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# Analysis of vertebral chemistry to assess stock structure in a deep-sea shark, Etmopterus spinax

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**Abstract :**

Deep-sea sharks play a valuable ecological role helping maintain food web balance, yet they are vulnerable to commercial fishing because of slow growth rates and low reproductive capacity. Overfishing of sharks can heavily impact marine ecosystems and the fisheries these support. Knowledge of stock structure is integral to sustainable management of fisheries. The present study analysed vertebral chemistry using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) to assay concentrations of 7Li, 23Na, 24Mg, 55Mn, 59Co, 60Ni, 63Cu, 66Zn, 85Rb, 88Sr, 138Ba and 208Pb to assess stock structure in a deep-sea shark, Etmopterus spinax, in Norwegian and French waters. Few studies have applied this technique to elasmobranch vertebrae and the present study represents its first application to a deep-sea shark. Three stocks were identified at the regional scale off western Norway, southern Norway, and France. At finer spatial scales there was evidence of strong population mixing. Overall, the general pattern of stock structure outlined herein provides some indication of the spatial scales at which stocks should be viewed as distinct fisheries management units. The identification of an effective multi-element signature for distinguishing E. spinax stocks utilizing Sr, Ba, Mg, Zn and Pb and the methodological groundwork laid in the present study could also expedite future research into stock structure for E. spinax and deep-sea elasmobranchs more generally.

**Keywords** : deep-sea, LA-ICP-MS, shark, stock structure, vertebral chemistry

## INTRODUCTION

Deep-sea sharks perform a valuable ecological function maintaining the balance of food webs that support fisheries, however they are heavily impacted by sustained commercial fishing pressure (Neiva et al., 2006; Xavier et al., 2012). These species are slow growing and late maturing with low fecundities, limiting their capacity to rebound from population impacts such as overfishing (Coelho and Erzini, 2008; Simpfendorfer and Kyne, 2009). Information about their biology and habitat use would be useful to inform management, but is limited due to logistical difficulties in studying live specimens associated with the great depths at which they live (Neiva et al., 2006).

The reproductive capacity of deep-sea squalid sharks such as *Etmopterus spinax* is often constrained by low fecundity and long reproductive cycles, making them particularly vulnerable to population impacts such as overfishing and requiring effective management. A prerequisite for effective management is to define stock boundaries to delimit harvestable units and determine spatial scales at which fisheries can best be managed (Fowler et al., 2005; Haddon, 2007; Secor, 2013). However, little is known about population structuring in deep-sea sharks (Veríssimo et al., 2011). This information is important for fisheries management where the delineation of stock boundaries provides a tool to distinguish groups of fish affected by stressors like fishing pressure and which recognises stock boundaries may not be contiguous units but rather comprise aggregations of spatially separated populations connected by migration (Haddon, 2007; Secor, 2013).

Migration in marine species can be assessed using artificial, genetic or chemical tags to trace movements between populations. Artificial tags are fastened to captured individuals before release and reveal movements upon recapture or via transmission to receivers. However, artificial tags are typically unsuitable for deep-sea species due to high mortality arising from rapid temperature or pressure changes involved in capture and release (Kyne and

Simpfendorfer, 2007). Genetic tags have also been widely applied to assess population/stock structure in marine species (e.g. Ovenden et al., 2015). However, genetic tags are more informative for long term gene flow patterns across generations (genetic connectivity) than movements of individuals at ecological timescales within generations (demographic connectivity); the latter are more informative for stock management (Hellberg et al., 2002; Thorrold et al., 2002).

Natural element and isotope tags (henceforth 'natural tags') are found in calcified body parts of aquatic organisms (e.g. otoliths of bony fishes, statoliths of cephalopods, shells of molluscs and vertebrae of elasmobranchs) (Campana, 1999). They comprise chemical signatures absorbed from the ambient environment and are stored in concentrations that can reflect environmental element loads (Thorrold et al., 2002; Gillanders, 2009). Differences in elements between areas can result from differences in nutrient input emerging from variation in tides, hydrology, underlying geology, precipitation, upwelling and terrestrial inputs (Elsdon et al., 2008). By incorporating elements from the surrounding environment in concentrations reflective of environmental exposure, natural tags can help identify groups of fish that spend time in waters of similar chemistry and inform about population boundaries, movements and population connectivity (Gillanders and Kingsford, 1996; Elsdon et al., 2008).

In contrast to highly crystallised aragonitic teleost otoliths, elasmobranch vertebral centra are composed of cartilaginous tissue surrounded by an extra-cellular matrix mineralised by crystals of calcium phosphate hydroxyapatite (Dean and Summers, 2006). The relatively poorly crystallised apatite of elasmobranch vertebral centra is not analogous to the highly crystallised aragonite of otoliths and therefore can be expected to behave differently. Nonetheless, apatite accretion of elasmobranch centra forms a permanently mineralised marginal crust that remains metabolically inert and unaltered throughout an individual's lifetime (Doyle, 1968; Clement, 1992); and is thus suitable for elemental analysis. In this way the apatite of elasmobranch centra differs from the transitional hydroxyapatite of teleost bone that is reworked (Clement, 1992; Ashhurst, 2004). This chemical stability of elasmobranch vertebrae is an important distinction, as earlier work suggested that elasmobranch centra do not comprise a 'closed' system (Welden et al., 1987); inferring the potential for chemical alteration through leaching etc. However, direct histological examination has found no evidence of reworking of vertebral material in elasmobranch centra (Clement, 1992) and the retention of vertebral bomb radiocarbon signatures throughout the lives of elasmobranchs (Campana et al., 2002) support the closed system hypothesis and suggest the suitability of these structures for elemental analyses (Hussey et al., 2012; Smith et al., 2013; Kerr and Campana, 2014).

Elasmobranch vertebral centra can incorporate trace elements via substitution of elements that are similar to calcium at concentrations that may reflect their abundance in the ambient environment (Edmonds et al., 1996; Tillett et al., 2011), however the exact mode of inclusion for particular elements requires further study. Studies involving synthetic hydroxyapatites and apatite of other marine taxa suggest the principal mode of inclusion is via direct substitution for Ca for elements including Ba (Wells et al., 2000), Cd (Bigi et al., 1991; Wells et al., 2000), Fe (Pon-On et al., 2008), Li (Mayer et al., 1986), Mg (Aoba et al., 1992), Mn (Pon-On et al., 2008), Pb (Bigi et al., 1991) and Sr (Schoenberg, 1963; Wells et al., 2000), while Zn is included through entrapment in interstitial spaces (Tang et al., 2009). Natural tags in elasmobranch vertebrae may be informative for assessing stock boundaries when assayed at the growing vertebral edge, whose chemistry corresponds to site of capture (Izzo et al., in press). Experimental evidence indicates such signatures may be accumulated in elasmobranchs after as little as three weeks residency in a particular area (Werry et al., 2011).

### *Ecology of E. spinax*

*Etmopterus spinax*, the velvet belly lanternshark, is a small, bioluminescent shark reaching around 50 cm total length (TL) and 11 years in age and inhabits the continental slope and shelf to depths of approximately 2200 m (Sion et al., 2004; Gennari and Scacco, 2007; Aranha et al., 2009). Currently listed as Near Threatened in the Northeast Atlantic (Coelho et al., 2009), *E. spinax* is a common bycatch species with the catch routinely discarded in commercial deep sea trawl and longline fisheries targeting species such as northern prawn (*Pandalus borealis*), Norway lobster (*Nephrops norvegicus*), red shrimp (*Aristeus antennatus*) and European hake (*Merluccius merluccius*) (Coelho and Erzini, 2008; Aranha et al., 2009). Commercial landings of velvet belly lanternshark have declined since the 2010 EU regulation of zero total allowable catch (TAC) came into force; however it is likely that discards have increased (ICES, 2014).

Little is known about the ecology or movements of *E. spinax* throughout its range. Diet differs among regions, although crustaceans, teleost fishes and cephalopods appear to form important components across the species range (Serena et al., 2006; Fanelli et al., 2009) and ontogenetic shifts in diet from crustaceans to teleosts and cephalopods have been reported (Neiva et al., 2006; Fanelli et al., 2009). Depth segregation is reported by size (and to a lesser extent by sex), with size increasing with depth (Massutí and Moranta, 2003; Serena et al., 2006). Juveniles are distributed in shallower waters that serve as nursery areas, while gravid females undertake pupping migrations into shallower waters and mature males and non-gravid females remain offshore (Sion et al., 2004; Coelho and Erzini, 2010). Females have been reported to dominate depths >600 m (Coelho and Erzini, 2010).

Late term gravid females occur in summer months (Coelho and Erzini, 2008; Aranha et al., 2009) and pups are born at around 9 cm TL with mean fecundity around 8 pups (Coelho and Erzini, 2008). Length at maturity has been recorded to vary among regions from

25 to 28 cm TL for males and 30 to 34 cm TL for females (Coelho et al., 2010). While sex ratio has been reported to favour females in the Atlantic (~2:1) (Coelho and Erzini, 2005; Aranha et al., 2009), they have been reported approximately equal in the Mediterranean up to 30 cm TL after which females dominate and reach greater lengths than males (Sion et al., 2004; Serena et al., 2006). The aplacental viviparous reproductive cycle may last 2 to 3 years with breeding thought to occur in winter months (Coelho and Erzini, 2008), when sex segregation could be expected to be less apparent as mature females mix with males in deep water breeding grounds.

### *Aims*

Elemental analysis of elasmobranch vertebrae to answer ecological questions is a relatively novel technique, used to assess stock structure in only two known studies to date (Schroeder et al., 2010; Izzo et al., in press), though it has been used more widely to investigate natal signatures (Tillett et al., 2011; Lewis et al., 2016) and as an environmental tracer (Werry et al., 2011; Scharer et al., 2012; Smith et al., 2013). The present study analysed vertebral chemistry of *Etmopterus spinax* as a means of investigating stock structure in a deep-sea shark for the first time, seeking to assess both temporal variation in elemental concentrations over sampling years and spatial variation among sampling sites and regions.

## MATERIALS AND METHODS

### *Specimen collection*

Specimens of *E. spinax* were collected as bycatch from annual demersal trawl surveys assessing French fish stocks (two locations in October-November 2013) and the Norwegian shrimp fishery (four locations in January-February 2014) (Fig. 1, Table 1). Additional samples from Langesund (Norway) were obtained from annual recreational fishing competitions in August 2012 and 2013 (Table 1). Samples obtained in these two years provided an opportunity to assess temporal variation in vertebral chemistry and therefore validate comparisons of elemental signatures collected over multiple years. A section ($n$ = 3 to 6) of pre-dorsal vertebrae were dissected and stored in ethanol. Where possible specimen total length (TL in cm) and sex (based on the presence of external sexual organs) were recorded (Table 1). Environmental data were only recorded for samples from the Norwegian shrimp survey and showed little variation (temperature ranged from 6 to 8°C and salinity was constant at ~35 ppt).

*Vertebral preparation*

Vertebral centra were separated and cleaned of adjoining tissue before being oven dried at 50$^o$C for 24 h (Fig. 2a). One vertebra per individual was embedded in an epoxy resin (Epofix, Struers) spiked with 40 ppm indium ($^{115}$In), which was used as a resin indicator when undertaking elemental analyses. Embedded vertebrae were sectioned sagittally into 500 µm thick sections using a low speed diamond saw (Isomet, Buehler) (Fig. 2b). Sections were wet polished using progressively finer grades of lapping film (30, 9 and 3 µm) before being rinsed in ultrapure water and air dried. Sections were then mounted onto glass microscope slides using In-spiked thermoplastic glue (Crystalbond™ 509). Slides were stored separately in snap lock bags and cleaned with ethanol before elemental analysis.

*Elemental analysis*

Vertebral element composition was quantified using an Agilent 7500cs inductively coupled plasma-mass spectrometer (ICP-MS) coupled to a New Wave Nd Yag 213 nm UV laser (housed at Adelaide Microscopy). Laser operating parameters were maintained throughout all ablations (see Supplementary materials Table S1). It was the intention of the present study to relate elemental signatures to age and so investigate life time elemental histories and population connectivity. However, vertebrae did not have visible age increments (Fig. 2), which made it impossible to relate elemental profiles to age with confidence, despite many efforts using various techniques in attempts to elicit age increments. Ablations therefore consisted of discrete (40 µm) transects at the vertebral edge and were assumed to represent the region of capture (Ashford et al., 2005).

Elements to be analysed were selected on the basis of use in previous studies investigating both vertebral chemistry in elasmobranchs and otolith chemistry in deep-sea bony fishes. Concentrations were measured for the following elements: $^{7}$Li, $^{23}$Na, $^{24}$Mg, $^{55}$Mn, $^{59}$Co, $^{60}$Ni, $^{63}$Cu, $^{66}$Zn, $^{85}$Rb, $^{88}$Sr, $^{138}$Ba and $^{203}$Pb. Concentrations of $^{43}$Ca and $^{115}$In were also measured to provide the basis of element:Ca ratios for statistical analysis and to exclude any non-vertebral material respectively.

National Institute of Standards and Technology (NIST) glass reference standard 612 (values given in Pearce et al., 1997) was ablated before, after and periodically throughout each ablation session to measure instrument drift and precision. All elements were within precision thresholds (coefficients of variation < 10%), with the exception of Na and Mn which were omitted from subsequent analyses. Raw count data were converted to elemental concentrations (in ppm) using the Glitter software program Version 3.0 (http://www.glitter-gemoc.com/) and normalised to Ca (in mmol mol$^{-1}$) in Microsoft Excel.

*Statistical analysis*

Data were quality filtered by removing outliers with elemental concentrations in excess of three standard deviations from the mean (McCune et al., 2002). Such outliers are commonplace in carbonate element analysis and may reflect instrumental noise rather than ecologically relevant values (Smith et al., 2013). In total, 13 values were identified as outliers and omitted. Element data were log($x$+1) transformed and fit to an Euclidean distance resemblance matrix using the Primer software program Version 6 (http://www.primer-e.com/). Element concentrations were analysed individually and as a multi-element signature using single factor permutational univariate and multivariate analyses of variance (ANOVA) respectively with site/region and gender as fixed factors (Anderson, 2001). For all tests, 4999 unrestricted permutations and Monte Carlo simulations of the data were performed.

Preliminary analyses indicated that vertebral chemistry did not differ among sharks caught in Langesund in 2012 and 2013 (see Supplementary materials Table S2). Hence, samples collected from all years were used in the spatial analyses (Table 1), with sampling site as a fixed factor. Where significant differences were found among sampling sites, *post hoc* pairwise *t*-tests were used to determine which sites differed. Although sex data were not complete for all datasets (Table 1), sex was investigated as a cofactor in spatial analyses where available ($n$ = 125).

For the multi-element signature, stepwise discriminant function analysis (DFA) was used to remove redundant elements contributing little discriminatory power using the SPSS

Statistics software package Version 20 ([www.ibm.com/software/au/analytics/spss/products/statistics/](www.ibm.com/software/au/analytics/spss/products/statistics/)). Canonical analysis of principle coordinates (CAP, Anderson and Willis, 2003), using a leave one out data fitting approach, was used to assess spatial discrimination among sampling sites. On the basis of CAP classification success and pairwise comparisons between sites, broader spatial regions sharing similar elemental signatures were identified. Spatial differences among regions were assessed using the same multivariate analysis of variance and multivariate discriminant analyses outlined above.

## RESULTS

### *Spatial variation among sampling sites*

The multi-element signature and the individual element:Ca ratios for Mg, Zn, Sr, Ba and Cu differed significantly among sampling sites (Table 2). *Post hoc* canonical analysis of principal coordinates (CAP) for the multi-element signature suggested the Brest (France), Biscay (France) and Bergen (Norway) sites differed from the other sampling sites, which generally overlapped (Fig. 3). Total correct classification of sites based on the multi-element signature was only 39%, however classification success differed among sites ranging from 0% at Skagerrak East to 60% at Biscay.

Mean element:Ca ratios for each site suggested Sr as a potentially useful indicator for spatial variation with high mean values for western Norwegian sites and lower concentrations in eastern Norway and France (Fig. 4). Variance in Mg:Ca, Zn:Ca and Cu:Ca was high (Fig. 4). Mean concentrations for Ba appeared similar among all sites except Biscay (France) in the far south of the study area, which had significantly lower Ba concentrations (Fig. 4).

*Post hoc* pairwise analyses revealed that Flekkefjord (Norway) differed from all other sampling sites for the multi-element signature and Mg:Ca, while differences between Flekkefjord and other sites for other elements were less uniform (see Supplementary materials Table S3, Fig. 4). Bergen (western Norway) differed from most sites for Zn:Ca and Sr:Ca. Langesund (eastern Norway) and both French sites were similar for Sr:Ca, however they differed from each other and most other sites for Cu:Ca (only Skagerrak West and Flekkefjord were similar to Langesund for Cu:Ca). In addition to Cu:Ca, the two French sites differed from each other only for Ba:Ca, whereby Biscay differed from all other sites for Ba:Ca.

There was no significant difference in the multi-element signature based on gender ($F(1,113) = 0.4$, $p = 0.63$) or interactions between site and gender ($F(5,113) = 1.6$, $p = 0.15$), indicating females had not spent more or less time within sites than males (i.e. they had taken on similar signatures).

### *Spatial variation among regions*

Based on results of fine scale spatial variation among sampling sites, three broad geographical regions were identified: Western Norway (Bergen: $n = 17$), Southern Norway (all other Norwegian sites (4): $n = 85$), and France (both French sites: $n = 43$). Element:Ca ratios differed significantly among regions for the multi-element signature and for each of Zn, Sr and Ba (Table 2, Fig. 5). Pairwise analyses indicated the multi-element signature and Zn:Ca differed among all regions with the exception of Southern Norway and France, Sr:Ca differed among all regions, and Ba:Ca differed between Southern Norway and France (Table 3, Fig. 5). At the regional scale the multi-element signature did not differ between sexes

($F(1,119) = 0.4$, $p = 0.61$), nor was there a significant interaction between region and gender ($F(2,119) = 2.4$, $p = 0.07$).

Stepwise omission of elements contributing no discriminatory power using DFA gave rise to a refined multi-element signature comprising Sr, Ba, Mg, Zn and Pb concentrations that accounted for 100% of modelled variation among samples. Total CAP classification success for the multi-element signature was greater (64%) for sampling regions than for individual sampling sites (39%), and overlap among sampling regions was reduced, though still apparent (Fig. 3). Classification to region of capture was: Western Norway = 47%; Southern Norway = 68%; France = 60%.

## DISCUSSION

Knowledge of stock structure is integral to determining appropriate spatial scales for fisheries management units (Compagno and Fowler, 2005; Haddon, 2007; Secor, 2013). Despite this, shark stock structures remain poorly understood. The present study suggests that trace element signatures in the vertebrae of *E. spinax* can be used to distinguish stocks of the species at regional scales.

### *Fine scale stock structuring*

Among sampling sites, the finest spatial scale assessed, sites that differed for the multi-element signature also differed for Mg:Ca, suggesting that Mg:Ca was the principal driver of differentiation at the fine scale. The multi-element signature was not powerful enough to distinguish most sampling sites at such fine scales and low classification success suggested considerable overlap among sampling sites within regions.

Magnesium is conservative in seawater, with its concentration varying with salinity (Quinby-Hunt and Turehian, 1983). The very low Mg:Ca for samples from Flekkefjord (Norway), which differentiated it from other sites, may thus result from its location at the mouth of a deep river-fed fjord. Magnesium has been used to trace movements of elasmobranchs along salinity gradients (Tillett et al., 2011; Werry et al., 2011) and is generally higher in freshwater than in seawater (McMahon et al., 2013). Magnesium values at Flekkefjord may therefore differ to other populations due to freshwater input from the nearby fjord driving down ambient Mg concentrations. It was also the shallowest site (mean depth: 252 m) for which depth data were available. The small mean body length of individuals from Flekkefjord (24 cm TL) and its relatively shallow depth may be suggestive of it being a nursery area, since depth related segregation has been reported in the species, with adults migrating to deeper waters and pregnant females moving into shallower waters to pup (Coelho et al., 2010).

There was considerable evidence for population mixing among sampling sites. For example, no individuals from Skagerrak East could be successfully classified to their location of capture . Low classification success has been attributed to population mixing in fish (Rooker et al., 2008; Geffen et al., 2011). Skagerrak East was the deepest Norwegian sampling site (mean depth: 493 m) and had the largest mean body length (35 cm TL) of sites sampled by trawl net for which a complete set of size data were available. The large mean length, near sexual parity (M:F 14:11, Table 1), greater depth and evidence for population mixing at Skagerrak East suggest that this may be a breeding area frequented by migrating adults. This is supported by the fact that *E. spinax* are thought to breed during winter months (Coelho and Erzini, 2008), which corresponds with the sampling period.

Sharks from Langesund had the largest mean body length (45 cm TL); however the collection method (angling) may have given rise to a size bias favouring larger individuals as has been recorded in comparisons of longline and trawl net sampling in related smooth lanternsharks, *Etmopterus pusillus* (Xavier et al., 2012). Further sampling may therefore be required to gain insights into demographic structure at Langesund that are representative of the entire population. There were however a number of large pregnant females ranging from 45 to 50 cm TL containing embryos, some of which were aborted post-capture. Since sampling at Langesund occurred in summer, when pupping may occur (Aranha et al., 2009), Langesund may be a pupping ground or a pre-pupping aggregating site for pregnant females; this is also a relatively shallow area (200-300 m) and gravid females have been found at shallower depths, potentially related to pupping movements (Coelho and Erzini, 2010).

### *Broad scale stock structuring*

Stock structure became more apparent at the broader regional scale. Previous studies have indicated wide variability in the spatial scales at which elemental signatures in calcified structures can be used to identify groups of fish (Gillanders, 2002; Bergenius et al., 2005; Smith, 2013). This may arise from factors including local geochemistry, oceanography, hydrology or terrestrial inputs influencing water chemistry in different ways at different spatial scales (Bergenius et al., 2005). The extent of variation in water chemistry will therefore determine the spatial scales at which elemental signatures differ, such that spatially significant differences may become more apparent at broader scales in relatively homogenous waters than in waters with steep chemistry gradients such as estuarine-marine transition zones.

While the use of elemental signatures at fine scales may be useful for assessing stock structure in sedentary, site-attached species, such as reef-dwelling fish (e.g. Bergenius et al., 2005), assessment of elemental signatures at broader regional scales may be more informative for stock structure in wider ranging species (Smith, 2013). In the present study, total classification success for the multi-element signature increased considerably at the regional scale compared to the fine scale among sampling sites. It was comparable to that recorded in other studies involving predominantly marine fish including the investigation of reef specific self-recruitment of neon damselfish (*Pomacentrus coelestis*) on the Great Barrier Reef (Patterson et al., 2004), natal homing and population mixing in bluefin tuna (*Thunnus thynnus*) during trans-Atlantic migrations (Rooker et al., 2008), and stock structure in adult Australasian snapper (*Pagrus auratus*) in South Australia (Fowler et al., 2005), lending support to the suitability of this method for assessing broad scale stock structure in *E. spinax*.

While it is not necessary to quantify the mineral sources and environmental influences that give rise to spatial variation in natural tags (it suffices that they are distinctly different among regions: Thorrold *et al.* 1998; Campana 2005), speculation on such drivers may be informative. In the present study Sr followed a declining trend from Western Norway > Southern Norway > France. Variation in Sr:Ca can indicate salinity gradients in estuarine-marine transition zones (Scharer et al., 2012), however in strictly marine environments Sr may be associated with deep water or upwelling (de Villiers, 1999). This stems from the life cycle of protozoan *Acantharia*, which dwell in the upper water column depleting it of Sr in the synthesis of celestite ($SrSO_4$) skeletons, with Sr remineralised at depth upon their decay (De Deckker, 2004). High Sr:Ca in Western Norway may therefore reflect upwelling in the exposed waters off western Norway driven by prevailing northerly winds (Helle, 1978; Asplin et al., 1999) potentially transporting Sr lateral to the coast, while Southern Norway is sheltered from these winds by land masses. Low Sr:Ca in French sharks may reflect low ambient Sr concentrations possibly driven by prevailing downwelling in the Bay of Biscay (Borja and Collins, 2004; Batifoulier et al., 2012).

High Ba concentrations have been associated with riverine plumes transporting terrestrial sediments or upwelling from areas where Ba enriched sediments have settled at depth (Kingsford and Gillanders, 2000; Elsdon and Gillanders, 2005). The high Ba:Ca in South Norway may be driven by the Baltic Current discharging through the Skagerrak, bringing brackish water from the Baltic Sea loaded with sediments of terrestrial origin from the many rivers feeding this basin (Sætre and Ljøen, 1972). Conversely, the sampling sites comprising the French region are exposed to a general poleward movement of warm slope water at depth originating along the Portuguese and North African coasts (Pingree and Le Cann, 1990; Pingree and Le Cann, 1992), which may contain less Ba of terrestrial origin than the Baltic Sea with its high freshwater input and may explain the low Ba:Ca in French sharks.

The three regional stocks suggested here were supported by DFA which refined the multi-element signature to five element:Ca ratios (Sr, Ba, Mg, Zn and Pb) describing 100% of modelled variation among stocks. Defining the drivers for regional differences in elemental signatures is complex due to the interaction of numerous environmental (e.g. geology, oceanography, hydrology or pollution) and biological variables (e.g. genotype, phenotype or condition) . Nevertheless, a pattern has emerged of three potential stocks (Western Norway, Southern Norway and France) at the regional level with evidence for juveniles showing a degree of site fidelity and considerable adult population mixing within regions.

### *Fisheries management implications*

In spite of a zero TAC management policy for *E. spinax* in French and Norwegian waters, discards are thought to have increased in recent years (ICES, 2014). Given the Near Threatened status of *E. spinax* in the Northeast Atlantic and its valuable ecological function, it may be timely to develop strategies to manage fisheries bycatch impacts on this species. The present study indicates stocks of *E. spinax* should be managed at the regional scale. In Norwegian waters in particular there is evidence for two potential stocks, one centred offshore from Bergen off Western Norway and one off Southern Norway, to which consideration should be given for independent management. In the broader context, the monitoring and management of *E. spinax* stocks at regional scales over hundreds of kilometres could give rise to issues of transnational cooperation in the management of this species (Curtin and Prellezo, 2010); however, more information is required about stock structure throughout the range of the species, particularly in the intervening space between Norway and France and further south into Portuguese and Mediterranean waters.

## *Conclusion*

The present study has shown vertebral chemistry analysis to be a promising technique to assess stock structure in the deep-sea elasmobranch, *E. spinax*. A multi-element signature assaying vertebral concentrations of Sr, Ba, Mg, Zn and Pb can be employed to discriminate stocks at regional scales with a relatively high degree of confidence comparable to that in other studies of marine species. In particular, the existence of three stocks is suggested in the area sampled: Western Norway, Southern Norway and France, suggesting that stocks should be managed at broad regional scales. At finer spatial scales this technique was less effective at distinguishing among sampling sites within these regions, potentially suggesting a high degree of population mixing. Potential future applications such as the mapping of nursery and breeding areas and assessment of their relative contributions to *E. spinax* recruitment could also be of assistance to fisheries managers in conserving stocks of this ecologically valuable, yet vulnerable species.

*Supplementary materials*

Supplementary material is available at the *ICESJMS* online version of the manuscript.

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**Table 1.** Summary of sampling information and biological data. Sampling site information includes: site name, site code (used herein), country, GPS coordinates, year of collection, mean depth (in m), and sample size (N). Biological data includes: sex ratio (Male:Female), mean total length (TL ± standard deviation) and minimum and maximum total lengths (TL range).

| **Site** | **Code** | **Country** | **Latitude** | **Longitude** | **Year** | **Depth** (m) | **N** | **Sex ratio** M:F | **TL (± SD)** (cm) | **TL range** (cm) |
|---|---|---|---|---|---|---|---|---|---|---|
| Bergen | Berg | Norway | 59°40'48.00"N | 4°6'30.00"E | 2014 | 269 | 17 | 8:9 | 22.5 (± 7.3) | 13–44 |
| Flekkefjord | Flek | Norway | 58°9'51.78"N | 6°32'35.07"E | 2014 | 252 | 9 | 5:4 | 24.3 (± 8.3) | 14–41 |
| Skagerrak West | SkaW | Norway | 57°44'3.88"N | 8°31'6.47"E | 2014 | 298 | 14 | 10:4 | 32.1 (± 6.7) | 19–43 |
| Skagerrak East | SkaE | Norway | 57°51'56.40"N | 9°8'34.12"E | 2014 | 493 | 25 | 14:11 | 35.2 (± 5.9) | 24–48 |
| Langesund | Lang | Norway | 58°44'31.12"N | 9°54'29.44"E | 2012 | 264† | 17 (15) | 7:8* | 44.1 (± 4.7)* | 39–51* |
| | | | | | 2013 | 264† | 20 (8) | 2:6* | 46.8 (± 3.7)* | 42–51* |
| Brest | Brest | France | 48°11'45.88"N | 8°25'86.64"W | 2013 | 412 | 23 (17) | 11/6 | 34.8 (± 3.7)* | 26–41* |
| Biscay | Bisc | France | 43°96'63.18"N | 2°15'87.11"W | 2013 | 496 | 20 | 9/11 | 31.1 (± 4.7)* | 21–39* |

† denotes depth data were unavailable and were acquired from http://www.geoplaner.com/ using GPS data.

* denotes incomplete dataset (bracketed N indicates samples for which data were available).

**Table 2**. Single-factor permutational ANOVA results comparing element:Ca concentrations in *E. spinax* vertebrae among sampling sites and regions. Multi = multi-element signature, Res = residual, df = degrees of freedom, MS = mean square, and P = probability. Significant differences are bolded.

| | **Site** | | | **Region** | | |
|---|---|---|---|---|---|---|
| **Element** | **df** | **MS** | **P** | **df** | **MS** | **P** |
| Multi | 6 | 2.714 | **<0.001** | 2 | 2.538 | **0.014** |
| Res | 138 | 0.606 | | 142 | 0.667 | |
| Li:Ca | 6 | <0.001 | 0.253 | 2 | <0.001 | 0.378 |
| Res | 138 | 0.001 | | 142 | <0.001 | |
| Mg:Ca | 6 | 2.056 | **<0.001** | 2 | 1.442 | 0.055 |
| Res | 138 | 0.422 | | 142 | 0.477 | |
| Co:Ca | 6 | <0.001 | 0.317 | 2 | <0.001 | 0.398 |
| Res | 138 | <0.001 | | 142 | <0.001 | |
| Ni:Ca | 6 | <0.001 | 0.258 | 2 | <0.001 | 0.61 |
| Res | 138 | <0.001 | | 142 | <0.001 | |
| Zn:Ca | 6 | 0.295 | **0.004** | 2 | 0.675 | **<0.001** |
| Res | 138 | 0.077 | | 142 | 0.077 | |
| Rb:Ca | 6 | <0.001 | 0.123 | 2 | <0.001 | 0.182 |
| Res | 138 | <0.001 | | 142 | <0.001 | |
| Sr:Ca | 6 | 0.162 | **<0.001** | 2 | 0.263 | **0.001** |
| Res | 138 | 0.035 | | 142 | 0.037 | |
| Ba:Ca | 6 | <0.001 | **0.001** | 2 | <0.001 | **<0.001** |
| Res | 138 | <0.001 | | 142 | <0.001 | |
| Pb:Ca | 6 | <0.001 | 0.721 | 2 | <0.001 | 0.239 |
| Res | 138 | <0.001 | | 142 | <0.001 | |
| Cu:Ca | 6 | 0.198 | **0.016** | 2 | 0.157 | 0.11 |
| Res | 138 | 0.007 | | 142 | 0.074 | |

**Table 3.** Pairwise comparisons between sampling regions based on element:Ca ratios in the vertebrae of *E. spinax* (refer to Fig. 5). Pairwise tests were conducted for the multi-element signature and individual elements whose concentrations differed among sites. Multi = multi-element signature, *t* = t value, and P = probability. Significant differences (P < 0.05) in element:Ca ratios between regions are bolded. Regions are Western Norway (WN), Southern Norway (SN), and France (F).

| **Element:Ca** | **Multi** | | **Zn:Ca** | | **Sr:Ca** | | **Ba:Ca** | |
|---|---|---|---|---|---|---|---|---|
| **Regions** | *t* | P | *t* | P | *t* | P | *t* | P |
| WN, SN | 2.298 | **0.016** | 3.464 | **0.002** | 2.089 | **0.038** | 1.012 | 0.317 |
| WN, F | 2.737 | **0.003** | 3.44 | **<0.001** | 4.447 | **<0.001** | 1.502 | 0.14 |
| SN, F | 0.866 | 0.413 | 0.478 | 0.64 | 2.368 | **0.02** | 3.727 | **<0.001** |

**Figure 1.** Map showing sites where samples were collected in Norwegian and French waters. Refer to Table 1 for detailed sampling information.

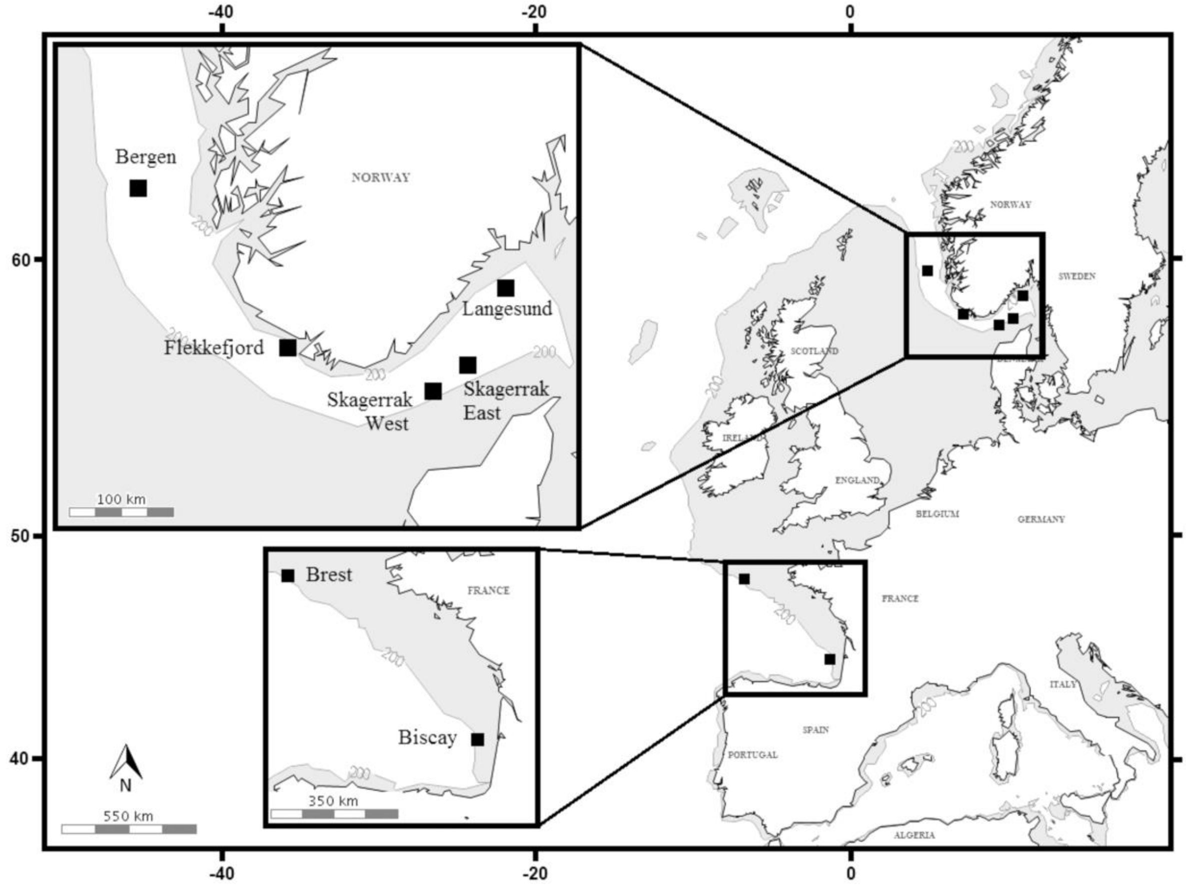
**Figure 2.** Whole vertebrae (A) were sectioned sagittally through the *centrum focus* for elemental analysis (B). Short transects at the edge (circled "*s*") were ablated to analyse elemental signatures from areas of most recent growth before capture.

**Figure 3.** Canonical analysis of principle coordinates (CAP) plot showing dissimilarity among sampling sites for the multi-element vertebral signature of *E. spinax*. French sites are grey, Bergen is solid black and remaining Norwegian sites are open. Refer to Table 1 for site codes.

**Figure 4.** Mean sampling site element:Ca ratios in the vertebrae of *E. spinax* (with standard errors) for: Mg (A), Zn (B), Sr (C), Ba (D), and Cu (E). Bars below *x*-axis indicate regional groupings. Letters above columns indicate similar means (*t*-test, $P < 0.05$) (Table S3). Note *y*-axis differs among all panels.

**Figure 5.** Mean sampling region element:Ca ratios in the vertebrae of *E. spinax* (with standard errors) for: Zn (A), Sr (B), and Ba (C). Letters above columns indicate similar means (*t*-test, $P < 0.05$) (Table 3). Note *y*-axis differs among all panels.

Fig. 1



-40
-20
0
60
50
40
Bergen
NORWAY
Langesund
Flekkefjord
200
Skagerrak West
Skagerrak East
100 km
Brest
FRANCE
200
Biscay
350 km
N
550 km
NORWAY
SWEDEN
SCOTLAND
DENMARK
IRELAND
ENGLAND
BELGIUM
GERMANY
FRANCE
ITALY
SPAIN
PORTUGAL
ALGERIA

Fig. 2

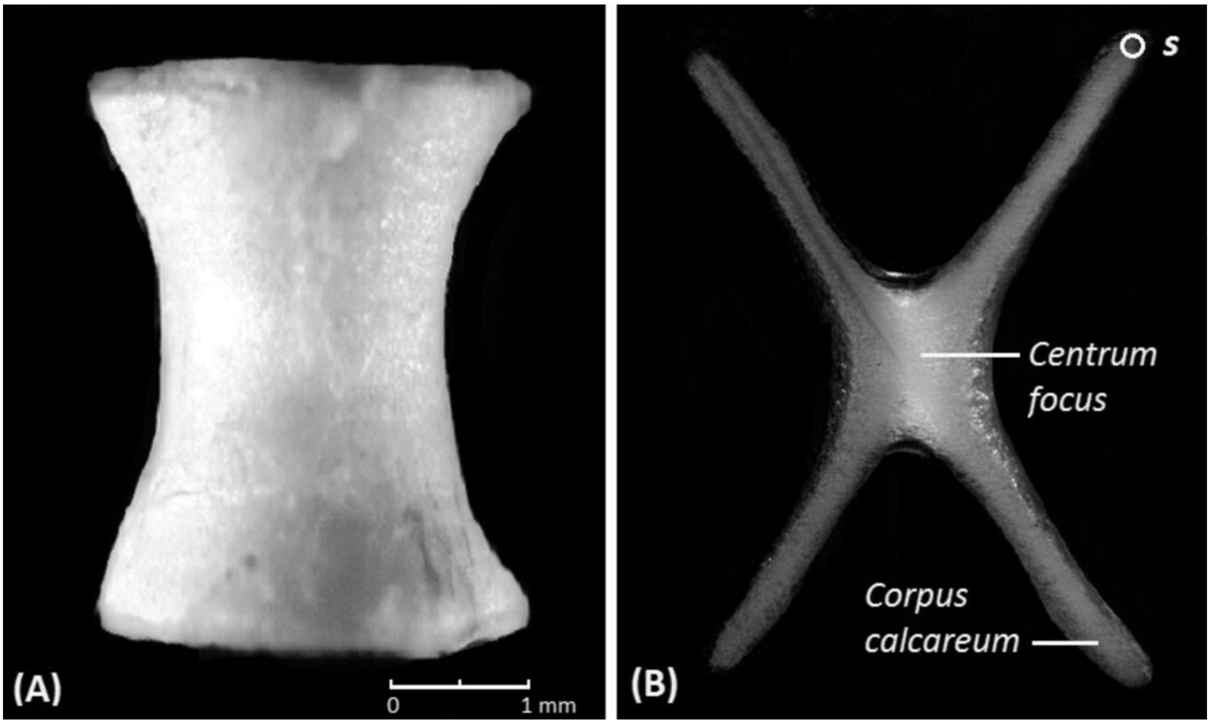


Fig. 3

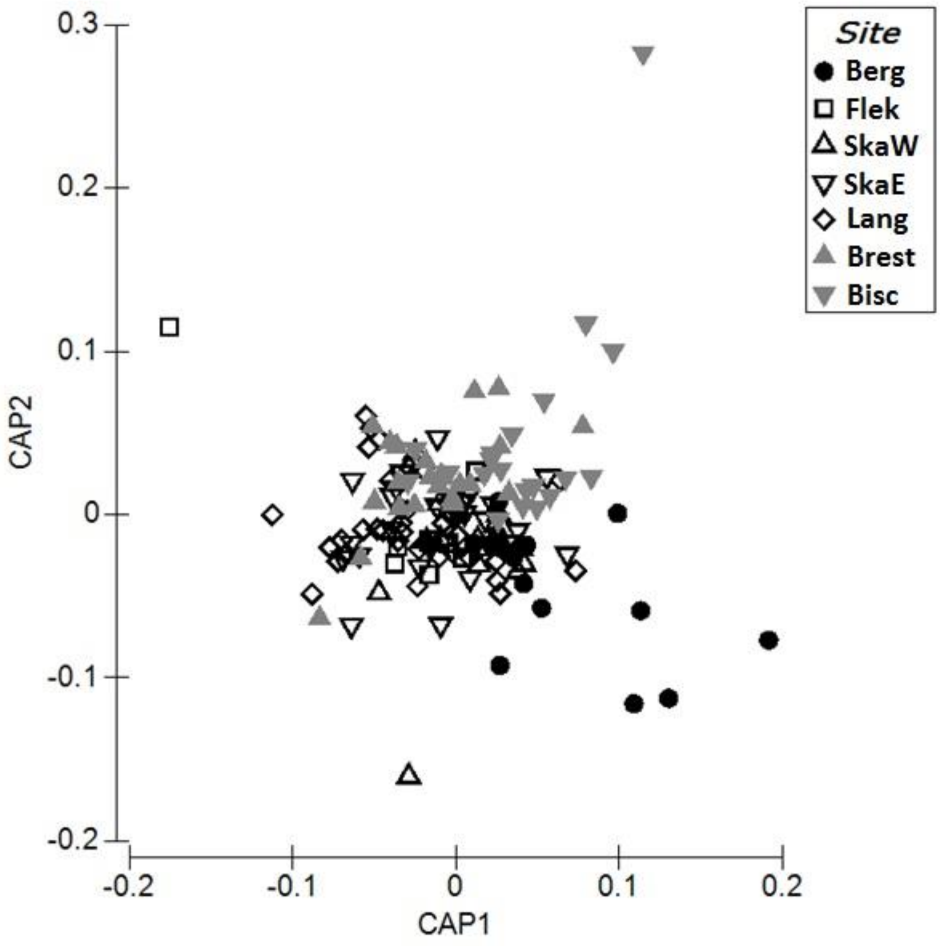

Site
Berg
Flek
SkaW
SkaE
Lang
Brest
Bisc
0.3
0.2
0.1
0
-0.1
-0.2
CAP2
-0.2
-0.1
0
0.1
0.2
CAP1

Fig. 4

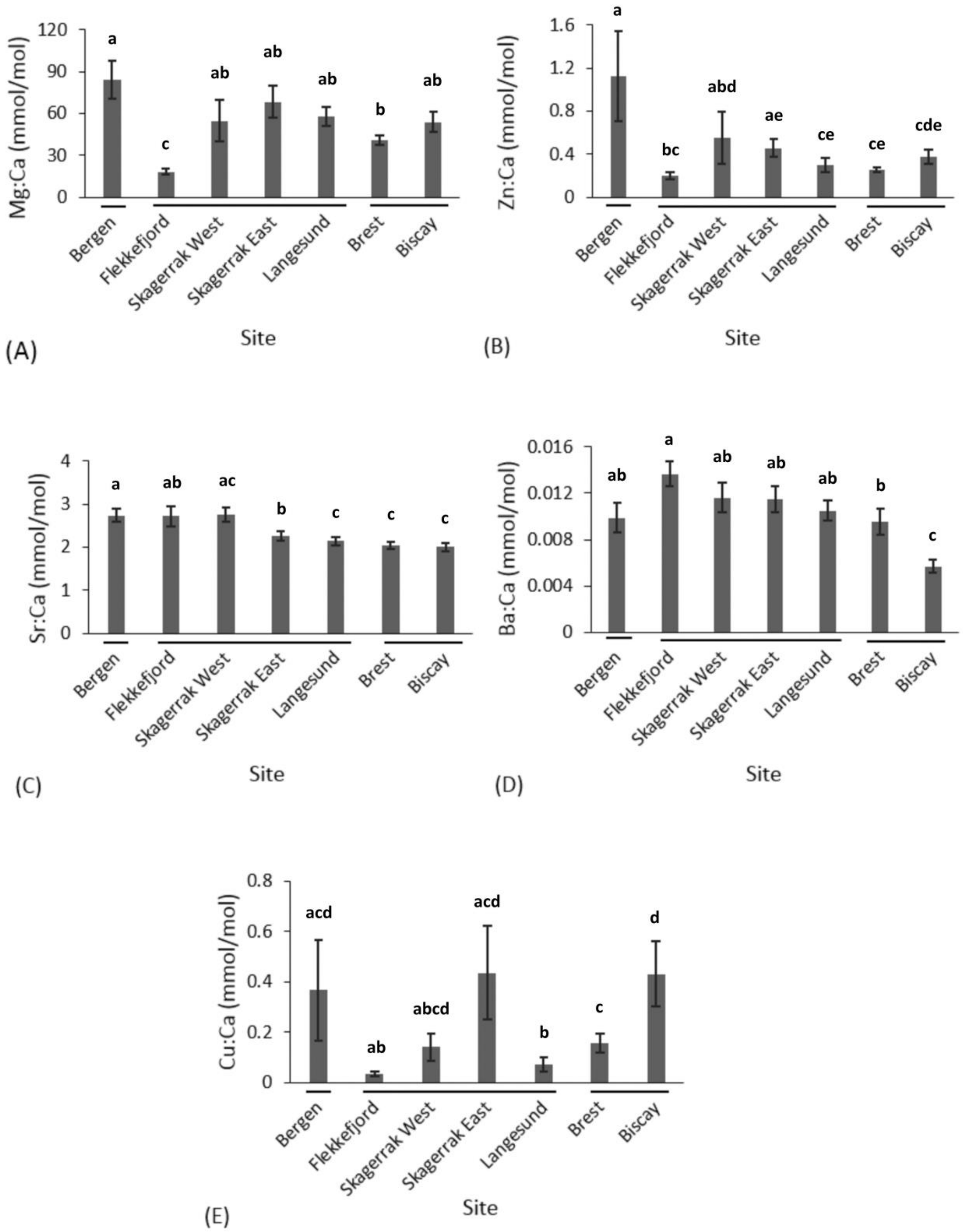
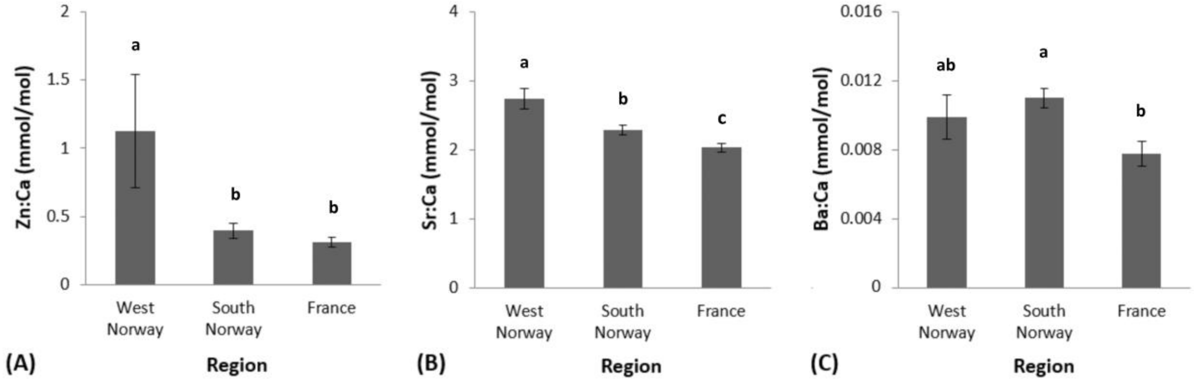

Mg:Ca (mmol/mol)
0
30
60
90
120
a
c
ab
ab
ab
b
ab
Bergen
Flekkefjord
Skagerrak West
Skagerrak East
Langesund
Brest
Biscay
Site
(A)
Zn:Ca (mmol/mol)
0
0.4
0.8
1.2
1.6
a
bc
abd
ae
ce
ce
cde
Bergen
Flekkefjord
Skagerrak West
Skagerrak East
Langesund
Brest
Biscay
Site
(B)
Sr:Ca (mmol/mol)
0
1
2
3
4
a
ab
ac
b
c
c
c
Bergen
Flekkefjord
Skagerrak West
Skagerrak East
Langesund
Brest
Biscay
Site
(C)
Ba:Ca (mmol/mol)
0
0.004
0.008
0.012
0.016
ab
a
ab
ab
ab
b
c
Bergen
Flekkefjord
Skagerrak West
Skagerrak East
Langesund
Brest
Biscay
Site
(D)
Cu:Ca (mmol/mol)
0
0.2
0.4
0.6
0.8
acd
ab
abcd
acd
b
c
d
Bergen
Flekkefjord
Skagerrak West
Skagerrak East
Langesund
Brest
Biscay
Site
(E)

Fig. 5


Zn:Ca (mmol/mol)
2
1.5
1
0.5
0
a
b
b
West Norway
South Norway
France
(A)
Region
Sr:Ca (mmol/mol)
4
3
2
1
0
a
b
c
West Norway
South Norway
France
(B)
Region
Ba:Ca (mmol/mol)
0.016
0.012
0.008
0.004
0
ab
a
b
West Norway
South Norway
France
(C)
Region