

## European hake (*Merluccius merluccius*) stock structure in the Mediterranean as assessed by otolith shape and microchemistry

Beatriz Morales-Nin<sup>a,\*</sup>, Sílvia Pérez-Mayol<sup>a</sup>, Kirsteen MacKenzie<sup>b</sup>, Ignacio A. Catalán<sup>a</sup>, Miquel Palmer<sup>a</sup>, Thibault Kersaudy<sup>b</sup>, Kélig Mahé<sup>b</sup>

<sup>a</sup> IMEDEA (UIB/CSIC), C/ Miquel Marqués 21, 07190 Esporles, Illes Balears, Spain

<sup>b</sup> IFREMER, Channel and North Sea Fisheries Research Unit, Fisheries laboratory, 62321 Boulogne-sur-mer, France

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### ABSTRACT

The European hake *Merluccius merluccius* is the third most valuable species for the North-East Atlantic and the Mediterranean fisheries. European hake has been rated as *overexploited* in the Mediterranean, thus careful management is advisable. Mediterranean hake is well-differentiated from Atlantic hake, but sub-population structure within the Mediterranean, and how this structure could be translated into stocks (operative management units), is still an elusive topic. Otolith shape and chemistry (concentration of trace elements) have been systematically used to distinguish fish stocks. Our aim was therefore to assess the discrimination capability (assigning fish to the correct geographical unit) of otolith shape and microchemistry at two geographical scales within the Mediterranean: (1) the official geographical subareas (GSAs), and (2) three larger units previously suggested by genetic markers (i.e., Western Mediterranean, Adriatic with Central Mediterranean, and Eastern Mediterranean). Two complementary analyses were completed because shape is more easily analyzed than chemistry. First, a large sample of juvenile hake ( $n = 1656$ ) from 40 Mediterranean GSAs subunits was used for shape analysis. Second, a subsample of those fish ( $n = 154$ ) from 10 GSAs was analyzed for both otolith shape and microchemistry. Irrespective of the type of data (shape and/or chemistry) and geographical scale (GSAs versus the 3-units), between-unit differences were always statistically significant. However, according to the large within-unit variability, discrimination capability was always poor but better at the GSA scale, and even better when both shape and microchemistry were combined. Moreover, unsupervised clustering methods (the number and limits of the units are data-driven and not a priori defined as above) failed to find an optimal structure. Overall, these results are fully compatible with the hypothesis of a continuous gradient, within which discrete spatial units cannot be safely recognized.

### 1. Introduction

The European hake *Merluccius merluccius* (L., 1758) is exploited by both North-East Atlantic (Casey and Pereiro, 1995) and Mediterranean fisheries (Abaunza et al., 2001; Ardizzone and Corsi, 1997; Oliver and Massutí, 1995). This fish is the third most valuable species in economic terms in the Mediterranean (FAO, 2020), and is mainly sold fresh in Italian and Spanish markets (Casey and Pereiro, 1995). In the Mediterranean, hake is primarily caught by bottom trawlers that operate over the continental shelf and slope and by gillnetters and set bottom longliners (STECF, 2022a, 2022b).

European hake has been rated as *overexploited* (FAO, 2020) thus

careful management is advisable, which implicitly implies to identify proper operative management units (i.e., stocks). Stocks are ideally geographically discrete units, in the sense that the population dynamics of a given stock should remain largely unaffected by other surrounding stocks, which implies negligible migration, almost complete self-recruiting and low between-fish variability in life history parameters (Begg and Waldman, 1999; Begg et al., 1999). However, violations of those assumptions are common due to spatially-poor information and can imply severe consequences for the sustainability of fish and fisheries (Cadrin, 2020).

In the Mediterranean, stock assessments and management plans are implemented at spatial management units that can group several

\* Corresponding author.

E-mail addresses: [beatriz@imedea.uib-csic.es](mailto:beatriz@imedea.uib-csic.es) (B. Morales-Nin), [silvia@imedea.uib-csic.es](mailto:silvia@imedea.uib-csic.es) (S. Pérez-Mayol), [kirsteen.mackenzie@ifremer.fr](mailto:kirsteen.mackenzie@ifremer.fr) (K. MacKenzie), [ignacio@imedea.uib-csic.es](mailto:ignacio@imedea.uib-csic.es) (I.A. Catalán), [palmer@imedea.uib-csic.es](mailto:palmer@imedea.uib-csic.es) (M. Palmer), [Thibault.Kersaudy@ifremer.fr](mailto:Thibault.Kersaudy@ifremer.fr) (T. Kersaudy), [Kelig.Mahe@ifremer.fr](mailto:Kelig.Mahe@ifremer.fr) (K. Mahé).

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Geographical Subareas (GSA). GSAs have been delimited by the General Fisheries Commission for the Mediterranean (GFCM), a region that, together with the Black Sea, FAO considers as a major fishing area. In most cases, Mediterranean hake management plans occur at the level of sub-region (e.g., Western Mediterranean) that can comprise several GSAs. Therefore, management plans are adopted by countries or other management entities, for example the GFCM or the EU, because in many situations the GSA, despite covering often only a portion of a stock, may encompass international waters. However, the delineation of these areas into stocks (as defined above) is not clear.

Many methods have been used for delineating stock boundaries, including genetic markers, tagging, life history, parasites, morphometry, demography, etc. (Artetxe-Arrate et al., 2019; Mares-Mayagoitia et al., 2021; Reiss et al., 2009), and citations therein). Otolith features (mainly shape and trace elements) have also been used extensively (Kerr and Campana, 2014). Otolith shape is species-specific (Koken, 1994) and seems unaffected by short-term changes in fish condition (Campana and Casselman, 1993) or by environmental variations (Campana, 1999). Regarding trace elements, water chemistry and other environmental drivers affect elemental incorporation (Elsdon and Gillanders, 2004; Gillanders, 2005; Walter and Thorrold, 2006), although other biological processes may also modify trace elements incorporation (Catalán et al., 2014; Elsdon et al., 2008). Trace elements are therefore considered natural tags, provided that newly deposited otolith material is not reabsorbed and that otoliths continue to grow throughout the lifespan (Campana, 1999).

The existence of different hake stocks (here forth in the sense of a relatively closed population) in European waters has previously been suggested on the basis of many of the methods listed above (Alheit and Pitcher, 1995; Cipriani et al., 2018; Inada, 1981; Lundy et al., 1999; Roldán et al., 1998). Specifically, North-East Atlantic and Mediterranean Sea populations have consistently been considered separate units (Abaunza et al., 2001; Cimmaruta and Bondanelli, 2005; Milano et al., 2014). Milano et al. (2014) also described a finer-scale significant genetic population structure using SNPs that revealed a strong differentiation among Western, Central and Eastern Mediterranean.

Although otolith shape and/or trace element composition have shown potential for discriminating hake sub-populations, for identifying migration routes, and for describing life history traits (Lombarte et al., 2003; Morales-Nin et al., 2005, 2014; Pira et al., 2005; Swan et al., 2006; Tanner et al., 2012; Tomás et al., 2006) hake stock structure within the Mediterranean remains elusive because of the limited geographical coverage and sample sizes of these studies. Our aims here are therefore: (1) to describe otolith shape and trace elements composition from a large sample of fish, covering the entire Mediterranean; and (2) to compare the discrimination capability (i.e., assigning new fish to the correct unit) at two very different geographical scales within the Mediterranean. These scales are: (1) the geographical subareas subunits (henceforth GSAs; FAO, 2009), which can be aggregated to different degrees for implementing EU Multiannual Management plans (MAPs) and GFCM recommendations, which act as a MAP (STEFCF, 2020, 2022a, 2022b), and (2) three larger areas suggested by previous works (i.e., Western Mediterranean Sea, Adriatic Sea with Central Mediterranean Sea, and Eastern Mediterranean Sea; Maurin, 1965; Milano et al., 2014; Orsi-Relini et al., 2002; Spedicato et al., 2022). Here, we test the hypothesis that a particularly coherent structure (higher classification power) will emerge by analysis from two geographical perspectives: individual GSAs vs the three large areas as delineated by MED\_UNITS genetic study (Spedicato et al., 2022).

## 2. Material and methods

### 2.1. Fish sampling

The initial sample set was composed of 1868 European hake samples, hereafter the *large sample*, from 40 subunits resulting from a

geographical partitioning of the 27 Mediterranean geographical subareas by resolution of GFCM (FAO, 2009) (Fig. 1). To test and put into context the within-Mediterranean coherence, 196 additional fish from 4 ICES areas (NE Atlantic; Fig. 1) were added for comparative purposes. However, statistical analyses (Section 2.4) were restricted to the Mediterranean fish. The samples were provided by the MED\_UNITS project consortium and were collected during the MEDITS: Mediterranean Trawl Survey (Bertrand et al., 2002; Spedicato et al., 2019); EVHOE: Evaluation Halieutique Ouest de l'Europe (Laffargue et al., 1987); scientific trawl surveys and biological sampling under the Data Collection Framework (DCF) (<https://datacollection.jrc.ec.europa.eu/>) and Data Collection Reference Framework (DCRF) (<https://www.fao.org/gfcm/data/dcrf/ru/>).

Due to reported ontogenetic changes in hake otoliths (Morales-Nin et al., 2014) and the possible influence of maturity stage on both shape and chemistry, only juvenile hake were studied (females <28 cm TL and males <16 cm TL). The use of immature fish is also supported by previous works (Lombarte and Castellón, 1991; Lombarte et al., 2003) where immature otoliths of different species of *Merluccius* showed higher influence of external factors (especially temperature) than in mature specimens. Moreover, Recasens et al. (1998) reported a shorter depth range (less environmental variability) in juvenile than in adult specimens of *Merluccius merluccius*.

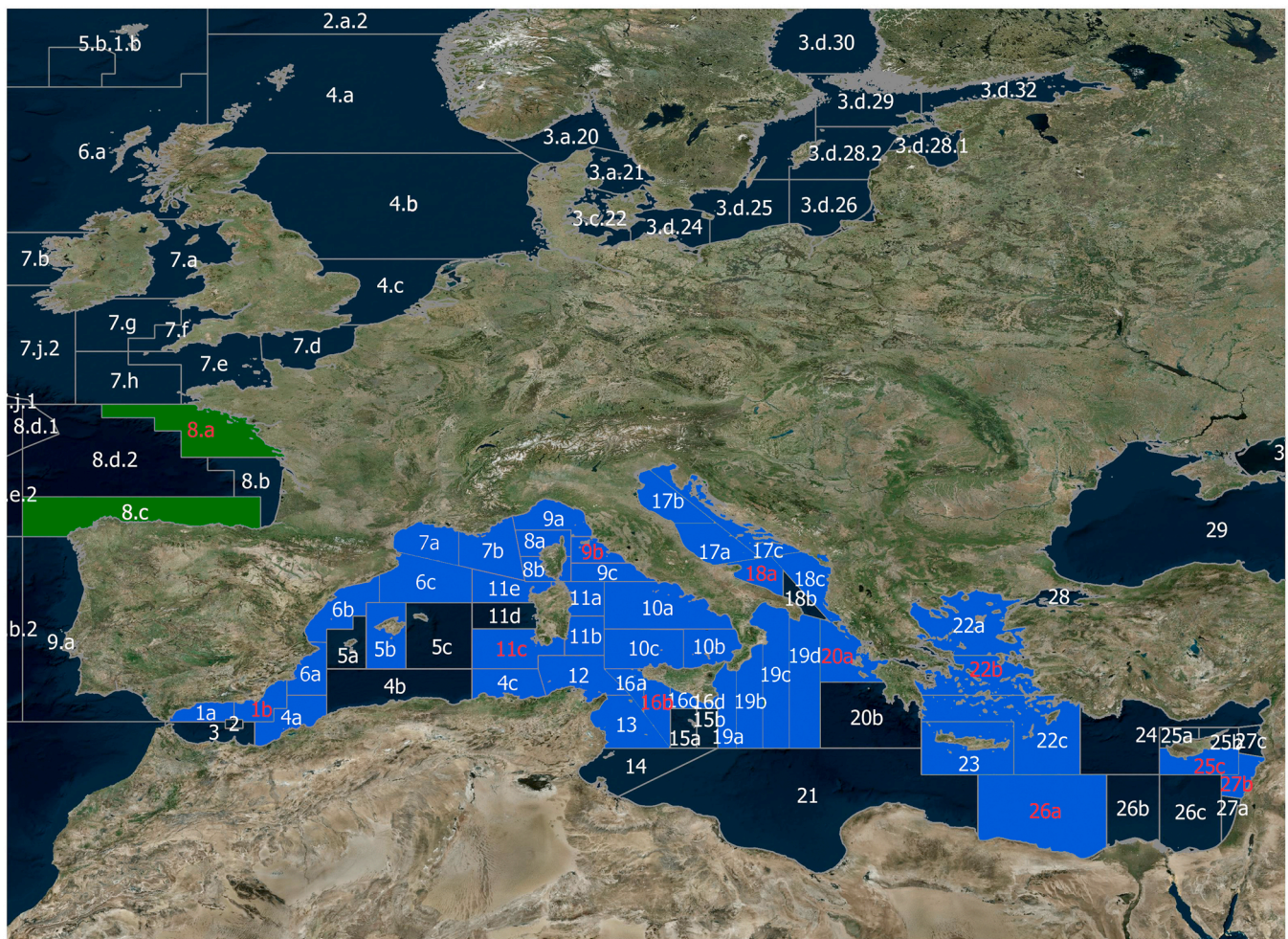
A subsample of the females of the *large sample* was used for microchemistry analysis. This subsample (hereafter the *small sample*, N = 154) comprised between 15 and 25 fish from each of 10 GSAs subunits (1b, 9b, 11c, 16b, 18a, 20a, 22b, 25c, 26a, and 27b) and one Atlantic division (VIIIa) (Fig. 1). As the complexity of microchemistry analyses heavily restricts sample size, the potential confounding effects of sex cannot be accounted for; thus, we a priori selected only immature females (L50 35.8 cm for females, Recasens et al., 2008). Therefore, we assumed that immature individuals of both sexes would show the same spatial pattern. Additionally, 49 European hake samples from ICES area VIIIc (Galician Coast; Fig. 1), from biological samplings from commercial fisheries in the framework of the Spanish funded project DREAMER (CTM2015-66676-C2-1-R), were also considered for comparative purposes (i.e., no shape data were available for them).

### 2.2. Otolith shape

The otolith outline for each fish was described using Elliptic Fourier Descriptors (EFDs). The resulting matrix containing the EFDs (as columns) for each otolith (as rows) was submitted to a principal components analysis to reduce dimensionality. Provided that neither directional asymmetry between right and left otoliths (Díaz-Gil et al., 2015; Mahé et al., 2019; Palmer et al., 2010), nor sexual dimorphism were found, hereafter only the left otolith was analyzed, and sex was ignored. The shape data collection and pre-processing process are fully detailed in Supplementary material S1. After pre-processing, the *large sample* matrix used for classification and multivariate analyses of Mediterranean fish using otolith shape (see Section 2.4) was composed of 1656 otoliths (rows) and 11 principal components (columns) from 40 GSA subunits in order to have better spatial resolution.

### 2.3. Otolith microchemistry

Otoliths were prepared under clean conditions and analyzed using a CETAC Teledyne Technologies Laser Ablation System LSX-213 G2 + coupled to a Thermo-Finnigan Element XR Inductively Coupled Plasma-Mass Spectrometer (LA-ICP-MS). We analyzed the otolith edge area only, because it is assumed that this area summarizes the environmental conditions experienced by a fish just before it was captured. A suite of 27 different isotopes was determined, but after a complex data pre-processing (Martí-Puig et al., 2019) and a rigorous quality control (fully detailed in Supplementary material S2), the *small sample* matrix used for classification and multivariate analyses of otolith shape and



**Fig. 1.** European hake sample origin. Mediterranean management units analyzed in the large sample (blue background) and in the small sample (red code). Atlantic ICES areas are also indicated (green background). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

microchemistry (see Section 2.4) was composed of 154 otoliths (rows) and seven elements ( $^{23}\text{Na}$ ,  $^{24}\text{Mg}$ ,  $^{43}\text{Ca}$ ,  $^{44}\text{Ca}$ ,  $^{55}\text{Mn}$ ,  $^{88}\text{Sr}$  and  $^{138}\text{Ba}$ ) from 10 GSA subunits.

#### 2.4. Classification and multivariate analyses

The resulting matrices (i.e., the *large sample* matrix of shape and the *small sample* matrix of shape+microchemistry) were analyzed following two complementary statistical strategies (classification and MANOVA), which were each completed at two spatial scales (GSAs versus 3-areas).

Linear discriminant analysis (*mass* library from the R package) was used as a classification method because preliminary trials demonstrated that it performs better than alternative classification methods (as in Jones et al., 2017; details provided in Supplementary materials). Classification success was assessed using leave-one-out cross-validation. In addition to success rate, predicted versus observed unit membership were compared using the kappa coefficient (Artetxe-Arrate et al., 2019). Moreover, actual classification rates were then compared with the baseline success after randomly shuffling the unit membership of fish (30 iterations), which allowed comparison of success rates obtained with different sample sizes and numbers of units. Finally, between-unit differences were assessed using MANOVA as implemented in the *rda* library from the *vegan* package (Oksanen et al., 2020). Variables were standardized (i.e., mean-subtracted and divided by standard deviation) thus the same weight is given to any variable. Significance of

between-unit differences was assessed after 1000 permutations.

In addition to the analyses above, unsupervised clustering methods were used as an alternative to the two a priori defined spatial scales above (GSAs versus 3-areas). These are data-driven methods because the number and limits of the units are not a priori defined. Here, the optimal number of clusters that minimizes within-unit variability was assessed using the elbow method (Matson and Gertseva, 2020) using the k-mean algorithm on the between-fish Euclidean distance matrix.

### 3. Results

#### 3.1. Large sample (shape only)

For the *large sample* (1656 otoliths), the classification success at the GSA level was 17 %, while this figure reached 54 % after pooling GSAs into three larger units (Western, Central and Eastern Mediterranean). Note, however, that the crude value of classification success heavily depends on the number of units considered. In our case, the classification success at the GSA level was five times greater than expected by chance after accounting for this effect, while it was very close to that expected by chance at the 3-units spatial level. The kappa coefficient displayed the same pattern, and the value at the GSA level was better ( $z = 34.2$ ) than at the 3-units level ( $z = 9.0$ ). MANOVA results strongly supported the existence of shape differences at the two spatial levels (Table 1).

**Table 1**

Results of the classification and multivariate analyses completed. Note that (1) classification success was better at the GSA level, (2) combining shape and chemistry improves classification success, and (3) between-unit differences are always significant, irrespective of the sample size and the type of data.

SAMPLE AND DATA TYPE		CLASSIFICATION			MANOVA (model/residual)			
		Success ( %)	Times better than by chance (95 % CI)	Kappa / z value	df	Variance	F	Prob
40 GSAs	All fish (1656 otoliths)	17.0	5.9 (4.7–6.4)	0.144/34.2	39/1616	1.46/9.53	6.38	0.001
Shape								
3-units		51.4	1.04 (1.04–1.05)	0.129/9.0	2/1653	0.16/10.83	12.8	0.001
10 GSAs	Subsample (154 otoliths)	35.7	3.4 (2.2–5.1)	0.279/10.1	9/144	1.72/8.27	3.33	0.001
Shape								
3-units		45.4	1.4 (1.1–1.8)	0.181/3.17	2/151	0.41/9.58	3.29	0.001
10 GSAs	Chemistry	29.2	3.0 (1.9–5.6)	0.205/7.45	9/144	1.51/ 5.48	4.41	0.001
Shape								
3-units		55.8	1.8 (1.5–2.5)	0.338/5.95	2/151	0.79/6.20	9.70	0.001
10 GSAs shape+chemistry	Chemistry	44.1	4.5 (2.7–9.9)	0.375/13.6	9/144	3.23/13.76	3.76	0.001
3-units shape+chemistry		59.7	1.7 (1.4–2.5)	0.396/6.97	2/151	1.21/15.78	5.81	0.001

To better visualize the otolith shape differences, the averaged outlines of Atlantic, Western, Central and Eastern Mediterranean were compared (Fig. 2). Some shape differences could be identified between Atlantic and Mediterranean otoliths, whereas otoliths within the Mediterranean were more similar. Atlantic otoliths of immature specimens were more elongated with respect to Mediterranean individuals. Differences between Mediterranean specimens were mainly located in the *postrostrum*, more pointed in the Western and more rounded in the Eastern specimens, and intermediate in the Central area.

3.2. Small sample (shape and chemistry)

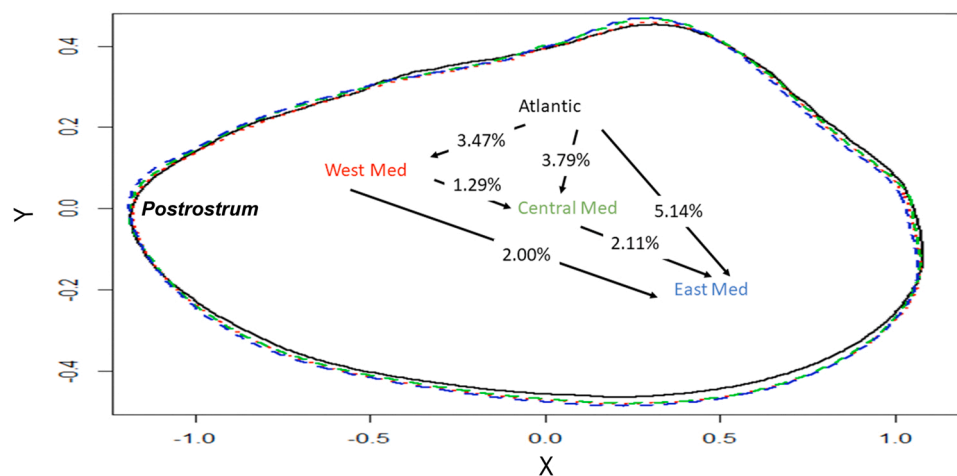
3.2.1. Otolith microchemistry

From the 27 measured isotopes, those retained after quality controls were: <sup>23</sup>Na, <sup>24</sup>Mg, <sup>43</sup>Ca, <sup>44</sup>Ca, <sup>55</sup>Mn, <sup>88</sup>Sr and <sup>138</sup>Ba. The incorporation of the recently deposited material in the otolith edge showed some trends (Fig. 3), and the high levels of <sup>55</sup>Mn and <sup>24</sup>Mg from GSA 20a are particularly noteworthy. <sup>24</sup>Mg, <sup>55</sup>Mn, and <sup>138</sup>Ba concentrations in otoliths seem to be genetically determined (Clarke et al., 2011). The Sr:Ca ratio is affected by stock, salinity and temperature, Ba:Ca by salinity and stock, while Mg:Ca is only affected by temperature (Barnes and Gilanders, 2013). The lower <sup>88</sup>Sr values in GSA22b and 26a might be related to lower salinity caused by water masses from the Black Sea and the River Nile. Other elements do not show marked differences and are

probably under physiological control (Hüssy et al., 2021).

3.2.2. Classification and MANOVA

Classification success at the two spatial scales considered (GSAs versus 3-units) and using (1) shape only, (2) chemistry only, and (3) shape and chemistry is summarized in Table 1. Again, success rate per se may be meaningless because it heavily depends on the number of units considered. Concerning the data type, the highest classification success was achieved after combining shape and chemistry. Regarding the spatial level, and similarly to the *large sample* data set above, the highest success was achieved at the GSA level, while the success at the 3-units level was only slightly higher than expected by chance. For the GSA level, using shape+chemistry success reached 44.1 % of correct classification, which is 4.5 times higher than expected by chance. Kappa coefficient showed the same trend (Table 1). However, even in the best cases, classification success should be considered poor, as a new fish will be attributed to the wrong unit most of the time. This poor prediction capability is related to the large within-unit variability found in relation to the between-unit variability. As an example, Fig. 4 shows the large amount of overlapping observed at GSA level for the trace element composition, but shape and shape+chemistry display the same pattern (not shown). Note that between-GSA similarity seems at least partially related to a longitudinal gradient. The general pattern is that the closer two GSAs are to each other, the more similar are the otoliths. However,



**Fig. 2.** Differences between four identified stocks units shown by average shape reconstruction for each group based on EFDs. Percentages are the average percentages of non-overlapping surface between the two reconstructed otolith shapes at the individual level (Atlantic: black line; West Med: red dotted line; Central Med: green dotted line; East Med: blue dotted line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

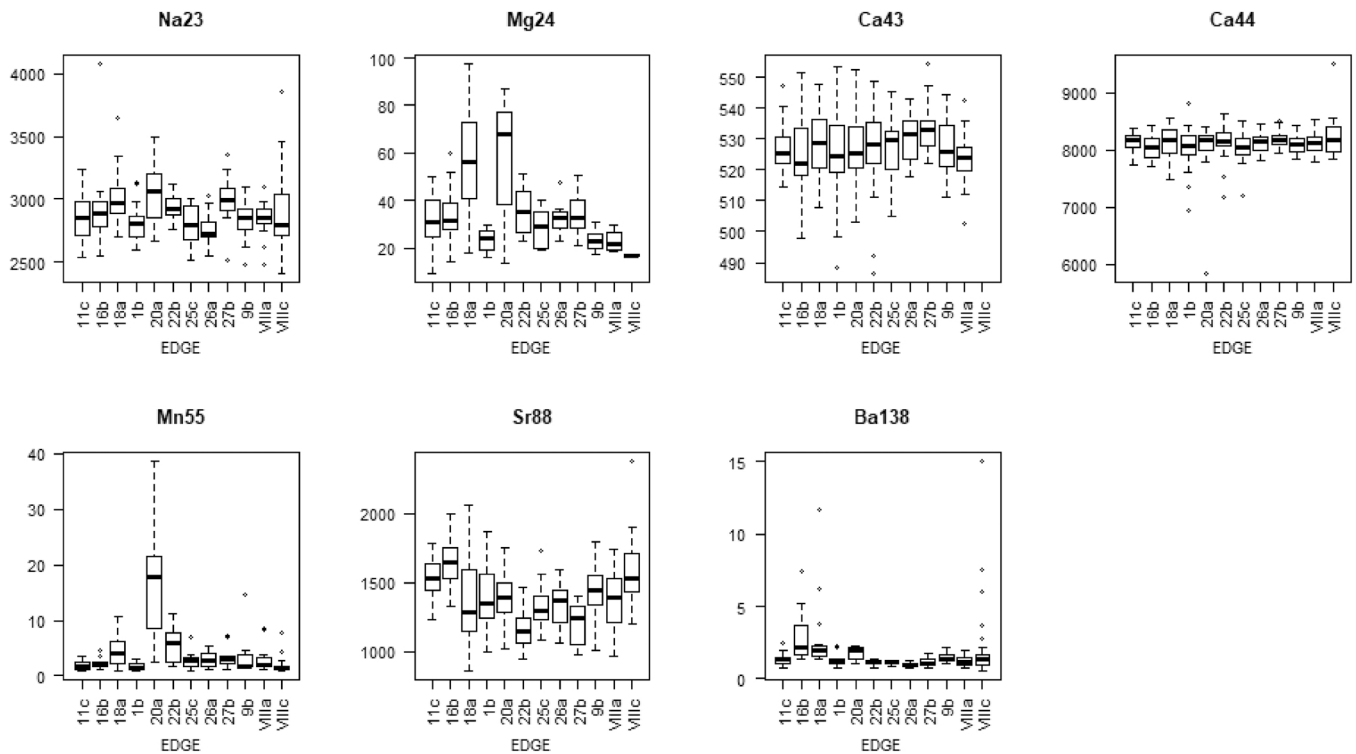


Fig. 3. Between- and within-unit variability in the element concentration (ppm) for the seven elements retained after quality control. Units 11c to 9b correspond with the Mediterranean GSAs subunits, and VIIIa and VIIIc to the Atlantic divisions.

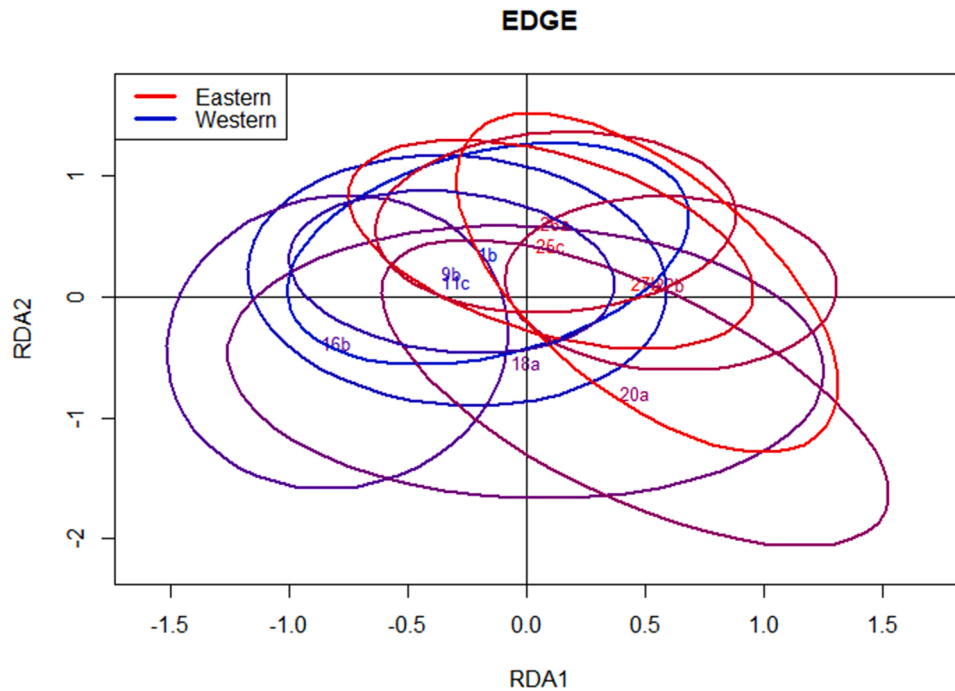


Fig. 4. Ellipses including 95 % of the otoliths from each GSA. Colors denote GSA Easternness. The axes correspond to the scores on the first two principal components of the ordination constrained by GSA-membership. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

some pairs of geographically distant GSAs (notably, Gibraltar/Alboran (GSA 1b) and Cyprus (GSA 25c)) break this general pattern and are more similar than expected given its geographical distance. In contrast, MANOVA results strongly supported the existence of differences at the two spatial levels, either using shape or chemistry individually or in

combination (Table 1).

### 3.3. Optimal number of clusters

While the two sections above focus on a priori defined units (either

GSA or 3-units), here we looked for a data-driven number of classes (units) within which items (i.e., fish) were optimally classified. According to the elbow method, when plotting the number of clusters against the summed within-cluster sum of squares, the optimal number of clusters is identified as a sudden slope change, from which any further increase in the number of clusters implies a negligible decrease of within-cluster variability (i.e., fish within units are homogeneous). However, the two data sets displayed the same pattern: the larger the number of clusters, the smaller the within-cluster variability was, showing no sign of reaching an asymptotical minimum nor an elbow (Fig. 5). These patterns suggest that GSAs, if taken as operative management units, do not hold in terms of stock coherence.

#### 4. Discussion

Previous studies suggested that European hake population is not homogeneous within the Mediterranean. The existence of several stocks at different spatial scales has been proposed (Casey and Pereiro, 1995; Cresson et al., 2015; Morales-Nin et al., 1998; Swan et al., 2006), generally defining three main groups (Western, Central and Eastern Mediterranean). In these studies, otolith chemistry was shown to successfully predict unit membership for up to 65.5 % of the fish (Swan et al., 2006), with up to 90 % accuracy achieved when combining trace elements and  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values (Tanner et al., 2012; this figure includes some Atlantic samples). A similar 3-units stock structure has been reported for other species such as sardine *Sardina pilchardus* (Jemaa et al., 2015), cephalopods (Keller et al., 2017), and bogue *Boops boops* (Mahé et al., 2019).

It could be argued that European hake stock structure remained previously unresolved because the number of fish and the spatial coverage analyzed in the above references were limited. Unfortunately, the results reported here cannot precisely delineate stock geographical

boundaries in the Mediterranean Sea despite analyzing many fish from the entire geographical gradient and using two otolith markers (i.e., shape and chemical composition) that have been used successfully for many species and regions.

When comparing the two spatial scales used herein, it should be noted that the crude value of classification success (i.e., assigning new fish to the correct unit) depends on the underlying stock structure and on the number of units considered. For example, the success rate achieved for the *large sample* at GSA level was only 17 % but rose to 51 % when considering the 3-units. However, the success rate at GSA level for the *small sample* was over five times higher than expected by chance, while the successful assignment was close to that expected by chance when considering the 3-units structure.

Concerning the type of otolith markers (shape versus chemistry), the best classification success was achieved after combining shape and chemistry (4.5 times better than chance), which is larger than the corresponding figures for shape alone (3.4 times) and chemistry alone (3.0 times). However, the different complexity and cost of the two methods suggests that such a combined strategy would be desirable but may be logistically unaffordable in some cases. In any case, the multidisciplinary analysis of stock structure should be the way forward, as different methods form a puzzle of different temporal resolutions in how the populations are structured in space (Cadriin, 2020).

Irrespective of the spatial unit considered and the type of phenotypic marker, the crude values of classification success reported here are always low. Thus, predicting unit membership of new fish will be poor, and identifying clear-cut spatial units with otolith markers will be problematic. More efficient and accurate stock characterization may be obtained from integrated studies of otolith morphometry and chemistry (Biolé et al., 2019; Longmore et al., 2010; Soeth et al., 2019). However, discriminant power will depend not only on the traits used; larger discrimination is expected when shape and/or chemical composition are homogeneous at the within-unit scale but display clear-cut differences at the between-unit scale (Longmore et al., 2010; Rodrigues Maciel et al., 2021).

In the case of the European hake, the low classification success seems to be related to the large within-unit variability: fish within GSAs tend to display heterogeneous element composition and shape (Figs. 3 and 4). This larger within-unit variability than between-unit variability (i.e., the differences among individuals from the same area were at least equivalent to the differences between areas), is something also observed for the genetic variability (Spedicato et al., 2022). Thus, it seems that fish at different locations experience different abiotic environmental (e.g., differences in temperature and salinity) and biotic scenarios (e.g., prey availability) that are expected to affect otolith shape and chemistry, which are also expected to be affected by individual genotype, and by interactions during the ontogenetic trajectory (Burke et al., 2008; Swan et al., 2006; Vignon, 2018; Vignon and Morat, 2010). Ontogenetic changes in otolith shape may arise from variations in growth too, which are related to ontogenetic changes in habitat quality or with developmental processes, such as sexual maturity (Campana and Casselman, 1993; Cardinale et al., 2004; Monteiro et al., 2005; Simoneau et al., 2000).

Here we have accounted for some of the processes above: first, the ontogenetic effects on otolith shape have been minimized because only juvenile fish were considered, and the fish size effect has been removed prior to shape analyses. Additionally, sex was not found to affect hake otolith shape, as has been seen in other species, e.g. silver hake *Merluccius bilinearis* (Bolles and Begg, 2000), Atlantic mackerel *Scomber scombrus* (Castonguay et al., 1991), haddock *Melanogrammus aeglefinus* (Begg et al., 2001), lake trout *Salvelinus namaycush* (Simoneau et al., 2000), Atlantic cod *Gadus morhua* (Cardinale et al., 2004), blue whiting *Micromesistius poutassou* (Mahé et al., 2016a) and swordfish *Xiphias gladius* (Mahé et al., 2016b). The existence of otolith shape asymmetry can also result in misclassification (Mahé et al., 2019), but no directional bilateral asymmetry was found in the European hake.

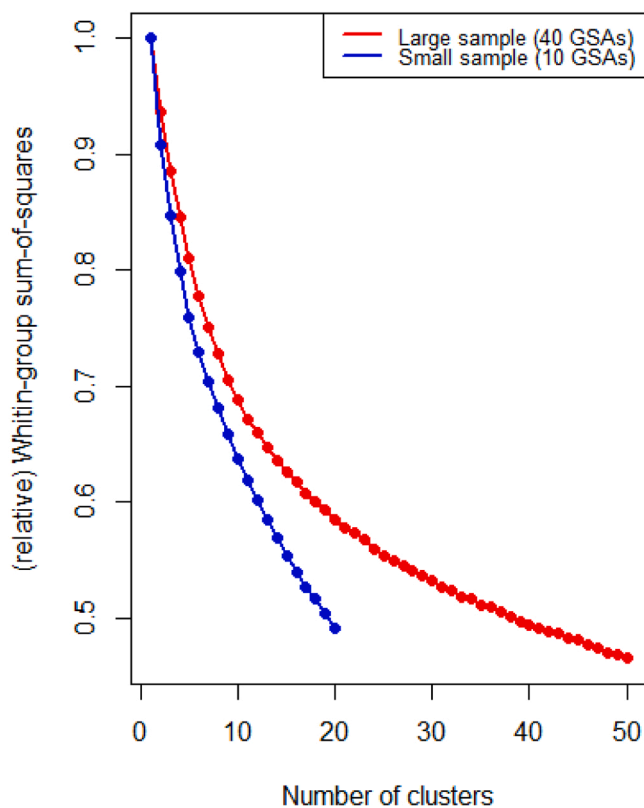


Fig. 5. Elbow plot (number of clusters versus within-cluster sum of squares) for large (40 GSAs) and small (10 GSAs) samples. Note that any asymptotical minimum is reached.

Even after accounting for the processes above, other confounding factors may remain when otolith shape and/or chemistry are used to identify stock structure. The causes of the large heterogeneity found for hake cannot be determined with the methods applied here, but connectivity (migrations through any ontogenetic stage) and/or genetic drift between neighbouring GSAs may result in the establishment of a stable genetic gradient (Spedicato et al., 2022) when coupled with the longitudinal environmental gradient reported for the Mediterranean. Factors controlling hake sub-population structure appear linked to environmental features, notably hydrological processes. Although the Mediterranean is connected to the Atlantic Ocean and Black Sea, intangible structures such as the Almeria-Oran front are known to frequently separate the stocks of a large number of species between the Atlantic and the Mediterranean (e.g., Bacha et al., 2014; Heras et al., 2019). Likewise, the Sicily Channel is a known physical barrier between the western and the eastern basins of the Mediterranean Sea because of its relatively shallow depth and its peculiar circulation pattern (Skliris, 2014).

The existence of an underlying, continuous genetic and phenotypic gradient impedes the clear-cut stock delineation and is the worst scenario for supporting simple management measures, which otherwise seems urgent for a resource rated as overexploited (FAO, 2020). Stock-specific management strategies would be debatable when the management units proposed do not guarantee that population dynamics for a given stock remain largely independent of the surrounding stocks (Lindgren et al., 2022). A high classification rate based on phenotypic features has been used as surrogate for supporting a given stock structure, but the results reported here for the European hake are not fully consistent with the two spatial scales considered. Conversely, the shape and chemistry patterns seem to better fit a continuous, longitudinal gradient.

Nevertheless, some relevant conclusions emerge from the results reported here. Management plans based on three large units (i.e., Western, Central and Eastern Mediterranean) are not strongly supported with respect to management at smaller units. The current stocks are managed in a somewhat intermediate way: some stock assessments comprise several GSAs (e.g., 1, 5, 6, 7 or 8–11) whereas other individual GSAs have their own assessments (e.g., 20). Given the results of this study, it seems reasonable to develop assessments and management plans that include spatially explicit local specificities (i.e., population dynamics and fishing effort at spatial units even smaller than GSAs), nursery areas and global connectivity. Specifically, overly coarse management measures (e.g., equal effort reduction in large areas) may lead to economic collapse for some local fleets without achieving an ecological sustainability of the resource. The fishery of the Balearic Islands could be an example: it has been largely reduced in the last decades (78 %; Vaquer-Suñer and Barrientos, 2021); thus additional reductions supported by hake population trend at a larger spatial scale could imply its economic collapse, with no effects on the hake status. Contrarily, adaptive spatial management has been proposed as a fruitful management avenue. This approach requires rich data (e.g., dynamic area-based management strategy, Hilborn et al., 2021) including better knowledge of the fish biology, continuous monitoring of catch and effort, operative tracking of the fleet and predictive capability of population demography, operational capability for adopting immediate measures, and enforcement capability. These data and capabilities are available in some Mediterranean areas, and some works have shown promising results on how to manage the European hake that could be adapted to a lack of strong stock structure (Colloca et al., 2015; Tserpes et al., 2008). Khoukh and Maynou (2018) and Sala-Coromina et al. (2021) showed with a realistic model and data of fishermen catch how the closure of a hake nursery area could improve the biology and economy of the fishery, within a Management Evaluation Strategy (MES) framework.

As indicated above, some stock assessments and Management Plans comprise several GSAs. Aiming at incorporating spatial structure for

certain vital phases, a framework has been established in the Adriatic Sea (FAO, 2021) for protecting a large nursery area of European hake, Norway lobster (*Nephrops norvegicus*) and other species. Further, including synchrony studies at a demographic level would help identify the correctness of the spatial structure of the assessments (Ostman et al., 2017). In general, adopting spatial management plans that incorporate bioeconomic tools and biological population structure, and conducting a series of simulation scenarios for management and testing assumptions are promising avenues for managing demersal stocks (Cadrin, 2020; Kerr et al., 2017; Spedicato et al., 2022).

Our results, from phenotypic analyses of otoliths, are based on the integration of several drivers into the chemistry or shape of calcified structures, including inter-individual intrinsic physiological differences (e.g., genetically-driven) and responses to existing local conditions. Environmental gradients such as temperature or salinity are important in the Mediterranean (Spedicato et al., 2022), and can shape phenotypic responses without implications for stock delineation. Contrary to the hypothesis that further data are needed to delineate the stocks is that hake does not show a clear-cut stock structure in the Mediterranean; this is expected in species with high connectivity. Hake are expected to display high connectivity as larvae are spawned both in summer and winter, with a higher larval duration than average coastal fishes (Arneri and Morales-Nin, 2000; MacPherson and Raventós, 2001). Further, spawning does not occur close to retention areas but over large areas of the shelf (García-Fernández et al., 2021; Olivar et al., 2003), thereby increasing dispersion, and the movement patterns of adult fish within the Mediterranean are currently not well-known.

We provide evidence that a relatively continuous gradient, rather than a clear-cut separation of hake stocks, is a plausible hypothesis for the Mediterranean hake population structure, according to single and combined phenotypic otolith information, based on a larger sample size than previously reported. This information is consistent with previous work, including genetic evidence suggesting three large potential stocks without strong classification power (Spedicato et al., 2022). We advocate for continuous impulse of dynamic spatial management plans that incorporate several spatial scales of information, from local to regional, to ensure both the sustainability of the resource and the profitability of small fleets.

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#### CRedit authorship contribution statement

**Beatriz Morales-Nin:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition. **Silvia Pérez-Mayol:** Methodology, Formal analysis, Resources, Writing – original draft, Writing – review & editing, Visualization. **Kirsteen MacKenzie:** Methodology, Formal analysis, Resources, Writing – review & editing. **Ignacio A. Catalán:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition. **Miquel Palmer:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Thibault Kersaudy:** Resources. **Kélig Mahé:** Conceptualization, Methodology, Formal analysis, Resources, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fishres.2022.106419.

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