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Extensive larval dispersal and restricted movement of juveniles on the nursery grounds of sole in the Southern North Sea

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Abstract :

Connectivity between spawning and nursery grounds influences the colonization, replenishment and resilience of populations of marine organisms. Connectivity rate, measured as the exchange of individuals between spawning and nursery grounds, is therefore a crucial determinant of stock size. However, connectivity of early-life stages is hard to explore due to sampling limitations and insufficient knowledge on potential larval sources. Here we present new insights into pre- and post-settlement dispersal of the common sole (Solea solea L.) at a spatial scale of 5–500 km in the Southern North Sea. Patterns at a scale of <100 km were considered local, whereas patterns further than 100 km were considered regional. Multi-elemental signatures of the otolith edge of 213 juvenile sole were used to discriminate at 79% of overall accuracy three main nursery grounds in the Southern North Sea, namely UK coast, Belgian coast and Dutch Wadden Sea. Interregional differences in otolith composition (especially for Mg, Mn and Ba) suggest that sole migration following settlement is limited in the Southern North Sea. Elemental signatures of the same fish indicated mixing during larval dispersal. Each nursery ground recruited an important mix of juveniles from three of the four chemically distinct natal sources identified from the larval otolith signatures. However the percentage of correct regional re-assignment varied from 67 to 80% with a maximum in the Wadden Sea. The results contributed to the validation of biophysical models of larval drift. Our findings support decision making for both fisheries management and marine spatial planning at the national and European level.

Highlights

► In the North Sea, juvenile otolith signatures differ at the regional scale (100 km). ► Movement appears to be limited once the juveniles have reached a nursery ground. ► Local nursery areas receive larvae from four chemically distinct natal sources. ► Mixed larval contributions to nurseries point to an extensive larval dispersal.

Keywords : Connectivity, Early-life history, North Sea, Otolith elemental composition, Trace elements, *Solea solea*

1. Introduction

Connectivity, i.e. the exchange of larvae, juveniles or adults among geographically separated groups (Palumbi 2004), drives colonization, enables replenishment and promotes resilience of populations to disturbances (Botsford et al. 2001, Cowen et al. 2007). In marine organisms with a planktonic larval phase, connectivity between spawning and nursery grounds is one of the main drivers of recruitment (Harden-Jones 1968, Rijnsdorp et al. 1992). Spawning ground origin and dispersal pathways may impact the quality of the larval pool and thereby modulate juvenile survival (Pineda et al. 2007, Shima & Swearer 2010). In addition, anthropogenic activities or environmental changes of the spawning grounds may modify connectivity and therefore impact supply to the nursery grounds (Lacroix et al. 2018). Therefore, understanding connectivity at the scale of dispersal is paramount to apply adapted management measures for metapopulation persistence (Batista et al. 2015, Krueck et al. 2017). Connectivity may be more important than habitat quality for the design of Marine Protected Areas (MPAs, OSPAR 2013). This information feeds the increasing calls for prioritizing locations that are self-replenishing, interconnected, and/or important larval sources (Krueck et al. 2017). However, while the efficiency of MPA positioning depends on the quality of input data regarding fish dispersal (Batista et al. 2015), data on fish movements at the larval and juvenile stages remain difficult to compile.

The distribution and dispersal of flatfishes have been investigated extensively, both through sampling and tagging (Burt & Millner 2008, Dorel et al. 1991, Gibson 2015), the analysis of life history traits (Randon et al. 2019), and the use of biological tags like otolith microchemistry (Darnaude & Hunter 2018, Hunter et al. 2003). The ecology of the common sole (*Solea solea* L., 1758; Soleidae) has been particularly well studied (Amara et al. 2007, Fincham et al. 2013, Marchand 1991). The species mainly spawns from March to June along the shores of the North East Atlantic Ocean (Ellis et al. 2012, Rijnsdorp et al. 1992). After hatching, the pelagic larvae drift in the water column for about one month before settling in a shallow coastal or estuarine nursery (Russell 1976, van der Land 1991). Estimates of larval dispersal range between 80 and 300 km depending on the spawning ground, with an average drift of about 150 km as estimated from biophysical modeling and genetic markers (Dorel et al. 1991, Kotoulas et al. 1995, Lacroix et al. 2013). Yet, empirical evidence of connectivity between sole spawning and nursery grounds is scarce (Morat et al. 2014). Similarly, little is known about the movement of juvenile sole in the months following settlement. Population

structuring among the nursery grounds and the adult feeding grounds of sole has been characterized through otolith chemistry along the coasts of the Atlantic Ocean and the Mediterranean Sea (Leakey et al. 2009, Tanner et al. 2012). However, this information is limited in the North Sea (Cuveliers et al. 2010). The distribution of sole nurseries tends to be continuous along the North Sea coasts (Ellis et al. 2012, Rijnsdorp et al. 1992) and biophysical modeling suggested a mixed contribution of several spawning grounds to most juvenile habitats (Lacroix et al. 2013, Savina et al. 2010). However, a recent shift was observed in the distribution of North Sea sole (Engelhard et al. 2011), attributed to a shift in the distribution of its spawning and nursery grounds (Ellis et al. 2012). In the future, a longer larval dispersal due to colder temperatures experienced by earlier hatched larvae could also impact the distribution (Lacroix et al. 2018). These recent changes, probably caused by both climatic and fishing pressures, call for a focus on the early-life connectivity of sole in the area. Understanding the patterns of dispersal and their causes is of primary importance because climate change modeling at the level of the North Sea predicts an increase in larval recruitment for some fish species. Strong regional differences among nursery grounds may characterize the shifts in larval recruitment (Lacroix et al. 2018).

This study measures connectivity between the spawning and nursery grounds of sole in the Southern North Sea using otolith microchemistry. Otoliths are calcified structures in the inner ear which grow with regular increments and incorporate time-delimited information (Campana 1999). For example, when juvenile fish settle on the nursery ground, the high metabolic impact of metamorphosis is recorded in otolith growth (De Pontual et al. 2000), with a distinct separation between increments deposited during the pelagic larval stage from those formed after benthic settlement. Revealing chemical differences between pre- and post-settlement regions of the otolith may inform about dispersal at the larval stage but also about fish movements within and between nursery ground areas. The chemical composition of otoliths has already been proven effective to infer differences in spawning origin and to discriminate among nursery grounds (e.g., Gibb et al. 2017, De Pontual et al. 2000). Although small-scale migration patterns in coastal zones remain challenging to trace with otolith microchemistry because of the short residence time of water (Tanner et al. 2012), estuaries often carry strong chemical signals, at small and large spatial scales (Gillanders & Kingsford 2003, Di Franco et al. 2012, De Pontual et al. 2000).

The North Sea is particularly suited for otolith elemental studies of fish spatial origin and movement because of its chemistry. The area is one of the most polluted seas of the world (Grizzetti et al. 2017). Its coasts receive runoff water that carries the chemical signature of local river catchments (De Witte et al. 2016, Hamer et al. 2006). Onshore-offshore gradients in relation to the distance from riverine input are observed for certain elements. We focused on seven coastal areas used as nursery ground by sole in the Southern North Sea, spread along the UK, Belgian and Dutch coastlines. Distances between sampling locations range from 5 to 500 km. We tested whether the otoliths of juvenile sole from these sampling locations could discriminate multi-elemental signatures. The composition of the larval part of the otoliths was also used to estimate the number of chemically distinct spawning grounds (further referred to as natal sources) that supply each location with larvae. We then quantified the extent of local (<100 km) pre- and post-settlement dispersal by comparing the elemental signatures from three key life history periods: the pelagic larval phase, the few weeks after metamorphosis (post-settlement), and the few weeks on the nursery ground preceding capture.

2. Material and methods

2.1 Sample collection

Age-0 and age-1 sole belonging to five cohorts, hereafter referred to as settled juveniles, were collected between 2006 and 2016 on three major Southern North Sea nursery grounds (Ellis et al. 2012, Rijnsdorp et al. 1992) which correspond to our 'sampling regions' (Fig. 1 and Table 1): (1) the UK nursery ground (UK), sampled near Sizewell power station and in the Thames Estuary, (2) the Belgian nursery ground (BE), sampled at four locations: B1, B2, B3, and B4, which includes juveniles caught at Zandvliet (ZANj07) in the Scheldt Estuary, and (3) the Wadden Sea nursery ground (WA) along the Dutch coast (sampled at Balgzand (BALj06) and in the Ems-Dollard Estuary. At all sites, a 3 m beam trawl with cod end mesh size of 10 mm was used to catch the fish. Sampling covered several years but only two nursery grounds (B3 and B6) were sampled in consecutive years (in 2013-2014). At all sites, juvenile sole were captured between the end of May and October, except for the Sizewell power station which was sampled in March. Water temperature was measured on site on the day of

sampling. All fish were measured and weighed (standard length to the nearest mm and total weight to the nearest g; Suppl. Fig. 1) and kept frozen until dissection.

In order to have a sufficient number of fish per site to enable discrimination, nearby nursery grounds in each region were grouped, resulting in seven geographically distinct nursery areas, hereafter referred to as 'sampling locations'. The closest sampling locations were 5 km apart (along the Belgian coast) and distance between the furthest sampling locations was 500 km. Therefore, patterns at a scale of less than 100 km (i.e. the full extent of the Belgian coastline) were considered local, whereas patterns at a scale wider than 100 km (i.e. among the four sampling regions) were considered regional.

2.2 Otolith extraction and preparation

All equipment for otolith extraction and handling was acid washed in a 10% nitric solution prior to use. Only the left sagittal otoliths were used in this study because the chemical composition, shape and mass of otolith is asymmetric in flatfish (Mille et al. 2015, Mérigot et al. 2007). Otoliths were extracted and weighed to the nearest 0.005 mg and fish age-class (0 or 1) was determined by macroscopic examination. To remove any surface contamination, the otoliths were rinsed with distilled water and 0.1% nitric acid, and sonicated in vials filled with ultrapure water. Otoliths were then mounted in molds, covered with epoxy resin and transversely cut through the nucleus using diamond blades mounted on an ISOMET Buehler precision saw. The nucleus was exposed in thin sections on one side by polishing the otoliths cuts. They were sonicated in ultrapure water for a second time, left to dry for 24 h under a class 100 laminar flow hood and glued on a glass slide for later analysis. Otoliths from different sampling locations were mixed randomly on each slide to minimize session effects.

2.3 Otolith elemental analysis

Otolith elemental composition was determined by laser ablation inductively-coupled plasma mass spectrometry (LA-ICPMS) using a Nu Instruments ATTOM ES High resolution ICP-MS coupled to a Resonetics RESOlution M-50 193 nm excimer laser ablation system with helium as carrier gas. Otolith material was ablated along the same

transect for all fish from the core to the dorsal edge. The laser beam diameter was set at 64 µm and analyses were made with an energy of 90 mJ (Attenuated 50%), a frequency of 7 Hz and at a speed of 5 µm.s^{-1}. A total of 26 isotopes were measured (Suppl. Table 1). For some elements, two isotopes were initially quantified to test for possible mass interference. Precision and accuracy were measured using National Institute of Standards and Technology (NIST) 610, FEBS and NIES standards of known composition. Standards were measured at least every 15 measures for accuracy and precision calculations. NIST 612 was used for calibration. The chemical signal was processed with Iolite software (Paton et al. 2011). Data below the Limit of Detection (LOD = 3 x standard deviation of the blanks, Anstead et al. 2015) were set to zero (average missing percentage per element was 2.1 ± 3.7%; Suppl. Table 1). After visual inspection for outliers, missing values, interference between isotopes and precision and accuracy (Suppl. Table 2), the nine most reliable elements (^{23}Na, ^{26}Mg, ^{55}Mn, ^{63}Cu, ^{66}Zn, ^{85}Rb, ^{86}Sr, ^{137}Ba, ^{208}Pb, see Panfili et al. 2002) were kept for further analyses. All element concentrations were normalized to ^{43}Ca (the internal standard) and expressed in µmol mol^{-1} for Mg/Ca, Mn/Ca, Cu/Ca, Zn/Ca, Rb/Ca, in mmol mol^{-1} for Na/Ca, Fe/Ca and Sr/Ca, and in µmol mol^{-1} for Pb/Ca.

2.4 Selection of early life history zones and signal processing

Primary increments have been validated to be daily in larval and juvenile sole, and characteristic marks represent hatching, first feeding and metamorphosis (Amara et al. 1998, Lagardère & Troadec 1997, Paoletti, pers. comm.). Three zones along the transect analyzed were isolated to measure the chemical signal at different life history stages: (1) the 'larval' area just outside the core, reflecting the signature of the fish natal source, (2) the 'post-settlement' portion just after the metamorphosis mark reflecting the signature of the nursery ground colonized at benthic settlement, and (3) the 'otolith edge', i.e. the portion laid down during the last weeks before capture and reflecting the signature of the sampling location (Fig. 2). On average, the three zones were all 77 ± 5 µm long, which corresponds to 2-3 weeks of signal integration depending on fish age and growth rate (Lagardère & Troadec 1997). They were respectively positioned at 50 µm from the edge of the otolith, 30 µm from the settlement mark and 50 µm from the primordium (as the core area is 40 ± 10 µm long for sole, Lagardère et al. 1995). Measurements for identifying the life history periods were made on images captured using a Leica M125 microscope (objective 10x).

2.5 Data analysis

2.5.1 Spatio-temporal variation in elemental signatures on the nursery grounds

The concentrations of the nine elements retained for this study did not meet assumptions of normality and homoscedasticity (Shapiro-Wilk and Bartlett tests) even after $\log_{10}$ transformation. Therefore, the temporal stability in the chemical signal was tested for the two sites sampled in 2013 and 2014 (B2 and B6) through Wilcoxon tests and correction of the significance levels using a Benjamini-Hochberg correction for multiple testing. A MANOVA between multi-elemental fingerprints, time (year) and space (sampling region) was performed to test the effect of space and time on elemental composition. Spatio-temporal variation associated with the multi-elemental signature at the sampling location was also visually assessed using a Principal Component Analysis (PCA). The effect of fish length was investigated using Spearman rank correlation for each element within each sampling region and location. Spatial variation in otolith signature was investigated using the Kruskal Wallis test (for single elements) followed by a *post hoc* comparison (Dunn test) to determine which elements contributed to the differences among sampling locations. For this, we used the chemical signatures recorded on the edge of the otolith for all fish, as they reflect the time and location of capture.

Spatial discrimination success among sampling locations (based on otolith edge signatures) was also assessed using the random forest (RF) algorithm (Breiman 2001). RF requires no *a priori* on the distribution of the data and allows to significantly improve discrimination power when using otolith multi-elemental data (Mercier et al. 2011). Details of the method are available in Tournois et al. (2017). First, all possible combinations of elements out of the nine retained were tested to identify the combination allowing to achieve the best discrimination accuracy using RF. For this, data were centered and reduced to give the same weight to all elements in the spatial discrimination. To avoid circular reasoning, 75% of the signatures were randomly selected to build and “train” each possible combination of elements ('RF classifier') while the remaining 25% were used to test its reliability in re-assigning signatures to the correct origin. In each case, 500 classification trees and 1000 iterations were used and minimum, maximum and average overall discrimination accuracies were calculated to assess the value of each RF classifier for spatial discrimination as in Tournois et al.

(2017). Once the list of elements leading to the best discrimination (i.e. the optimal RF classifier) were identified, classification accuracies and the True Skill Statistics (TSS, Allouche et al. 2006) were calculated for each sampling location. The accuracy represents the total number of fish correctly reclassified to their sampling location while TSS also accounts for true negative prediction. TSS ranges from -1 to +1, where +1 represents 100% of correct prediction of presence or absence in a given habitat, 0 indicates random predictions and -1 indicates 100% incorrect predictions. Finally, the contribution of each element to the spatial discrimination was assessed by calculating the mean decrease in global Gini Index (GI, Breiman 2001) after its removal from the optimal classifier. GI ranges from 0 (when all elements equally contribute to the total discrimination) to 100 (when a single element contributes to 100% of the total discrimination). The higher the decrease in GI when one element is removed from the classifier, the more that element is essential for the discrimination. The percentage of assignment was calculated, with 100 iterations, assigning fish to each sampling location, including the capture location, based on their edge signature. Average percentage of re-assignment was then calculated for each sampling location and each sampling region.

2.5.2 Investigation of potential migration after settlement

Once validated based on otolith edge signature (see *section 2.5.1*), the optimal RF classifier was used to identify the nursery area most likely occupied by the juvenile sole just after metamorphosis. This also enabled detection of any migration among sampling locations after the benthic settlement. For this, the optimal RF classifier was trained with all otolith edge signatures and used to assign each otolith post-settlement signature to its most likely location of origin (among the seven sampled). The percentage of assignment was calculated, with 100 iterations, assigning fish to each sampling location, including the capture location, based on their post-settlement signature. Average percentage of re-assignment was then calculated for each sampling location and each sampling region.

2.5.3 Natal sources

Clustering analysis was performed on the otolith region corresponding to the larval phase (see *section 2.4*) to estimate the number of larval origins (hereafter referred to as 'natal sources') contributing to the focal nursery grounds as well as any connectivity patterns between them. Larval clusters were defined based on the material outside the core area as the core is under maternal influence and enriched in some elements associated with physiological changes rather than with environmental signals (e.g. Ruttenberg et al. 2005, Brophy et al. 2003). Larval clusters were defined using Euclidian distance by unsupervised K-means clustering (i.e. without taking sampling location into account). The optimal number of clusters was determined using the *NbClust* package in R (Charrad et al. 2014) which compares 30 different types of clustering indices. Data was centered and reduced because k-means clustering tends to give more weight to large clusters and is less likely to identify small larval sources due to its sphericity assumption.

3. Results

Otolith elemental signatures were successfully measured for 213 juvenile sole covering the seven sampling locations and three regions (Table 1). Nine elements (Na, Mg, Mn, Cu, Zn, Rb, Sr, Ba, Pb) were regularly detected at all sampling locations with high precision and accuracy; they were retained for statistical analyses (Suppl. Table 3a). The mean percentage of data below the LOD was ≤ 4% for all elements, except for Li (57%), Co (61%), Cd (96%) and Pb (11%), the first three of which were excluded from further analyses (Suppl. Table 1). The relative standard deviation for NIST 612 was ≤ 6 for most elements, with the exception of Li (15), Na (8), Mg (7) and Fe (10). The recovery rates and relative standard deviations of other standards are listed in Suppl. Table 2. Correlations between fish length and otolith element concentrations within regions or locations varied in no particular direction and were rarely significant after correction for multiple testing (Suppl. Table 4) indicating no significant effect of fish length on the otolith concentrations measured.

3.1 Spatial variation in the elemental signature at the sampling location

Discrimination accuracy among all sampling locations was highest (79%) when eight of the nine elements (Na, Mg, Mn, Cu, Rb, Sr, Ba and Pb) were included in the RF classifier. Hence, most elements are informative for discriminating among the seven sampling locations in the Southern North Sea (Fig. 3). Using this optimal RF classifier, the accuracy of correct assignment for otolith edge signatures was >80% for the UK and Wadden Sea nursery grounds, but slightly lower for those along the Belgian coast where the average classification accuracy was 75.8 ± 9.9% (Table 2). TSS were all positive, highest for the Wadden Sea (0.86) and UK (0.76) but still good for all sampling locations for the Belgian nursery ground (0.69 ± 0.1). Contributions to nursery discrimination varied among elements. Mean decrease in Gini index indicated that Mg, Mn and Ba were the three most important elements for classification accuracy, achieving an accuracy of 57% using just those three elements (Fig. 3). Otolith edges, representing the chemical signatures of the sampling locations, were significantly different in Na (KW = 60.87, 13, $p < 0.001$), Mg (131.24, 6, $p < 0.001$), Mn (95.77, 6, $p < 0.001$), Cu (91.20, 6, $p < 0.001$), Zn (119.77, 6, $p < 0.001$), Rb (67.92, 6, $p < 0.001$), Sr (66.587, 6, $p < 0.001$), Ba (113.00, 6, $p < 0.001$) and Pb (65.27, 6, $p < 0.001$). Mg, Mn and Ba concentrations were significantly lower in UK samples, compared to the Wadden Sea and most Belgian sampling locations (exceptions were the low Ba concentrations in B2 and B3). The Wadden Sea was characterized by the highest average concentration and the highest variability in Mn concentrations compared to the other nursery grounds, and high Ba concentrations (similar to B1 and B4). The Belgian nursery ground was characterized by the highest Mg and Pb concentrations, and Mn levels in between UK and the Wadden Sea (Fig. 4).

Incorrect assignments were low for the UK and the Wadden Sea nursery grounds (<26%; Table 3). However, Belgian sampling locations were often confounded with each other with re-assignment errors of 0.6 to 32.4% (Table 3). These errors were due to similarities in otolith concentrations for Mn (B1-B2-B3-B4), Rb (B1-B2-B3), Pb (B1-B2-B4), Mg, Cu, and Sr (B2-B3-B4) or Na, and Ba (B1-B4 and B2-B3, Fig. 4). Assignment errors were highest for the sole from B4 (32.4% in B1) and B2 (26.0%). Assignment errors to another region were also common for sole juveniles captured on the Belgian nursery ground. For example 14.3% of B3 juveniles were assigned to the UK nursery ground and 26.8% of those from B4 were assigned to the Wadden Sea nursery ground. UK and B3 were similar in Na, Cu, Rb, Ba and Pb concentrations (Fig. 4, Table 3). The Wadden Sea and the Belgian nursery grounds had similar Cu, Sr, and Ba signatures. Only 14.9% of the juveniles captured on the UK nursery were assigned

to the Belgian nursery (8.7% to B3 in particular) and 11.5% to the Wadden Sea nursery. Similarly, only 14.5% of the juveniles captured on the Wadden Sea nursery ground were assigned to the Belgian nursery (especially to B2 and B3).

3.2 Temporal variation in elemental signatures at the sampling location

Differences in otolith signatures among sampling locations might partly result from temporal variations in local environmental conditions. Both year and sampling region were significant (MANOVA, df = 4, F = 28.54, $p < 0.001$ and df = 2, F = 18.86, $p < 0.001$, respectively). Interestingly, some chemical elements –like Mn and Ba– were more impacted by year of sampling than other elements (Fig. 5). The first and second principal components together explained 54.1% of the variance. The first axis, which was mainly determined by Mg, Cu and Pb (in growing order of importance), and the second axis, which was mainly determined by Mn, Ba and Sr, overlapped between the three sampling regions. The Wadden Sea was the most distinct nursery ground, characterized by high Mn and Ba, and low Sr and Cu concentrations, while UK was characterized by low Mg and high Sr concentrations.

Interannual variation in otolith edge signatures were observed at both B3 and B6 sampling locations. Differences between 2013 and 2014 signatures were significant ($p < 0.001$) at both locations for four elements (Mg, Mn, Cu and Pb; Mg (207, $p < 0.001$), Mn (172, $p < 0.01$), Cu (31.5, $p < 0.01$), and Pb (38.5, $p < 0.01$) for B3; Mg (253, $p < 0.001$), Mn (265.5, $p < 0.001$), Cu (54, $p < 0.001$), and Pb (272, $p < 0.001$) for B6.) out of nine. Other elements were not significantly different between years: Zn (72.5, $p = 0.176$), Rb (163.5, $p = 0.014$), Sr (154, $p = 0.040$), Ba (106, $p = 0.983$) for B3; Na (W = 84, $p = 0.023$), Rb (129, $p = 0.438$), Sr (98, $p = 0.072$) for B6. Additionally, significant interannual variations in Na signatures (W = 192.5, $p < 0.001$) were observed at B3, while the concentrations at B6 showed significant variations in Zn (W = 306, $p < 0.001$) and Ba (W = 279.5, $p < 0.001$).

3.3 Prediction of movement after settlement

Post-settlement signatures of most juveniles were assigned to a sampling location within the region of capture (79.3%), or even to the capture location itself (46.1%). The

observation suggests that the movements of juvenile sole on the nursery ground are restricted after settlement (Table 4). However, post-settlement signatures of some fish were assigned to another region than the final capture location. For example, 54.0% of the juveniles captured on the UK nursery ground had post-settlement signatures assigned to the Belgian nursery ground (mostly to B3, Table 4). These assignments could be explained by similarities in Na, Cu, Rb, Sr, Ba, and Pb concentrations on the Belgian and UK nursery grounds leading to error in classification accuracy (Table 3, Fig. 4). Similarly, 24.3% of the juveniles captured in the Wadden Sea were assigned post-settlement to the Belgian nursery. On the other hand, a lower proportion of juveniles captured on the Belgian nursery ground were assigned to the UK coast (14.7%) or to the Wadden Sea (19.4%, Table 4). The post-settlement otolith signatures from the juveniles captured on the UK nursery grounds and assigned to the Belgian nursery (especially B3) were high in Mg and low in Ba and Pb (Fig. 6), similarly to those recorded in the otolith edges of juveniles from B1. Similarly, some post-settlement signatures from juveniles captured in the Wadden Sea nursery ground were assigned to the Belgian nursery ground (especially B2), due to the higher values for Mg, Mn, Cu, and Pb.

3.4 Natal sources

K-means clustering identified four clusters of chemically distinct larval signatures (Fig. 7), suggesting four natal sources for the Southern North Sea nursery grounds. Sources 1 and 3 were the main contributors to the Belgian nursery ground; they were characterized by the highest concentrations in Mn and Ba for source 1, and low concentrations in Mg, Cu, and Rb, and the lowest Na concentrations for source 3 (Fig. 7, Suppl. Fig. 2 and Suppl. Table 3b). Source 4 was characterized by the lowest Mn and Ba concentrations. Its contribution to the Belgian and the UK nursery grounds was overall lower but it represented the most abundant source of juveniles at two of the four Belgian sampling locations for some sampling years (B3 in 2014 and Zandvliet in 2007). The UK nursery grounds primarily received individuals from source 3, while Wadden Sea received juveniles from all three other sources (1, 2 and 4). However, source 2 only supplied the Wadden Sea nursery ground, mostly Balgzand.

4. Discussion

Spatial differences in otolith elemental chemistry discriminated juvenile sole on the UK and the Wadden Sea nursery grounds and indicated some overlap between both nursery grounds and the Belgian nursery ground. Post-settlement signatures were mostly assigned to the closest sampling location. This suggests limited movement of juvenile sole after settlement on the nursery grounds, provided there is little to no temporal variability in signatures between the time at settlement and the time at capture. The mixed contributions of four chemically distinct natal sources to the three focal regions in the Southern North Sea points toward an extensive connectivity during larval dispersal.

Strong chemical differentiation on the nursery grounds and limited movement after settlement

Despite the challenge of working at a small spatial scale (5 km between the closest sampling locations) and in a coastal area with various terrestrial influences, the chemical signatures of eight elements provided high nursery-specific assignment levels for the juvenile sole. Assignment rates to the nursery grounds within the same region (76.1%) were comparable with previous studies (Cuveliers et al. 2010: 88%, Tanner et al. 2012: 71-80%). Eight elements were selected by the optimal RF classifier to achieve the highest accuracy, but the first five elements were sufficient to achieve an assignment accuracy of 70%. Errors in assignment were low for the UK and the Wadden Sea nursery grounds (<26%). In contrast, Belgian sampling locations were often confounded with each other due to similarities in otolith concentrations for several elements. Assignment errors to another region were also common for sole juveniles captured on the Belgian nursery ground. The UK and the Belgian nursery grounds were similar in Na, Cu, Rb, Ba and Pb concentrations while the Wadden Sea and the Belgian nursery grounds had similar Cu, Sr, and Ba signatures. Freshwater sources entering the Southern North Sea are the estuaries of the Thames, Scheldt, Meuse, Rhine and Ems-Dollard rivers. The high assignment success we found might be attributed to the chemical composition of these rivers as each drains basins with a distinct geology: Mesozoic Alp geology for the Rhine, Eocene deposits of the Scheldt, Paleocene deposits for the Thames, and Quaternary deposits for the Ems and Dollard (Hartmann et al. 2014, Preusser 2008, Yang & Nio 1989).

In addition to geo-specific river signatures, other influences may be attributed to the pollution history and differences in fish ecophysiology (see below). The North Sea is one of the most polluted seas world-wide due to the high level of industrialization and urbanization (Grizzetti et al. 2017). Despite a decreasing trend in metal concentrations due to strict regulations since the 1980s (Emeis et al. 2015), local concentrations in sediment and suspended matter remain high (De Witte et al. 2016). In this study otolith Sr concentrations were lowest in the Wadden Sea and Ba concentrations highest in estuarine environments (i.e. Zandvliet, Balgzand and the Ems-Dollard Estuary). The patterns of otolith Sr and Ba usually matched the salinity gradients (Campana 1999, Leakey et al. 2009). Zandvliet is situated in the brackish part of the Scheldt Estuary where salinity reaches a value of about 6 (Le Cozr et al. 2017). Similarly, Balgzand and the Ems-Dollard Estuary are low salinity nursery grounds. Additional sources of Ba include terrestrial runoff, groundwater, pollution and remobilization from sediments (Hamer et al. 2006). A recent meta-analysis showed that otolith Sr and Ba concentrations may be influenced by intrinsic factors such as diet, condition and ontogeny and additional extrinsic factors such as the ecological niche (Izzo et al. 2018), supporting former evidence (Sturrock et al. 2012). Balgzand and the Ems-Dollard Estuary exhibited similar concentrations for most elements although they were located in different tidal basins of the Wadden Sea and were sampled eight years apart. For example, both locations exhibited high Mn concentrations that may reflect enrichment of the Wadden Sea in dissolved and particulate Mn compared to the surrounding water masses and/or hypoxic conditions (Limburg et al. 2015). Pb, Cu and Zn, which are associated with pollution, were higher in sole otoliths from the Belgian coast compared to the Thames Estuary, while Rb levels, which are associated with ingested plastic pollution (Lavers & Bond 2016), were similar. The eastern Belgian coast is influenced by the highly industrialized Scheldt Estuary, with continuing and past traces of heavy metal pollution (Zwolsman et al. 1996), whereas the inner Thames Estuary has been rehabilitated (Andrews & Rickard 1980, Grizzetti et al. 2017). Despite the distance between the two UK sampling locations, the chemical similarities were supported by the northeast residual current (Grizzetti et al. 2017).

Similarity in chemical signatures between recently settled juveniles and juveniles captured later on in the same nursery ground points to limited movement after settlement. In this study random forest (RF) classification of the post-settlement signatures assigned 21% of the juveniles to another sampling region than that of their final capture. The value fits with the overall observation that juvenile flatfish remain

localized (within 3-40 km) on the nursery grounds (Le Pape & Cognez 2016). However, the post-settlement signatures depend on the local water characteristics which might change over time (Chang & Geffen 2013). Most assignments of post-settlement signatures to another sampling region might be linked to the spatial accuracy of the optimal RF classifier. Indeed, juveniles that settled in the Belgian nursery might have moved after settlement to be captured off the Dutch coast (and *vice versa*). Instead, otolith microchemistry might be influenced by time and physiological processes (Chang & Geffen 2013, Reis-Santos et al. 2012, Sturrock et al. 2012). For example, high Sr levels are associated with high growth rates during metamorphosis (De Pontual et al. 2000). We therefore specifically looked at elements outside the nucleus and the metamorphosis zone (after the Sr peak) to avoid measuring chemical variation associated with ontogeny. However, temporal variability in the microchemical signal might be driven by seasonal or interannual variation (Tanner et al. 2012). Although most studies found a significant effect of season or year on the microchemical signal, temporal variation did not hinder spatial discrimination, just as confirmed in the present study (Cuveliers et al. 2010, Reis-Santos et al. 2012, Tanner et al. 2012).

The interannual variation in chemical signature at two Belgian sampling stations was significant for several chemical elements and year had a significant effect overall when considering all sampling regions and chemical elements. This suggests that temporal variation has to be taken into account to use otolith signatures for geolocation. But it could also mean that different sources supply the nursery throughout the years. Biophysical models point at considerable interannual variation of larval connectivity, which correlates with interannual recruitment variability (Bolle et al. 2009, Lacroix et al. 2013, 2018). Although recruitment variability has been attributed to winter mortality in adult sole (Rijnsdorp et al. 1992), overall variation in recruitment, which might be affected by hydrodynamics and life history traits, remains incompletely understood. It is a common thread in the knowledge on many fish stocks (Cury et al. 2014).

Identification of chemically distinct natal sources and larval dispersal

The identification of the natal sources contributing to each nursery ground is key for understanding the dynamics of the population structure. Here, the eight most reliable elements to discriminate juvenile sole grouped in four chemically distinct sources. Each

nursery ground received individuals from three of the four chemically distinct natal sources, albeit in different proportions. Sources 1, 3 and 4 contributed to the UK and Belgian nursery grounds, while source 1, 2 and 4 contributed to the Wadden Sea nursery ground. Hence, empirical observations on juvenile sole point in the direction of local recruitment supplemented with external input. This is exactly what a biophysical model of larval sole predicted: a mixed input from several spawning grounds to most North Sea nursery grounds (Lacroix et al. 2013). Interestingly, the model predicted that the UK nursery ground is mostly self-recruiting, similar to what we observe. The Wadden Sea was supplied uniquely and almost exclusively at Balgzand by source 2. This phenomenon is observed in some of the modeled years by Lacroix et al. (2013). Important is that the absence of a source on a nursery ground might also be linked to interannual fluctuations in the contributions of the various spawning grounds (Lacroix et al. 2013).

The spawning location is tightly linked to the nursery grounds. The spawning grounds of North Sea sole are located more inshore and in shallower waters compared to other flatfish (Rijnsdorp et al. 1992). Flatfish optimize settlement success in two ways. Firstly, successful spawning ground locations have been selected over time and flatfish have a high fidelity to their spawning ground (Hunter et al. 2003). One of the most important requirements of a successful spawning ground is the presence of suitable hydrographic conditions to transport eggs and larvae to the nursery ground (Symonds & Rogers 1995). Secondly, settlement can be delayed up to three weeks (Marchand 1993 for common sole). Factors triggering the delay are linked to terrestrial and benthic chemical cues, and freshwater input (Dixson et al. 2011, Freckelton et al. 2017, Kerstan 1991). The absence of a source 3 signature in the northeast and the presence of some individuals with a source 3 signature on the UK and the Belgian nursery grounds might suggest that source 3 is linked to the English Channel spawning ground, as was predicted by modeling (Lacroix et al. 2013). In the English Channel and Southern North Sea a northeast residual current has been well described, although its intensity may vary among years (Sentchev et al. 2006). In addition, source 3 resembles the UK nursery signature as it is characterized by low Mg, Cu, and the lowest Na concentrations. Higher Ba concentrations in source 3 compared to the UK nursery ground suggest that the spawning ground linked to source 3 is located offshore. Source 1, characterized by high Na, Mg, Mn, and Ba and low Cu concentrations, contributed a lot to the Belgian nursery but also to the Wadden Sea nursery. Source 1 could be located close to the Belgian coast as its signature resembles the signature of the

Belgian nursery with high Na, Mg, Mn, and also high Ba concentrations. Zandvliet and Balgzand, two locations under estuarine influence, were each supplied by a single natal source (source 4 and 2, respectively). The specific signatures of Zandvliet and Balgzand might be an artefact of the low sample size because the K-means clustering method used is sensitive to sample size and less likely to identify small larval clusters.

Several spawning grounds contribute to the sole nursery grounds in the Southern North Sea, according to our results. This is a validation of several model predictions (Barbut et al. 2019, Bolle et al. 2009, Lacroix et al. 2013, Savina et al. 2010). Biophysical individual-based models are arguably one of the best tools to explore transport dynamics of flatfish larvae (Huret et al. 2013) as they allow predictions over a large spatial extent and a high temporal frequency, far more than can be realized empirically. However, the lack of precise information on key parameters for larval survival such as swimming capability, mortality and pelagic larval duration hamper the parametrization of behavior routines of individual-based models. Although the biological parametrization of these models includes many assumptions, the current parametrization seems sufficient for a semi-quantitative assessments of dispersal. A next step would be to confirm the modelled variation between years. Interannual variation in hydrodynamics and life history dynamics considerably impact recruitment (Gibson 2015). Connectivity research based on microchemistry requires the establishment of an extensive chemical baseline from putative sources (i.e. larvae in the spawning grounds and juveniles on the nursery grounds) to re-assign individuals of unknown origin (Gillanders et al. 2005). Establishing an extensive baseline is not always feasible due to spatio-temporal sampling limitations and knowledge gaps. However, statistical tools incorporating uncertainty and enabling assignment to missing sources provide an a useful supporting tool. For example, Bayesian models provide uncertainty of assignment estimates (Reis-Santos et al. 2018). Estimating the potential number of sources would help to proceed during the validation step (i.e. know better how many spawning sources to sample) and allow hypothesis-testing of the contribution of different spawning grounds to focal nursery grounds using other methods such as genetic markers (Reis-Santos et al. 2018). Nevertheless, using Bayesian models does not eliminate the need to validate the contribution of each spawning ground to the nursery grounds. Determining the chemical signal of larvae on the spawning grounds is key to validating connectivity patterns.

Important larval dispersal supports the observation that the population genetic structure of sole in the North Sea is homogeneous (Cuveliers et al. 2012, Diopere et al. 2018) and could come from a high level of gene flow. However, larvae move less than might be expected from advection. For example, plaice larvae are retained on the spawning grounds due to nycthemeral migration (Fox et al. 2009) and selective tidal transport (Rijnsdorp et al. 1985). Hence, despite the homogenous genetic structure, some structure is present with fish adapted to the local environment (Diopere et al. 2018). Limited differences in natal sources were observed between the UK and the Belgian nursery grounds, compared to the Wadden Sea. Differences in natal sources were also present within the Wadden Sea nursery ground. So, the main drivers of otolith chemistry are operating at a local scale of approximately 100 km, or even less in the case of the Wadden Sea, as supported by assignment. This finding is consistent with an average dispersal distance of sole of about 150 km based on tagging studies (Burt & Millner 2008), biophysical modeling (Barbut et al. 2019) and genetic markers (Diopere et al. 2018, Kotoulas et al. 1995). Field surveys reported comparable dispersal distances between spawning and nursery grounds (80-100 km, Dorel et al. 1991). Although local settlement is a common feature of many marine species (Pinsky et al. 2017) sole has a good dispersal potential. Interestingly, dispersal potential might be linked to spawning date. For example, summer spawning flatfish, such as sole, turbot and brill, disperse less further than winter spawning flatfish such as common dab, European flounder and European plaice (Barbut et al. 2019).

The shift in location of the spawning and nursery grounds, and spawning season may lead to important changes in connectivity. Global change has been affecting fish and plankton communities in the North East Atlantic Ocean over the last 20 years such that the ranges of many species have shifted northward (Beaugrand et al. 2002, McLean et al. 2018). The spawning season of sole has shifted to earlier in the year (Fincham et al. 2013) but the distribution of adults has shifted southward. The most likely reason is that sole have shifted to deeper waters (Engelhard et al. 2011). Although spawning and nursery ground locations have not shifted yet, modeling predicts geographical shifts in connectivity and larval recruitment at the nursery grounds due to climate change (Teal et al. 2012, Lacroix et al. 2018).

Conclusions

This study provides new evidence on the early-life connectivity of sole, during the stages when most of the realized dispersal is assumed to take place in the species (Batista et al. 2015, Krueck et al. 2017). The connectivity of sole is extensive at the larval stage and limited at the juvenile stage. The high spatial discrimination of the microchemical signal of sole otoliths identified several natal sources and connectivity patterns in the southern North Sea. The new evidence on the local connectivity dynamics of the early-life stage of sole is consistent with the output of several biophysical models of larval dispersal (Barbut et al. 2019, Bolle et al. 2009, Lacroix et al. 2013). This confirms that elemental analysis is a valuable tool for species with extended larval dispersal and low genetic differentiation. Understanding the directionality of flow between larval sources (i.e. spawning grounds) and sinks (i.e. nursery grounds), and connectivity patterns is crucial for the management of the intensively fished stocks (ICES 2018) and the proper design and functioning of Marine Protected Areas (Botsford et al. 2001, Gaines et al. 2010). Several questions related to the population dynamics and ecology of sole remain to be addressed. For example what is the contribution of interannual variation to dispersal, what is the role of (sub)adult dispersal, what is the age-specific mortality rate, and what is the contribution of phenotypic plasticity and selection to local environmental pressures.

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Figures and tables

Figure 1: Map of the Southern North Sea showing the sampling locations for juvenile sole (n = 213) off the British (UK), Belgian (BE) and Dutch Wadden Sea (WA) coasts. Codes for sampling locations include the station name (e.g. "B02" for "B02j13"), the life stage of the fish ("j" for juvenile) and the year of sampling ("13" for 2013). See also Table 1.

Figure 2: Transversal section of an otolith of juvenile sole (transmitted light, x80 magnification) showing the laser ablation transect and selections representing the larval (core), post-settlement and nursery ground (edge) signatures representing the chemical fingerprints of the habitats occupied at each life history stage.

Figure 3: Effect of the number of elements included in the otolith signatures on the discrimination accuracy among the seven sampling locations based on the Random Forest (RF) approach. The gray area shows the interval between the minimum and maximum accuracies for each signature, while the mean accuracy (±SD) is depicted by the squares. The table shows the list of elements included in the optimal RF classifier ranked by the corresponding mean decrease in the Gini index (importance for spatial discrimination).

Figure 4: Box plots of the elemental composition (expressed as ratios to Ca) of juvenile sole otoliths at all sampling locations (see Table 1). For each sampling location, the solid line and the box correspond to the median and the interquartile range, respectively. Different letters indicate groups of significantly different signatures based on Dunn's post hoc test ($p < 0.05$). The concentration of each element was normalized to ^{43}Ca (the internal standard) and expressed in µmol mol^{-1}, except for Na/Ca and Sr/Ca (in mmol mol^{-1}) and Pb/Ca (in pmol mol^{-1}).

Figure 5: Correlation biplot based on principal component analysis (PCA) of the elemental profiles of the otoliths of 213 juvenile sole. The position of the elemental vectors is indicated with respect to the first two principal components (PC1, PC2), which together explain 55% of the variance. The individual elemental profiles are organized by sampling region (UK, Belgian and Wadden Sea nursery).

Figure 6: Elemental composition (expressed as ratios to Ca) of juvenile sole otoliths in the transect selections representing settlement (dark grey) and sampling locations (light grey, see Table 1). For each sampling location, the solid line and the box correspond to the median and the interquartile range, respectively. Different letters indicate groups with significantly different signatures based on Dunn's post hoc test ($p < 0.05$). The concentration of each element was normalized to ^{43}Ca (the internal standard) and expressed in µmol mol^{-1}, except for Na/Ca and Sr/Ca (in mmol mol^{-1}) and Pb/Ca (in pmol mol^{-1}).

Figure 7: Assignment (%) of juvenile sole to the four natal sources based on the chemical variation in larval otolith signatures, by K-mean clustering. For each sampling site the pie chart shows the relative contribution of each natal source (source 1: n = 57, source 2: n = 16, source 3: n = 85, source 4: n = 55).

Table 1: Number of sole juveniles analyzed for otolith elemental composition per sampling location and region (UK, Belgian and Dutch Wadden Sea nursery grounds), including the date of sampling, geographical coordinates and the sea surface temperature at the time of sampling (NA = Not Available).

Table 2: Classification accuracy and True Skill Statistics (TSS) of each sampling location when using the optimal RF classifier for spatial discrimination of sole nurseries.

Table 3: Errors in re-assignment to sampling location (see Table 1) based on multi-elemental otolith edge signatures. Each row shows the average percentage of individual sole from each location assigned to each sampling location by the optimal Random Forest (RF) classifier (see Fig. 3). Numbers on the diagonal indicate individuals successfully re-assigned to their sampling location. Boxes group the sampling locations belonging to the same sampling region.

Table 4: Assignment of otolith settlement signatures to sampling location (see Table 1) using the Random Forest (RF) classifier optimal for sampling location discrimination. Numbers on the

diagonal indicate individuals successfully re-assigned to sampling location as defined in Table 1. Boxes group the sampling locations belonging to the same sampling region.

Supplementary material

Supplementary Figure 1: Box plots of the standard length of the juveniles sampled for each of the four sampling regions. The solid line and the box correspond to the median and the interquartile range, respectively. Different letters indicate groups of significantly different signatures based on Dunn's post hoc test ($p < 0.05$).

Supplementary Figure 2: Box plots of the elemental composition of the larval signatures of juvenile sole otoliths (see Suppl. Table 3). For each natal source, the solid line and the box correspond to the median and the interquartile range, respectively. Different letters indicate groups of significantly different signatures based on Dunn's post hoc test ($p < 0.05$). The concentration of each element was normalized to ^{43}Ca (the internal standard) and expressed in µmol mol^{-1}, except for Na/Ca and Sr/Ca (in mmol mol^{-1}) and Pb/Ca (in pmol mol^{-1}).

Supplementary Table 1 Limit Of Detection (LOD = 3 x standard deviation of the blanks) and percentage of missing values for each element after setting negative LOD values to zero. The concentration of each element was normalized to ^{43}Ca (the internal standard) and expressed in µmol mol^{-1}, except for Na/Ca, Fe/Ca and Sr/Ca (in mmol mol^{-1}) and Pb/Ca (in pmol mol^{-1}). Only elements in bold were retained for final analyses.

Supplementary Table 2: Accuracy and precision of the ICPMS measurement of standard reference material (FEBS, NIST610, NIES) over all analysis sessions in this study (NA = missing value, NCV = Non Communicated Value). Recovery rate is the ratio of the measured concentration to the certified or reference concentration (in %). Relative standard deviation is the ratio of the standard deviation to the average concentration (in %). Elements in bold were retained for final analyses.

Supplementary Table 3: (a) Mean elemental signature (± standard deviation) for each sampling location (see Table 1, otolith sampling location signature). (b) Mean elemental signature (± standard deviation) of the four natal sources identified by K-means clustering based on the larval signature (source 1: n = 57, source 2: n = 16, source 3: n = 85, source 4: n = 55). For the list of sampling locations see Table 1. The concentration of each element was normalized to ^{43}Ca (the internal standard) and expressed in µmol mol^{-1}, except for Na/Ca and Sr/Ca (in mmol mol^{-1}) and and Pb/Ca (in pmol mol^{-1}).

Supplementary Table 4: Spearman rank correlations (upper table) obtained between body size and elements, and associated p values (lower table) for (a) each sampling region and (b) each sampling location. Significant values (alpha level 0.05) are reported before (in bold) and after (underlined) Bonferroni correction for multiple testing.

File: Delerue-Ricard_JSR_MS_20191127_tables1-4

Extensive larval dispersal and restricted movement of juveniles on the nursery grounds of sole in the Southern North Sea

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Table 1

Region	Sampling location	Station	Sampling size	Sampling date
UK coast (n = 32)	UK	GPRj16	19	14/03/2016
	UK	CHAj07	13	01/08/2007
Belgian coast (n = 122)	B1	B01j14	16	15/09/2014
	B2	B02j13	15	10/09/2013
	B2	B03j13	21	28/08/2013
	B2	B03j14	10	26/05/2014
	B3	B06j13	18	09/09/2013
	B3	B06j14	17	16/09/2014
	B4	B08j14	17	10/10/2014
	B4	ZANj07	8	01/10/2007
Wadden Sea (n = 51)	Balgzand	BALj06	14	01/08/2006
	Ems-Dollard estuary	NL1j14	16	16/09/2014
		NL2j14	21	23/09/2014

Table 2

	UK	B1	B2	B3
Accuracy	80.7	71.9	78.9	69.3
TSS	0.76	0.69	0.73	0.66

Assigned Location / Sampling Location	UK	B1	B2	B3	B4	WA	Region	Average assig
UK	73.6	0.4	4.7	8.7	1.2	11.5	73.6	55.3%
B1	6.5	34.7	15.3	6.2	32.4	4.9	88.5	per sampling lo
B2	8.6	2.2	59.6	12.5	4.2	12.9	78.5	
B3	14.3	0.9	26.0	41.8	0.7	16.3	69.4	
B4	6.6	13.9	9.4	1.4	41.8	26.8	66.6	76.1 %
WA	5.4	0.6	6.5	6.2	1.3	80.1	80.1	per sampling r

Table 3

Table 4

Assigned location / Settlement signature at the sampling location	UK	B1	B2	B3	B4	WA	Region	Av
UK	35.1	11.4	10.0	26.5	6.1	11.0	35.1	
B1	3.8	25.0	6.3	33.7	31.3	0.0	96.2	
B2	0.0	19.9	56.0	11.2	4.3	8.5	91.5	
B3	2.9	17.0	17.4	43.7	14.2	2.9	94.3	
B4	8.0	8.0	20.0	16.0	40.0	8.0	84.0	
WA	0.6	5.1	11.4	3.8	3.8	75.1	75.1	

Highlights:

- In the North Sea, juvenile otolith signatures differ at the regional scale (100 km)
- Movement appears to be limited once the juveniles have reached a nursery ground
- Local nursery areas receive larvae from four chemically distinct natal sources
- Mixed larval contributions to nurseries point to an extensive larval dispersal