## Abstract

Deep-sea sharks play a valuable ecological role helping maintain food web balance, yet they are vulnerable to commercial fishing due to 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 $^{7}$Li, $^{23}$Na, $^{24}$Mg, $^{55}$Mn, $^{59}$Co, $^{60}$Ni, $^{63}$Cu, $^{66}$Zn, $^{85}$Rb, $^{88}$Sr, $^{138}$Ba and $^{203}$Pb 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 utilising 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**: stock structure, shark, deep-sea, vertebral chemistry, LA ICP-MS.

## 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

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

67 Natural element and isotope tags (henceforth 'natural tags') are found in calcified
68 body parts of aquatic organisms (e.g. otoliths of bony fishes, statoliths of cephalopods, shells
69 of molluscs and vertebrae of elasmobranchs) (Campana, 1999). They comprise chemical
70 signatures absorbed from the ambient environment and are stored in concentrations that can
71 reflect environmental element loads (Thorrold et al., 2002; Gillanders, 2009). Differences in
72 elements between areas can result from differences in nutrient input emerging from variation
73 in tides, hydrology, underlying geology, precipitation, upwelling and terrestrial inputs
74 (Elsdon et al., 2008). By incorporating elements from the surrounding environment in
75 concentrations reflective of environmental exposure, natural tags can help identify groups of
76 fish that spend time in waters of similar chemistry and inform about population boundaries,
77 movements and population connectivity (Gillanders and Kingsford, 1996; Elsdon et al.,
78 2008).

79 In contrast to highly crystallised aragonitic teleost otoliths, elasmobranch vertebral
80 centra are composed of cartilaginous tissue surrounded by an extra-cellular matrix
81 mineralised by crystals of calcium phosphate hydroxyapatite (Dean and Summers, 2006). The
82 relatively poorly crystallised apatite of elasmobranch vertebral centra is not analogous to the
83 highly crystallised aragonite of otoliths and therefore can be expected to behave differently.
84 Nonetheless, apatite accretion of elasmobranch centra forms a permanently mineralised
85 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).

111 *Ecology of E. spinax*

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

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

133 Late term gravid females occur in summer months (Coelho and Erzini, 2008; Aranha
134 et al., 2009) and pups are born at around 9 cm TL with mean fecundity around 8 pups
135 (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°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/). 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.

294 Magnesium is conservative in seawater, with its concentration varying with salinity
295 (Quinby-Hunt and Turehian, 1983). The very low Mg:Ca for samples from Flekkefjord
296 (Norway), which differentiated it from other sites, may thus result from its location at the
297 mouth of a deep river-fed fjord. Magnesium has been used to trace movements of
298 elasmobranchs along salinity gradients (Tillett et al., 2011; Werry et al., 2011) and is
299 generally higher in freshwater than in seawater (McMahon et al., 2013). Magnesium values at
300 Flekkefjord may therefore differ to other populations due to freshwater input from the nearby
301 fjord driving down ambient Mg concentrations. It was also the shallowest site (mean depth:
302 252 m) for which depth data were available. The small mean body length of individuals from
303 Flekkefjord (24 cm TL) and its relatively shallow depth may be suggestive of it being a
304 nursery area, since depth related segregation has been reported in the species, with adults
305 migrating to deeper waters and pregnant females moving into shallower waters to pup
306 (Coelho et al., 2010).

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

317 Sharks from Langesund had the largest mean body length (45 cm TL); however the
318 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

343 informative for stock structure in wider ranging species (Smith, 2013). In the present study,
344 total classification success for the multi-element signature increased considerably at the
345 regional scale compared to the fine scale among sampling sites. It was comparable to that
346 recorded in other studies involving predominantly marine fish including the investigation of
347 reef specific self-recruitment of neon damselfish (*Pomacentrus coelestis*) on the Great Barrier
348 Reef (Patterson et al., 2004), natal homing and population mixing in bluefin tuna (*Thunnus*
349 *thynnus*) during trans-Atlantic migrations (Rooker et al., 2008), and stock structure in adult
350 Australasian snapper (*Pagrus auratus*) in South Australia (Fowler et al., 2005), lending
351 support to the suitability of this method for assessing broad scale stock structure in *E. spinax*.

352 While it is not necessary to quantify the mineral sources and environmental influences
353 that give rise to spatial variation in natural tags (it suffices that they are distinctly different
354 among regions: Thorrold *et al.* 1998; Campana 2005), speculation on such drivers may be
355 informative. In the present study Sr followed a declining trend from Western Norway >
356 Southern Norway > France. Variation in Sr:Ca can indicate salinity gradients in estuarine-
357 marine transition zones (Scharer et al., 2012), however in strictly marine environments Sr
358 may be associated with deep water or upwelling (de Villiers, 1999). This stems from the life
359 cycle of protozoan *Acantharia*, which dwell in the upper water column depleting it of Sr in
360 the synthesis of celestite ($SrSO_4$) skeletons, with Sr remineralised at depth upon their decay
361 (De Deckker, 2004). High Sr:Ca in Western Norway may therefore reflect upwelling in the
362 exposed waters off western Norway driven by prevailing northerly winds (Helle, 1978;
363 Asplin et al., 1999) potentially transporting Sr lateral to the coast, while Southern Norway is
364 sheltered from these winds by land masses. Low Sr:Ca in French sharks may reflect low
365 ambient Sr concentrations possibly driven by prevailing downwelling in the Bay of Biscay
366 (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.


## Acknowledgements

'Tusen takk' to the fine people at the Institute of Marine Research in Norway who kindly provided a place on their research vessel *Håkon Mosby* to collect samples. Special thanks to Trude Thangstad, Heidi Gabrielsen and Lise Heggebakken. We thank the Norwegian Shark Alliance (HAI Norge) for the provision of samples and resources, as well as Giovanni Romagnoni, Veronica Ranza and Øyvind Kleiv for sampling. The EVHOE 2013 campaign directors from Ifremer: Jean-Pierre Leauté, Lionel Pawlosky and Michèle Salaun, as well as the crew of the French scientific vessel *Thalassa* are thanked for their contribution to the sample collection. Thanks also to Aoife McFadden, Ben Wade and Ruth Williams at Adelaide Microscopy for lending their assistance and expertise. Funding was provided via a Playford Memorial Trust scholarship and an Australian Research Council (ARC) linkage grant (LP120100652).

# REFERENCES


Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology, 26: 32-46.

Anderson, M. J., and Willis, T. J. 2003. Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. Ecology, 84: 511-525.

Aoba, T., Moreno, E., and Shimoda, S. 1992. Competitive adsorption of magnesium and calcium ions onto synthetic and biological apatites. Calcified Tissue International, 51: 143-150.

Aranha, A., Menezes, G., and Pinho, M. R. 2009. Biological aspects of the velvet belly lantern shark, *Etmopterus spinax* (Linnaeus, 1758) off the Azores, North East Atlantic. Marine Biology Research, 5: 257-267.

Ashford, J. R., Jones, C. M., Hofmann, E., Everson, I., Moreno, C., Duhamel, G., and Williams, R. 2005. Can otolith elemental signatures record the capture site of Patagonian toothfish (*Dissostichus eleginoides*), a fully marine fish in the Southern Ocean? Canadian Journal of Fisheries and Aquatic Sciences, 62: 2832-2840.

Ashhurst, D. E. 2004. The cartilaginous skeleton of an elasmobranch fish does not heal. Matrix Biology, 23: 15-22.

Asplin, L., Salvanes, A. G. V., and Kristoffersen, J. B. 1999. Nonlocal wind-driven fjord–coast advection and its potential effect on plankton and fish recruitment. Fisheries Oceanography, 8: 255-263.

Batifoulier, F., Lazure, P., and Bonneton, P. 2012. Poleward coastal jets induced by westerlies in the Bay of Biscay. Journal of Geophysical Research: Oceans, 117: 1-19.

Bergenius, M. A., Mapstone, B. D., Begg, G. A., and Murchie, C. D. 2005. The use of otolith chemistry to determine stock structure of three epinepheline serranid coral reef fishes on the Great Barrier Reef, Australia. Fisheries Research, 72: 253-270.

Bigi, A., Gandolfi, M., Gazzano, M., Ripamonti, A., Roveri, N., and Thomas, S. A. 1991. Structural modifications of hydroxyapatite induced by lead substitution for calcium. Journal of the Chemical Society, Dalton Transactions: 2883-2886.

Borja, A., and Collins, M. 2004. Oceanography and marine environment in the Basque Country, Elsevier, Amsterdam. 616 pp.

Campana, S. E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Marine Ecology Progress Series, 188: 263-297.

Campana, S. E. 2005. Otolith science entering the 21st century. Marine and Freshwater Research, 56: 485-495.

Campana, S. E., Natanson, L. J., and Myklevoll, S. 2002. Bomb dating and age determination of large pelagic sharks. Canadian Journal of Fisheries and Aquatic Sciences, 59: 450-455.

Clement, J. 1992. Re-examination of the fine structure of endoskeletal mineralization in chondrichthyans: implications for growth, ageing and calcium homeostasis. Marine and Freshwater Research, 43: 157-181.

Coelho, R., and Erzini, K. 2005. Length at first maturity of two species of lantern sharks (*Etmopterus spinax* and *Etmopterus pusillus*) off southern Portugal. Journal of the Marine Biological Association of the United Kingdom, 85: 1163-1165.

Coelho, R., and Erzini, K. 2008. Life history of a wide-ranging deepwater lantern shark in the north-east Atlantic, *Etmopterus spinax* (Chondrichthyes: Etmopteridae), with implications for conservation. Journal of Fish Biology, 73: 1419-1443.

Coelho, R. Blasdale, T., Mancusi, C., Serena, F., Guallart, J., Ungaro, N., Litvinov, F., Crozier, P. & Stenberg, C. 2009. *Etmopterus spinax*. The IUCN Red List of Threatened Species 2009: e.T161388A5412576. http://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T161388A5412576.en. Downloaded on 22 July 2016

Coelho, R., and Erzini, K. 2010. Depth distribution of the velvet belly, *Etmopterus spinax*, in relation to growth and reproductive cycle: The case study of a deep-water lantern shark with a wide-ranging critical habitat. Marine Biology Research, 6: 381-389.

Coelho, R., Rey, J., Gil de Sola, L., de Carvalho, J. F., and Erzini, K. 2010. Comparing Atlantic and Mediterranean populations of the velvet belly lanternshark, *Etmopterus spinax*, with comments on the efficiency of density-dependent compensatory mechanisms. Marine Biology Research, 6: 373-380.

Compagno, L., and Fowler, S. 2005. Sharks of the world, Collins, London. 368 pp.

Curtin, R., and Prellezo, R. 2010. Understanding marine ecosystem based management: A literature review. Marine Policy, 34: 821-830.

De Deckker, P. 2004. On the celestite-secreting Acantharia and their effect on seawater strontium to calcium ratios. Hydrobiologia, 517: 1-13.

de Villiers, S. 1999. Seawater strontium and Sr/Ca variability in the Atlantic and Pacific oceans. Earth and Planetary Science Letters, 171: 623-634.

Dean, M. N., and Summers, A. P. 2006. Mineralized cartilage in the skeleton of chondrichthyan fishes. Zoology, 109: 164-168.

Doyle, J. 1968. Ageing changes in cartilage from *Squalus acanthias* L. Comparative Biochemistry and Physiology, 25: 201-206.

Edmonds, J. S., Shibata, Y., Lenanton, R. C. J., Caputi, N., and Morita, M. 1996. Elemental composition of jaw cartilage of gummy shark *Mustelus antarcticus* (Gunther, 1870). Science of the Total Environment, 192: 151-161.

Elsdon, T. S., and Gillanders, B. M. 2005. Alternative life-history patterns of estuarine fish: Barium in otoliths elucidates freshwater residency. Canadian Journal of Fisheries and Aquatic Sciences, 62: 1143-1152.

Elsdon, T. S., Wells, B. K., Campana, S. E., Gillanders, B. M., Jones, C. M., Limburg, K. E., Secor, D. H., et al. 2008. Otolith chemistry to describe movements and life-history parameters of fishes: Hypotheses, assumptions, limitations and inferences. Oceanography and Marine Biology: An Annual Review, 46: 297-332.

Fanelli, E., Rey, J., Torres, P., and Gil de Sola, L. 2009. Feeding habits of blackmouth catshark Galeus melastomus Rafinesque, 1810 and velvet belly lantern shark *Etmopterus spinax* (Linnaeus, 1758) in the western Mediterranean. Journal of Applied Ichthyology, 25: 83-93.

Fowler, A. J., Gillanders, B. M., and Hall, K. C. 2005. Relationship between elemental concentration and age from otoliths of adult snapper (*Pagrus auratus*, Sparidae): Implications for movement and stock structure. Marine and Freshwater Research, 56: 661-676.

Geffen, A. J., Nash, R. D., and Dickey-Collas, M. 2011. Characterization of herring populations west of the British Isles: An investigation of mixing based on otolith microchemistry. ICES Journal of Marine Science, 68: 1447-1458.

Gennari, E., and Scacco, U. 2007. First age and growth estimates in the deep water shark, *Etmopterus Spinax* (Linnaeus, 1758), by deep coned vertebral analysis. Marine Biology, 152: 1207-1214.

Gillanders, B. M. 2002. Temporal and spatial variability in elemental composition of otoliths: Implications for determining stock identity and connectivity of populations. Canadian Journal of Fisheries and Aquatic Sciences, 59: 669-679.

Gillanders, B. M. 2009. Tools for studying biological marine ecosystem interactions - natural and artificial tags. *In* Ecological Connectivity among Tropical Coastal Ecosystems, pp. 457-492. Ed. by I. Nagelkerken. Springer, Dordrecht.

Gillanders, B. M., and Kingsford, M. J. 1996. Elements in otoliths may elucidate the contribution of estuarine recruitment to sustaining coastal reef populations of a temperate reef fish. Marine Ecology Progress Series, 141: 13-20.

Haddon, M. 2007. Fisheries and their management. *In* Marine Ecology, 2 edn, pp. 515-532. Ed. by S. D. Connell, and B. M. Gillanders. Oxford University Press, Melbourne.

530 Hellberg, M. E., Burton, R. S., Neigel, J. E., and Palumbi, S. R. 2002. Genetic assessment of
531 connectivity among marine populations. Bulletin of Marine Science, 70: 273-290.
532 Helle, H. B. 1978. Summer replacement of deep water in Byfjord, Western Norway: Mass exchange
533 across the sill induced by coastal upwelling. Elsevier Oceanography Series, 23: 441-464.
534 Hussey, N., MacNeil, M., Olin, J., McMeans, B., Kinney, M., Chapman, D., and Fisk, A. 2012. Stable
535 isotopes and elasmobranchs: tissue types, methods, applications and assumptions. Journal
536 of Fish Biology, 80: 1449-1484.
537 ICES. 2014. Report of the Working Group for Elasmobranch Fishes (WGEF). ICES Document ICES CM
538 2014/ACOM:19. 887 pp.
539 Izzo C., Huveneers C., Drew M., Bradshaw C.J.A., Donnellan S.C., Gillanders B.M. (In press) Vertebral
540 chemistry demonstrates movement and population structure of bronze whaler sharks.
541 Marine Ecology Progress Series. DOI: 10.3354/meps11840.
542 Kerr, L. A., and Campana, S. E. 2014. Chemical composition of fish hard parts as a natural marker of
543 fish stocks. *In* Stock identification methods: Applications in fishery science, pp. 205-234. Ed.
544 by S. X. Cadrin, L. A. Kerr, and S. Mariani. Academic Press, San Diego.
545 Kingsford, M. J., and Gillanders, B. M. 2000. Variation in concentrations of trace elements in otoliths
546 and eye lenses of a temperate reef fish, *Parma microlepis*, as a function of depth, spatial
547 scale, and age. Marine Biology, 137: 403-414.
548 Kyne, P. M., and Simpfendorfer, C. A. 2007. A collation and summarization of available data on
549 deepwater chondrichthyans: biodiversity, life history and fisheries. Report by the IUCN Shark
550 Specialist Group for the Marine Conservation Biology Institute. pp. 1-137.
551 Lewis, J., Patterson III, W., Carlson, J., and McLachlin, K. 2016. Do Vertebral Chemical Signatures
552 Distinguish Juvenile Blacktip Shark (*Carcharhinus limbatus*) Nursery Regions in the Northern
553 Gulf of Mexico? Marine and Freshwater Research, 67: 1014-1022.
554 Massutí, E., and Moranta, J. 2003. Demersal assemblages and depth distribution of elasmobranchs
555 from the continental shelf and slope off the Balearic Islands (western Mediterranean). ICES
556 Journal of Marine Science: Journal du Conseil, 60: 753-766.
557 Mayer, I., Berger, U., Markitziu, A., and Gidalia, I. 1986. The uptake of lithium ions by synthetic
558 carbonated hydroxyapatite. Calcified Tissue International, 38: 293-295.
559 McCune, B., Grace, J. B., and Urban, D. L. 2002. Analysis of ecological communities, MjM software
560 design, Gleneden Beach, OR.
561 McMahon, K. W., Hamady, L. L., and Thorrold, S. R. 2013. Ocean ecogeochemistry: a review.
562 Oceanography and Marine Biology: An Annual Review, 51: 327-374.
563 Neiva, J., Coelho, R., and Erzini, K. 2006. Feeding habits of the velvet belly lanternshark *Etmopterus*
564 *spinax* (Chondrichthyes : Etmopteridae) off the Algarve, southern Portugal. Journal of the
565 Marine Biological Association of the United Kingdom, 86: 835-841.
566 Ovenden, J. R., Berry, O., Welch, D. J., Buckworth, R. C., and Dichmont, C. M. 2015. Ocean's eleven: a
567 critical evaluation of the role of population, evolutionary and molecular genetics in the
568 management of wild fisheries. Fish and Fisheries, 16: 125-159.
569 Patterson, H. M., Kingsford, M. J., and McCulloch, M. T. 2004. Elemental signatures of *Pomacentrus*
570 *coelestis* otoliths at multiple spatial scales on the Great Barrier Reef, Australia. Marine
571 Ecology Progress Series, 270: 229-239.
572 Pearce, N. J., Perkins, W. T., Westgate, J. A., Gorton, M. P., Jackson, S. E., Neal, C. R., and Chenery, S.
573 P. 1997. A compilation of new and published major and trace element data for NIST SRM
574 610 and NIST SRM 612 glass reference materials. Geostandards Newsletter, 21: 115-144.
575 Pingree, R., and Le Cann, B. 1990. Structure, strength and seasonality of the slope currents in the Bay
576 of Biscay region. Journal of the Marine Biological Association of the United Kingdom, 70:
577 857-885.
578 Pingree, R., and Le Cann, B. 1992. Three anticyclonic Slope Water Oceanic eddies (swoddies) in the
579 southern Bay of Biscay in 1990. Deep Sea Research Part A. Oceanographic Research Papers,
580 39: 1147-1175.

581 Pon-On, W., Meejoo, S., and Tang, I.-M. 2008. Substitution of manganese and iron into
582 hydroxyapatite: Core/shell nanoparticles. Materials Research Bulletin, 43: 2137-2144.
583 Quinby-Hunt, M., and Turehian, K. 1983. Distribution of elements in sea water. Eos, Transactions
584 American Geophysical Union, 64: 130-131.
585 Rooker, J. R., Secor, D. H., De Metrio, G., Schloesser, R., Block, B. A., and Neilson, J. D. 2008. Natal
586 homing and connectivity in Atlantic bluefin tuna populations. Science, 322: 742-744.
587 Sætre, R., and Ljøen, R. 1972. The Norwegian coastal current. In 'Proceedings of the Port and Ocean
588 Engineering under Arctic Conditions (POAC) Conference'. Technical University of Norway,
589 Trondheim. pp. 514-537.
590 Scharer, R. M., Patterson III, W. F., Carlson, J. K., and Poulakis, G. R. 2012. Age and growth of
591 endangered smalltooth sawfish (*Pristis pectinata*) verified with LA-ICP-MS analysis of
592 vertebrae. Plos One, 7: e47850.
593 Schoenberg, H. P. 1963. Extent of strontium substitution for calcium in hydroxyapatite. Biochimica et
594 Biophysica Acta, 75: 96-103.
595 Schroeder, R., Simpfendorfer, C., and Welch, D. J. 2010. Population structure of two inshore shark
596 species (*Sphyrna lewini* and *Rhizoprionodon acutus*) using laser ablation inductively coupled
597 plasma mass spectrometry (LA-ICPMS) along the east coast of Queensland, Australia. In
598 'Stock structure of exploited shark species in north eastern Australia'. Report to the Fisheries
599 Research & Development Corporation Project No. 2007/035. pp. 39-48
600 Secor, D. H. 2013. The unit stock concept: Bounded fish and fisheries. *In* Stock identification
601 methods: Applications in fishery science, pp. 205-234. Ed. by S. X. Cadrin, L. A. Kerr, and S.
602 Mariani. Academic Press, San Diego.
603 Serena, F., Cecchi, E., Mancusi, C., and Pajetta, R. 2006. Contribution to the knowledge of the biology
604 of *Etmopterus spinax* (Linnaeus 1758)(Chondrichthyes, Etmopteridae). *In* FAO Fisheries
605 Proceedings, pp. 388-394.
606 Simpfendorfer, C. A., and Kyne, P. M. 2009. Limited potential to recover from overfishing raises
607 concerns for deep-sea sharks, rays and chimaeras. Environmental Conservation, 36: 97-103.
608 Sion, L., Bozzano, A., D'Onghia, G., Capezzuto, F., and Panza, M. 2004. Chondrichthyes species in
609 deep waters of the Mediterranean Sea. Scientia Marina, 68: 153-162.
610 Smith, W. D. 2013. Vertebral elemental markers in elasmobranchs: Potential for reconstructing
611 environmental history and population structure. Oregon State University, Corvallis.
612 Smith, W. D., Miller, J. A., and Heppell, S. S. 2013. Elemental markers in elasmobranchs: Effects of
613 environmental history and growth on vertebral chemistry. Plos One, 8: e62423.
614 Tang, Y., Chappell, H. F., Dove, M. T., Reeder, R. J., and Lee, Y. J. 2009. Zinc incorporation into
615 hydroxylapatite. Biomaterials, 30: 2864-2872.
616 Thorrold, S. R., Jones, C. M., Swart, P. K., and Targett, T. E. 1998. Accurate classification of juvenile
617 weakfish *Cynoscion regalis* to estuarine nursery areas based on chemical signatures in
618 otoliths. Marine Ecology Progress Series, 173: 253-265.
619 Thorrold, S. R., Jones, G. P., Hellberg, M. E., Burton, R. S., Swearer, S. E., Neigel, J. E., Morgan, S. G.,
620 et al. 2002. Quantifying larval retention and connectivity in marine populations with artificial
621 and natural markers. Bulletin of Marine Science, 70: 291-308.
622 Tillett, B. J., Meekan, M. G., Parry, D., Munksgaard, N., Field, I. C., Thorburn, D., and Bradshaw, C. J.
623 A. 2011. Decoding fingerprints: Elemental composition of vertebrae correlates to age-related
624 habitat use in two morphologically similar sharks. Marine Ecology Progress Series, 434: 133-
625 143.
626 Veríssimo, A., McDowell, J. R., and Graves, J. E. 2011. Population structure of a deep-water squaloid
627 shark, the Portuguese dogfish (*Centroscymnus coelolepis*). ICES Journal of Marine Science:
628 Journal du Conseil, 68: 555-563.
629 Welden, B., Cailliet, G., and Russell, A. 1987. Comparison of radiometric with vertebral band age
630 estimates in four California elasmobranch. *In* The age and growth of fish, pp. 301-315. Ed. by
631 R.C. Summerfelt and G.E. Hall. Iowa State University Press, Ames, Iowa.

632 Wells, B. K., Bath, G. E., Thorrold, S. R., and Jones, C. M. 2000. Incorporation of strontium, cadmium,
633 and barium in juvenile spot (*Leiostomus xanthurus*) scales reflects water chemistry. Canadian
634 Journal of Fisheries and Aquatic Sciences, 57: 2122-2129.
635 Werry, J. M., Lee, S. Y., Otway, N. M., Hu, Y., and Sumpton, W. 2011. A multi-faceted approach for
636 quantifying the estuarine-nearshore transition in the life cycle of the bull shark, *Carcharhinus*
637 *leucas*. Marine and Freshwater Research, 62: 1421-1431.
638 Xavier, J. C., Vieira, C., Assis, C., Cherel, Y., Hill, S., Costa, E., Borges, T. C., et al. 2012. Feeding ecology
639 of the deep-sea lanternshark *Etmopterus pusillus* (Elasmobranchii: Etmopteridae) in the
640 northeast Atlantic. Scientia Marina, 76: 301-310.

641

642

643 **Table 1.** Summary of sampling information and biological data. Sampling site information includes: site name, site code (used herein), country,
644 GPS coordinates, year of collection, mean depth (in m), and sample size (N). Biological data includes: sex ratio (Male:Female), mean total
645 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* |

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

647 * 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** |

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

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

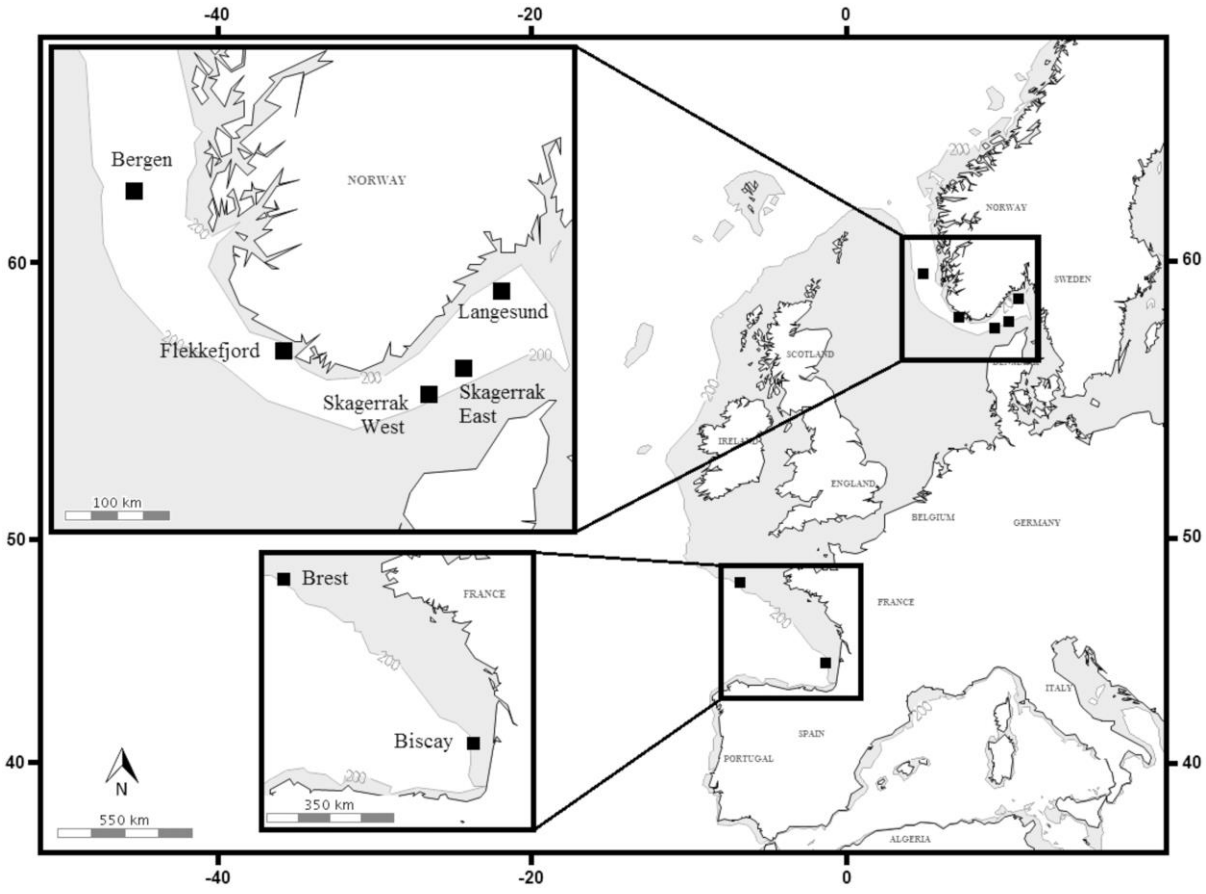
665 **Figure 3.** Canonical analysis of principle coordinates (CAP) plot showing dissimilarity
666 among sampling sites for the multi-element vertebral signature of *E. spinax*. French sites are
667 grey, Bergen is solid black and remaining Norwegian sites are open. Refer to Table 1 for site
668 codes.

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

673 **Figure 5.** Mean sampling region element:Ca ratios in the vertebrae of *E. spinax* (with standard
674 errors) for: Zn (A), Sr (B), and Ba (C). Letters above columns indicate similar means (*t*-test, P
675 $< 0.05$) (Table 3). Note *y*-axis differs among all panels.

676

677 Fig. 1

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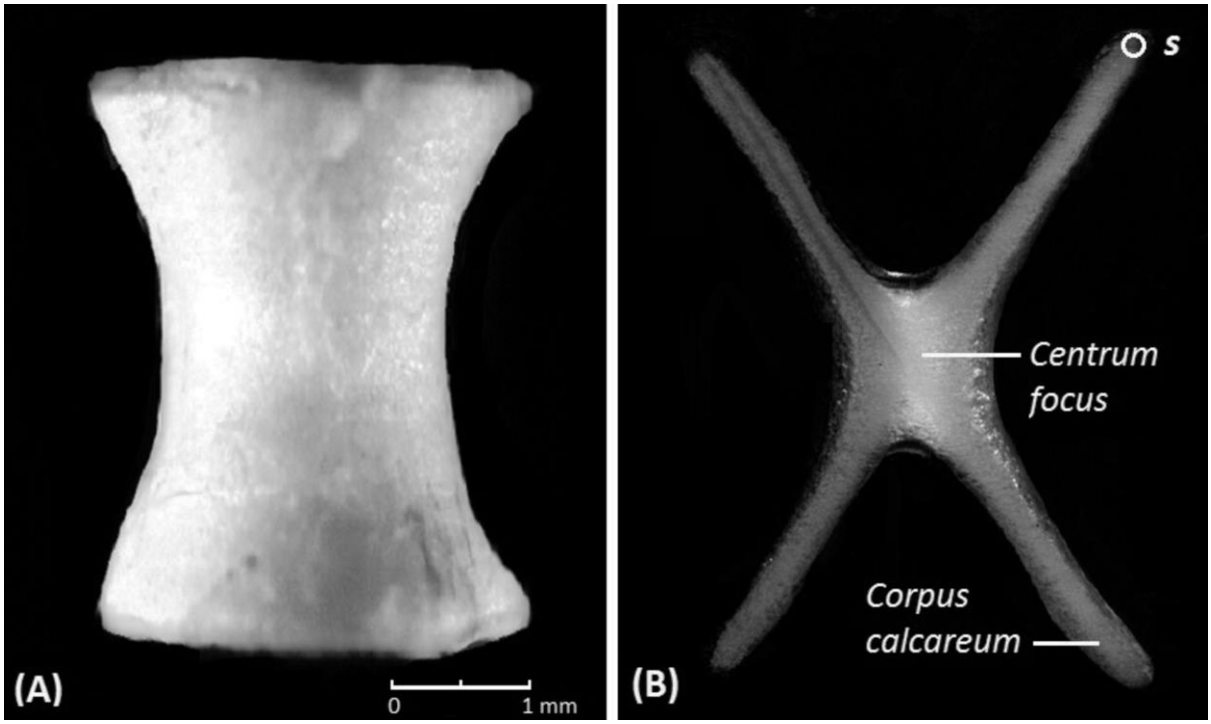
680 Fig. 2

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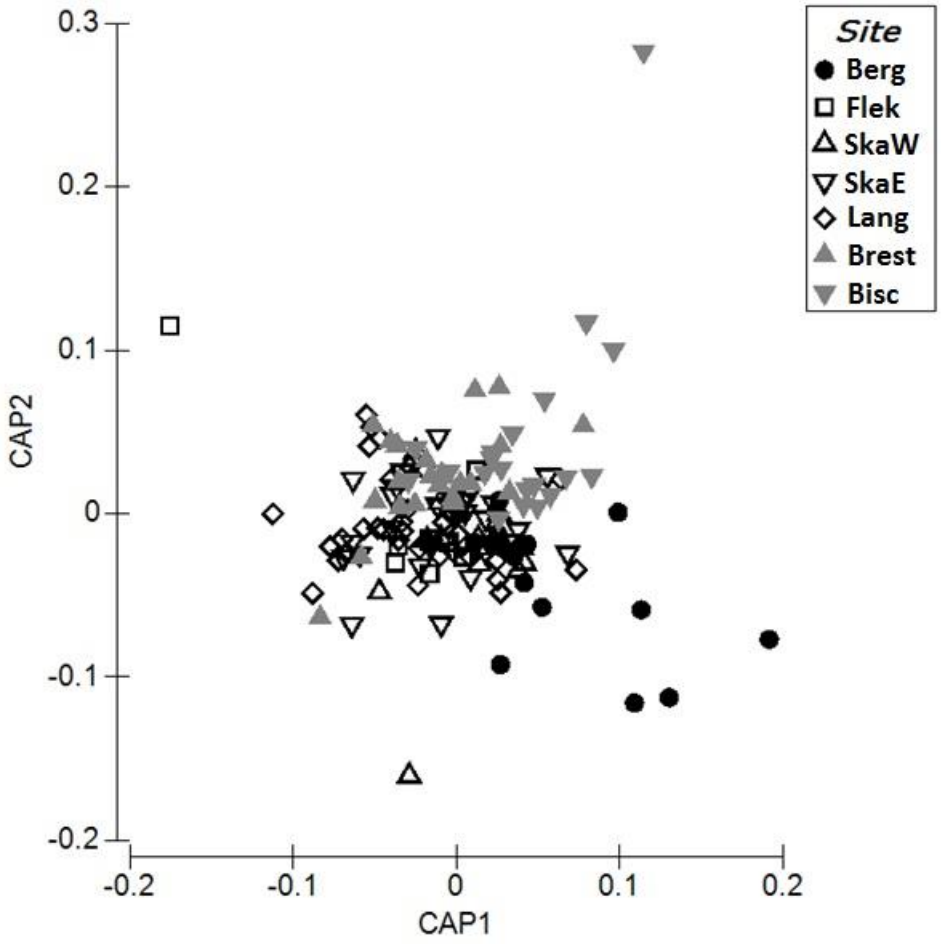
683 Fig. 3

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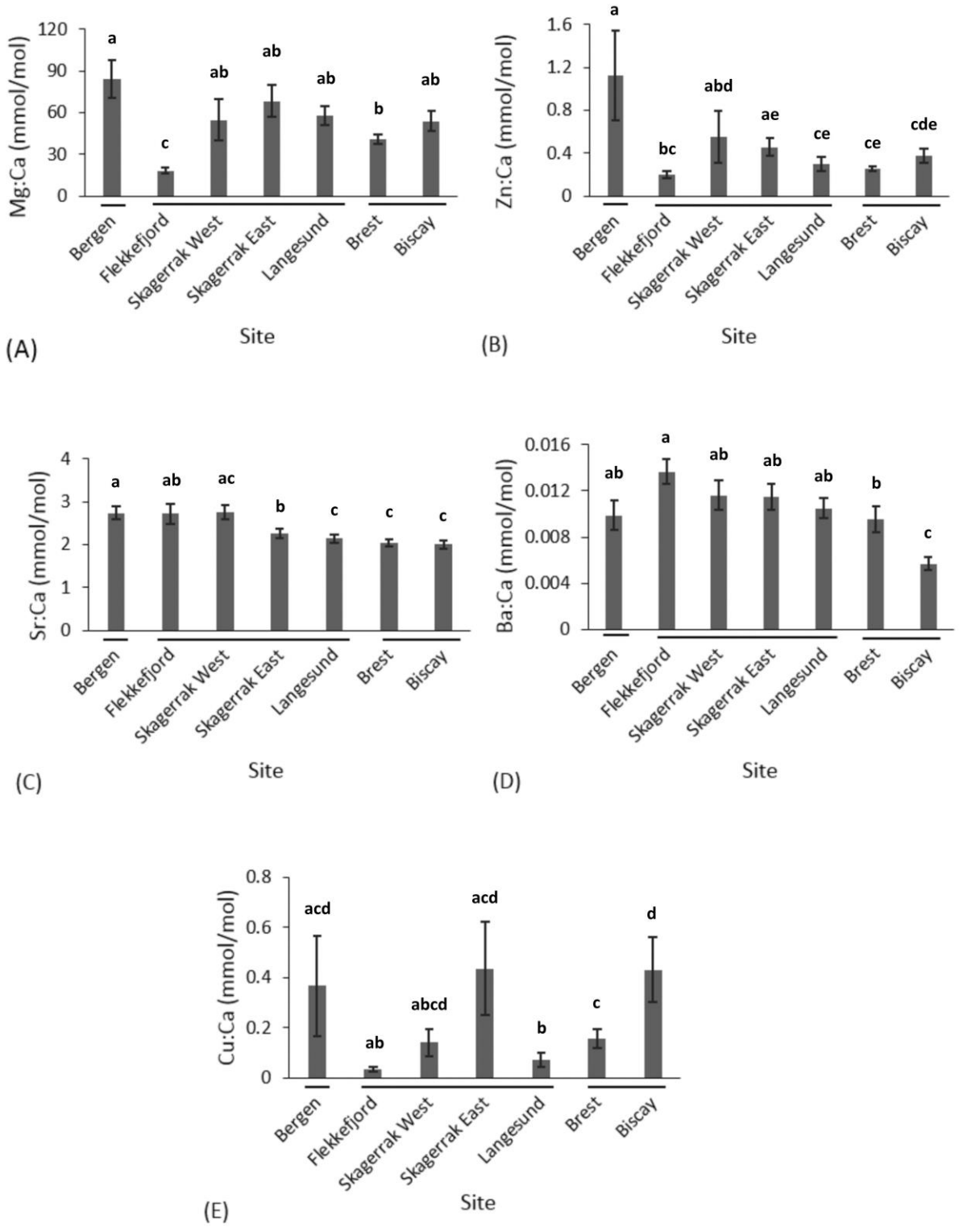
686 Fig. 4

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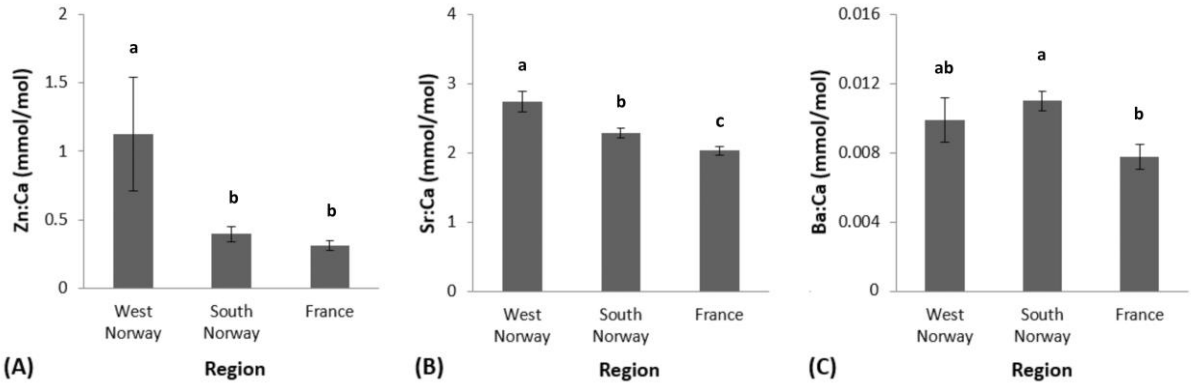
689 Fig. 5


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

690