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Otolith shape as a valuable tool to evaluate the stock structure of swordfish (*Xiphias gladius*) in the Indian Ocean

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Abstract

Swordfish (*Xiphias gladius*) is an oceanic-pelagic species currently fully exploited by several fisheries in the Indian Ocean, with suspicion of overexploitation in the southwest, but without a clear understanding of the real stock structure within this Ocean. Population structure of the Indian stock was studied in the western Indian Ocean using 395 individual samples collected from 2009 to 2014. Sagittal otoliths of the fish have been removed and shape analysis performed on these calcified pieces. Otolith morphometrics data and normalized Elliptical Fourier Descriptors (EFDs) were then extracted automatically by the dedicated image-analysis system TNPC. Preliminary, side effect was tested by Redundancy analysis (RDA) combined to permutation tests on 91 individual samples and showed no significant differences in the outline shape between the right and left otoliths. Consequently, 395 sagittal otoliths were used to identify stocks among several geographical areas (La Reunion, Mozambique channel, Rodrigues, South Africa, South Malagasy, Sri Lanka and Thailand) within the Indian Ocean. To investigate variations of otolith shape according to 4 explanatory variables, Principal Components Analysis (PCA) was applied to EFDs and a RDA with permutation tests was used on the first 7 PC selected by the broken-stick model. These tests demonstrated no significant effects, neither by sex ($p=0.121$), sampled year ($p=0.725$), or total length ($p=0.464$). Only, geographical area appeared to be significant ($p<0.05$). Regarding the relationship between the ratio otolith length/otolith width and the total length of fish, size effect was neither significant. Furthermore, Linear Discriminant Analysis (LDA) was performed and overall jackknifed classification success reached 30%. Finally, a clustering analysis has been realised using Ward's hierarchical algorithm, which discriminated 3 different groups; however each group was composed by some individual samples from all geographical areas. In conclusion, all these results did not show a clear geographical separation, which corroborate the recent genetic analysis at the Indian Ocean scale while identifying only a single swordfish stock component in this area.
Keywords

Keywords:

Xiphias gladius, otolith shape, Fourier descriptors, stock discrimination, Indian Ocean.

Introduction

Swordfish (*Xiphias gladius*) is a highly migratory oceanic species, currently fully exploited by several commercial fisheries in the Indian Ocean. It is commonly accepted that large pelagic species are lacking in spatial structure due to their cosmopolitan distribution, large population size, high fecundity and ability to easily migrate inter-ocean distances (Nakamura, 1985). On the basis of the last swordfish stock assessment of this species in the Indian Ocean(IOTC–WPB11 2013), levels of catches in the whole Indian Ocean for 2007–2011 (average of 21 916 tons) were considered below the estimated maximum sustainable yield (MSY; 29 900–34 200 tons). Nevertheless, when population structure was considered and when the assessment focused on the southwest Indian Ocean (SWIO) as an independent stock – a case requested by the Scientific Comity of the Indian Ocean Tuna Commission (IOTC; IOTC–SC16 2013) on the basis of the fishery data, most of the evidence indicated that the resource has been overfished in the past decade, with the current level of catches indicating a stock fully exploited (7 566 tons in 2011 with an estimated MSY: 7 100–9 400; IOTC–WPB11 2013). Such a result clearly emphasis the importance of better knowing the spatial structure of this species at the scale of the Indian Ocean, and its relation with adjacent oceans (i.e. Atlantic and Pacific).

Stock identification and spatial structure information provide a basis for understanding fish population dynamics and provides reliable resource assessment for fishery management (Reiss et al. 2009). Several techniques may be used to identify the stock limits, such as tagging experiments, analyses of spatial variation of genetic or morphometric markers, differentiation of life-history variables, parasites and contaminant concentrations (Pawson and Jennings 1996; Cadrin et al. 2014). A recent study on population genetics using multi-genetic marker approach and spatio-temporal analysis suggested there is a single panmictic population (i.e. a single stock) of swordfish in the Indian Ocean (Muths et al. 2013). This study also concluded in the need of using other stock discrimination approaches to unravel the real stock structure of this species.

In this study, swordfish stock structure was investigated using the shape of sagittal otoliths. Otolith morphology is influenced by biotic and abiotic factors (Cardinale et al. 2004; Capoccioni et al. 2011). Analysis of the outline of otoliths has previously been used for stock discrimination (haddock: Begg and Brown 2000; cod: Galley et al. 2006; Petursdottir et al. 2006, Stransky et al. 2008; striped red mullet: Benzinou et al. 2013). Based on the logistic and sample collection presented in Muths et al. (2013), this study focus on individuals sampled over a wide geographic area, from South Africa to Thailand, and otolith shape analysis was performed to discriminate components of swordfish in the Indian Ocean stock.

Materials and methods

Sample collection

Ethical approval was not required for this study, as all fish were collected as part of routine fishing procedures. Swordfish samples from South Africa to Thailand (Fig. 1; Table 1) were collected from 2009 to 2014 by onboard observers on commercial fishing vessels or at landing (with due care collecting the related fishing information). Information on sample location (exact latitude and longitude or 5° square) was systematically noted. Sex and maturity of the sampled individuals was determined by macroscopic examination of gonads. Only mature fish were included in this study to minimize the effect of sexual maturity which may change otolith shape (Cardinale et al. 2004). Sagittal otoliths were extracted from a total of 395 individuals ranging from 56 to 300 cm total length (mean 155.0.

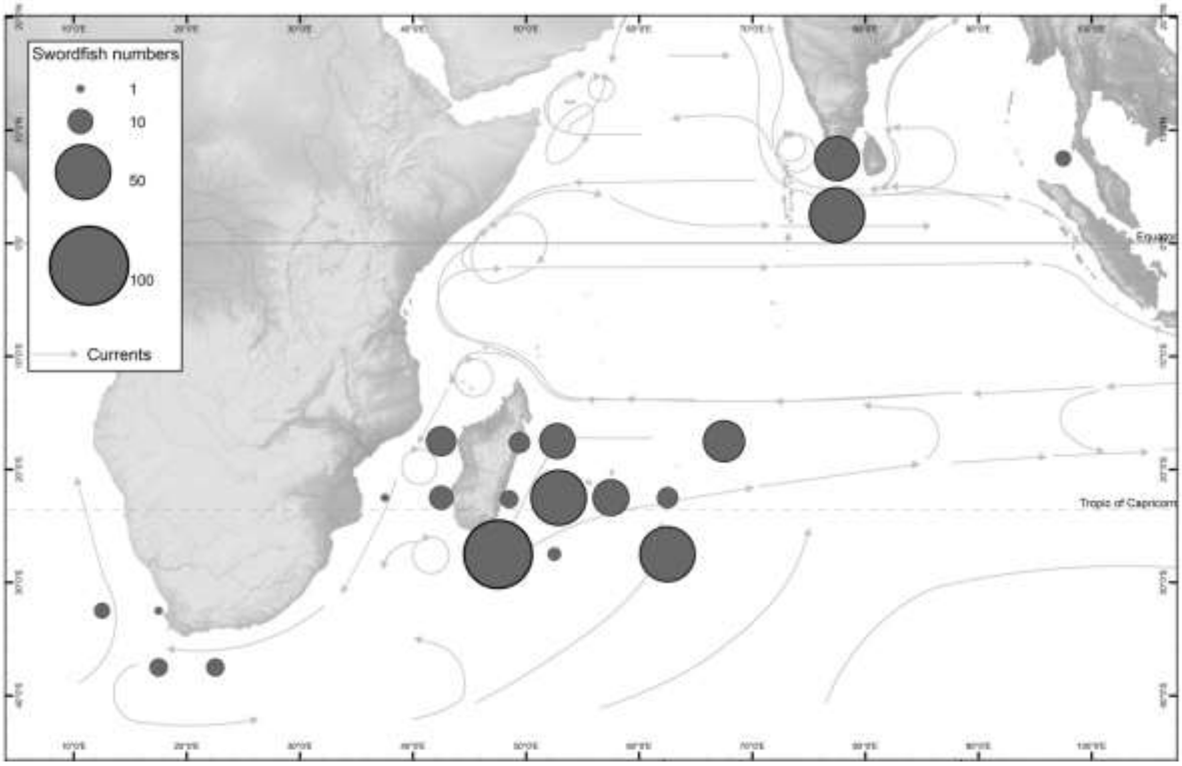

Figure 1. Map of location of swordfish samples collected between 2009 and 2014 in the Indian Ocean.

Table 1. Number of swordfish otolith samples by year, sex (Male, Femelle, Other : no identication of sex) and sampling area. Mean Total length characteristics by sampling area are given.

Sampling area	Total Length (cm)	Sex			Sampling year				Total
		Male	Female	Other	2009	2010	2013	2014	
La Réunion	143.0±23.0	38	59	10	61	46			**107**
Mozambique	135.1±38.6	11	10	13		34			**34**
Rodrigues	165.2±29.8	29	54	0	39	44			**83**
South Africa	185.9±36.9			20			5	15	**20**
South Malagasy	150.4±34.2	21	48	2	62	9			**71**
Sri Lanka	167.3±57.5	8	20	48	23	53			**76**
Thailand	98.2±20.3		3	1		4			**4**
Total	**155.0**±38.6	**107**	**194**	**94**	**185**	**190**	**5**	**15**	**395**

Otolith shape analysis

Sworfish otoliths present a large dorsal area, between the *excisura major* and the *excisura minor*, very distinct from the *antirostrum* (Fig. 2). Images of the whole left and right sagittal otoliths were scanned (Epson V750) under reflected light and stored with high resolution (3200 dpi). Image processing was performed using the image analysis system TNPC (Digital processing for calcified structures, version 7, www.tnpc.fr) with the *sulcus acusticus* facing up. In order to compare left and right otolith shapes, a mirror image of left otoliths was used.

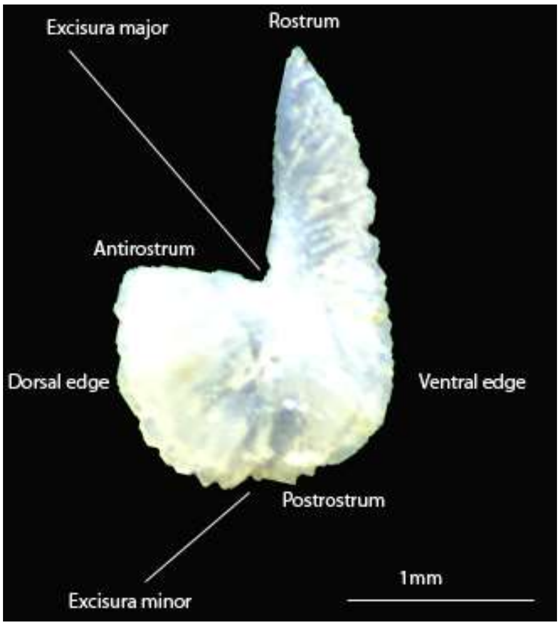

Figure 2. Photograph of a whole swordfish sagittal otolith.

Otolith length and width were measured and the contour of each otolith was extracted using the automatic threshold in the TNPC software. To describe otolith contours, Elliptic Fourier Analysis (EFA; Lestrel 1997) was carried out. For each otolith, the first 99 elliptical Fourier harmonics (Hi) were extracted and normalised with respect to the first harmonic using the TNPC software and were, thus, invariant to otolith size, rotation and starting point of the shape measurements (Kuhl and Giardina 1982). To determine the number of harmonics required to reconstruct the otolith outline, the Fourier Power (PF) was calculated for each individual otolith *k* as a measure of the amount of contour rebuilt by each harmonic:

$$F(n_k) = \sum_{HI=1}^{n_k} \frac{A_{\mathrm{HI}}^2+B_{\mathrm{HI}}^2+C_{\mathrm{HI}}^2+D_{\mathrm{HI}}^2}{2}$$

Where A_{HI}, B_{HI}, C_{HI} and D_{HI} are the parameters of the *HI*th harmonic and n_k is the total number of harmonics included. The value of n_k was chosen such that $PF(n_k)$ explains 99.99% of variance in contour coordinates or, in other words, such that shape is reconstructed at 99.99 (Lestrel 2008).

Statistical analyses

Preliminary, side effect was tested by Redundancy analysis (RDA) combined to permutation tests on 91 individual samples. To visualise differences in otolith shape between right and left sides, an average otolith shape of each side group was formed by the outline reverse Fourier transform.

To investigate potential explanations for otolith shape differences in more detail, multivariate analyses were carried out. To reduce the number of dimensions, Principal Components Analysis (PCA) was applied to EFDs of otolith contours (Rohlf and Archie 1984). In order to limit the number of principal components (PCs) to only significant PCs, the ‘broken stick method’ was applied (Legendre and Legendre 2012). This method assumes that, if the total variance (sum of the eigenvalues) is divided randomly among the various components, then the expected distribution of the eigenvalues will follow a broken-stick distribution. The contributions of original variables toward the corresponding eigenvectors (PC loadings) were analyzed to understand which shape features had more influence on each PC. Next, the Redundancy analyses (RDA) were carried out. RDA is an extension of multiple regressions to multivariate response data and an extension of principal component analysis (Legendre & Legendre 2012). The RDA aimed the variance explained by the explanatory variables of interest, i.e. total fish length, sex, sampling year and sampling year. A permutation test was used to test the significance of each explanatory variable. To determine whether otoliths collected in different sampling year could be distinguished based on their contour shapes, stepwise Linear Discriminant Analysis (LDA) was applied to select the discriminant variables among the Fourier harmonics (Rencher and Christensen 2012). To evaluate the resulting discriminant functions, the percentage correct classification of individuals to sampling year was calculated using jackknife cross-validation and Wilk’s lambda criteria (Klecka 1980). Finally, a cluster analysis was performed on the normalised Fourier harmonics to group individuals with similar otolith contour shapes. For this, Ward's hierarchical algorithm based on squared Euclidean distances was used.
All statistical analyses were performed using the ‘Vegan’ (Oksanen et al. 2013), ‘MASS’ (Venables and Ripley 2002), ‘CAR’(Fox and Weisberg 2011), ‘FactoMinR’ (Lê et al. 2008), ‘HH’ (Heiberger and Burt Holland 2004) and ‘Ellipse’ (Murdoch and Chow 1996) packages in the statistical environment R (R Core Team 2014).

Results

Among the 99 Fourier harmonics extracted to describe otolith contours, the first 43 harmonics explained more than 99.99% of the otolith variation and were thus used for the multivariate analysis. The Redundancy analysis (RDA) combined to permutation tests of both otoliths showed no significant difference between the left and right otoliths (Fig. 3).

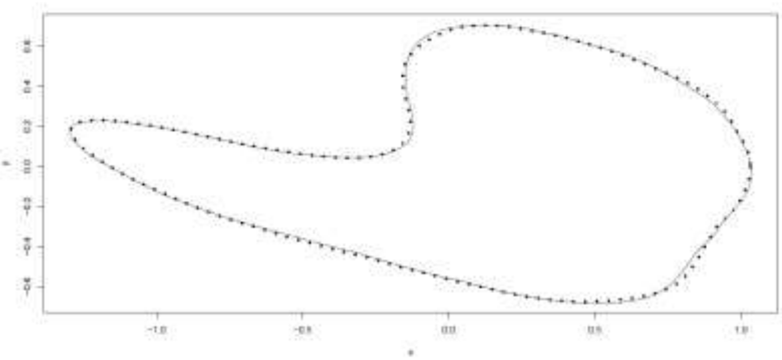

Figure 3. Mean otolith outline shapes formed with reverse Fourier transform of the outline using the first 43 harmonics showing the overlap and variations between right (dark grey dash line) and left (grey solid line) otolith shape of swordfish from the Indian Ocean (N=91).

As the left and right otoliths of swordfish were comparable, 304 right otoliths and 91 left otoliths were combined in this study. Principal Components Analysis of these first 43 Fourier harmonics showed that the first and the second PC accounted for 35.9% and 25.2% of the total variance respectively. Only the first seven PCs were significant as determined by their eigenvalues exceeding the threshold eigenvalue generated by the broken-stick model. The effect of total length (p=0.464), sex (p=0.121), sampling year (p=0.725) and sampling area (p=0.002) were tested by the RDA. Only sampling area was significant (p<0.05), sampling year was used as explanatory variable in the subsequent LDA with sex:sampling area and sampling year:sampling area combinations. The overall jackknife classification success was 30% (Table 1). The analysis showed significant differences among groups of swordfish sampled in different areas of Indian Ocean (Wilks'λ = 0.017; F=1.255; p=0.001). The misclassification percentage for each sampling area was explained by closed and distant areas (Table 1).

Table 1. Jackknifed correct classification matrix of the linear function discriminant analysis for mature swordfish (N=395) between sampling areas based on the first normalized 43 harmonics. The percentages in each row represent the classification into the sampling area given in columns (correct classification in grey square).

Sampling area	South Africa	South Malagasy	Mozambique	La Réunion	Rodrigues	Sri Lanka	Thailand	%
South Africa	4	3	2	1	9	1	0	20
South Malagasy	5	19	4	20	9	13	1	27
Mozambique	3	2	5	11	9	4	0	15
La Réunion	5	17	16	41	14	13	1	38
Rodrigues	2	12	8	23	28	10	0	34
Sri Lanka	2	12	14	13	12	21	2	28
Thailand	0	2	1	0	1	0	0	0

The hierarchical clustering analysis performed on the matrix of 43 Fourier harmonics identified three clusters of fishes (Fig. 4). All sampling areas were divided practically in the same way (Table 2).

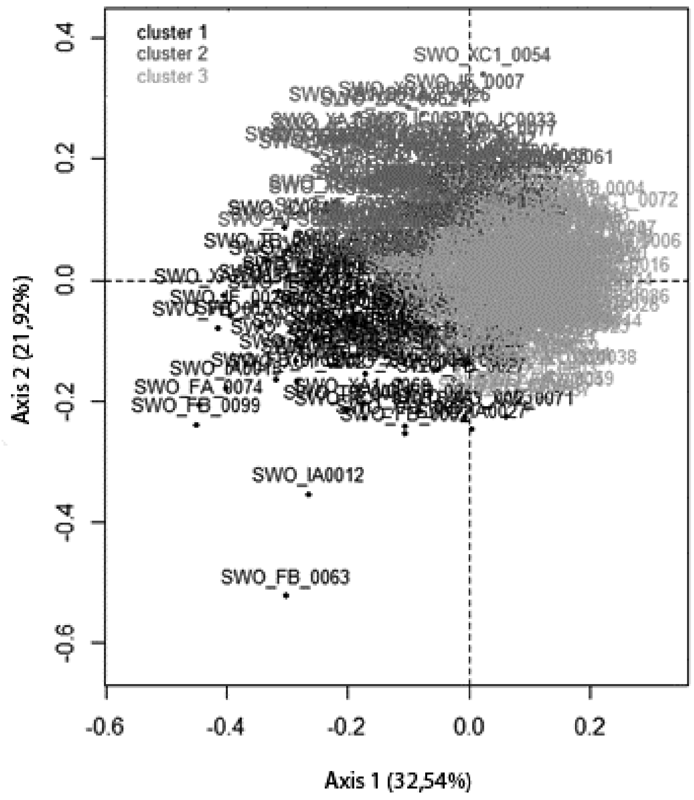

Figure 4. Average individuals from the three clusters identified by hierarchical cluster analysis using Ward's hierarchical algorithm based on the squared Euclidean distances for all swordfish (N=395) on the first 2 dimensions. The dots with sample numbers represent individuals.

Table 2. Classification matrix of the hierarchical clustering on principal components for mature swordfish (N=395) between Sampling areas based on the first normalized 43 harmonics.

Sampling area	**Cluster 1**	**Cluster 2**	**Cluster 3**	**Total**
South Africa	2	5	13	20
South Malagasy	11	20	40	71
Mozambique	3	13	18	34
La Reunion	20	23	64	107
Rodrigues	12	23	48	83
Sri Lanka	17	17	42	76
Thailand	1	1	2	4
Total	66	102	227	395

Discussion

The previous recent genetic study (Muths et al. 2013) identified difference between Atlantic and Indian Oceans swordfish stocks but did not linger on possible sub-divisions into Indian Ocean stock. Otolith shape analysis is an another efficient stock identification tool linked to genetic heterogeneity and the influence of environmental factors (Cadrin and Friedland 1999; Campana and Casselman 1993; Torres et al. 2000; Cardinale et al. 2004; Swan et al. 2006; Vignon and Morat 2010). The external contour of otoliths could be describe by several techniques as the basic descriptors (coefficient of form, roundness, circularity…), the geometric morphometric analyses (Ponton 2006; Ramirez-Perez et al. 2010; Vergara-Solana et al. 2013); wavelet functions (Parisi et al. 2005; Sadighzadeh et al. 2014); growth markers (Benzinou et al. 2013); and the geodesic method (Benzinou et al. 2013). Among these, the elliptical Fourier analysis remains the most widely used and powerful method to describe otolith external shape (Aguera and Brophy 2011; Capoccioni et al. 2011; Fergusson et al. 2011; Legua et al. 2013; Paul et al. 2013). This method has the advantage to be unaffected by short-term changes in fish condition (Campana and Casselman 1993) due to environmental variations (Campana 1999). Nevertheless, its biological interpretation is more complex than one based on linear morphometric descriptors (Stransky and MacLellan 2005). Some studies have combined the elliptical Fourier analysis and some basic descriptors of otolith contours to help interpretation of results (Campana and Casselman 1993; Begg and Brown 2000; Galley et al. 2006; Merigot et al. 2007; Fergusson et al. 2011; Legua et al. 2013).

On this study, side effect was tested first and there were no difference between right and left otoliths of swordfish. Same conclusion was reported for Bluefin tuna (*Thunnus thynnus*) tested by otolith morphological characteristics (Megalofonou 2006). The sampling used in this study was restricted to adult fish to avoid the confounding effect of allometric growth on otolith shape (Cardinale et al. 2004) and the sexual maturity effect, which could modify the outline contour of otoliths (Campana and Casselman 1993). Here , the effect of total length, sex and sampling year were not significant. The use of morphological otolith parameters (length and width) allowed us to observe the otolith shape, and conclude that it was comparable during all life stages of swordfish. Sexual dimorphism effect didn't affected significantly otolith shape of swordfish as shown in other studies for several species, e.g. Atlantic mackerel (*Scomber scombrus*, Castonguay et al. 1991), haddock (*Melanogramus* *aeglefinus*, Begg et al. 2000), lake trout (*Salvelinus namaycush*, Simoneau et al. 2000) and Atlantic cod (*Gadus morhua*, Cardinale et al. 2003). Contrary to others species, e.g. herring (*Clupea harengus*, Bird et al. 1986), orange roughy (*Hoplostethus atlanticus*, Gauldie and Jones 2000), and southern blue whiting (*Micromesistius australis*, Legua et al. 2013), there was significantly effect of sex on otolith shape. Effect of sampling year was not significant for the swordfish which corroborated others studies on different species, such as Atlantic salmon (*Salmo salar,* Friedland and Reddin 1994) and Atlantic cod (*Gadus morhua*, Campana and Casselman 1993). However, a season effect has been found for species carrying out large migrations, such as pacific sardine (*Sardinops sagax,* Felix-Uraga et al. 2004; Vergara-Solana et al. 2013). This was not tested in the present analysis due to a lack of sampling coverage across seasons.

Sampling area effect was significant ($p<0.05$). Moreover, the results of linear discriminating analysis indicated significant difference among groups of swordfish sampled in different areas of Indian Ocean. However, the misclassification distant percentage for each sampling area was explained by closed and distant areas. In this way, no geographical sub-structures are distinguishable in the Indian Ocean swordfish stock. The cluster analysis corroborates this result with 3 groups composed by the same sampling areas. Possible sources of misclassification in otolith shape analysis are individual variability and migration (Campana and Casselman 1993; Tracey et al. 2006).

Such a find is in agreement with what was previously found using several genetic markers and more than 2230 samples collected in the Indian Ocean and adjacent oceans. This study provides strong genetic evidence than swordfish from the SWIO region and the one from the rest of the Indian Ocean are part of the same genetic stock. However, this study also highlights that Atlantic and Indian oceans swordfish represent two distinct genetic stocks. This last point raises the question of the management of this resources between the International Commission for the Conservation of Atlantic Tunas than manage the Atlantic stocks and the IOTC that manages the Indian stock. As already suggested for the bigeye tuna (*Thunnus obesus*; Durand et al., 2005), it might be very interesting to investigate the patterns of habitat use of IO and AO of large pelagic fishes in the South African waters, with a special focus on the sex-biased dispersal.This study confirms the hypothesis that otolith shape analysis may assist the implementation of a consistent identification for exploited fish stocks and corroborate the result of recent genectic study.

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