Journal of the Marine Biological Association of the United Kingdom, 2011, 91(6), 1237–1242. © Marine Biological Association of the United Kingdom, 2010
doi:10.1017/S002531541000189X

Discriminating bluemouth, *Helicolenus dactylopterus* (Pisces: Sebastidae), stocks in Portuguese waters by means of otolith shape analysis

ANA NEVES[1], VERA SEQUEIRA[1], INÊS FARIAS[2], ANA RITA VIEIRA[2], RAFAELA PAIVA[1] AND LEONEL SERRANO GORDO[1]

[1]Faculdade de Ciências da Universidade de Lisboa, Departamento de Biologia Animal & Centro de Oceanografia, Bloco C2, Campo Grande, 1749-016 Lisboa, Portugal, [2]Unidade de Recursos Marinhos e Sustentabilidade, Instituto de Investigação das Pescas e do Mar (IPIMAR–INRB), Avenida Brasília, 1449–006 Lisboa, Portugal

The understanding of the stock structure of a species is essential to effectively manage fisheries. Otolith shape analysis has been successfully applied in stock identification using the elliptic Fourier analysis. This method was used to assess possible differences in the otolith shape of Helicolenus dactylopterus *caught around the Azores, Madeira and mainland Portugal. A total of 294 individuals ranging from 23 to 29 cm in total length, equally distributed by sex and area, were analysed. The multivariate analysis of variance performed on the otolith normalized elliptic Fourier descriptors (NEFDs) revealed significant differences for both area and sex, but no interaction was found between the two factors. In the canonical discriminant analysis an overall classification success of 69.4% and 66.7% was achieved for females and males respectively. These results suggest the usefulness of otolith shape analysis for stock differentiation of bluemouth from Portuguese waters.*

Keywords: bluemouth, *Helicolenus dactylopterus*, elliptical Fourier analysis, north-east Atlantic, otolith, shape analysis, stock discrimination

Submitted 7 May 2010; accepted 25 September 2010; first published online 15 December 2010

INTRODUCTION

The bluemouth, *Helicolenus dactylopterus* (Delaroche, 1809) (Pisces: Sebastidae), is a benthic deep-water scorpionfish found at depths of 200–1000 m. It is widespread in the eastern Atlantic Ocean, from Norway to South Africa, and around the Azores, Madeira and the Canary Islands (Hureau & Litvinenko, 1986). In Portuguese waters, *H. dactylopterus* is caught by a multispecies fishery that uses both bottom longline and trawl net (Figueiredo *et al.*, 1995) and is the most important commercial scorpaenid, with more than 2500 t landed annually in the past decade (DGPA, 2009). Despite its economic importance, very little information exists on its biology and in the early life stages in particular. Bluemouth is known to have internal fertilization the embryos being released in the early stage of development (Sequeira *et al.*, 2003) and larvae occur in the zooplankton.

The species live in strong association with submarine mountains in the vicinity of deep canyons (Figueiredo *et al.*, 1995) which may suggest a rather sedentary existence (Uiblein *et al.*, 2003), which can help constitute the local populations. In fact, tagging experiments around the Azores archipelago strongly suggest that adult fish lead a very sedentary life style, as many tagged specimens have been recaptured, after more than one year, exactly in the same places as they were originally caught and tagged (Aboim, 2005). The larval behaviour however is not known. The presence of seamounts and oceanic islands in combination with several patterns of circulation flow and water masses may contribute to generate trapped parcels of water around these features (e.g. Taylor columns on seamounts) acting as larval retention mechanisms (Rogers, 1994). On the other hand, it is known that many deep-water species present an extensive larval phase with high levels of dispersal and, as a result, they should exhibit a lack of stock structure on oceanic, regional and sub-regional scales (Aboim, 2005).

The stock structure of bluemouth in the north-eastern Atlantic is unknown. Several definitions of stock concept exist (e.g. Ihssen *et al.*, 1981; Hilborn & Walters, 1992) but its final application is to understand the stock structure of a species in order to properly assess and manage fish resources (Begg *et al.*, 1999). Each fish stock is identified on the basis of a number of distinctive characteristics that vary due to environmental and genetic factors. Although both genotypic and phenotypic approaches should be used to analyse the stock structure of a species, phenotypic differences can provide a firm basis for separate management units (Cadrin & Friedland, 1999) in part due to the broad number of techniques that can be used to identify stocks (Begg *et al.*, 1999). Otolith shape is one of these techniques that have been successfully used in stock identification (Waldman *et al.*, 1997)

Corresponding author:
A. Neves
Email: ananeves73@gmail.com

and, in comparison to other stock identification methods, is less subject to short-term variability caused by changes in feeding or spawning condition (Cadrin & Friedland, 2005).

Otolith shape is species-specific and the morphology can often vary geographically within a species (Lombarte & Lleonart, 1993). Several environmental factors alter the rate of otolith growth, which in turn may modify the otolith shape. Moreover different genotypes can also induce differences in otolith shape (Cardinale *et al.*, 2004). As long as genetic or environmental differences exist and the populations remain at least partially segregated, the otolith shape might vary (Campana & Casselman, 1993), which strengthens the validity of shape analysis for stock identification.

Otolith shape analysis has been successfully applied in stock identification using Fourier transformations of the outline coordinates (Bird *et al.*, 1986; Campana & Casselman, 1993; Friedland & Reddin, 1994), and more recently, the elliptic Fourier analysis (Tracey *et al.*, 2006; Mérigot *et al.*, 2007; Stransky *et al.*, 2008).

Elliptical Fourier functions represent a precise method for describing and characterizing outlines, efficiently capturing outline information in a quantifiable manner and thus extracting a significant percentage of biological information (Lestrel, 1989, 1997). The utilization of elliptical Fourier analysis surpasses some of the limitations of conventional Fourier analysis: equal divisions over the interval sampled; dependency of the coordinate system; and the difficulty of dealing with the outlines that curve back on themselves (Lestrel, 1989).

The main goal of this paper is to assess possible differences in the otolith shape of bluemouth caught in the Azores, Madeira and mainland Portugal using elliptical Fourier analysis and multivariate statistical analysis and therefore contribute to highlight the stock structure of this species in the north-eastern Atlantic.

MATERIALS AND METHODS

Sample collection

Bluemouth specimens were sampled from commercial landings of fishing vessels operating off mainland Portugal (Peniche), Madeira and Azores archipelagos (Figure 1) from November 2005 to March 2008. For each individual, total length (TL, ± 1 mm) and sex were recorded. Sagittal otoliths (hereafter referred to as otoliths) were taken as pairs, rinsed with water, air dried and stored in labelled plastic tubes.

A total of 294 individuals were analysed, 49 females and 49 males from each area. Males ranged from 23.6 to 29.8 cm in the Azores, from 23.1 to 29.7 cm in Madeira, and 23.5 to 29.9 cm in mainland Portugal, while females varied from 23.0 to 29.9 cm in the Azores, from 23.0 to 29.7 cm in Madeira, and from 23.1 to 29.8 cm in mainland Portugal.

In order to restrict the analysis to sexually mature individuals and minimize the variability caused by size-related effects, samples were restricted to fish within 23–29 cm TL range for each sex in the three areas, and whenever possible the same number of fish for each length-class (1 cm) was sampled for each area.

Due to the limited number of specimens obtained for Madeira archipelago and to guarantee a balanced number of observations, a total of 49 otoliths per sex and area were used.

Image and shape analysis

Digitized images for each right otolith were captured using a video camera (Leica DFC290) linked to a Wild stereomicroscope (6× magnification). Otoliths were immersed in a 1:1 glycerine–alcohol solution, positioned concave side up (sulcus acusticus down) and rostrum pointing to right before image capture under reflected light.

The elliptic Fourier approximation described by Kuhl & Giardina (1982) was used since it facilitates the analysis of two-dimensional forms (Lestrel, 1989). This procedure consists of the decomposition of a curve into a sum of harmonically related ellipses. Each harmonic yields four coefficients that are used as input variables for standard multivariate analysis (Baylac & Frieβ, 2005).

The SHAPE 1.3 program (Iwata & Ukai, 2002) was used to extract the elliptical Fourier descriptors (EFDs) from the contour shape of the otoliths. The ChainCoder package extracts the contour of an object from its digital image and stores the relevant information as chain-codes. The Chc2Nef package provides the normalized EFD coefficients (NEFDs) through a discrete Fourier transformation of the chain-coded contour, which causes the degeneration of the first three coefficients to fixed values: $a_1 = 1$, $b_1 = c_1 = 0$. For each otolith, 100 harmonics were generated.

While the accuracy of the otolith shape reconstruction increases with the number of descriptors used, it is often desirable to reduce the number of harmonics for multivariate analyses. To select the minimum number of harmonics for the best reconstruction of the otolith outline, the level of 99% accumulated variance was adopted as suggested by Stransky *et al.* (2008). The variance of each harmonic is proportional to its amplitude and provides a measure of the influence of each harmonic on the outline form (Lestrel, 1997). The variance for the *n*th harmonic is given by the expression:

$$S^2 = \frac{(a_n^2 + b_n^2 + c_n^2 + d_n^2)}{2}$$

where a_n, b_n, c_n and d_n are the Fourier coefficients of the *n*th harmonic. Then the cumulated variance is calculated as $S_c^2 = \sum_1^n S$.

A random subsample of 10 otoliths for each sex and area was used to define the adequate number of harmonics needed for the analysis.

Statistical methods

Multivariate analysis of variance (MANOVA) was used to analyse the effect of area and sex on the NEFDs. Canonical discriminant analysis (CDA) was then performed to detect morphometric differences in the otolith of bluemouth from the three areas and to investigate if otolith shape could be used to classify samples in terms of area of origin. The stepwise method was used to choose which variables to include in the discriminant functions. Jackknife cross-validation procedures were used to calculate an unbiased estimation of classification success.

All statistical analyses were performed in Statistica 9.0 except CDA that was made with SPSS 17.0. The significance level was set at 0.05 for all the statistical tests used.

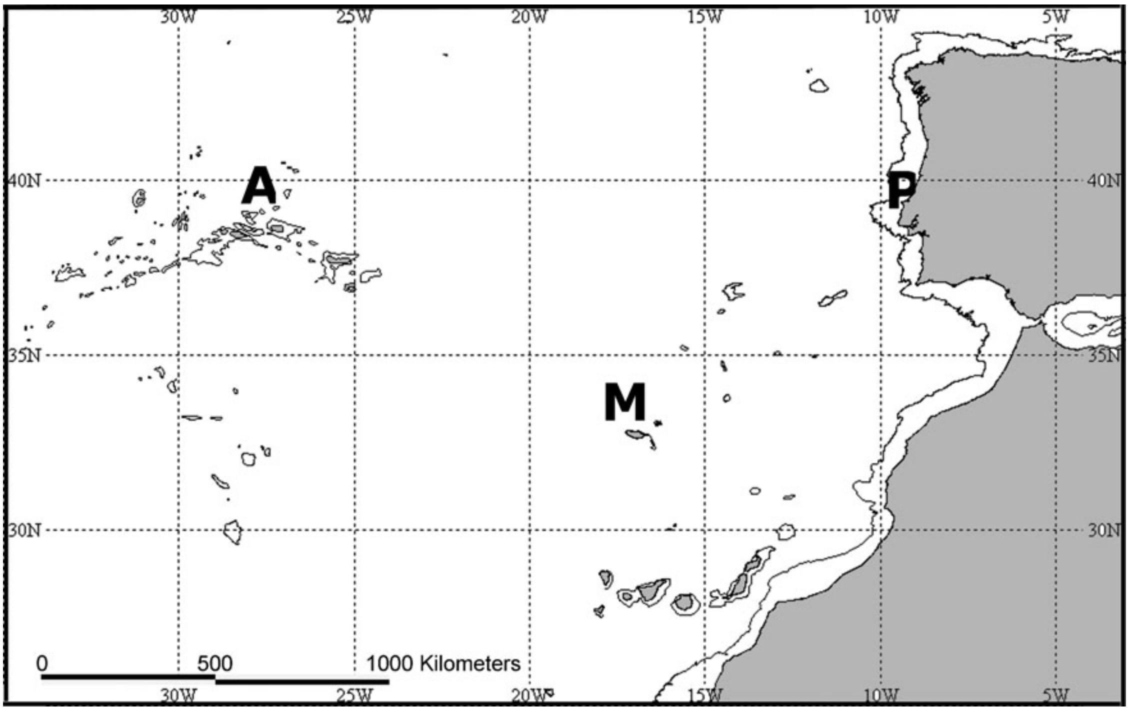

Fig. 1. Map with the sampling locations of bluemouth and the 1000 m isobath. A, Azores archipelago; M, Madeira archipelago; P, Peniche (mainland Portugal).

RESULTS

Image shape analysis

The mean and standard deviation of the cumulative variance of the harmonics are plotted in Figure 2. The otolith contours defined by 1, 5, 15 and 100 harmonics are also given.

A maximum of 15 harmonics (excluding the 1st harmonic) were needed to explain 99% of the otolith shape variation. Therefore, each individual was represented by the subsequent 61 coefficients (1 coefficient from harmonic 1 and 4 coefficients from harmonics 2–16).

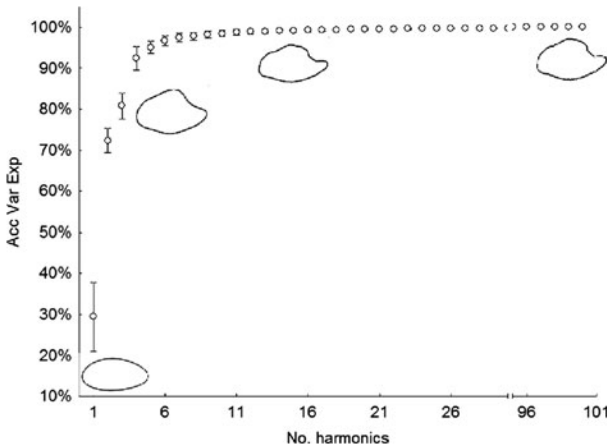

Fig. 2. Mean and standard deviation of the cumulative variance of 100 harmonics for 60 randomly selected otoliths. Acc Var Exp, accumulated percentage of variance explained by the *n*th harmonic. Otolith contours defined by 1, 5, 15 and 100 harmonics are also plotted.

Variance statistical analysis

The result of the MANOVA to compare the otolith NEFDs between the three areas and the sexes evidence statistically significant differences for both factors, but no interaction was found between them (Table 1). Since the effect of sex on the otolith shape was shown to be significant, all subsequent analyses were performed for each sex separately.

Canonical discriminant analysis

The variables used in the stepwise discriminant analysis are given in Table 2. For the analysis of females, 14 coefficients were used, while 17 were used in the analysis of males. Lower and higher order coefficients were relevant for the discrimination for both females and males.

The differences among areas evidenced by the MANOVA were also supported by the CDA. An overall classification success of 69.4% and 66.7% was achieved for females and males respectively (Table 2). The highest rate classification was found for females from mainland Portugal with 76% success, while females from Madeira and males from Madeira and mainland Portugal showed less discrimination

Table 1. Summary of multivariate analysis of variance (N = 294) on the otolith normalized elliptical Fourier descriptors coefficients from mainland Portugal, Madeira and Azores. Pillai, Pillai's test statistic; Wilks, Wilks's test statistic; df, degrees of freedom (numerator, nominator); *P*, *P* value.

	Pillai	Wilks	df	*P*
Area	0.871	0.306	122, 464	0.0000
Sex	0.277	0.723	61, 232	0.0121
Area : sex	0.440	0.606	122, 464	0.1566

https://doi.org/10.1017/S002531541000189X Published online by Cambridge University Press

Table 2. Jackknifed classification matrix of the discriminant analysis applied to the three areas for females and males. Percentages in rows represent the classification into the areas given in columns (correct classification in bold). Overall classification success: 69.4%, Wilk's $\lambda = 0.330$ (females); 66.7%, Wilk's $\lambda = 0.329$ (males).

	Females			**Males**		
	Azores	**Madeira**	**Mainland Portugal**	**Azores**	**Madeira**	**Mainland Portugal**
Azores	**67.3**	22.4	10.2	**69.4**	14.3	16.3
Madeira	26.5	**65.3**	8.2	22.4	**65.3**	12.2
Mainland Portugal	18.4	6.1	**75.5**	22.4	12.2	**65.3**
Variables	a1, c2, d3, a5, c5, a6, a7, b7, d7, a8, c8, d11, b14, c15			a1, d1, a2, b2, a3, d3, d4, b5, c5, a6, c6, d8, a10, c10, b13, a15, c15		

with 65%. The two discriminant functions were significant for the discrimination of sites in both females (f1: $\Lambda = 0.330$, $P < 0.0001$; f2: $\Lambda = 0.684$, $P < 0.001$) and males (f1: $\Lambda = 0.329$, $P < 0.0001$; f2: $\Lambda = 0.722$, $P < 0.001$). The score plots for the first two discriminant functions (Figures 3 & 4) show a separation between the three areas although some overlapping can be noticed. The first discriminant functions explained 70% of between group variance for females and 76% for males.

DISCUSSION

The results obtained with the otolith shape analysis suggest its usefulness as a stock differentiation tool. The MANOVA performed on the bluemouth otolith shape coefficients revealed differences between sexes and among the three areas of Portuguese waters, which were subsequently supported by the discriminant analysis with moderate classification success values but highly above the aleatory ones. Stransky (2005) found similar classification rates (72%–74% overall) between west, central and east regions in the North Atlantic for two *Sebastes* species closely related to bluemouth (*Sebastes marinus* (Linnaeus, 1758) and *S. mentella* Travin, 1951), although his study showed low discrimination when analysing the areas within these regions.

Nevertheless, some overlapping between areas can be noticed. Several confounding effects have been pointed out by Simoneau *et al.* (2000), namely: age, year-class and sex among others. In this work, the minimization of length effect was achieved restricting the length range as narrow as possible and analysing only adult individuals since Campana & Casselman (1993) found that otolith shape analysis might be of negligible value to stock identification if applied to sexually immature fish. Also the elliptical Fourier descriptors were normalized for otolith size, rotation and starting point (Kuhl & Giardina, 1982; Iwata & Ukai, 2002) in order to highly reduce these confounding factors in the analysis. The aggregation of data from different years is frequently used in otolith shape analysis studies (DeVries *et al.*, 2002; Tracey *et al.*, 2006; Farias *et al.*, 2009) and although it is referred to as a possible confounding effect, Friedland & Reddin (1994) and Campana & Casselman (1993) found no significance in the year effect on the otolith shape of Atlantic salmon and cod, respectively. Bergenius *et al.* (2006) even mention that it would be good practice to compare multiple cohorts collected

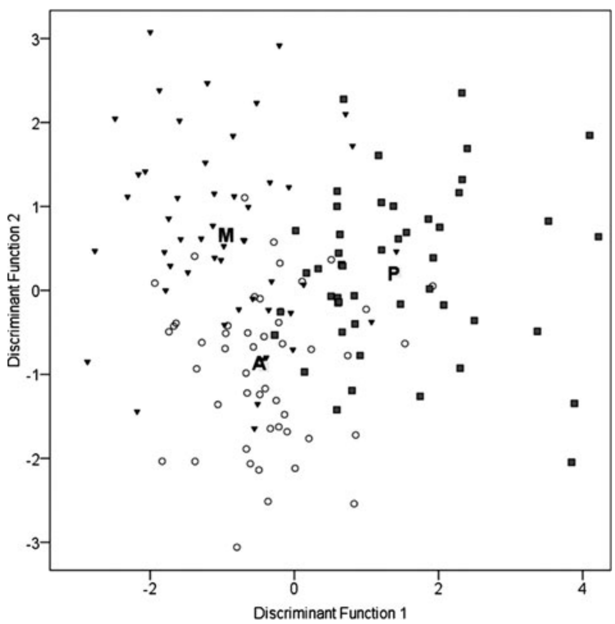

Fig. 3. Discriminant analysis function scores for female samples from the Azores (open circles), Madeira (black inverted triangles) and mainland Portugal (grey squares) based on 14 elliptical Fourier normalized descriptors of the first 15 harmonics. The letters indicate the class centroids.

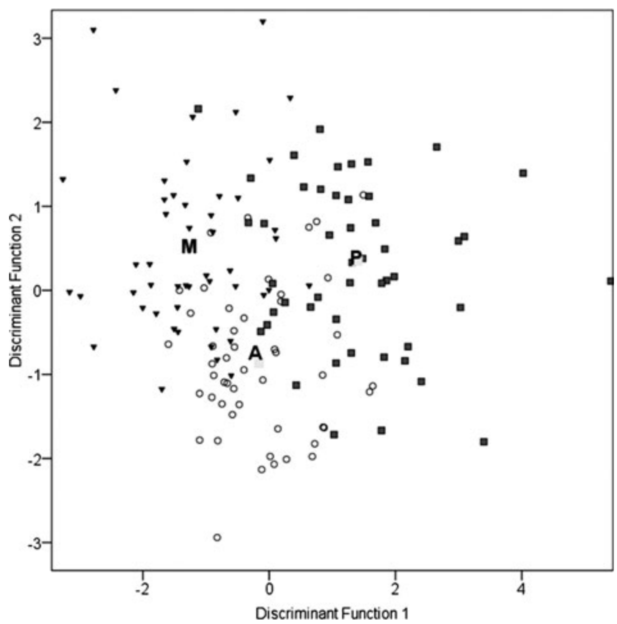

Fig. 4. Discriminant analysis function scores for male samples from the Azores (open circles), Madeira (black inverted triangles) and mainland Portugal (grey squares) based on 17 elliptical Fourier normalized descriptors of the first 15 harmonics. The letters indicate the class centroids.

https://doi.org/10.1017/S002531541000189X Published online by Cambridge University Press

over several years so as to minimize confounding spatial variation in otolith shape with particular times of sampling and derive a time-averaged assessment of the spatial structure of a stock. Sexes were also analysed separately to minimize the number of sources of uncertainty and to highlight the real differences in otolith shape between the three areas.

The bluemouth stock structure in the north-eastern Atlantic is still unknown. The present results on otolith shape analysis clearly show their usefulness in supporting the existence of more than one stock unit in the southern north-eastern Atlantic (although their use in safely classifying new individuals from unknown sources to a known area is insufficient) reinforcing the evidence for a stock structure of bluemouth shown by other techniques. In fact, Aboim (2005), using genetic studies with microsatellites markers, found evidence of some genetic differentiation between bluemouth of some areas in the north-eastern Atlantic. Swan *et al.* (2006), using otolith chemistry, showed that Portuguese mainland samples were more similar in composition to the Mediterranean groups than to the Azorean or Rockall Trough samples, being a possible explanation for the existence of more continental material or any other characteristic of the water mass. More recently, Sequeira *et al.* (2009) found significant differences for the growth rate of bluemouth populations of mainland Portugal and the Azorean waters. This probable stock differentiation is also supported by the species behaviour. *Helicolenus dactylopterus* dwell mostly around submarine mountains in the neighbourhood of deep canyons (Figueiredo *et al.*, 1995), and exhibit a rather sedentary behaviour (Uiblein *et al.*, 2003), so the possibility of the existence of local populations constituting different management stocks is much more likely to occur.

Therefore, and as stressed by Cardinale *et al.* (2004), otolith shape analysis can be seen as a complement technique towards an effective fisheries management. This methodology is also cheaper and/or more time efficient than several others (e.g. genetics, parasites and microchemical discrimination techniques).

Although other studies (parasites, more genetic techniques) should be implemented to clearly understand the stock structure of bluemouth in the north-eastern Atlantic, the available information indicates the existence of local populations of this species and the default management scenario should be to use a precautionary approach (e.g. to consider the three areas as separate stocks) to ensure resource sustainability and maintenance of genetic biodiversity (Begg & Waldman, 1999). A failure to recognize these stocks may lead to erroneous management actions, including the overexploitation of some stocks.

ACKNOWLEDGEMENT

This study was partially financed by the Fundação para a Ciência e Tecnologia (FCT) through the grant awarded to V. Sequeira (SFRH/BDD/22746/2005).

REFERENCES

Aboim M.A. (2005) *Population genetics and evolutionary history of some deep-sea demersal fishes from the Azores—North Atlantic*. PhD thesis. School of Ocean and Earth Sciences, University of Southampton, UK.

Baylac M. and Frieβ M. (2005) Fourier descriptors, Procrustes superimposition, and data dimensionality: an example of cranial shape analysis in modern human populations. In Slice D.E. (ed.) *Modern morphometrics in physical anthropology*. New York: Kluwer Academic/Plenum Publishers, pp. 145–165.

Begg G.A. and Waldman J.R. (1999) An holistic approach to fish stock identification. *Fisheries Research* 43, 35–44.

Begg G.A., Friedland K.D. and Pearce J.B. (1999) Stock identification and its role in stock assessment and fisheries management: an overview. *Fisheries Research* 43, 1–8.

Bergenius M., Gavin A.J., Begg A. and Mapstone B. (2006) The use of otolith morphology to indicate the stock structure of common coral trout (*Plectropomus leopardus*) on the Great Barrier Reef, Australia. *Fishery Bulletin* 104, 498–511.

Bird J.L., Eppler D.T. and Checkley D.M. (1986) Comparisons of herring otoliths using Fourier-series shape-analysis. *Canadian Journal of Fisheries and Aquatic Sciences* 43, 1228–1234.

Cadrin S.X. and Friedland K.D. (1999) The utility of image processing techniques for morphometric analysis and stock identification. *Fisheries Research* 43, 129–139.

Cadrin S.X. and Friedland K.D. (2005) Morphometric outlines. In Cadrin S.X, Friedland K.D. and Waldman J.R. (eds) *Stock identification methods: applications in fisheries science*. Amsterdam: Elsevier/Academic Press, pp. 173–183.

Campana S.E. and Casselman J.M. (1993) Stock discrimination using otolith shape analysis. *Canadian Journal of Fisheries and Aquatic Sciences* 50, 1062–1083.

Cardinale M., Doering-Arjes P., Kastowsky M. and Mosegaard H. (2004) Effects of sex, stock, and environment on the shape of known-age Atlantic cod (*Gadus morhua*) otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* 61, 158–167.

DeVries D., Grimes B. and Prager M. (2002) Using otolith shape analysis to distinguish eastern Gulf of Mexico and Atlantic Ocean stocks of king mackerel. *Fisheries Research* 57, 51–62.

DGPA (2009) *Recursos da pesca—série estatística*. Lisbon: DGPA. [In Portuguese.]

Farias I., Vieira A.R., Gordo L.S and Figueiredo I. (2009) Otolith shape analysis as a tool for stock discrimination of the black scabbardfish, *Aphanopus carbo* Lowe, 1839 (Pisces: Trichiuridae), in Portuguese waters. *Scientia Marina* 73 (Supplement 2), 47–53.

Figueiredo M.J., Figueiredo I. and Moura O. (1995) *Distribution, abundance and size composition of blackbelly rosefish* (Helicolenus dactylopterus) *and Mediterranean redfish* (Hoplostethus mediterraneus) *on the slope of the Portuguese south and southern west coasts.* ICES CM 1995/G:10.

Friedland K.D. and Reddin D.G. (1994) Use of otolith morphology in stock discriminations of Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* 51, 91–98.

Hilborn R. and Walters C.J. (1992) *Quantitative fisheries stock assessment. Choice, dynamics and uncertainty*. London: Chapman and Hall, 570 pp.

Hureau J.C. and Litvinenko N.I. (1986) Scorpaenidae. In Whitehead P.J.P., Bauchot M.-L., Hureau J.-C., Nielsen J. and Tortonese E. (eds) *Fishes of the north-eastern Atlantic and the Mediterranean*. Rome: FAO, pp. 1211–1229.

Ihssen P.E., Brooke H.E., Casselman J.M., McGlade J.M., Payne N.R. and Utter F.M. (1981) Stock identification: materials and methods. *Canadian Journal of Fisheries and Aquatic Sciences* 38, 1838–1855.

Iwata H. and Ukai Y. (2002) SHAPE: a computer program package for quantitative evaluation of biological shapes based on elliptic Fourier descriptors. *Journal of Heredity* 93, 384–385.

Kuhl F.P. and Giardina C.R. (1982) Elliptic Fourier features of closed contour. *Computer Graphics and Image Processing* 18, 236–258.

Lestrel P.E. (1989) Method for analysing complex two-dimensional forms: elliptical Fourier functions. *American Journal of Human Biology* 1, 149–164.

Lestrel P.E. (1997) *Fourier descriptors and their applications in biology*. Cambridge: Cambridge University Press, 466 pp.

Lombarte A. and Lleonart J. (1993) Otolith size changes related with body growth, habitat depth and temperature. *Environmental Biology of Fishes* 37, 297–306.

Mérigot B., Letourneur Y. and Lecomte-Finiger R. (2007) Characterization of local populations of the common sole *Solea solea* (Pisces, Soleidae) in the NW Mediterranean through otolith morphometrics and shape analysis. *Marine Biology* 151, 997–1008.

Rogers A.D. (1994) The biology of seamounts. *Advances in Marine Biology* 30, 305–350.

Sequeira V., Figueiredo I., Muñoz M. and Gordo L.S. (2003) New approach to the reproductive biology of *Helicolenus dactylopterus*. *Journal of Fish Biology* 62, 1206–1210.

Sequeira V., Neves A., Vieira A.R., Figueiredo I. and Gordo L.S. (2009) Age and growth of bluemouth, *Helicolenus dactylopterus*, from the Portuguese continental slope. *ICES Journal of Marine Science* 66, 524–531.

Simoneau M., Casselman J. and Fortin R. (2000) Determining the effect of negative allometry (length/height relationship) on variation in otolith shape in lake trout (*Salvelinus namaycush*), using Fourier-series analysis. *Canadian Journal of Zoology* 78, 1597–1603.

Stransky C. (2005) Geographic variation of golden redfish (*Sebastes marinus*) and deep-sea redfish (*S. mentella*) in the North Atlantic based on otolith shape analysis. *ICES Journal of Marine Science* 62, 1691–1698.

Stransky C., Murta A.G., Schlickeisen J. and Zimmermann C. (2008) Otolith shape analysis as a tool for stock separation of horse mackerel (*Trachurus trachurus*) in the Northeast Atlantic and Mediterranean. *Fisheries Research* 89, 159–166.

Swan S.C., Geffen A.J., Morales-Nin B., Gordon J.D.M., Shimmield T., Sawyer T. and Massutí E. (2006) Otolith chemistry: an aid to stock separation of *Helicolenus dactylopterus* (bluemouth) and *Merluccius merluccius* (European hake) in the Northeast Atlantic and Mediterranean. *ICES Journal of Marine Science* 63, 504–513.

Tracey S.R., Lyle J.M. and Duhamel G. (2006) Application of elliptical Fourier analysis of otolith form as a tool for stock identification. *Fisheries Research* 77, 138–147.

Uiblein F., Lorance O. and Latrouite D. (2003) Behaviour and habitat utilisation of seven demersal fish species on the Bay of Biscay continental slope, NE Atlantic. *Marine Ecology Progress Series* 257, 223–232.

and

Waldman J.R., Richards R.A., Schill W.B., Wirgin I. and Fabrizio M.C. (1997) An empirical comparison of stock identification techniques applied to striped bass. *Transactions of the American Fisheries Society* 126, 369–385.

Correspondence should be addressed to:

A. Neves
Faculdade de Ciências da Universidade de Lisboa
Departamento de Biologia Animal & Centro de Oceanografia
Bloco C2, Campo Grande, 1749-016 Lisboa, Portugal
email: ananeves73@gmail.com

https://doi.org/10.1017/S002531541000189X Published online by Cambridge University Press