound an estimate of average Me:Ca; all calcium readings indicated even ablation across the otolith surface. All elements were expressed as μm/mol or mm/mol (depending on quantity) and then expressed as a ratio to calcium. Four locations on the otolith were compared using averaged LA-ICP-MS data points (Figure 2): (1) the 'early life' period, which was defined as the average of the first 50 Me:Ca data points of the transect, through the primordium region ('average core', both 24 and 32 μm); (2) the 'late life prior to capture' encompassed an average of the last 50 data points of the transect ('average edge', both 24 and 32 μm); (3) average of separate edge ablation with 24 μm ('total edge load', only 24 μm); and (4) an average of 150 data points of the entire transect ('total load', both 24 and 32 μm). This method ensured no

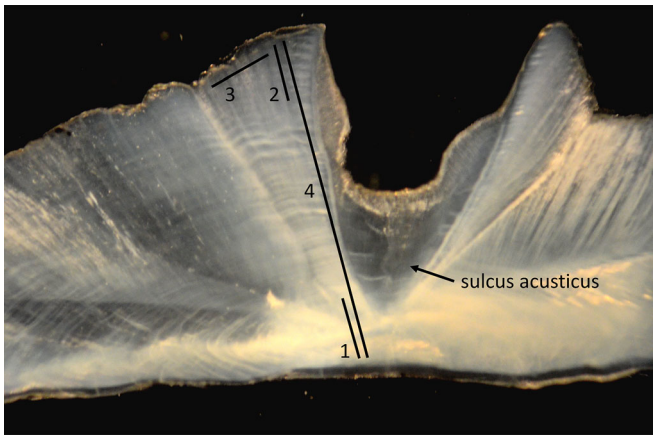


**FIGURE 2** *Etelis coruscans* otolith transect magnified and photographed with transmitted and reflected light. The approximate areas of the LA-ICP-MS transects (24 and 32 μm ablation mask sizes) and the edge measurement (24 μm) are indicated. The approximate locations of calculated averages are depicted with (1) the average of the first 50 data points of the transect (average core), (2) the average of the last 50 data points of the transect (average edge), (3) average of the separate edge measurements (total edge load) and (4) an average of 150 data points of the entire transect (total load)

unequal weighting of points among samples but does not take into account differences in age and growth among individuals. For each EEZ and each method there were three replicate otoliths. The average core measurement would have included the first several years, including the larval and juvenile portions of the lifespan. The average edge would have included several years before capture, presumably in the environment of the EEZ it was captured in. The available information on adult movements of *Etelis* spp. indicate high site attachment (Weng, 2013). The justification for using averaged values was to broadly compare how regions of the otolith may assist in the detection of spatial differences, and to understand how location on the otolith may change estimates, perhaps averaging to environmental differences with respect to age.

## 2.4 | Statistical treatment of data

To investigate the relative variation for each species, it was necessary to assess the natural variation among individual otolith samples as residual variance. Averages for all groups of solution-based and LA-ICP-MS data were evaluated by a coefficient of variation (CV) based on single element concentration ratios, where the standard deviation over the mean was expressed as a percentage for untransformed data. Between methods, greater variability among samples can aid discrimination or add additional noise at the EEZ level. Furthermore, specific groups of otolith elemental ratios were evaluated by a linear regression to see if proportional variance trends were similar between methods for core versus whole (solution-based) and average core and average total (LA-ICP-MS) samples. Data were Box–Cox transformed, centred and scaled (package caret; Kuhn, 2017) and a coefficient of determination ($R^2$) indicated the proportion of unexplained variance among measurements.

It is important for both univariate regression analyses and multivariate analyses such as multivariate analysis of variance (MANOVA) and linear discriminant function analysis (LDFA) that data were transformed, scaled and centred to meet assumptions of normality and homogeneity of variance. A Box–Cox power transformation was generally more effective than $\log(x + 1)$ transformation for data to conform with multivariate normality and has been recommended in other otolith studies (Walther *et al*., 2017). Otolith chemistry data can be highly variable and specific elemental ratios are often non-normal and positively skewed (right-tailed). When select elements were not multivariate normal, they were removed. Pairs of elemental concentrations were also compared within a group of measurements (*e.g*., core, whole, average core, average edge) and for correlations greater than 0.7 one or both elements were removed from subsequent multivariate analysis. Elements were considered separate and independent for univariate analyses. Data were tested using Shapiro–Wilk's tests for normality, Mardia's test for multivariate normality (package MVN; Korkmaz *et al*., 2014) and visually investigated with QQ plots and histograms. For some regressions, specific data points were removed and analyses retested, and overall there were few statistical outliers, but they were kept for the benefit of equal sample sizes (for parametric tests) and all assumptions were considered reasonably met.

## 2.5 | Investigating age effects

Specific elements may be differentially incorporated into otoliths over time and may be correlated with the age of the individual fish. To evaluate if age correlated with elements in the otolith, the age of each individual fish was included in a linear regression with the elemental ratios for each group of measurements. Age was independently estimated from annual increment counts from the individual's other otolith (Williams *et al*., 2015). The distribution of age within each group was significantly different from normal for *Etelis boweni* samples only and this was corrected for by a square-root transformation for LA-ICP-MS data (both measured from 32 and 24 μm mask sizes) and by a Tukey's Ladder of Powers transformation for solution-based whole otolith samples (rcompanion package; Mangiafico, 2017) when a square-root transformation was insufficient to meet assumptions. Fish were all adults at capture, but differences in age among samples were due to the selection of individuals based on fork-length comparisons and not age, which was not known at the time of selection. Each elemental ratio from each group of measurements was plotted in a linear model against the variable age (or transformed age) to look for significant relationships. Some stock structure investigations have found significant element–otolith weight relationships (Campana, 2005), but due to the moderate sample size, as well as the fact some otoliths were chipped, otolith weight was determined to not be a reliable measurement, and element–otolith weight relationships were not investigated.

## 2.6 | Single-element otolith variation among multiple EEZs

To evaluate whether single elements were responsible for some of the variation between EEZs, solution-based ICP-MS samples were analysed using a generalized linear model with the factors Species ($a = 2$), EEZ ($b = 5$) and Measurement (core versus whole) as fixed factors for averaged elemental ratio for both species combined (five EEZs for balanced design), and follow-up models for each species individually with the factors EEZ and Measurement (six and five EEZs depending on the species). Since each of the dissolved otoliths came from different fish, samples were treated as independent and data were Box–Cox transformed, centred and scaled. Normality was assessed by Shapiro–Wilk's test and homogeneity of variance by Levene's test.

LA-ICP-MS data were treated similarly, but as separate measurements (core, edge) were not from independent fish, there were two key differences. First, we used a regression between core and edge measurements to determine the coefficient of determination ($R^2$) between samples. Second, instead of a linear model, a linear mixed-effects model (analogous to a repeated-measures ANOVA) was used to capture the variance within individual fish. Data were similarly Box–Cox transformed, centred and scaled, then tested for block within-block interactions with a Tukey test [residualPlots, car package (Fox & Weisberg, 2011), none of which were significant and therefore there was no evidence of such an interaction], assumptions of normality (Shapiro–Wilk's) and homogeneity of variance (Levene). For each Me:Ca, two models were compared using crossed factors EEZ, Species and Measurement, and then for each species separately, with only factors EEZ and Measurement. Models were compared using Akaike information criterion corrected for small sample size (AICc) values and this procedure was repeated for 24 and 32 μm LA-ICP-MS averaged data. To evaluate the attributes of the other types of averaged measurements, we ran similar linear mixed-effects models to compare 'total edge' and 'average edge' (both 24 μm). For the final comparison, we looked for spatial variation across the averaged data from the entire transect ('total load', 24 and 32 μm) for variation at the EEZ level only.

## 2.7 | Classification to EEZs for multiple stocks for two species

To assess how well the combined elemental concentrations were able to successfully classify membership to the correct EEZ, average concentrations of multiple elements were analysed using linear discriminant function analysis (LDFA) and multivariate analysis of variance (MANOVA). Discriminant function analysis maximizes the differences between groups using the standardized predictors (in this case average Me:Ca values), then predicted data were compared to the original discriminant function assignments to show where and if there were any misclassifications or commonly mistaken groups. In this study, classic discriminant function was preferable to the jack-knife cross-validation, which can be less accurate in calculating the resubstitution error with relatively small datasets (Moran, 1975; Zollanvari *et al.*,

**TABLE 2** Coefficient of variation for trace elements from solution-based and LA-ICP-MS methods for two species (*Etelis coruscans* and *Etelis boweni*) to compare the variability between measurements (samples from multiple Exclusive Economic Zones are pooled by method)

| | *Etelis coruscans* ($n = 18$) | | | | | | | | *Etelis boweni* ($n = 15$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Solution-based ICP-MS | | LA-ICP-MS (24 μm) | | | LA-ICP-MS (32 μm) | | | Solution-based ICP-MS | | LA-ICP-MS (24 μm) | | | LA-ICP-MS (32 μm) | | |
| | Core | Whole | Average core | Average edge | Total edge | Core | Edge | Total load | Core | Whole | Core | Edge | Total edge | Total load | Core | Edge | Total load |
| Ba:Ca | 52.3 | 15.3 | 44.5 | 26.1 | 34.4 | 27.3 | 24.0 | 20.4 | 19.6 | 43.2 | 91.9 | 40.7 | 43.4 | 35.8 | 61.4 | 29.3 | 26.9 |
| Sr:Ca | 9.9 | 14.7 | 16.6 | 22.4 | 25.6 | 13.5 | 22.0 | 6.08 | 10.5 | 21.9 | 11.9 | 24.1 | 22.4 | 17.2 | 11.5 | 19.9 | 18.1 |
| Mg:Ca | 58.6 | 48.2 | 78.5 | 56.8 | 50.7 | 47.7 | 50.4 | 39.5 | 40.0 | 50.1 | 25.7 | 27.7 | 22.0 | 17.1 | 31.7 | 44.8 | 23.8 |
| Mn:Ca | 22.4 | 17.5 | 56.6 | 38.2 | 80.3 | 37.7 | 29.9 | 28.5 | 12.7 | 38.6 | 66.9 | 59.3 | 66.2 | 61.8 | 54.7 | 74.0 | 55.7 |
| Li:Ca | | | 137.2 | 197.8 | 167.3 | 153.7 | 100.5 | 178.7 | 135.5 | | 26.7 | 30.0 | 29.1 | 22.0 | 49.7 | 33.9 | 33.0 |
| Fe:Ca | 4.6 | 1.1 | 113.5 | 59.8 | 41.4 | 55.1 | 103.5 | 30.1 | 46.4 | 2.7 | 71.1 | 55.2 | 59.0 | 58.7 | 44.4 | 66.5 | 56.8 |
| Cu:Ca | 66.2 | 25.8 | 118.4 | 138.0 | 69.5 | 74.3 | 84.5 | 49.4 | 66.4 | 88.1 | 28.0 | 26.5 | 46.5 | 21.3 | 34.8 | 37.2 | 30.1 |
| Ni:Ca | 60.5 | 41.9 | 52.0 | 51.1 | 66.3 | 47.1 | 40.8 | 37.7 | 40.4 | 47.2 | 19.6 | 39.9 | 25.4 | 18.6 | 31.8 | 34.3 | 24.2 |
| Zn:Ca | | | 144.8 | 76.1 | 59.1 | 101.8 | 180.3 | 78.5 | 95.5 | | 31.8 | 54.7 | 108.5 | 34.7 | 64.2 | 49.1 | 51.3 |

*Note*: ICP-MS, inductively coupled plasma mass spectrometry; LA-ICP-MS, laser ablation inductively coupled plasma mass spectrometry. Coefficient of variation values are shaded according to high values of variation (>80%, dark green), moderate (40%–80%, medium green) and low (<40%, light green).

**TABLE 3** Variation in solution-based ICP-MS otolith chemistry for two deepwater snapper species (*Etelis coruscans and Etelis boweni*)

| | | Both species | | | | *Etelis coruscans* | | | | *Etelis boweni* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Element | Source of Variation | Degrees of freedom (Df) | Mean squares (MS) | F value | *p* value | Df | MS | F | *p* value | Df | MS | F | *p* value |
| Ba:Ca | EEZ | 4 | 3.78 | 4.60 | <0.01** | 5 | 1.72 | 1.85 | 0.14 | 4 | 3.13 | 5.38 | <0.01** |
| | Core *vs.* whole | 1 | 0.15 | 0.19 | 0.67 | 1 | 0.50 | 0.54 | 0.47 | 1 | 3.03 | 5.18 | <0.05* |
| | Interaction | 4 | 0.66 | 0.81 | 0.53 | 5 | 0.74 | 0.80 | 0.56 | 4 | 0.42 | 0.72 | 0.59 |
| | Residual | 50 | 0.82 | | | 24 | 0.93 | | | 20 | 0.58 | | |
| Sr:Ca | EEZ | 4 | 3.79 | 5.38 | <0.01** | 5 | 3.18 | 7.66 | <0.001*** | 4 | 3.67 | 8.20 | <0.001*** |
| | Core *vs.* whole | 1 | 3.34 | 4.74 | 0.03 | 1 | 0.15 | 0.36 | 0.55 | 1 | 4.60 | 10.29 | <0.01** |
| | Interaction | 4 | 1.34 | 1.90 | 0.12 | 5 | 1.80 | 4.34 | <0.01** | 4 | 0.19 | 0.43 | 0.79 |
| | Residual | 50 | 0.70 | | | 24 | 0.42 | | | 20 | 0.45 | | |
| Mg:Ca | EEZ | 4 | 1.21 | 1.21 | 0.32 | 5 | 0.88 | 0.86 | 0.52 | 4 | 0.72 | 1.09 | 0.39 |
| | Core *vs.* whole | 1 | 1.86 | 1.86 | 0.18 | 1 | 0.63 | 0.61 | 0.44 | 1 | 9.37 | 14.13 | <0.01** |
| | Interaction | 4 | 0.56 | 0.55 | 0.70 | 5 | 1.05 | 1.02 | 0.43 | 4 | 0.87 | 1.32 | 0.30 |
| | Residual | 50 | 1.00 | | | 24 | 1.03 | | | 20 | 0.66 | | |
| Mn:Ca | EEZ | 4 | 2.49 | 3.33 | <0.05* | 5 | 2.41 | 7.85 | <0.001*** | 4 | 1.94 | 3.22 | <0.05* |
| | Core *vs.* whole | 1 | 8.87 | 11.87 | <0.01** | 1 | 10.61 | 34.52 | <0.001*** | 1 | 7.30 | 12.11 | <0.01** |
| | Interaction | 4 | 0.70 | 0.94 | 0.45 | 5 | 0.99 | 3.21 | <0.05* | 4 | 0.47 | 0.78 | 0.55 |
| | Residual | 50 | 0.75 | | | 24 | 0.31 | | | 20 | 0.60 | | |
| Cu:Ca | EEZ | 4 | 1.05 | 1.04 | 0.40 | 5 | 0.83 | 1.01 | 0.44 | 4 | 0.49 | 0.37 | 0.83 |
| | Core *vs.* whole | 1 | 0.53 | 0.52 | 0.47 | 1 | 4.75 | 5.75 | <0.05* | 1 | 0.46 | 0.35 | 0.56 |
| | Interaction | 4 | 0.88 | 0.87 | 0.49 | 5 | 1.25 | 1.52 | 0.22 | 4 | 0.02 | 0.01 | 1.00 |
| | Residual | 50 | 1.01 | | | 24 | 0.83 | | | 20 | 1.33 | | |
| Fe:Ca | EEZ | 4 | 1.24 | 1.82 | 0.14 | 5 | 1.36 | 25.71 | <0.001*** | 4 | 1.11 | 12.09 | <0.001*** |
| | Core *vs.* whole | 1 | 16.60 | 24.27 | <0.001*** | 1 | 22.14 | 417.34 | <0.001*** | 1 | 17.92 | 195.75 | <0.001*** |
| | Interaction | 4 | 0.81 | 1.18 | 0.33 | 5 | 0.95 | 17.99 | <0.001*** | 4 | 1.21 | 13.16 | <0.001*** |
| | Residual | 50 | 0.68 | | | 24 | 0.05 | | | 20 | 0.09 | | |
| Zn:Ca | EEZ | 4 | 4.03 | 5.42 | <0.01** | 5 | 2.23 | 5.01 | <0.01** | 4 | 1.37 | 1.19 | 0.34 |
| | Core *vs.* whole | 1 | 0.61 | 0.83 | 0.37 | 1 | 4.96 | 11.17 | <0.01** | 1 | 0.41 | 0.36 | 0.56 |
| | Interaction | 4 | 1.29 | 1.74 | 0.16 | 5 | 1.65 | 3.71 | <0.05* | 4 | 0.05 | 0.04 | 1.00 |
| | Residual | 50 | 0.74 | | | 24 | 0.44 | | | 20 | 1.15 | | |

*Note*: Combined univariate elemental concentrations for two species and also separate species elemental concentrations were analysed with a two-factor analysis of variance (ANOVA). Prior to ANOVA, data was Box–Cox transformed, centred and scaled. EEZ, Exclusive Economic Zone; ICP-MS, inductively coupled plasma mass spectrometry.

2009). LDFA outperforms machine-learning methods as long as parametric assumptions are met (Jones *et al.*, 2016). For all LDFA analyses, elemental concentrations that were multivariate normal and indicated no collinearity between pairs of elements were used as covariates (four to nine elements) with equal prior probabilities of class membership for all EEZs. Separate LDFAs were run for each group of samples (*i.e.*, core and whole solution-based ICP-MS, average core and average edge LA-ICP-MS samples for both 24 and 32 μm measurements, function lda in package MASS; Venables & Ripley, 2002). For each group, the predicted values were graphed by the first two linear discriminants and the between-group variance (proportion explained) was reported.

MANOVA tests the differences between linear combinations of multiple measured variables based on a variance–covariance

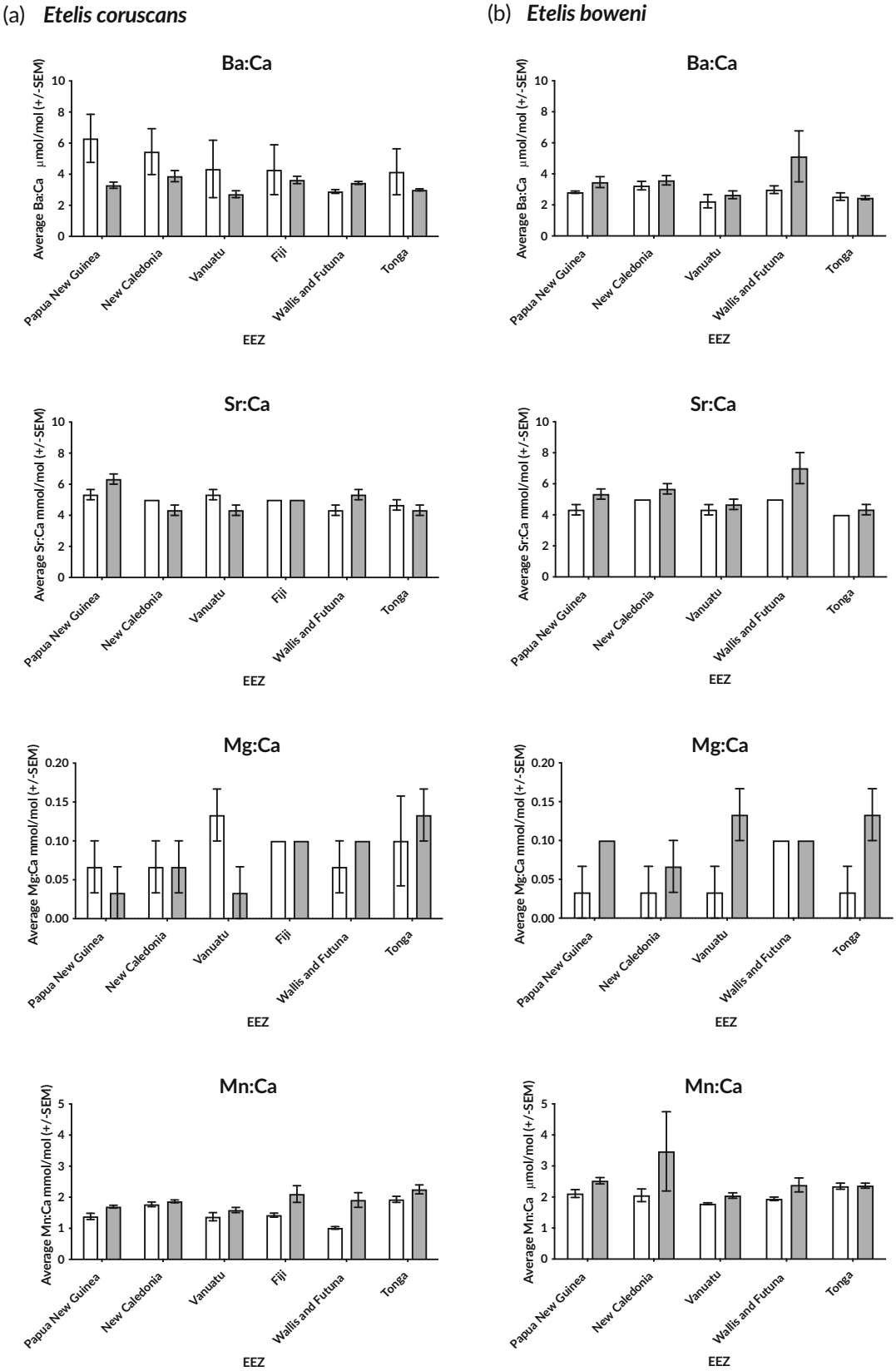


**FIGURE 3** Variation in trace metal concentrations for (a) *Etelis coruscans* and (b) *Etelis boweni* among multiple locations (six and five Exclusive Economic Zones, respectively) for selected elements Ba:Ca, Sr:Ca, Mg:Ca and Mn:Ca (mean concentration ± standard error of the mean) in solution-based ICP-MS whole otolith chemical analyses. There are no error bars where all three replicates had the same value. ☐ core; ▭ whole

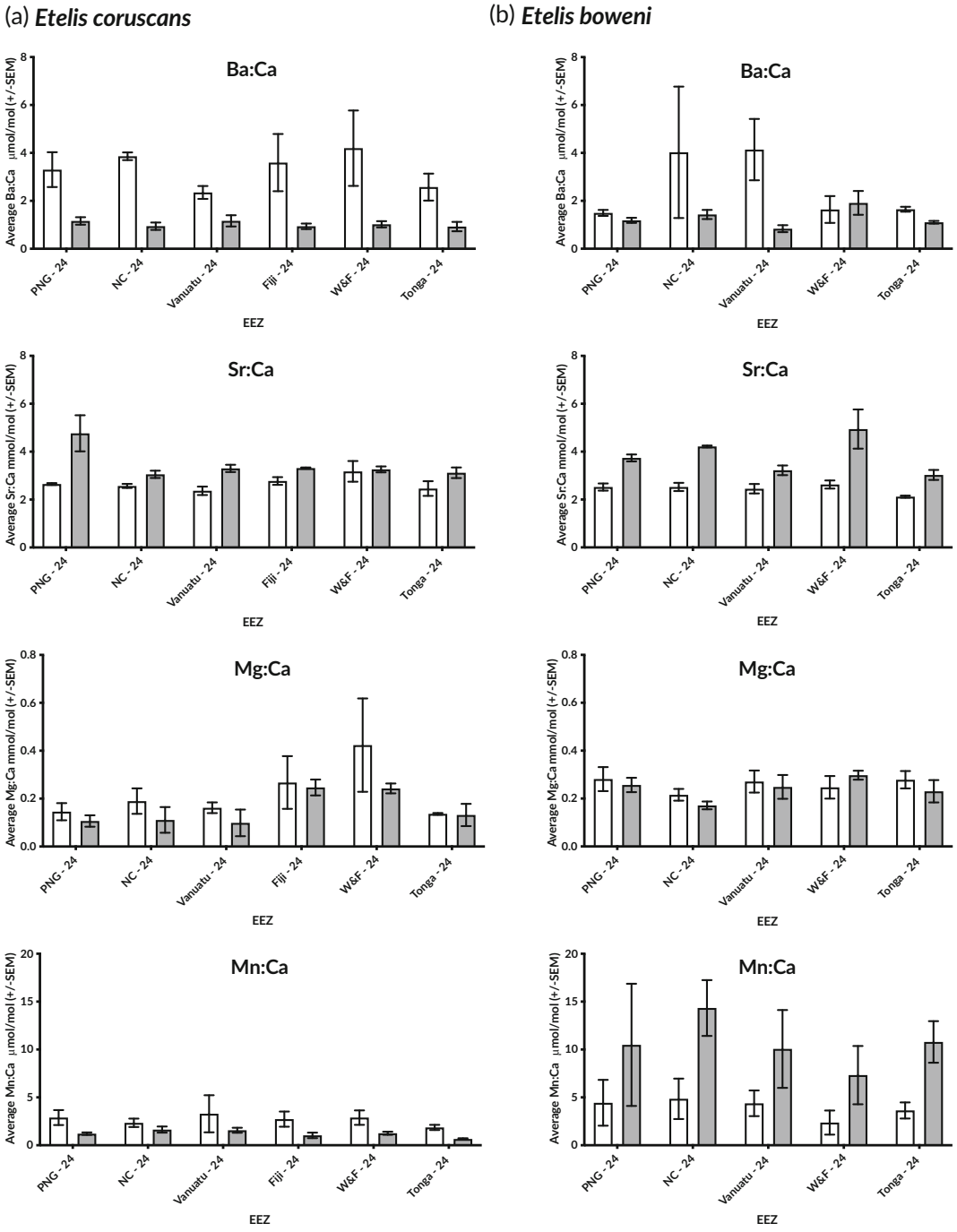


**FIGURE 4** Sampling across the otolith (core-to-edge; refer to Figure 2, location 4) showed distinct differences between species and capture locations and the magnitude of elemental concentration between average core (refer to Figure 2, location 1) and edge (refer to Figure 2, location 2) LA-ICP-MS (24 µm) measurements for two species of deepwater snapper (*Etelis coruscans* and *Etelis boweni*). ▭ average core; ▭ average edge

matrix. MANOVA determines where there are significant differences between the main effects and interactions of the independent variables (univariate analyses) as well as the importance of the dependent variable. Individual MANOVAs were run according to measurement type, with the same number of covariates (four to nine elements) as the corresponding LDFA. For MANOVA, Pillai's test statistic is considered the most robust and powerful to detect multivariate differences and provides a highly conservative F-statistic (Olson, 1974).

## 3 | RESULTS

There were clear differences in variation among all samples regardless of location for both methods (solution-based ICP-MS and LA-ICP-MS) and this pattern was consistent between species. Furthermore, among-sample variability was similar across all methods (Table 2). *E. coruscans* had greater variability among otolith samples for both methods. Fe:Ca, Zn:Ca, Cu:Ca and Li:Ca demonstrated the highest

**TABLE 4** Variation in laser ablation inductively coupled plasma mass spectrometry otolith chemistry for two deepwater snappers *Etelis coruscans* and *Etelis boweni*

| | Both species | | | | | *Etelis coruscans* | | | | | *Etelis boweni* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Element** | **Source of variation** | **Degrees of freedom (Df)** | **Mean squares (MS)** | ***F* value** | ***p value*** | **Source of variation** | **Df** | **MS** | ***F* value** | ***p value*** | **Df** | **MS** | ***F* value** | ***p value*** |
| Ba:Ca | EEZ | 4,20 | 0.28 | 0.68 | 0.61 | EEZ | 5,12 | 0.14 | 0.52 | 0.75 | 4,10 | 0.23 | 0.40 | 0.80 |
| | Measurement | 1,20 | 27.68 | 66.47 | **<0.001***** | Measurement | 1,12 | 26.72 | 96.59 | **<0.001***** | 1,10 | 6.47 | 11.20 | <0.01** |
| | Species | 1,20 | 0.32 | 0.77 | 0.39 | Interaction | 5,12 | 0.18 | 0.66 | 0.66 | 4,10 | 2.51 | 4.34 | <0.05* |
| | EEZ*Measurement | 4,20 | 0.61 | 1.46 | 0.25 | | | | | | | | | |
| | EEZ*Species | 4,20 | 0.15 | 0.37 | 0.83 | | | | | | | | | |
| | Measurement*Species | 1,20 | 4.13 | 9.92 | **<0.01*** | | | | | | | | | |
| | EEZ*Measurement*Species | 4,20 | 1.51 | 3.63 | <0.05* | | | | | | | | | |
| Sr:Ca | EEZ | 4,20 | 2.06 | 6.42 | **<0.01**** | EEZ | 5,12 | 1.11 | 2.34 | **0.11** | 4,10 | 1.29 | 6.46 | <0.01** |
| | Measurement | 1,20 | 31.16 | 97.19 | **<0.001***** | Measurement | 1,12 | 14.02 | 29.52 | **<0.001***** | 1,10 | 19.26 | 96.24 | **<0.001***** |
| | Species | 1,20 | 0.00 | 0.00 | 0.97 | Interaction | 5,12 | 0.80 | 1.69 | 0.21 | 4,10 | 0.14 | 0.71 | **0.60** |
| | EEZ*Measurement | 4,20 | 0.15 | 0.45 | 0.77 | | | | | | | | | |
| | EEZ*Species | 4,20 | 0.48 | 1.51 | 0.24 | | | | | | | | | |
| | Measurement*Species | 1,20 | 1.43 | 4.46 | **<0.05*** | | | | | | | | | |
| | EEZ*Measurement*Species | 4,20 | 0.71 | 2.22 | 0.10 | | | | | | | | | |
| Li:Ca | EEZ | 4,20 | 0.01 | 0.20 | 0.94 | EEZ | 5,12 | 0.02 | 0.06 | 1.00 | 4,10 | 0.08 | 0.19 | 0.94 |
| | Measurement | 1,20 | 0.31 | 5.92 | <0.05* | Measurement | 1,12 | 1.96 | 7.60 | **<0.05*** | 1,10 | 9.58 | 22.02 | **<0.001***** |
| | Species | 1,20 | 2.51 | 48.02 | **<0.001***** | Interaction | 5,12 | 0.54 | 2.09 | 0.14 | 4,10 | 0.39 | 0.90 | 0.50 |
| | EEZ*Measurement | 4,20 | 0.02 | 0.42 | 0.79 | | | | | | | | | |
| | EEZ*Species | 4,20 | 0.01 | 0.20 | 0.93 | | | | | | | | | |
| | Measurement*Species | 1,20 | 1.07 | 20.47 | **<0.001***** | | | | | | | | | |
| | EEZ*Measurement*Species | 4,20 | 0.15 | 2.93 | <0.05* | | | | | | | | | |
| Mg:Ca | EEZ | 4,20 | 1.13 | 2.58 | 0.07 | EEZ | 5,12 | 1.44 | 2.66 | 0.08 | 4,10 | 0.97 | 1.21 | 0.36 |
| | Measurement | 1,20 | 3.00 | 6.86 | **<0.05*** | Measurement | 1,12 | 2.98 | 5.49 | **<0.05*** | 1,10 | 0.55 | 0.69 | 0.42 |
| | Species | 1,20 | 6.22 | 14.21 | **<0.01**** | Interaction | 5,12 | 0.37 | 0.67 | 0.65 | 4,10 | 0.62 | 0.77 | **0.57** |
| | EEZ*Measurement | 4,20 | 0.16 | 0.37 | **0.82** | | | | | | | | | |
| | EEZ*Species | 4,20 | 0.77 | 1.76 | 0.18 | | | | | | | | | |
| | Measurement*Species | 1,20 | 1.35 | 3.08 | **0.09** | | | | | | | | | |
| | EEZ*Measurement*Species | 4,20 | 0.25 | 0.57 | 0.69 | | | | | | | | | |
| Mn:Ca | EEZ | 4,20 | 0.10 | 0.60 | 0.67 | EEZ | 5,12 | 0.82 | 1.30 | 0.33 | 4,10 | 0.03 | 0.49 | 0.74 |
| | Measurement | 1,20 | 0.51 | 3.20 | **0.09** | Measurement | 1,12 | 14.18 | 22.59 | **<0.001***** | 1,10 | 9.99 | 161.90 | <0.001*** |
| | Species | 1,20 | 4.27 | 26.66 | <0.001*** | Interaction | 5,12 | 0.33 | 0.53 | 0.75 | 4,10 | 0.11 | 1.83 | 0.20 |
| | EEZ*Measurement | 4,20 | 0.13 | 0.82 | 0.53 | | | | | | | | | |
| | EEZ*Species | 4,20 | 0.14 | 0.86 | 0.51 | | | | | | | | | |
| | Measurement*Species | 1,20 | 12.29 | 76.63 | **<0.001***** | | | | | | | | | |
| | EEZ*Measurement*Species | 4,20 | 0.13 | 0.81 | 0.53 | | | | | | | | | |

(Continues)

**TABLE 4** (Continued)

| | Both species | | | | | *Etelis coruscans* | | | | | *Etelis boweni* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Element | Source of variation | Degrees of freedom (Df) | Mean squares (MS) | *F* value | *p value* | Source of variation | Df | MS | *F* value | *p value* | Df | MS | *F* value | *p value* | |
| Cu:Ca | EEZ | 4,20 | 0.17 | 0.35 | 0.84 | EEZ | 5,12 | 0.15 | 0.31 | 0.90 | 4,10 | 0.58 | 0.61 | 0.66 | |
| | Measurement | 1,20 | 0.24 | 0.50 | 0.49 | Measurement | 1,12 | 0.20 | 0.43 | 0.52 | 1,10 | 0.00 | 0.00 | **0.95** | |
| | Species | 1,20 | 0.28 | 0.57 | 0.46 | Interaction | 5,12 | 0.35 | 0.73 | 0.62 | 4,10 | 0.47 | 0.50 | 0.74 | |
| | EEZ*Measurement | 4,20 | 0.56 | 1.16 | 0.36 | | | | | | | | | | |
| | EEZ*Species | 4,20 | 0.34 | 0.70 | 0.60 | | | | | | | | | | |
| | Measurement*Species | 1,20 | 0.21 | 0.43 | 0.52 | | | | | | | | | | |
| | EEZ*Measurement*Species | 4,20 | 0.23 | 0.47 | 0.75 | | | | | | | | | | |
| Fe:Ca | EEZ | 4,20 | 0.08 | 0.36 | 0.83 | EEZ | 5,12 | 0.55 | 0.66 | 0.66 | 4,10 | 0.02 | 0.26 | 0.90 | |
| | Measurement | 1,20 | 9.42 | 43.14 | **<0.001***** | Measurement | 1,12 | 2.01 | 4.86 | **<0.05*** | 1,10 | 17.20 | 192.71 | **<0.001***** | |
| | Species | 1,20 | 12.19 | 55.85 | **<0.001***** | Interaction | 5,12 | 0.69 | 0.83 | 0.55 | 4,10 | 0.09 | 0.97 | 0.46 | |
| | EEZ*Measurement | 4,20 | 0.28 | 1.30 | 0.31 | | | | | | | | | | |
| | EEZ*Species | 4,20 | 0.18 | 0.84 | 0.52 | | | | | | | | | | |
| | Measurement*Species | 1,20 | 1.63 | 7.45 | **<0.05*** | | | | | | | | | | |
| | EEZ*Measurement*Species | 4,20 | 0.18 | 0.81 | 0.54 | | | | | | | | | | |
| Ni:Ca | EEZ | 4,20 | 0.04 | 0.19 | 0.94 | EEZ | 5,12 | 0.06 | 0.14 | 0.98 | 4,10 | 0.50 | 0.61 | 0.67 | |
| | Measurement | 1,20 | 0.01 | 0.04 | 0.85 | Measurement | 1,12 | 0.06 | 0.13 | 0.72 | 1,10 | 0.00 | 0.00 | **0.95** | |
| | Species | 1,20 | 9.54 | 42.91 | **<0.001***** | Interaction | 5,12 | 0.32 | 0.74 | 0.61 | 4,10 | 0.68 | 0.83 | 0.54 | |
| | EEZ*Measurement | 4,20 | 0.07 | 0.34 | 0.85 | | | | | | | | | | |
| | EEZ*Species | 4,20 | 0.07 | 0.33 | 0.85 | | | | | | | | | | |
| | Measurement*Species | 1,20 | 0.05 | 0.24 | 0.63 | | | | | | | | | | |
| | EEZ*Measurement*Species | 4,20 | 0.38 | 1.73 | 0.18 | | | | | | | | | | |
| Zn:Ca | EEZ | 4,20 | 0.90 | 1.25 | 0.32 | EEZ | 5,12 | 0.73 | 0.79 | 0.58 | 4,10 | 0.23 | 0.40 | 0.81 | |
| | Measurement | 1,20 | 5.55 | 7.72 | **<0.05*** | Measurement | 1,12 | 2.51 | 2.73 | **0.12** | 1,10 | 2.71 | 4.73 | **0.05** | |
| | Species | 1,20 | 0.77 | 1.08 | **0.31** | Interaction | 5,12 | 0.82 | 0.89 | 0.52 | 4,10 | 0.23 | 0.40 | **0.80** | |
| | EEZ*Measurement | 4,20 | 0.82 | 1.15 | 0.36 | | | | | | | | | | |
| | EEZ*Species | 4,20 | 0.35 | 0.48 | 0.75 | | | | | | | | | | |
| | Measurement*Species | 1,20 | 0.45 | 0.62 | 0.44 | | | | | | | | | | |
| | EEZ*Measurement*Species | 4,20 | 0.74 | 1.03 | 0.42 | | | | | | | | | | |

*Note*: Combined univariate elemental concentrations for two species and also separate species elemental concentration ratios were analysed with linear mixed effects models for two otolith locations sampled from the LA-ICP-MS transect (average core, average edge). Data were Box–Cox transformed, centred, scaled and include Type III with estimated Kenward–Roger approximations for degrees of freedom. Values reported here are for 24 μm data and values in bold are significant for 32 μm data. EEZ, Exclusive Economic Zone; LA-ICP-MS, laser ablation inductively coupled plasma mass spectrometry.

variability among LA-ICP-MS samples, while some elements showed little variation among samples (Ba:Ca, Sr:Ca). In contrast, *E. boweni* had lower variability across all samples and elements, but the elements with the highest among-sample variability were Ba:Ca, Mn:Ca, Fe:Ca and Zn:Ca from LA-ICP-MS samples and Cu:Ca among solution-based ICP-MS otolith core samples.

The differences between methods were smaller than the differences between species and spatial patterns within each method, but there were very few notable differences. For some elements, such as Mn:Ca and Fe:Ca, solution-based analyses had lower core and whole elemental ratios than LA-ICP-MS measurements. For *E. boweni*, Mg:Ca and Ni:Ca had greater variability in solution-based measurements. Core measurements for both solution-based and LA-ICP-MS measurements were more variable than average edge or total edge measurements for some elements, but not consistently for both species, and these differences are explored in subsequent analyses.

## 3.1 | Investigating the effect of age

Few elements showed consistent evidence of a relationship with age, and the relationship was not consistent between species. Significant relationships were plotted (Supporting Information Figures S1 and S2), but $R^2$ values were low and ranged between 0.2 and 0.44 for univariate elements. For solution-based samples, Sr:Ca showed a slight positive relation with age in dissolved whole otolith measurements for both species ($p < 0.01$ for *E. coruscans* and *E. boweni*), with older individuals having higher concentration ratios. While this trend was consistent in LA-ICP-MS samples, the variation was also greater. Age effects in some cases have the potential to confound results for collections of fish from multiple locations, but in this case the results are inconclusive.

## 3.2 | Between-species variation and spatial variation: solution-based ICP-MS

Variation in Me:Ca ratios was detected among EEZs for both species, and differences in spatial discrimination were found between otolith core and whole otolith measurements analysed by solution-based ICP-MS. Both species showed some patterns of spatial variation of trace element ratios (Table 3 and Figure 3), but ranked values of ratios varied by species and section of the otolith for each element. There were some significant differences in Ba:Ca, Sr:Ca, Mn:Ca and Zn:Ca

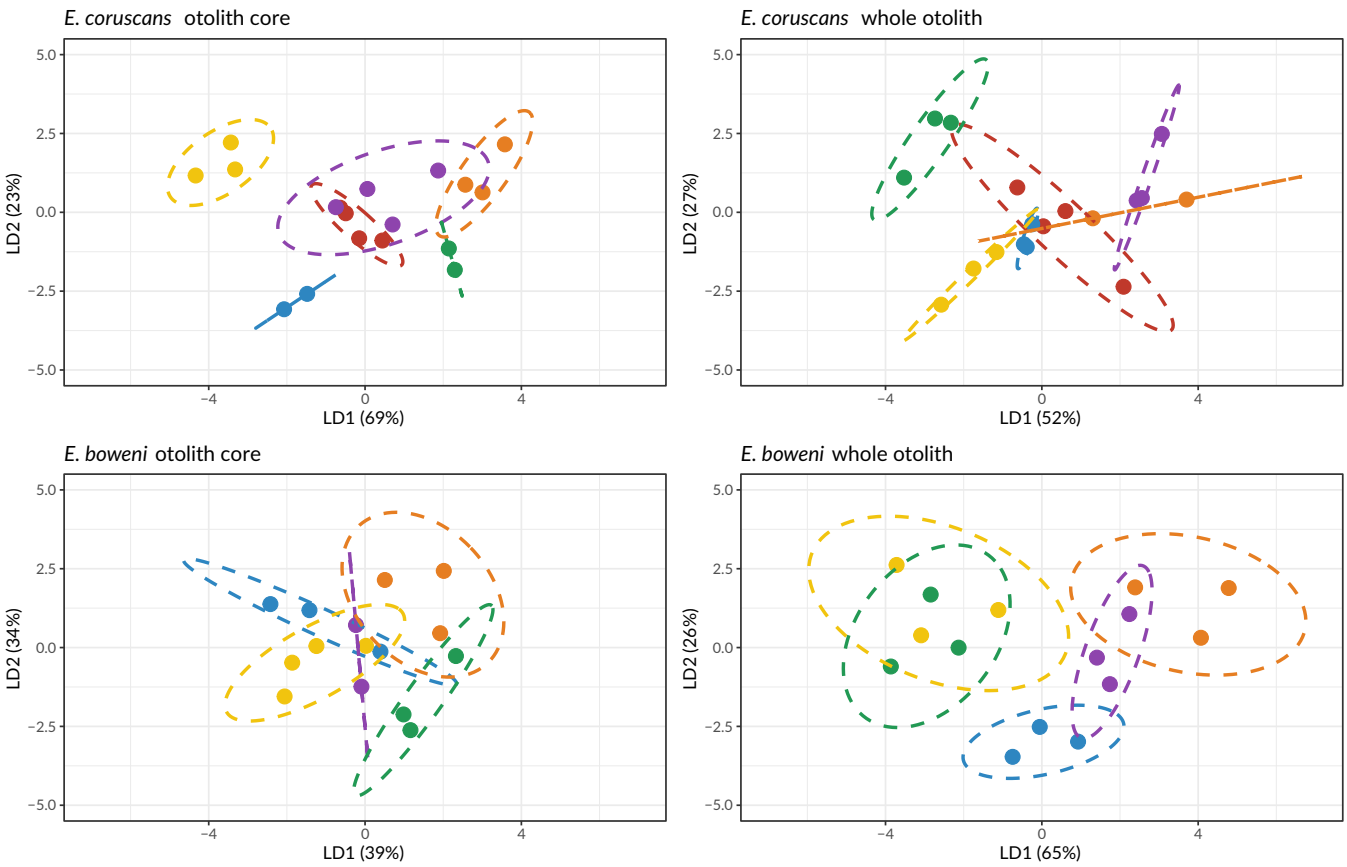


**FIGURE 5** Spatial separation of core (left) versus whole (right) otoliths resolved by solution-based ICP-MS for two species of eteline snappers (*Etelis coruscans* and *Etelis boweni*). Each plot shows predicted individual linear discriminant function scores incorporating trace elemental ratios, with separate Exclusive Economic Zone (EEZ) samples classified and 95% confidence ellipses showing the degree of overlap in elemental fingerprints. ●, Fiji (FJ); ●, Papua New Guinea (PG); ●, Vanuatu (VA); ●, New Caledonia (NC); ●, Tonga (TO); ●, Wallis and Futuna (WF)

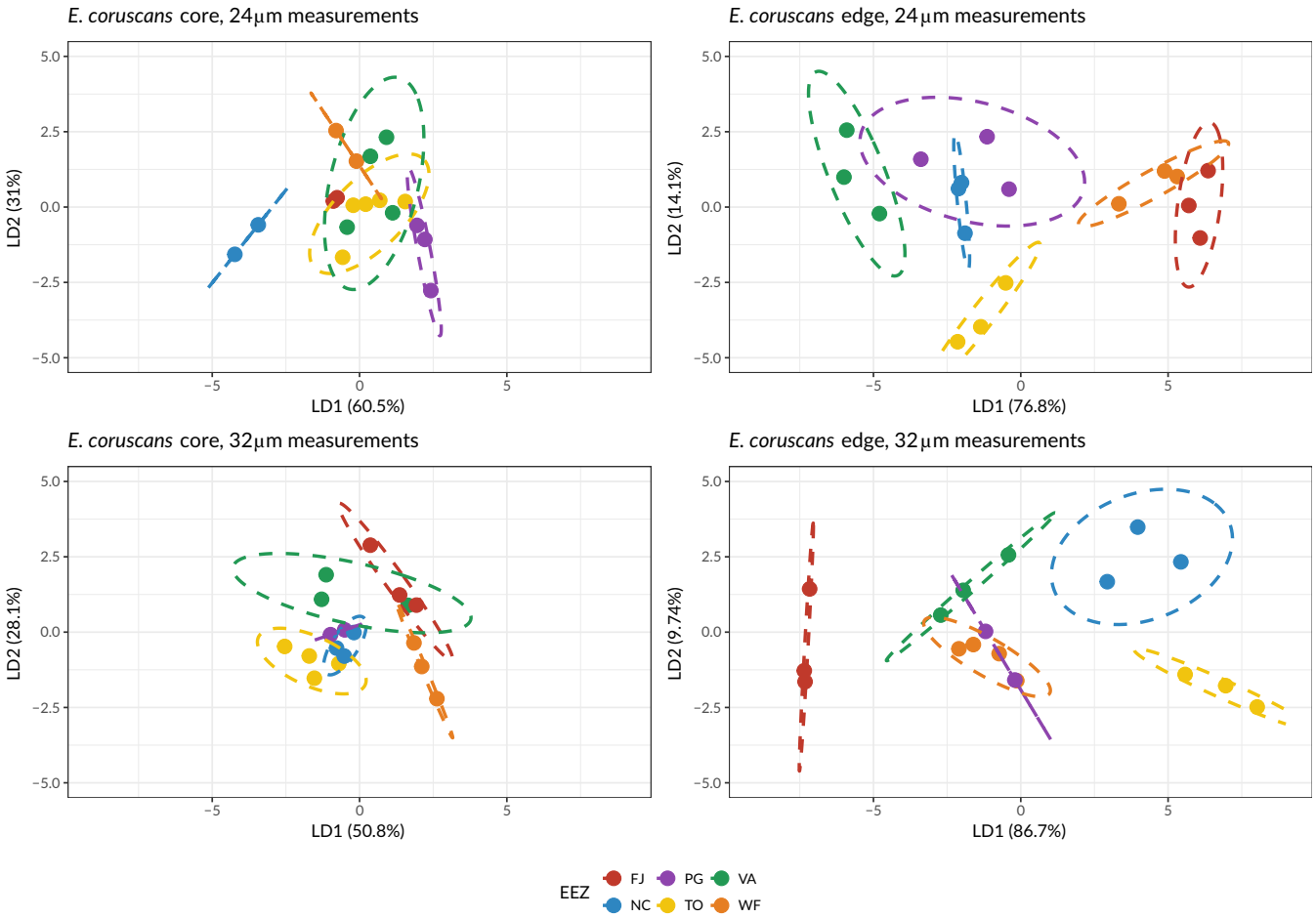


**FIGURE 6** Spatial separation of juvenile-core (left, refer to Figure 2, location 1) versus capture location-edge (right, refer to Figure 2, location 2) otoliths resolved by LA-ICP-MS for *Etelis coruscans*. Each plot shows separate linear discriminant function analyses incorporating trace elemental ratios of predicted group membership with separate Exclusive Economic Zone (EEZ) samples classified and 95% confidence ellipses showing the degree of overlap in elemental fingerprints. ●, Fiji (FJ); ●, Papua New Guinea (PG); ●, Vanuatu (VA); ●, New Caledonia (NC); ●, Tonga (TO); ●, Wallis and Futuna (WF)

among EEZs (two-way ANOVA). For instance, core samples from Vanuatu were significantly lower in Ba:Ca than New Caledonia (Tukey's HSD, $p_{adj} = 0.007$) and Papua New Guinea ($p_{adj} = 0.03$), samples from Papua New Guinea and Wallis and Futuna had significantly higher Sr:Ca than Tongan samples ($p_{adj} = 0.006$, $p_{adj} = 0.004$), while Vanuatu had lower Mn:Ca than Tonga ($p_{adj} = 0.04$).

Trace element concentrations of Mn:Ca and Fe:Ca were significantly higher in whole dissolved otoliths than in core samples from individuals collected from the same EEZ. No single elements varied significantly for the interaction of EEZ*Measurement area when samples from both species were combined, while a significant interaction was detected when species were analysed separately. The two-way fixed-factor ANOVA (EEZ*Measurement) demonstrated greater congruency between species for the elements Ba:Ca, Mg:Ca, Cu:Ca and Zn:Ca. Interestingly, some elements (Sr:Ca and Fe:Ca) may be incorporated differently by species. For these elements, the three-factor model (EEZ*Species*Measurement, not reported here) was the best-fit model with the lowest AICc values and the difference between models was highly significant.

For both species, there was significant variation between EEZs for most elements, and many elements had higher concentrations in the whole dissolved otolith than in dissolved cores. Where significant interactions existed, these were often caused by the rank of EEZ relative concentrations switching among core and whole samples.

## 3.3 | Ablation spot size and LA-ICP-MS discrimination

LA-ICP-MS transects for both species followed the same general pattern across locations for both ablation spot sizes, but there were differences in detection levels and magnitude (Figure 4, and Supporting Information Figures S3 and S4). The smaller ablation spot size (24 µm) had higher spatial resolution and slightly higher average concentrations than 32 µm measurements. For most elements, the differences between locations on the otolith (core versus edge) were consistent between the measurements. For some elements (*e.g.*, Mn:Ca) the differences between core and edge were

significantly different in magnitude for the smaller ablation spot size (Supporting Information Figures S3 and S4). Ablation datasets were longer for smaller ablation sizes, resulting in more data points than the larger laser ablation spot. As long as the detection of elements remains high, this may increase the detection of elemental variation spatially on the otolith.

### 3.4 | Between-species and spatial variation: LA-ICP-MS

Average core and edge LA-ICP-MS measurements showed clear differences among multiple elements, but these differed for the two species sampled. LA-ICP-MS showed the differences within the life-history transect (*i.e.*, the differences between core and edge) were greater than the spatial variation *per se* for the majority of univariate analyses (Table 4 and Figure 4). Overall, Ba:Ca and Mg:Ca showed consistently higher magnitude in the earlier life history, while more Sr:Ca was incorporated in the later life history for both species (Figure 4). Mg:Ca and Mn:Ca had higher concentration ratios for both species compared to solution-based ICP-MS samples (Figures 3 and 4), and *E. boweni* had higher Mn:Ca edge concentrations than *E. coruscans*. Several elements (Ba:Ca, Sr:Ca, Li:Ca, Mn:Ca, Fe:Ca) had significant interactions at the level of Measurement*Species, indicating that the differences in concentrations of these elements between the otolith core and edge were not consistent between species. The differences between the levels evaluated here (EEZ, averaged Measurements and Species) were mostly consistent between both ablation sizes. Coefficient of determination (or the proportion of the variance between core and edge measurements) assessed the independence of the measurements and revealed few strong or consistent correlations between 24 and 32 μm measurements (Supporting Information Table S1 and Figure S5). High coefficients may indicate that high or low core measurements produce corresponding high or low edge measurements.

Although the otolith chemistry along the edge of the otolith may show different spatial patterns, few differences in the placement of laser-ablated measurements for either species were observed (*i.e.*, Fe:Ca for *E. coruscans*, Fe:Ca and Mn:Ca for *E. boweni*; Supporting Information Table S2) when comparing the average edge measurement to the total edge (Figure 2; measurement 2 versus 3) showing overall congruency among the EEZ differences (Supporting Information Figures S6 and S7). Most differences between edge measurements were not significant and much smaller in magnitude compared to the differences between average core and average edge measurements.

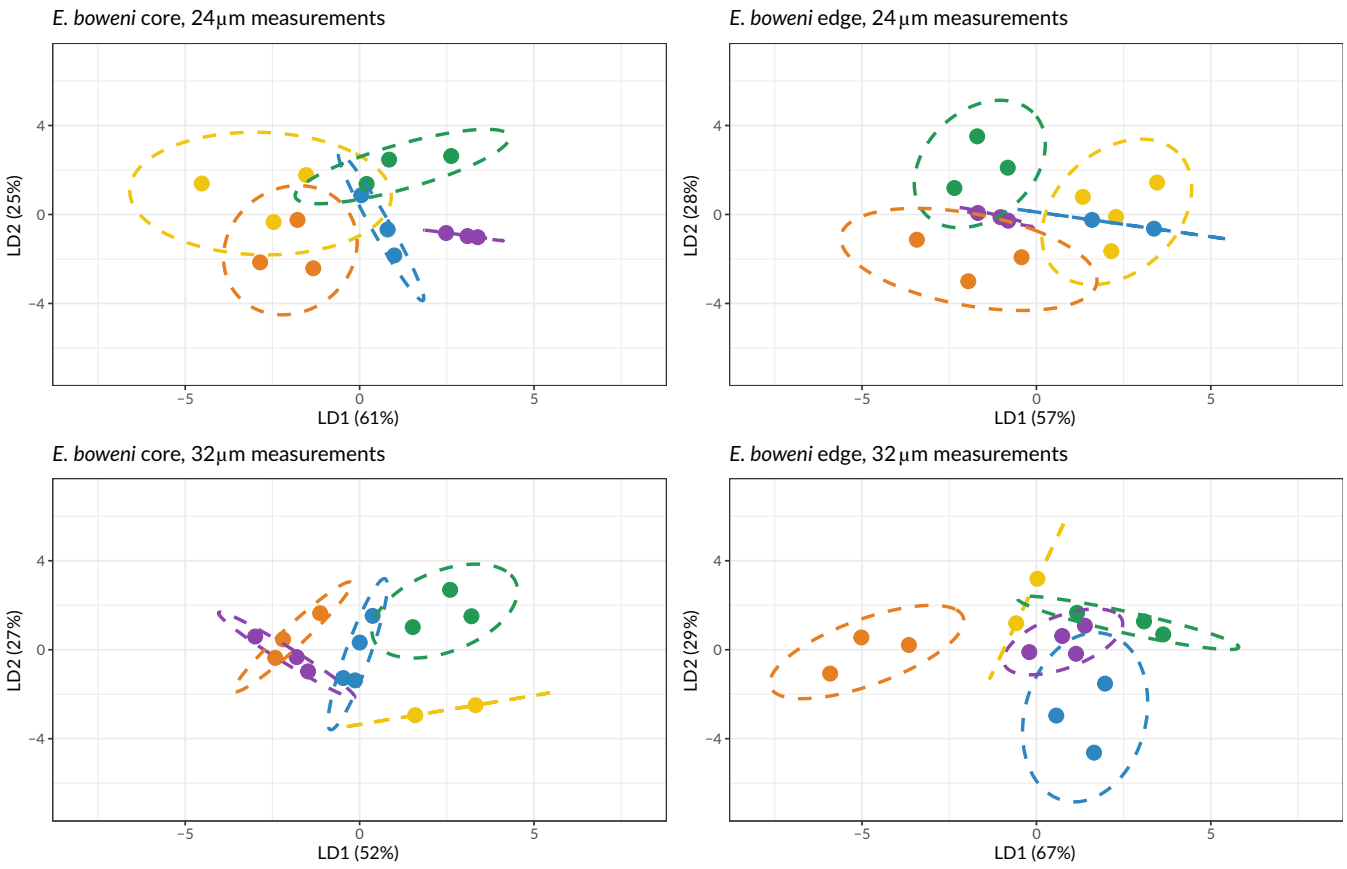


**FIGURE 7** Spatial discrimination of juvenile-core (left, refer to Figure 2, location 1) versus capture location-edge (right, refer to Figure 2, location 2) otoliths resolved by LA-ICP-MS for *Etelis boweni*. Each plot shows separate linear discriminant function analyses incorporating trace elemental ratios of predicted group membership with separate Exclusive Economic Zone (EEZ) samples classified and 95% confidence ellipses showing the degree of overlap in elemental fingerprints. ●, New Caledonia (NC); ●, Papua New Guinea (PG); ●, Tonga (TO); ●, Vanuatu (VA); ●, Wallis and Futuna (WF)

**TABLE 5** LDFA shows classification accuracy by multiple-element ICP-MS models

| Solution-based ICP-MS | | | Mardia's test[a] | Multivariate analysis of variance (MANOVA) | | | | | Linear discriminant function analysis (LDFA) | |
|---|---|---|---|---|---|---|---|---|---|---|
| Species | Sampling method | Elements included (#) | *p value* | Source of variation | Degrees of freedom (Df) | Pillai's test | Approx. *F value* (numerator Df/denominator Df) | *p value* | Elements (%) | Elements with age (%) |
| *Etelis coruscans* | Core | Ba, Mg, Mn, Zn (**4**) | 0.43 | EEZ | 5,12 | 2.03 | 2.48 (20/48) | **0.005 | 77.8 | |
| | Whole | Ba, Sr, Mg, Mn, Fe, Cu, Zn (**7**) | 0.45 | EEZ | 5,12 | 2.68 | 1.65 (35/50) | 0.05 | 83.3 | 88.9 |
| *Etelis boweni* | Core | Ba, Mg, Mn, Cu, Zn (**5**) | 0.86 | EEZ | 4,10 | 2.17 | 2.13 (20/36) | *0.02 | 93.3 | |
| | Whole | Ba, Mg, Mn, Fe, Cu, Zn (**6**) | 0.86 | EEZ | 4,10 | 2.46 | 2.13 (24/32) | *0.02 | 100 | 100 |
| LA-ICP-MS | | | | | | | | | | |
| *Etelis coruscans* | 24 μm – Total | Ba, Sr, Li, Mg, Mn, Fe, Zn (**7**) | 0.08 | EEZ | 5,12 | 2.12 | 1.08 (35/50) | 0.40 | 83.3 | 83.3 |
| | 24 μm – Core | Ba, Li, Mg, Mn, Fe, Ni (**6**) | 0.36 | EEZ | 5,12 | 1.77 | 1.00 (30/55) | 0.49 | 72.2 | |
| | 24 μm – Edge | Ba, Sr, Li, Mg, Mn, Fe, Ni, Cu, Zn (**9**) | 0.23 | EEZ | 5,12 | 2.91 | 1.24 (45/40) | 0.25 | 88.9 | 100 |
| | 32 μm – Total | Ba, Sr, Li, Mg, Mn, Fe, Ni, Cu, Zn (**9**) | 0.39 | EEZ | 5,12 | 2.66 | 1.01 (45/40) | 0.49 | 88.9 | 88.9 |
| | 32 μm – Core | Ba, Li, Mg, Mn, Fe, Ni, Cu, Zn (**8**) | 0.65 | EEZ | 5,12 | 1.96 | 0.72 (40/45) | 0.85 | 66.7 | |
| | 32 μm – Edge | Ba, Li, Mg, Mn, Fe, Ni, Cu, Zn (**8**) | 0.07 | EEZ | 5,12 | 2.56 | 1.18 (40/45) | 0.29 | 94.4 | 94.4 |
| *Etelis boweni* | 24 μm – Total | Ba, Sr, Li, Mg, Mn, Ni, Zn (**7**) | 0.82 | EEZ | 4,10 | 2.68 | 2.03 (28/28) | *0.03 | 100 | 100 |
| | 24 μm – Core | Ba, Sr, Mg, Mn, Ni, Cu, Zn (**7**) | 0.94 | EEZ | 4,10 | 2.48 | 1.63 (28/28) | 0.10 | 100 | |
| | 24 μm – Edge | Ba, Li, Mg, Mn, Ni, Cu, Zn (**7**) | 0.27 | EEZ | 4,10 | 2.45 | 1.58 (28/28) | 0.12 | 100 | 100 |
| | 32 μm – Total | Ba, Sr, Mg, Mn, Zn (**5**) | 0.57 | EEZ | 4,10 | 1.79 | 1.46 (20/36) | 0.16 | 80.0 | 80.0 |
| | 32 μm – Core | Ba, Sr, Li, Mg, Mn, Fe, Ni, Zn (**8**) | 0.56 | EEZ | 4,10 | 2.40 | 1.12 (32/24) | 0.39 | 93.3 | |
| | 32 μm – Edge | Ba, Sr, Mg, Mn, Cu, Zn (**6**) | 0.12 | EEZ | 4,10 | 2.13 | 1.52 (24/32) | 0.13 | 93.3 | 93.3 |

*Note*: Two sampling methods were compared for spatial separation and resolution: solution-based ICP-MS and LA-ICP-MS. Further comparisons included core or whole (solution-based ICP-MS), and aperture of the laser ablation mask and the location of the measurement from the otolith transect (LA-ICP-MS). Both solution-based and LA-ICP-MS measurements for two deepwater snapper species (*Etelis coruscans* and *Etelis boweni*) show the classification percentage to the correct EEZ. Age of the specimen was included as a covariate for some of the LDFA models to see if classification accuracy changed. Elemental measurements were Box–Cox transformed, scaled and centred. Elements included in the models conformed with multivariate normality, elemental ratios were assumed independent and certain elements were removed if highly correlated (Pearson's $r > 0.7$). EEZ, Exclusive Economic Zone; ICP-MS, inductively coupled plasma mass spectrometry; LA-ICP-MS, laser ablation inductively coupled plasma mass spectrometry; LDFA, linear discriminant function analyses.

[a]Mardia's test for multivariate normality was adjusted for small samples ($n < 20$), nonsignificant values showed data were multivariate normal.

By testing if the position of the edge measurements affected comparisons, we could determine with greater confidence that temporal differences such as the year of capture or growth inconsistencies are not masking the spatial resolution. These results indicate that the edge measurement differences were not consequential to the interpretation of edge otolith chemistry for spatial discrimination at this scale.

The differences within the life-history transects were better for spatial separation than the average of the entire transect ('total load'), which showed no significant separation for most elements among the EEZs investigated (Supporting Information Table S3 and Figure S8). Similar to the dissolution of the whole otolith in solution-based ICP-MS, the effect of averaging 150 data points may diminish the ability to detect differences, and variation in the life history may be better spatially resolved by separate measurements.

### 3.5 | Elemental fingerprints by EEZ

Both solution-based ICP-MS and LA-ICP-MS methods detected variation in elemental fingerprints, but the patterns were not consistent between species or methods. Solution-based ICP-MS showed more overlap between EEZs for core samples than whole otoliths for *E. boweni* than for *E. coruscans* (Figure 5) with linear discriminants 1 and 2 combined describing 72.8%–91.9% of the multivariate variance. For *E. coruscans*, whole otolith samples indicated that Vanuatu was separate from other locations, and core measurements indicated that Tonga and New Caledonia samples were separate from other groups. Whole otolith samples of *E. boweni* indicated two separate groups, with Tonga and Vanuatu sharing greater similarities in otolith chemistry than Papua New Guinea, New Caledonia, and Wallis and Futuna, which shared some overlap in chemical composition. In contrast, the elemental compositions of the otolith cores did not differ among EEZ locations for *E. boweni*.

LA-ICP-MS methods generally yielded similar results to solution-based ICP-MS, with considerably more overlap in average core samples than average edge samples, and the first two linear discriminants accounted for 78.9%–96.4% of the information for *E. coruscans* (Figure 6) and 79.1%–96.2% for *E. boweni* (Figure 7). There were few consistent differences in LDFAs comparing 24 and 32 μm ablation sizes, but there was clearer separation along LD1 for *E. coruscans* evident in these small sample sizes for both ablation sizes. This may be interpreted as Tonga and Fiji having more distinct stocks for *E. coruscans*, and Wallis and Futuna more clearly separated from other EEZs for *E. boweni*.

Greater classification accuracy was achieved with LA-ICP-MS, but both solution-based and LA-ICP-MS analyses yielded high classification accuracy (Table 5), with classification success ranging from 67% to 100%. In general, LA-ICP-MS models included more elements and performed slightly better than solution-based comparisons. Models that incorporated age as a covariate had marginal improvement on the model's predictive ability, often not changing the classification accuracy. The average edge LA-ICP-MS measurements had the greatest classification accuracy (89%–100%), while average core had the overall lowest (67%–100%). There were some minor differences with ablation size, but these were smaller differences in accuracy than between models of different measurements.

Multivariate analysis of variance (MANOVA) results indicated few significant differences among the measurements sampled. Both core and whole samples for *E. boweni* and core samples for *E. coruscans* were significantly different among solution-based ICP-MS comparisons. For almost all LA-ICP-MS samples, MANOVA results proved to be poor in resolving differences among EEZs for LA-ICP-MS methods, and only average total load measurements were significantly different among EEZs for the smaller ablation size for one species.

## 4 | DISCUSSION

The focus of the study was to determine the method that would give the best resolution of differences in elemental chemistry, which could assist with the stock discrimination of two species of deepwater snappers. There were significant differences in the otolith chemistry between species caught in different locations, which may be indicative of geographic heterogeneity among EEZs. This study provides initial evidence that geochemical signatures may be used to distinguish the spatial structure within metapopulations for deepwater fish species over a broad region in the Pacific. The important finding that otolith chemistry varies between closely related species in the same environment emphasizes the importance of accounting for species-specific variability in metapopulation structure when evaluating stock structure for multiple species within a single fishery. Furthermore, the differences between areas sampled on the otolith, representing various life-history stages, varied significantly within an individual, so care must be taken to further resolve how these differences in life history are reflected when using otolith chemistry to delineate stock boundaries. For regional stock identification of deepwater snapper, multivariate fingerprints for both solution and laser-based ICP-MS methods discriminated among fish caught from six Pacific Island nations. This may be due to microhabitat differences between species (benthic versus nektonic for adult *E. coruscans* and *E. boweni*, respectively) that influence diet and growth.

The observed differences in otolith chemistry may not be solely due to spatial differences, as there are a number of potentially confounding factors that were not controlled for in this study. For example, otoliths were collected over a 3-year period, which may have introduced additional variability among individuals. Simultaneous sampling of otoliths over the spatial scale of this study would be desirable and would have minimized the possible confounding factor of time. However, such simultaneous sampling is very difficult for these relatively remote fisheries with limited resources for research. Further sampling of contextual information, such as water chemistry, over the same spatial scale as otoliths were collected would have improved our understanding of deepwater environments and could have been correlated with otolith chemistry. Nevertheless, this study provided important information that allowed us to compare different methods, which might be useful for species from lesser known ecosystems.

There are relative advantages and disadvantages to using solution or laser-based ICP-MS methods, which should be carefully considered when designing studies for stock discrimination. Solution-based methods may be faster for large sample sizes (*e.g.*, Kingsford *et al.*, 2009) and between locations where chemical signatures have clear differences, but the results may be coarser and lack temporal resolution of where elemental ratios differ along the otolith. This may limit the degree of interpretation and the questions solution-based methods can answer. Dissolving the whole or part of the otolith may conceal subtle differences and some trace elements (*e.g.*, Fe:Ca measurements were at or below detection limits for solution-based samples) that are in low concentrations and are limited to comparisons of elements measured in the certified reference material. An assumption of whole otolith analyses is that larval dispersal or seasonal adult migration (*i.e.*, stock-mixing) as a small part of the total otolith will not confound the signatures of discrete stocks (Thorrold and Swearer, 2009). Solution-based methods are considerably less demanding in analysis time and post-processing time but require fastidious laboratory preparation and protocols. The advantages of LA-ICP-MS include the ability to look at the patterns across the otolith transect, which when sampled from the core to the edge corresponds with the fish's lifespan. Transects are useful as otoliths are 'superior chronological records' (Kerr & Campana, 2014), with detailed and spatially refined results over a spectrum of spatial scales. Average edge measurements presumably sampled the last few years of life prior to capture and there may be inconsistent otolith growth around the edge, which has been found in other species (*e.g.*, snapper *Chrysophrys auratus* and sand flathead *Platycephalus bassensis*; Hamer & Jenkins, 2007). Post-processing LA-ICP-MS data is time-consuming, but transect patterns can confirm groups with different life histories (*e.g.*, Burns *et al.*, 2020; Secor *et al.*, 2001), strengthening the evidence that groups form different metapopulations.

While a wholly marine fish may not have the same magnitude of differences as fishes experiencing riverine or estuarine influences, average core and edge samples were sufficient to reveal some separation between locations. It is important to remember that otolith chemistry has limited interpretation on the temporal stability of stock structure, as even occasional movements into different environments may potentially introduce detectable differences into the otolith chemistry (Campana, 2005). However, we can infer that individuals with overlapping chemical signatures (*e.g.*, core signatures) come from more similar environments, which cannot definitively state, nor rule out, a common source population or different location origin with similar water chemistry (Campana, 2005). Otolith morphological studies of *E. boweni* have demonstrated that the otolith does not grow at a constant rate along all dimensions (Smith, 1992). It is important to maintain the same transect or sampling location for otolith chemical analyses, which was done in this study. Since fishery sampling can be limited year to year by funding and time, the edge comparison showed that the differences in edge measurements were less significant, meaning if multiple year-classes are sampled it would not affect the regional discrimination. The visualization of the transect from the core to the edge revealed how stable edge measurements are over time, therefore the 'edge' exhibits stable elemental ratios over several years before capture and is a useful area of the otolith for spatial resolution (Avigliano *et al.*, 2017; Campana, 2005; Tanner *et al.*, 2011). The implication for broad-range studies is that these methods can potentially be used over longer time-spans and multiple-year classes. In this study, we used a sampling window between 2012 and 2015 as variability over interannual time scales is an important consideration in otolith chemistry analyses (Walther & Thorrold, 2009). Resolution and classification accuracy may be improved with larger sample sizes and less coarse data reduction techniques (*i.e.*, averaging). Comparing differences in the ablation spot sizes was useful to know as the 'stretch' of data points is wider with the smaller ablation spot, therefore accentuating the temporal differences better, while also slightly increasing the magnitude of these measurements and detection of rarer elements. This can help in minimizing errors in assigning life-history stages with specific places along the otolith elemental transect, ideal for combining otolith chemistry and microstructure analyses (*e.g.*, Sih & Kingsford, 2015). Other comparisons of ablation spot size found ablation sizes (100 *vs.* 32 μm) had similar measured concentrations in the elements with strong signals (*i.e.*, Ba:Ca, Mn:Ca), however, and larger ablation size reduced some of the 'noise' for elements with weaker signals (*i.e.*, Cu:Ca, Limburg, 2018).

The magnitude of change between the 'core' and the rest of the otolith indicates the early life physiology or environment is different than later life stages for both of the species investigated. This may be useful in future studies to assess natal origin, to estimate larval dispersal distances and to generalize connectivity patterns. Deepwater snappers exhibit long pelagic larval stages (*e.g.*, *Pristipomoides* spp. 8–26 weeks; Leis & Lee, 1994; Moffitt & Parrish, 1996), which may explain the similarity in core signatures. As larvae and pelagic juveniles, deepwater snappers could be encountering more uniform conditions as they travel large distances with the currents for multiple months, resulting in highly overlapping elemental fingerprints.

We investigated the effects of age on otolith chemistry because age can affect the time of exposure to different water chemistry (Kerr & Campana, 2014) such that elemental concentrations vary with fish size (Edmonds *et al.*, 1989). We found inconclusive evidence for significant correlations between fish age and trace element concentrations in the otolith, but this should be investigated further. This may be due to small sample sizes and the confounding effects of pooling multiple locations where age, growth, size and environmental variation may occur. Otolith chemistry can vary at spatial scales of tens to hundreds of kilometres (Dorval *et al.*, 2005; Gillanders & Kingsford, 2000; Thorrold & Swearer, 2009) and temporal scales of seasons to years (Campana *et al.*, 2000; Gillanders, 2001) so it is important to design the study to avoid confounding spatial and temporal factors that can influence otolith chemistry. It would be a sensible precaution to test for age-related differences in whole otolith chemistry with larger sample sizes. Accordingly, should they arise, size-related effects on elemental signatures within stocks could be statistically removed (Campana, 2005). Recent studies have found sex-specific and regional growth differences for *E. carbunculus* (Williams *et al.*, 2017), which may affect some elements' incorporation. Differences in growth and reproduction should be included as an additional layer of information

in stock separation estimates as differences in demographics are important for metapopulation-based models. For instance, differences in growth may translate to differences in otolith chemistry. Also, for species where known spawning migrations occur (*e.g.*, eels, groupers), these movements may confound elemental signatures for individuals that have reached spawning age.

Overall, the between-species differences were smaller than the location differences in the multivariate fingerprints, meaning the patterns were similar over the same spatial scale for both species. Investigating the trace element composition of otoliths has broad implications for using otolith chemistry as 'natural tags' over regional spatial scales (thousands of kilometres) and mixed-species fisheries. Otolith chemistry has successfully been used to discriminate stocks of shallow-water and pelagic species over broad spatial scales, and over varying physical, chemical, latitudinal and longitudinal gradients. The results from this study indicate that otolith chemistry may discriminate among stocks of eteline snappers (or similar deepwater species), for which the data on movements and migrations are limited, and life-history transitions still remain key knowledge gaps. There will be spatial differences for each species, but if within species the physiology and responses to environmental factors vary, different elemental fingerprints will be detected for each species at different spatial scales.

Determining which elements offer the most discriminatory power is also important, as all elements can contribute to the whole elemental signature to resolve population structure, but individual elements incorporate differently into the otolith and the mechanisms behind this are still not well understood. Thresher and Proctor (2007) hypothesized that the ontogenetic variability in Sr would be due to behavioural and ecological factors; it provided clear differences in spatial structure despite the presumed homogeneity in the deep marine environment. Differences in growth rates may also influence Mg and Ba concentrations in fish otoliths [see Kerr & Campana (2014) for some examples]. Similarly, reproduction may influence elemental composition of otoliths (Fuiman & Hoff, 1995). This study indicates that elemental inclusion varies across the otolith but is not uniform in pattern for all the elements studied here. From LA-ICP-MS transects, Ba:Ca was often higher in earlier stages and Sr:Ca was higher in later stages. Where these changes occur along the transect may also point to important environmental or demographic changes in the life history of the fish. These important distinctions were not evident in dissolved otoliths because otolith material across all life stages is pooled into a single sample for analysis. Interspecific variation was also observed for Mn:Ca measurements, with *E. boweni* exhibiting higher concentrations than *E. coruscans*.

Future otolith chemistry studies for eteline snappers would benefit from incorporating some of the potential sources of variation affecting either water chemistry or physiology. A major assumption of this study was that factors driving the changes in otolith chemistry (*e.g.*, water chemistry, diet or the environmental history) would be sufficiently different spatially and relatively temporally stable for the period of capture locations analysed. Some elemental differences are expected to be species-specific due to diet or physiology (Sturrock *et al.*, 2014). If spatial effects are greater, then latitudinal, longitudinal or oceanographic mechanisms may have greater effect sizes than local factors. It was assumed that these species would be exposed to similar water chemistry and environmental conditions. However, it was not possible to collect water samples at the times and locations fish were collected to test this hypothesis. Furthermore, to be representative of the environment these fishes inhabit, water samples would have to be collected at great depths (>200 m for capture depths). Not much is known about variability in water chemistry at these depths and at spatial scales of hundred to thousands of kilometres in the Pacific, although it is presumed that local oceanographic processes (*e.g.*, nutrient upwelling) could be operating that may produce differences in water chemistry that are sufficient for discrimination. Diet may influence elemental signals (*e.g.*, Doubleday *et al.*, 2013; Sanchez-Jerez, 2002) and variation in food sources among EEZs may contribute to spatial variation in signatures, although in experiments diet often has less influence than water chemistry on element uptake (Walther & Thorrold, 2006). The information on species-specific diet of deepwater fish species is often summarized from limited samples at disparate locations, and not throughout the species' distribution (*e.g.*, Haight *et al.*, 1993; Parrish, 1987). Deepwater snappers are known to feed on a wide range of pelagic and benthic fish and invertebrate groups. Feeding studies in Hawaii indicate that *E. coruscans* and *E. carbunculus* are mainly piscivorous, while other deepwater species from the *Pristipomoides* genus primarily eat zooplankton (Haight *et al.*, 1993) and there is some evidence of diet-partitioning among *Pristipomoides* species in the Mariana Archipelago (Seki and Callahan, 1988). Only recently has *E. boweni* been distinguished from *E. carbunculus* (Andrews *et al.*, 2014, 2016). In Hawaii, where some of the trophic comparisons have been made, only *E. carbunculus* occurs, whereas *E. boweni* and *E. carbunculus* co-occur throughout the remainder of the Indo-Pacific distribution. There are considerable biological differences between these species (Williams *et al.*, 2017), so it is likely that there are physiological and dietary differences reflected in the otoliths between *E. coruscans* and *E. boweni* as well. Diet-based influences are expected to influence Ba and Sr in the otolith and are less likely to affect elements Mg, Mn, Ca and Cu (Kerr & Campana, 2014). There are also differences in the otolith chemistry based on the sex and age of the fish, which could be taken into account. Physiological controls regulating otolith uptake of elements found elements such as Mn, Cu, Zn, Sr and Ca were under greater physiological control while elements including Ba, Mg and Li were not as heavily regulated (Sturrock *et al.*, 2014). These differences may be important as recent demographic studies demonstrate subregional differences in maturity for the pygmy ruby snapper, *E. carbunculus*, caught from the Main Hawaiian Islands compared to the Northwest Hawaiian Islands, which may be due to environmental influences or differing fishing histories between the two fishery management areas (DeMartini, 2017).

We demonstrated that the otolith elemental chemistry can discriminate otolith chemical signatures among deepwater fishes from multiple EEZs. Both solution-based and laser ablation methods were capable of showing spatial differences in elemental fingerprints of two species of *Etelis* with high levels of classification accuracy. However, LA-ICP-MS methods had the added advantage of displaying multiple

life-history stages along a single transect, allowing for more detailed temporal resolution of elemental changes within individuals and multiple comparisons for classification to EEZ. This study provides initial evidence that there may be spatial separation of stocks among some EEZs, and this information may enhance management of eteline snapper fisheries in the Pacific. To facilitate future research on eteline snappers, the results from this study provide a protocol of methodology that can have broader applicability for investigating the stock structure of deepwater fishes.

## AUTHOR CONTRIBUTIONS

T.L.S., A.J.W. and M.J.K. conceived the study idea and sampling design. T.L.S. and Y.H. discussed the laboratory protocols. T.L.S. prepped otolith samples and ran the LA-ICP-MS analyses. Y.H. performed the solution-based analyses. T.L.S. completed the data post-processing and statistical analysis with feedback from A.J.W and M.J.K. T.L.S. wrote the manuscript draft and all authors contributed to the manuscript. Laboratory funding from grants to T.L.S. and M.J.K.

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