


# Size-effect, asymmetry, and small-scale spatial variation in otolith shape of juvenile sole in the Southern North Sea

Sophie Delerue-Ricard  · Hanna Stynen · Léo Barbut · Fabien Morat · Kelig Mahé · Pascal I. Hablützel · Kris Hostens · Filip A. M. Volckaert

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**Abstract** While otolith shape analysis can provide a valuable tool for discriminating between fish populations, factors which may influence otolith shape, such as the effect of size, directional asymmetry in growth, and local environmental conditions, are often unknown. Here, we analyzed differences in otolith shape across three size classes of age-0 common sole *Solea solea* L. from nursery grounds off the Belgian coast and in the Wadden Sea. Across size classes, form-factor decreased and roundness remained

consistently high in both nursery grounds, while ellipticity increased in the Belgian nursery. Directional asymmetry between left and right otoliths measured by Fourier coefficients accounted for 0.96 and 7.2% of the variance when comparing otoliths overall, and for each size class, respectively. Within the Belgian nursery, results were consistent across sampling years and locations. In addition, otolith shape was marginally different between nursery grounds, but highly variable within nursery grounds. A small divergent group, which seems partly related to fish size, was noted at both spatial and temporal scales. Based on these results and before embarking on a study of population structure using otolith shape in age-0 common sole, we recommend testing for directional asymmetry and fish size effects across the entire region of interest.

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S. Delerue-Ricard (✉) · H. Stynen · L. Barbut · P. I. Hablützel · F. A. M. Volckaert  
Laboratory of Biodiversity and Evolutionary Genomics (LBEG), KU Leuven, Charles Deberiotstraat 32, Box 2439, 3000 Louvain, Belgium  
e-mail: Sophie.DelerueRicard@kuleuven.be

S. Delerue-Ricard · K. Hostens  
Institute for Agricultural and Fisheries Research, Ankerstraat 1, 8400 Ostend, Belgium

L. Barbut  
Operational Directorate Natural Environment (OD Nature), Royal Belgian Institute of Natural Sciences (RBINS), Gulledele 100, 1200 Brussels, Belgium

F. Morat  
PSL Research University: EPHE-UPVD-CNRS, USR3278 CRIOBE, 66860 Perpignan, France

F. Morat  
Laboratoire d'Excellence «CORAIL», BP 1013 Papetoai, 98729 Moorea, French Polynesia

K. Mahé  
Ifremer, Fisheries Laboratory, Sclerochronology Centre, 150 quai Gambetta, BP 699, 62321 Boulogne, France

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## Introduction

Coastal ecosystems play a key role as nursery grounds for juvenile fish (Costanza et al., 1998) because of their high productivity. However, coastal nursery grounds have a discontinuous distribution, and experience increasing fragmentation due to anthropogenic pressures, which can result in changes of metacommunity diversity and dynamics (Jung et al., 2017). In addition, habitat heterogeneity within nursery grounds may influence the spatio-temporal dynamics of fish populations on a small spatial scale (Le Pape et al., 2003).

Various indirect methods are available to assess the spatial population dynamics of the early-life stages of marine fish, such as modeling of larval transport, comparison of parasite communities, analysis of genetic differentiation, chemical composition and shape of fish otoliths, or tagging of the late juvenile stages (Pawson & Jennings, 1996; Koubbi et al., 2006; Cadrin et al., 2014; Neves et al., 2018). Despite the diversity of methods, population structure and connectivity patterns between and within nursery grounds remain challenging to evaluate (Kaplan et al., 2017). Moreover, each tool may integrate information at specific, yet different spatial and temporal scales. Biophysical modeling of larval transport and otolith shape variation focus on ‘ecological’ time scales (Thorrold et al., 2001; Lacroix et al., 2013), while genomic tools have been applied to measure population structure over both short ‘ecological’ and longer ‘evolutionary’ time scales (Pinsky et al., 2017). Yet, advanced genomic tools work best with extensive genomic background information on the species of interest and well-preserved DNA.

Otoliths are calcified structures residing in the inner ear of fish, growing with the constant deposition of successive calcium carbonate layers. As they grow,

otoliths incorporate time-delimited information that can be used to describe the development and ambient environmental conditions experienced by the individual. In addition, otolith shape is a useful and well-established tool to discriminate between species and stocks (Campana & Casselman, 1993). However, ontogenetic development affects otolith shape through changes in growth and metabolism, especially during sexual maturity (Cardinale et al., 2004; Mérigot et al., 2007; Hüsey, 2008). During the early-life stages, otoliths evolve from circular to more complex shapes (Lagardère & Troadec, 1997; Hüsey, 2008), which may limit the utility of otolith shape as stock marker for immature fish or fish of different age classes. Moreover, left and right otoliths may be different, i.e., directionally asymmetrical, particularly in flatfishes (Mille et al., 2015). Environmental and anthropogenic pressure may cause stress-induced changes such as increased levels of directional asymmetry (Gagliano & McCormick, 2004), which is disadvantageous because it interferes with hearing and orientation (Anken et al., 2002; Lychakov & Rebane, 2005).

Most studies have compared intraspecific differences in otolith shape either over large distances (> 500 km, e.g., Vieira et al., 2014), across oceanographic barriers (Tuset et al., 2003), or between habitats (Vignon & Morat, 2010; Morat et al., 2014). It is not yet clear what can be learned from otolith shape at small spatial scales and in the absence of strong oceanographic dispersal barriers. Here, we used otolith shape analysis to investigate small-scale nursery structure of the flatfish sole *Solea solea* (Linnaeus, 1758; Soleidae) in the shallow subtidal of the Southern North Sea. Sole is less abundant than European plaice (*Pleuronectes platessa* L.) or dab (*Limanda limanda* L.) in the study area, but it has a high commercial value, contributing to regionally and locally important demersal fisheries. To date, connectivity patterns between spawning and nursery grounds remain unclear (Burt & Millner, 2008; Lacroix et al., 2013). In the Southern North Sea, sole displays peak spawning from April to June. After hatching, the pelagic larvae drift for ~ 1 month in the water column before settling in a nursery (Russell, 1976; van der Land, 1991). Genetic data from adult sole suggest isolation by distance along the Atlantic coast, and weak population structure within the Southern North Sea (Cuveliers et al., 2012; Diopere et al., 2018), with a mean dispersal distance estimated at 150 km

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*Present Address:*

P. I. Hablützel

Flanders Marine Institute (VLIZ), Wandelaarkaai 7, 8400 Ostend, Belgium

(Kotoulas et al., 1995; Lacroix et al., 2013). Within the nursery grounds, age-0 sole travel shorter distances, between 10 and 30 km (Le Pape & Cognez, 2016). Nevertheless, small-scale spatio-temporal variation in connectivity between and within nursery grounds has, to the best of our knowledge, not been investigated empirically. The degree of isolation between and within nursery grounds may be estimated from the variation in otolith shape. Being able to delineate the smallest level of spatial resolution is an important step to measure connectivity and to better understand recruitment patterns.

In the current study, we address three key questions to determine whether otolith shape can be used to assess small-scale spatial patterns in juvenile flatfish: (1) What is the effect of fish size on otolith shape? (2) What is the effect of directional asymmetry between left and right otoliths? and (3) Is there spatio-temporal variation in the otolith shape of age-0 sole?

## Materials and methods

We investigated variation in otolith shape between two subsequent years and at two spatial scales within the dispersal range of sole larvae, to better understand the resolution of otolith shape variation between and within nursery grounds in the Southern North Sea. Juvenile sole were sampled at the regional scale (between nursery grounds, 400 km distance) from the Belgian and Wadden Sea (The Netherlands) nursery grounds in 2014 (BE2014 and NL2014). Juvenile sole were sampled at the local scale (within nursery) from the eastern and the western shallow subtidal coastal zones of the Belgian nursery in 2013 and 2014 (BE2013 and BE2014, up to 40 km distance between sampling sites). The discriminatory power of otolith shape was tested using Fourier coefficients and shape indices (see further). In addition, asymmetry between left and right otoliths and correlations between the shape indices and fish size were estimated using the combined data from all fish from the three datasets (BE2013, BE2014, NL2014).

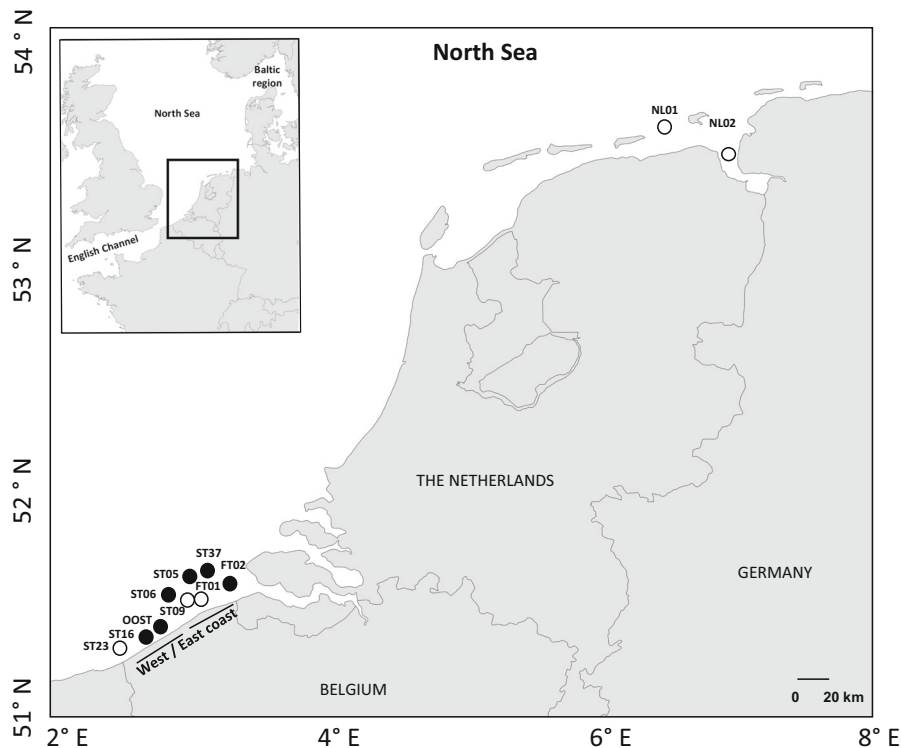
### Sample collection

Juvenile sole were sampled off the Belgian coast from late August to late September in 2013 and from mid-September to mid-October in 2014 (Fig. 1; Table 1)

and at two stations in the Wadden Sea in September 2014. At each site, specimens were collected by beam trawling either on board of RV *Simon Stevin* (B-FishConnect project campaign), RV *Belgica* (Belgian Demersal Young Fish Survey, DYFS), or RV *Stern* (Dutch DYFS). Sea surface temperature (SST) was measured at each site at the time of collection. Each fish was measured to the nearest mm [standard length (SL)]. Sagittal otoliths were extracted from a total of 314 individuals ranging from 52 to 102 mm SL. Finally, each otolith was cleaned, sonicated, and then stored dry in plastic vials. For this study, age-0 sole were used. Fish age was confirmed by the absence of an annual ring in the otolith. To assess variation in otolith shape associated with fish size, the 314 individuals were divided into three SL size classes, L1 (52–76 mm,  $n = 105$ ), L2 (76–82 mm,  $n = 105$ ), and L3 (82–102 mm,  $n = 104$ ).

### Otolith shape indices and Fourier coefficients

Left and right sagittae were placed on a microscope slide with a black background, positioned with the *sulcus acusticus* oriented towards the observer and the posterior side oriented to the top. External transmitted light sources were used and adjusted to illuminate the otoliths. High-contrast images were produced using an Olympus ColorView digital microscope camera linked to an Olympus BX51 microscope ( $\times 20$  magnification). Images were then processed with the TNPC 7 software ('Digital Processing for Calcified Structures'; [www.tnpc.fr](http://www.tnpc.fr)) to extract the following morphometric parameters: surface area of the otolith ( $A_o$ ), otolith perimeter ( $P_o$ ), maximum length ( $L_o$ ), and width ( $O_w$ ) to the nearest  $10^{-2}$  mm. Based on these measurements, form-factor, roundness, circularity, rectangularity, and ellipticity were calculated as in Tuset et al. (2003). Form-factor estimates surface area irregularity and has a maximal value of one in the case of a perfect circle. Roundness and circularity describe the proximity of shape to a circle and have minimal values of 1 and 12.57, respectively. The closer both indices approach the minimal value, the closer the shape of the otolith is to a perfect circle. Rectangularity gives the proportion of the length and width with respect to the area and has a maximal value of one in case of a perfect square. Ellipticity describes the proportion of change in the different axes (Tuset et al., 2003).



**Fig. 1** Map of the sampling stations of age-0 sole on the Belgian and the Wadden Sea nursery grounds in 2013 and 2014

In addition to the use of shape indices, otolith contours were described by elliptic Fourier descriptors (EFDs) which were obtained with TNPC 7 software. For each otolith, the first 99 elliptical Fourier harmonics were extracted and normalized with respect to the first harmonic. Hence, they were invariant to otolith size, rotation, and starting point of the contour description (Kuhl & Giardina, 1982). To determine the number of elliptical Fourier harmonics required to reconstruct the otolith outline, the Fourier power (FP) spectrum was calculated for each individual otolith. For the  $n$ th harmonic, FP is given by the equation:

$$FP_n = (A_n^2 + B_n^2 + C_n^2 + D_n^2)/2,$$

where  $A_n$ ,  $B_n$ ,  $C_n$ , and  $D_n$  are the Fourier coefficients of the  $n$ th harmonic.

Cumulative FP ( $FP_c$ ) was calculated by summing the FP of each harmonic:

$$FP_c = \sum_1^n FP_n.$$

The number of harmonics was chosen such that the mean cumulated FP reached 99.99%; hence shape was reconstructed at 99.99% (Gonzalez-Salas & Lenfant, 2007; Mérigot et al., 2007). The first harmonic was not considered for further analysis (except for reconstructing average shape), because it had already been used for normalization, and because it would dominate shape reconstruction and mask the information derived from the other harmonics (Crampton, 1995).

#### Statistical analysis

First, a pilot experiment was conducted to assess the consistency of our methodology with regard to otolith position and lighting. Ten randomly chosen otoliths were repositioned and four replicate pictures were taken. A dendrogram analysis was performed to test the extent of differences between images of the same otolith and differences between images of different otoliths.

**Table 1** Number of fish analyzed per station and per nursery (Belgian coast and Wadden Sea), including the date of sampling, GPS coordinates, sea surface temperature at the time of sampling, and name of sampling survey (Demersal Young Fish Survey, DYFS, and B-FishConnect)

Year	Area	Coast side	Station	Sample size	Sampling date	GPS coordinate	Sea surface temperature (°C)	Survey	
2013	Belgium	West	OOST	14	28/08/2013	51.23 N, 2.80 E	19.3	B-FishConnect	
			ST16	30	10/09/2013	51.19 N, 2.70 E	18.8	DYFS	
		East	ST09	33	09/09/2013	51.35 N, 3.00 E	19.5	DYFS	
			ST05	33	12/09/2013	51.45 N, 3.01 E	17.7	B-FishConnect	
			ST37	29	12/09/2013	51.48 N, 3.14 E	17.8	DYFS	
			ST06	14	13/09/2013	51.38 N, 2.85 E	17.7	DYFS	
				FT02	20	24/09/2013	51.43 N, 3.31 E	16.9	DYFS
				Total	173				
2014	Belgium	West	ST23	30	15/09/2014	51.13 N, 2.70 E	18.1	DYFS	
			ST09	27	16/09/2014	51.35 N, 3.00 E	18.5	DYFS	
		East	FT01	23	10/10/2014	51.35 N, 3.10 E	16.9	B-FishConnect	
			Total	80					
	Wadden Sea		NL01	34	16/09/2014	53.48 N, 6.49 E	18.2	DYFS	
			NL02	27	23/09/2014	53.35 N, 6.97 E	18.1	DYFS	
			Total	61					

Multi-collinearity between shape indices was assessed by Pearson correlation. Only form-factor, roundness, and ellipticity were kept for further analyses. Shape indices were compared between length classes, the two nursery grounds, and sampling years. Differences in the mean values of shape indices between each size class and each dataset were assessed using non-parametric *k*-sample Anderson–Darling tests under the null hypothesis that all samples originated from the same distribution.

Before analyzing asymmetry levels and spatio-temporal variation in the Fourier coefficients, principal component analysis (PCA) was applied to the Fourier coefficients to avoid collinearity of shape descriptors, and to reduce the number of dimensions while retaining the majority of the variance (Rohlf & Archie, 1984). Only principal components (PCs) with eigenvalues higher than the mean of all eigenvalues were retained to remove PCs associated with noise. Fourier coefficients were significantly correlated with fish size. Residuals of the PCs of Fourier coefficients have been used to remove the effect of fish size, as in Mahé et al. (2016).

Partial redundancy analysis (pRDA) tests were performed with the PCs of the Fourier coefficients as response variables to explore the effect of otolith side,

between- and within-nursery spatial variation, year, coastal region, and SST. RDA is an extension of multiple regression analysis to multivariate response data (Legendre & Legendre, 2012). In all pRDAs, SL was used to correct for fish size, and a permutation test was used to assess the significance of the explanatory variables. A pRDA was performed to test the effect of otolith side (left/right) on shape. To visualize and quantify differences between left and right otoliths, the average otolith shape of each side was also built by outline reverse Fourier transformation, as in Mille et al. (2015).

Dendrograms were produced by ascending hierarchical classification (AHC) to verify the PCA clustering of individuals at the regional and local scales. AHC maximizes the similarities within clusters and differences between clusters of similar otolith shapes. To compute the AHC, the function ‘HCPC’ based on Ward’s distance was used.

pRDA was also used to determine regional and local scale (between and within nursery) annual differences in otolith shape. We used multiple linear regressions to test at the regional scale the effect of nursery, sampling date, and SST on the Fourier coefficient PCs, and to test at the local scale the effect of year, coastal zone (eastern/western), and SST on the

same PCs. We determined the variance explained by each explanatory variable. Only the left otoliths were used in the RDAs. Distribution of SL was compared using a randomization test. All statistical analyses were performed using the *ade4*, *FactoMineR*, *vegan*, and *stats* packages in the statistical environment R (R Development Core Team, 2011).

The outlines of the mean Fourier coefficients (prior to size correction) were plotted as an overlay image to visualize differences in otolith shape between left and right otoliths. The same overlay was used to visualize differences between left otoliths of the Belgian and Wadden Sea nursery grounds, and between left otoliths of the two PCA clusters (Mille et al., 2015).

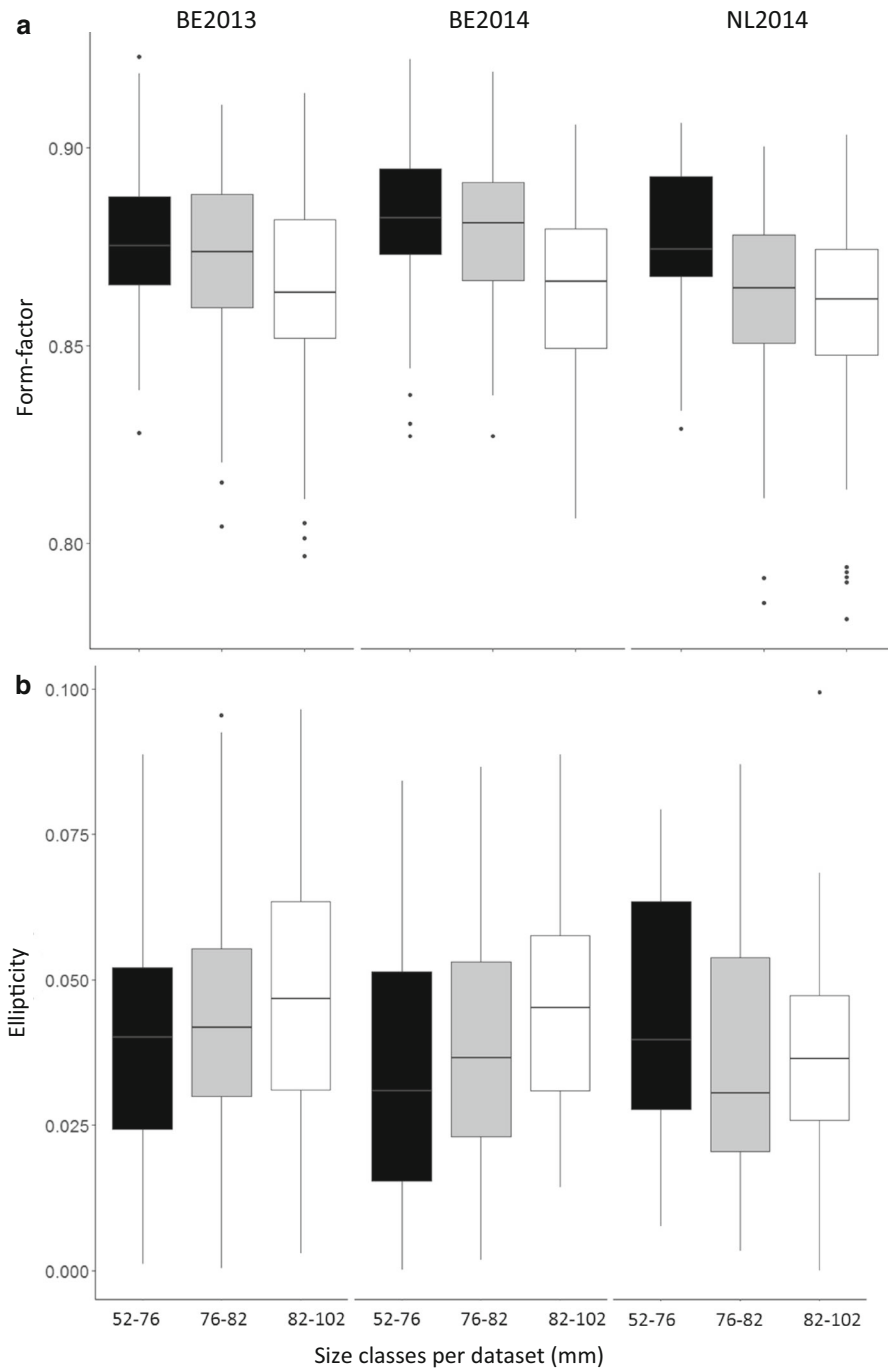
## Results

Cluster analysis showed that Fourier coefficients extracted from replicated images of the same otolith were consistently grouped together and our measures were accurate (Supplementary Fig. S1). Otolith form-factor, ellipticity, and roundness were not redundant (Pearson correlation test,  $P > 0.05$ ) and were kept for further analysis. For each dataset (BE2013, BE2014 and NL2014), form-factor significantly decreased with increasing SL (Fig. 2a; Table 2). Only in the Belgian nursery, ellipticity was significantly different between length classes for both sampling years (Fig. 2b). Roundness was generally close to one and did not vary significantly between size classes or nursery grounds but varied between sampling years in the Belgian nursery (Supplementary Fig. S2). In addition, otoliths sampled in 2014 off the Belgian coast were significantly more circular than in the Wadden Sea, and also significantly more circular and rounder than otoliths sampled in 2013 on the Belgian nursery as shown by form-factor variations (Table 2).

The Fourier coefficients differed significantly between left and right otoliths ( $n = 314$ ,  $P < 0.01$ ). This directional asymmetry explained almost 6% of the variance (Table 3) and was spread homogeneously across the otolith outline (Fig. 3a). Although the overall average directional asymmetry was quite small when all datasets and size classes were pooled (0.96% overall), asymmetry was much higher when each dataset and size classes were examined separately (mean asymmetry for each dataset was 7.2%). Left otoliths of sole (blind side) were larger than right

otoliths (eyed side) and were thus kept for further analysis.

Among the 99 Fourier harmonics extracted to describe the left otolith contour, the first 22 harmonics explained more than 99.99% of the variance and were thus retained for multivariate analyses. Small differences in average shape (1.2% overall) were visible between the Belgian and Wadden Sea nursery grounds based on the reconstructed average otolith shape (Fig. 3b). The variance in otolith shape explained by fish size ( $P = 0.086$  and  $0.004$  for NL–BE2014 and BE2013–2014, respectively) was removed (see “Materials and methods,” “Statistical analysis” sections for more details). The first and second axes from a PCA comparing the Belgian and Wadden Sea nursery grounds in 2014 accounted for 30.2 and 28.6% of the total variance, respectively. At the local scale (within the Belgian nursery) in 2013 and 2014, the first and second PCs accounted for 31.8 and 28.6% of the total variance, respectively. Ten PCs had eigenvalues above the mean eigenvalue, and accounted for 91.0% of the variance when comparing shapes at both nursery grounds in 2014, and 90.6% when comparing shapes in the Belgian nursery between years. Both PCA plots showed considerable variation in otolith shape both at the regional and local scale, and it was not possible to detect PCA clusters by nursery or by year (Fig. 4a, b). However, two distinct clusters were observed at both geographical scales and during both sampling years based on the first two PCs. These clusters were also supported in both cases by clustering dendrograms (Supplementary Fig. S3). A small number of fish, belonging to both the Belgian and Wadden Sea nursery grounds (Fig. 4a) and both 2013 and 2014 Belgian samples (Fig. 4b), clearly diverged in otolith shape. This divergent group of fish consisted of approximately 10% of the individuals from NL2014, 10% of BE2014, and 16% of the BE2013, independent of SL size class. Shape diverged in two areas on both the posterior or anterior side of the otolith and accounted for 11.5% of shape variation (Fig. 3c). For the 2014 dataset, fish from the divergent group were significantly longer (SL 72–95 mm) than those from the majority cluster (SL 57–93 mm, Fig. 5; randomization test for NL–BE2014:  $n = 141$ ,  $W = 1,221$ ,  $P = 0.041$ ). In the Belgian nursery, the average SL of the divergent group was not significantly longer than that of the majority cluster for the



**Fig. 2** Boxplot of form-factor (**a**) and ellipticity (**b**) for 314 age-0 sole juveniles of sole of three size classes for each dataset ( $n = 173$  for BE2013,  $n = 80$  for BE2014,  $n = 61$  for NL2014)

local dataset (Fig. 5; randomization test for BE2013–2014;  $n = 253$ ,  $W = 4,258$ ,  $P = 0.525$ ).

RDA was used to correlate the observed pattern of otolith shape with potential explanatory variables

(Table 3). At the regional scale, otolith shapes in the Belgian and Wadden Sea nursery grounds differed significantly, even though that difference was only marginally significant ( $P = 0.04$ ) and explained less

**Table 2** Summary of the mean value of the shape indices (form-factor, ellipticity, and roundness) per size class for each dataset ( $n = 173$  for BE2013,  $n = 80$  for BE2014,  $n = 61$  for NL2014) and comparisons of each shape index between thethree different size classes (L1–3) for each dataset and for the comparisons of all age-0 sole together at the regional (Belgian vs. Wadden Sea nursery) ( $n = 141$ ) and at the local scale (Belgian 2013 vs. 2014) ( $n = 253$ )

Shape index	Dataset	L1 (52–76 mm)	L2 (76–82 mm)	L3 (82–102 mm)	L1–L2–L3 <i>P</i> value	All sizes	<i>P</i> value
Form-factor	BE2013	0.876	0.871	0.864	< 0.001	NL2014 versus BE2014	< 0.001
	BE2014	0.883	0.879	0.865	< 0.001		
	NL2014	0.874	0.860	0.855	< 0.01		
Ellipticity	BE2013	0.039	0.043	0.048	0.016	NL2014 versus BE2014	0.723
	BE2014	0.034	0.038	0.047	0.012		
	NL2014	0.042	0.036	0.037	0.122		
Roundness	BE2013	0.947	0.946	0.939	0.354	NL2014 versus BE2014	0.807
	BE2014	0.961	0.960	0.943	0.081		
	NL2014	0.946	0.958	0.953	0.102		

**Table 3** Summary of partial redundancy analysis (pRDA) for asymmetry ( $n = 314$ ) and for spatio-temporal variations at the regional scale (Belgian vs. Wadden Sea nursery) ( $n = 141$ )and at the local scale (eastern vs. western coastal zones of the Belgian nursery and 2013 vs. 2014) ( $n = 253$ )

Hypothesis	Factors	df	<i>P</i> values	$R^2_{\text{adjusted}}$ (%)
Asymmetry: all otoliths	Otolith side	1	0.01	5.8
Regional differences: NL–BE2014	Nursery	1	0.04	0.6
	Sampling date	1	0.41	< 0
	SST	1	0.49	< 0
Local differences: BE2013–2014	Year	1	0.08	< 0
	Sampling date	1	0.34	< 0
	East/west coastal regions	1	0.70	< 0
	SST	1	0.19	< 0

Degrees of freedom (df), significance values, and the variance explained by each variable ( $R^2$ ) are included

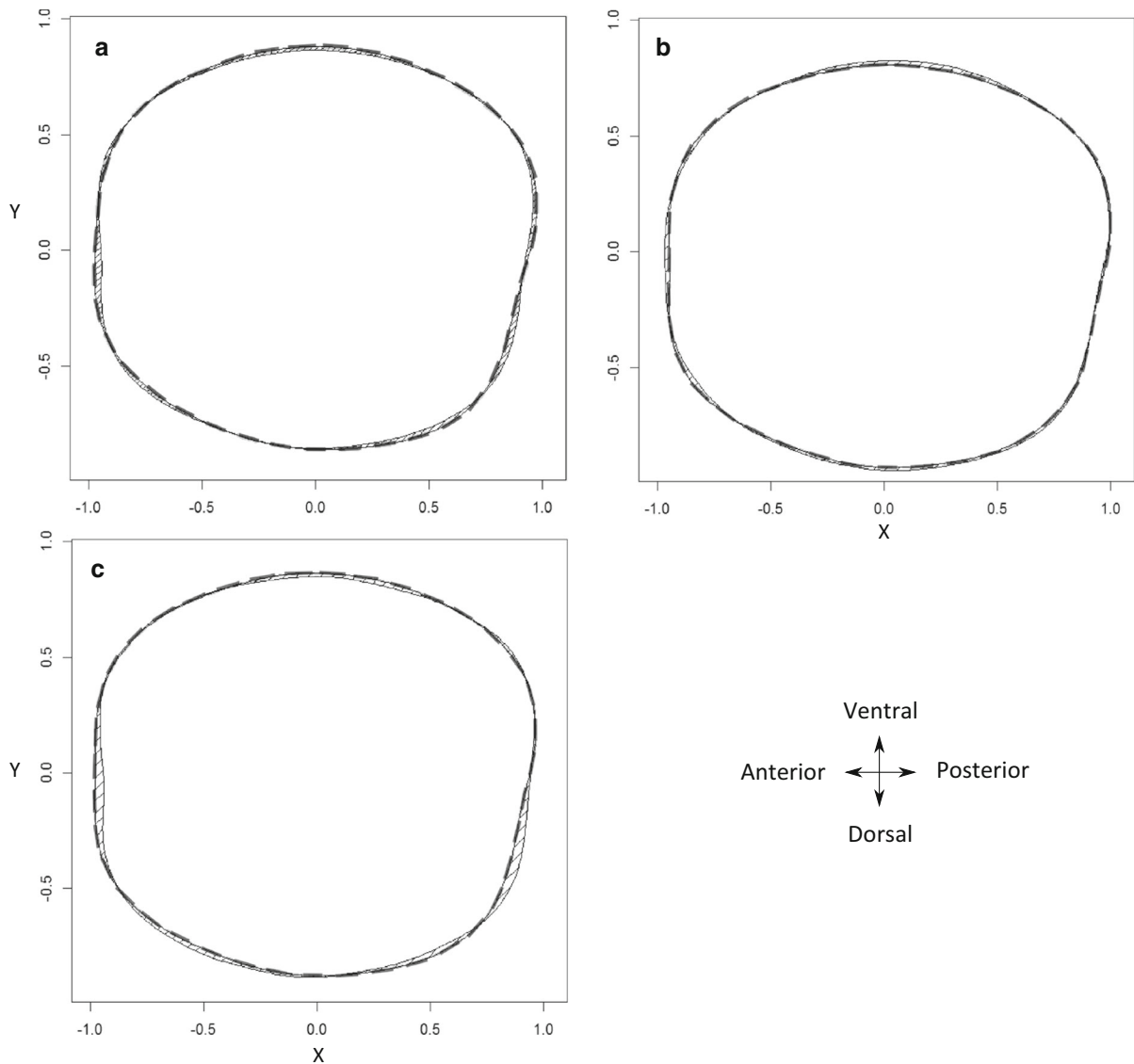
than 1% of the variance. At the local scale of the Belgian nursery, neither an effect of sampling year nor of coastal zone (eastern/western) on otolith shape was detected. Both SST and sampling date did not significantly explain variation in otolith shape, irrespective of the spatial scale considered.

## Discussion

Analysis of otolith shape on age-0 sole collected in the Southern North Sea revealed five interesting results.

Otolith shape, as described by shape indices and Fourier coefficients, was influenced by fish size. Weak but significant directional asymmetry in otolith shape was observed between the left and right otoliths. Among sites, variation in otolith shape was small but significant at the regional scale (Belgian vs. Wadden Sea nursery grounds, 400 km distance). Such variation was absent at the local scale (eastern vs. western coastal zones of the Belgian nursery, 40 km distance) and between two consecutive years (2013 vs. 2014).





**Fig. 3** Reconstruction of average sagittal otolith shape for 314 age-0 sole based on elliptic Fourier descriptors as a function of **a** otolith side (left dotted vs. right full line), **b** geography (Belgian nursery dotted vs. Wadden Sea full line), and **c** PCA

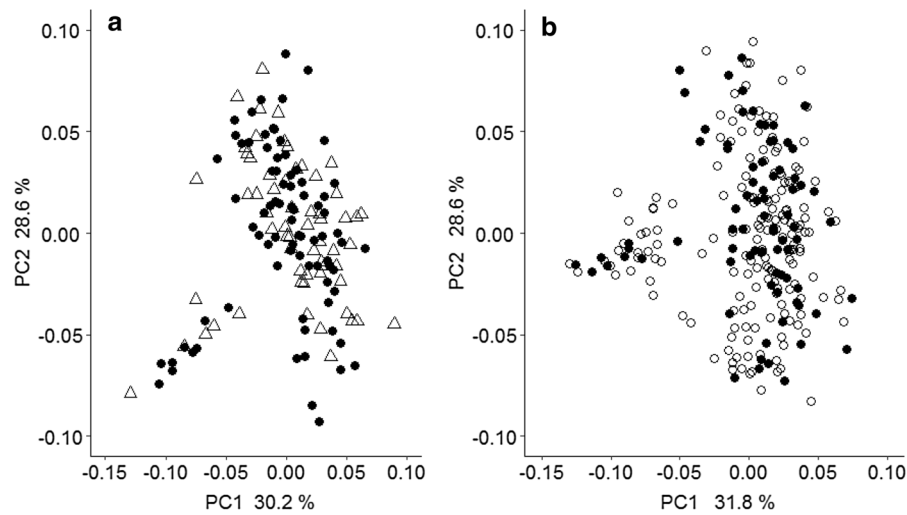
#### Impact of fish size and directional asymmetry on shape in age-0 sole

Several confounding factors were taken into account before considering which factors drive spatial and temporal variation of otolith shape. First of all, a single age group (age-0 sole) was considered. Secondly, we corrected Fourier coefficients for fish size (Campana & Casselman, 1993; Cardinale et al., 2004; Mérigot et al., 2007). Thirdly, variation due to methodology

clustering (Cluster 1 dotted vs. Cluster 2 full line see Fig. 4 for the clusters). Non-overlapping regions of the otolith have been crosshatched to show differences between otolith shape

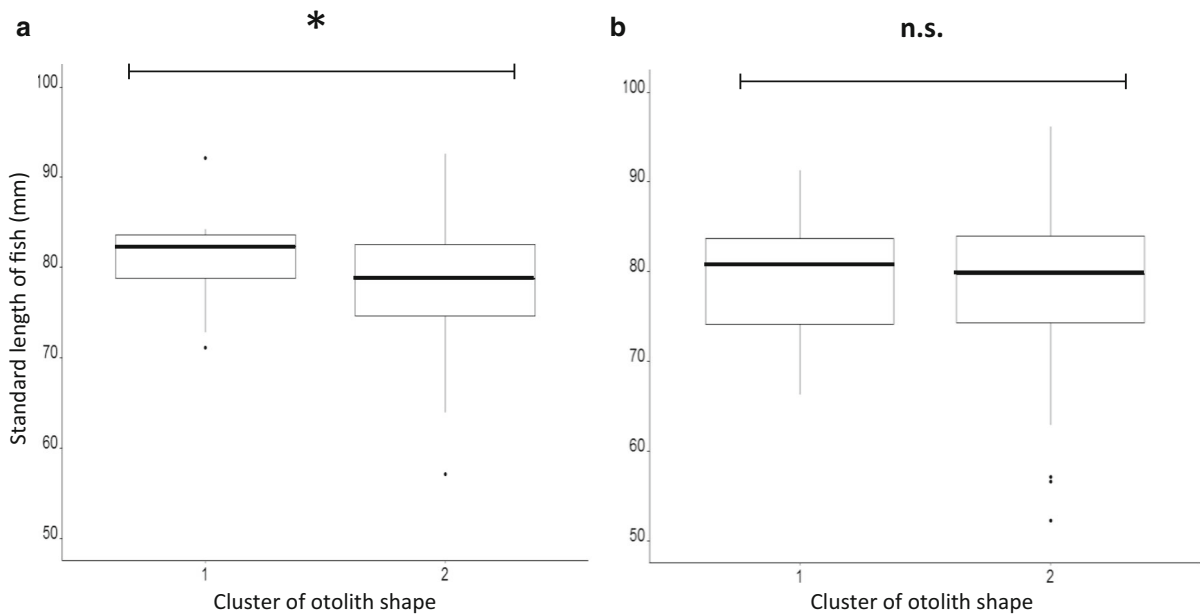
and individual variability was successfully addressed in a pilot experiment focusing on positioning and lighting methods of the photography.

Adult otoliths tend to be more complex in shape than juvenile otoliths (Capoccioni et al., 2011; Morat et al., 2017). Directional asymmetry in shape, mass, and chemical composition have been observed between the left and right otoliths in adult and juvenile flatfish but never quantified in juveniles (Mérigot



**Fig. 4** Principal component analysis of Fourier coefficients of otoliths of juvenile sole at **a** the regional scale [Belgian coast (filled circles) vs. Wadden Sea nursery grounds (open

triangles)], and **b** at the local Belgian scale for the year 2013 (open circles) versus 2014 (filled circles)



**Fig. 5** Size distribution of the standard length (in mm) of the two clusters of sole sampled in 2014 on the Wadden Sea and Belgian nursery grounds (**a**) and on the Belgian nursery in 2013 and 2014 (**b**). The average standard length was significantly

different between the two clusters (as indicated by the star in **(a)** for the nursery comparison while it was not significantly (**n.s.**) different between the two clusters on the Belgian nursery (**b**))

et al., 2007; Mille et al., 2015). This study demonstrates that directional asymmetry is already present in age-0 flatfish. Our results suggest that asymmetry builds up over time because of differential growth between the larger otolith on the blind side versus the

smaller otolith on the eyed side. Still, the limited variation observed between both otolith sides supports Mille et al. (2015) suggestion of the presence of an evolutionary selection pressure against asymmetry to avoid negative effects on fish hearing and equilibrium.

Our study establishes that, similarly to adults (Mille et al., 2015), left and right otoliths should not be used interchangeably in shape analyses of juvenile sole. This lack of interchangeability is likely for all other analyses carried out on otoliths, such as chemical asymmetry (Morat et al., 2014). Moreover, fish size should always be accounted for because it affects otolith shape even in age-0 fish. Our results are consistent with Mapp et al. (2017), who showed that including size information increases the power of stock discrimination based on juvenile otolith shape. The form-factor decreased with increasing size in both nursery grounds and during both sampling years, while ellipticity increased in the Belgian nursery. Roundness did not vary significantly either with fish size or between nursery grounds. The only difference in roundness we could detect was in the Belgian nursery, where roundness was lower in 2013 than in 2014. Overall, roundness was higher than observed in Mediterranean samples (Mérigot et al., 2007), which might be due to higher growth rates in the Mediterranean Sea compared to the North Sea sole (Morat, 2011). In summary, we show that otoliths of age-0 sole in the Southern North Sea were relatively round, directionally asymmetrical, and that area irregularity increased during the first year of life.

#### Small differences between nursery grounds

Our results show that we can detect variation in otolith shape at relatively small spatial scales. Overall, the average shape differed more between nursery grounds than between otolith sides. However, these differences disappear when size classes are taken into account and asymmetry measured within each. Assuming comparable environmental conditions at the Belgian and Wadden Sea nursery grounds (OSPAR, 2000), one might expect a comparable nursery ground environment impact on otolith shape, which we do not observe. Thus other factors, such as genetic background, spawning location, and dispersal may also be important in influencing otolith shape (Lombarte et al., 2003; Cardinale et al., 2004; Capoccioni et al., 2011; Mapp et al., 2017). This observation is supported by the similar levels of variation in otolith shape between nursery grounds and between the two successive sampling years. Genetic levels are expected to vary only to a limited extent within the Southern North Sea, because sole exhibit an almost homogeneous

population structure (Cuveliers et al., 2012; Diopere et al., 2018). At the same time, earlier microchemical studies point to a reduced mobility and site fidelity of age-0 sole in the Southern North Sea (Cuveliers et al., 2010; Le Pape & Cognez, 2016). Differences in otolith shape and microchemistry between two ‘adjacent’ nursery grounds support some degree of natal site fidelity, which is not unexpected given the temporally stable location of the spawning grounds of sole.

#### A divergent group of age-0 sole at both nursery grounds

We noticed two distinct groups of otolith shapes in both PCA analyses and clustering dendrograms. This result raises the question as to whether these individuals, representing 10 and 16% of all age-0 sole screened, respectively, experienced a somewhat different environment or have a different origin. Fish of the divergent group exhibited a larger size than most other fish. However, the divergent group was sampled across sampling dates (spanning over 1 month), suggesting that shape differences are not linked to age differences between individuals.

The environmental conditions experienced during juvenile dispersal depend on spawning ground and planktonic drift. According to the biophysical model of Lacroix et al. (2013), larvae arriving at the Belgian and Wadden Sea nursery grounds originate from the spawning grounds off the Belgian coast, the Eastern English Channel, and the Thames Estuary. Between-year connectivity levels are comparable, although the relative proportions of source populations vary. Building on the results of the present study, other approaches would be required to identify larval origin. For example, spawning time can be estimated from otolith microstructure and used to back-calculate larval origin (Amara et al., 1993). Shape differences might be related to residence time on the nursery ground (Delerue-Ricard, in prep.). Additionally, otolith microchemistry is effective in resolving small-scale spatial differences. Based on otolith microchemistry, Cuveliers et al. (2010) were able to distinguish nursery grounds of sole from the Thames Estuary, Belgian coast, and Wadden Sea. However, chemical signatures of spawning grounds of sole in that area have yet to be explored. In summary, complementary information on otolith chemistry, biophysical modeling, or a combination using the

aforementioned techniques, should reveal the origin of spawning, and the dispersal pathways (Campana, 2005; Morat et al., 2014). Such information would contribute to a better understanding of the connectivity patterns of sole between spawning and nursery grounds.

### The importance of spatial scale

Understanding local population dynamics is crucial, especially for coastal habitats, which are key nursery grounds for many marine species. Over the last decades, changes in food webs and sheltering conditions provided by structuring benthos have dramatically affected nursery function along the North Sea coast (Van der Veer et al., 2011; Rabaut et al., 2013; Jung et al., 2017). We examined otolith shape across the Southern North Sea at a scale close to the average dispersal distance of sole (150 km, Kotoulas et al., 1995). Small, but significant differences were identified between two adjacent nursery grounds. Within each nursery ground, no differences in otolith shape were found, despite differences in habitat characteristics between the eastern and the western coastal zones of the Belgian nursery, with mostly muddy to sandy sediments in the east and sandy to muddy sediments in the west (Van Hoey et al., 2004). Each sediment type harbors a specific macrobenthic assemblage (Degraer et al., 2008; Rabaut et al., 2013), with a distinct prey spectrum for sole and potential influence on otolith shape. However, starvation experiments suggest that food quantity could be more important than food type to determine otolith shape. Thus, if juvenile sole would be restricted to one coastal nursery ground or region within the Belgian nursery, the limited effect of food type on otolith shape could partially explain the absence of variation within the Belgian nursery (Cardinale et al., 2004; Gagliano & McCormick, 2004; Hüseyin, 2008).

In conclusion, small-scale differences in otolith shape can be diagnostic and may play a role in population dynamic studies. During the first year of life of sole, otolith shape evolves from round to a more complex shape. Directional asymmetry between left and right otoliths is already present in age-0 sole and has therefore to be taken into account in flatfish juveniles and adults alike. Despite a large variation in otolith shape, a subtle but significant difference was observed at a scale of 400 km, i.e., the dispersal scale

of sole, which is a promising finding for the study of small-scale spatial patterns. At the local scale (within nursery grounds), no significant shape differences were noted between the eastern and the western coastal zones of the Belgian nursery, nor between 2013 and 2014. However, a number of samples showing a divergent otolith shape, probably linked to fish size, were found on both nursery grounds in both successive years. Complementary studies on the integration of high-resolution genotyping, elemental analysis of otoliths, and biophysical modeling of early-life stages are expected to provide additional evidence on the dispersal dynamics between spawning and nursery grounds.

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**Data availability** The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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