Elemental Inventory in Fish Otoliths Reflects Natal Origin of Atlantic Herring (*Clupea harengus*) From Baltic Sea Juvenile Areas

Dorothee Moll\(^1\,2\)*, Paul Kotterba\(^2\), Klaus Peter Jochum\(^3\), Lena von Nordheim\(^1\,2\) and Patrick Polte\(^1\)

\(^1\) Thünen Institute of Baltic Sea Fisheries, Rostock, Germany, \(^2\) Institute for Marine Ecosystem and Fisheries Science, University of Hamburg, Hamburg, Germany, \(^3\) Max Planck Institute for Chemistry, Department of Climate Geochemistry, Mainz, Germany

Despite centuries of human exploitation and research on Atlantic herring (*Clupea harengus*) in Europe, there is still much uncertainty on where major nursery areas are located. However, understanding the quantitative contribution of particular coastal systems to adult fish populations is of utmost importance to secure sustainable fish resources. Routinely, marker elements indicating certain hydrological conditions, which are incorporated into calcified structures, the so-called otoliths, are used to trace the origin of fish. However, as in the Baltic Sea, small and large scale salinity gradients potentially masking specific salinity signals. Based on the entire elemental inventory of the otolith core region, indicating the chemical signature of the spawning area, we developed a unique elemental fingerprinting index (EFI), allowing comparisons of multi-elemental chemical signatures from within and between herring juvenile areas. Our results show significantly distinct chemical “fingerprints” on the scale of particular bays and estuaries, which were not detectable with the usual marker elements. We further demonstrate that heavy metals levels drive the potential to distinguish natal origin of herring. These findings provide an essential baseline for further studies on the impact of small scale productivity for exploited fish resources and central components of marine food webs.

**Keywords:** *Clupea harengus*, Western Baltic Sea, inshore spawning areas, age-0 herring, otolith chemistry, elemental fingerprinting, LA-ICP-MS

**INTRODUCTION**

Coastal ecosystems are highly productive, serving as crucial spawning and juvenile habitats for many fish species worldwide (Blaber and Blaber, 1980; Orth et al., 1984; Shulman, 1985). At the same time, coastal systems are under increasing anthropogenic pressure, resulting in global losses of species biodiversity and habitat degradation (Lotze et al., 2006; Worm et al., 2006; Waycott et al., 2009). However, management of natural fish resources is widely limited to fishery regulations for adult stocks. Regional coastal zone management rarely includes a certain focus on important fish nursery areas although impacts can have drastic consequences on resilience and recruitment strength of a fish population. The definition after Beck et al. (2001) emphasizes the concept of a nursery...
area, revealing the importance to identify the quantitative contribution of particular areas to the adult population of fish or invertebrates. Accordingly, a nursery area is not just one of multiple areas inhabited by juveniles of a population, but is defined as an area, which contributes a greater than average number of individuals to the adult population (Beck et al., 2001; Dahlgren et al., 2006). According to these quantitative aspects, research is nowadays increasingly focused on studies on juvenile-adult habitat connectivity and dependency of fish species on certain habitats (Thorrold et al., 2001; Forrester and Swearer, 2002; Gillanders, 2002a). This connectivity and dependency aspect is highly relevant to evaluate the relation between habitat availability and fish population dynamics and at the same time exposed a great lack of knowledge on general life history and migratory patterns of economic important fish species with a great uncertainty of obligate or facultative habitat use (Able, 2005).

Otoliths are the calcified ear stones in teleost fish and were formerly used for annual age and growth estimations (Beamish, 1979; Hoyer et al., 1985). During the last decades the scope of otolith research further expanded and resulted in new scientific applications, such as otolith microstructure analyses for age estimates of early life stages on a daily scale (Nishimura and Yamada, 1984; Campana and Moksness, 1991), otolith shape analyses for stock identification (Campana and Casselman, 1993; Tracey et al., 2006) and chemical analyses of the otolith core region to investigate elemental signals and spatial differences in juvenile fish otoliths (De Pontual et al., 1999; Gillanders and Kingsford, 2003; Hamer et al., 2003). This otolith chemistry approach takes advantage of specific fish otolith characteristics, being metabolically inert, growing continuously without resorption and incorporating elements into their growing surface. Hence, these calcified structures reflect the physical and chemical characteristics of the ambient water, where the fish is residing during the early ontogeny (Campana, 1999). With the implementation of otolith microchemistry, a new age of fishery research was established, resulting in studies concerning stock discrimination analyses (Geffen et al., 2011; Heidemann et al., 2012) and fish migration patterns (Limburg, 1995; Barnett-Johnson et al., 2008), mainly based on elements indicating hydrologic conditions like strontium and barium, which are known as markers for salinity gradients (Kennedy et al., 2002; Elsdon and Gillanders, 2005). Further approaches to investigate habitat connectivity were predominately based on a few selected elements in tropic and sub-tropic fishes (Milton et al., 1997; Thorrold et al., 2001). Studies concerning the elemental inventory of fish otoliths and habitat connectivity of economically important fish species in the North Atlantic region, such as flatfishes, cod and herring, are rather scarce (but see Brophy et al., 2003; Vasconcelos et al., 2008; Thorrisson et al., 2011).

The Atlantic herring (Clupea harengus, L. 1958) is one of the most economically important marine fishery target species in the world (FAO, 2016). Moreover, this small pelagic fish is of high ecological relevance, linking different trophic levels in the marine food web (Casini et al., 2004; Möllmann et al., 2004). In the Baltic Sea, C. harengus is a highly migratory fish, accomplishing annual migrations between their feeding grounds in the Kattegat-Skagerrak and their coastal spawning grounds located in bays, estuaries and lagoons along the coastal Western Baltic Sea during spring, showing a distinct homing behavior (Wheeler and Winters, 1984; McQuinn, 1997). This herring population is of high economic value for the Western Baltic Sea region. Herring reproduction success is affected by multiple abiotic and biotic parameters (Holliday and Blaxter, 1960; Laine and Rajasilta, 1999; Peck et al., 2012; Kotterba et al., 2017), as well as by human-induced stressors, such as pollution (Dethlefsen et al., 1996; Incardona et al., 2012) or eutrophication (Aneer, 1987; Rahikainen et al., 2017; von Nordheim et al., 2018). Eutrophication processes in the past decades led to severe habitat degradation of important spawning areas in Baltic coastal waters (Kääriä et al., 1988; Kanstinger et al., 2016). Western Baltic herring recruitment decreased during the past two decades and remained on a low level ever since, reaching the lowest value of the time-series in 2017 (ICES, 2018). The reasons for this decline are still unclear, but evidence mounts for a synergy of eutrophication, climate change and resulting ecological cascades negatively affecting the survival of early life stages (Moll et al., 2018). However, quantitative studies on the contribution of single coastal juvenile habitats to the adult herring population are lacking, but are of vital importance to understand the impact of local hazards to fish population dynamics and to establish adequate coastal zone management strategies.

The objectives of this study are to identify differences in the elemental composition of age-0 herring otoliths from different inshore spawning areas along the Western Baltic coastline. Hypothesizing that herring otoliths from different juvenile areas differ in their elemental composition, we created an elemental fingerprinting index (EFI) based on a multi-element approach.
MATERIALS AND METHODS

Age-0 Herring Sampling
All four inner coastal sampling sites are anthropogenic-influenced spawning areas for the Western Baltic spring spawning herring population, located along the German coastline of the Western Baltic Sea. In June 2016, sampling was conducted in the eutrophic Schlei Fjord (SF: ∼54.62°N, 09.09°E) and in Kiel Canal (KC; ∼54.33°N, 09.72°E), an artificial waterway with heavy ship traffic, connecting the Baltic Sea and the North Sea. Furthermore, young-of-the-year (YOY) herring was sampled in the Warnow Estuary (WE; ∼54.12°N, 12.10°E), with its dockyard and landing pier for cruise liners and ferries, and the eutrophic Greifswald Bay (GB; ∼54.25°N, 13.50°E) (see Figure 1). YOY herring was caught by beach seine (7 m opening, 5 mm mesh size, wing height 1.5 m) in the SF sampling area and by purse seine (40 m length, 4 mm mesh size, 3 m height) in KC, both nets were pulled by two people. Sampling areas WE and GB were sampled with a ring trawl of 1 m diameter (1550 µm mesh size) onboard a research vessel. Hydrological parameters [sea surface temperatures (SST) and salinity] were either recorded with a hand held field probe (YSI Professional Plus) or a remotely operated CTD. The characteristics of each sampling site, including abiotic parameters during sampling, mean total length (TL mm) of YOY herring, as well as numbers of individuals per sampling site are summarized in Table 1. Herring were frozen immediately after the catch and were transported to the laboratory for otolith dissection.

Young-of-the-year herring sampling was conducted under current licenses for wild fish sampling according to Mecklenburg-Western Pomeranian (Germany) fishery law (§11 LFischG, Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei, Mecklenburg-Vorpommern).

Sample Processing
Prior to otolith dissection, microscope slides were washed in 2% nitric acid (Rotipuran Supra 69%, Carl Roth), were rinsed with ultrapure water and were dried under a flow cabin to keep them free from dust particles. Double-sided adhesive transparent pads, placed on the cleaned glass slides, were used as otolith fixing material (for further information, see Supplementary Figure 1). Herring otoliths were dissected under a microscope (Olympus SD 30) and sagittal otoliths were removed with non-metallic equipment to minimize potential contamination. After dissection, each otolith (either left or right sagittal otolith) was rinsed in ultrapure water several times to remove any adhesive tissue and 30 otoliths per sampling area were used for chemical analysis of habitat-specific signatures, except for GB (n = 15). The chosen sample size was above the commonly accepted standard for this kind of analysis (Gillanders, 2002a; Hamer et al., 2003) and was assumed to be adequate to test our hypotheses. Otoliths were transferred and placed on the fixing material. Microscope slides with attached otoliths were stored in a plastic vials for further processing in the laboratory.

Analytical Methods
Elemental composition of herring otolith core regions were analyzed with the laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) technique, using a ThermoFinnigan ELEMENT2 ICP-MS with a Nd:YAG UP213 laser ablation system at the Max Planck Institute of Chemistry in Mainz, Germany. The certified reference material NIST612 (National Institute of Standards and Technology) and the micro analytical reference material MACS3 (United States Geological Survey) were used for calibration (Jochum et al., 2007; Jochum et al., 2011) and were analyzed prior to sample measurement and reference material measurements were constantly repeated to ensure quality. Otolith core regions were ablated with 80 µm laser spot size at 60% power with a pulse repetition rate of 10 Hz and energy densities of ∼10.2 J cm⁻². Dwell durations of otoliths were operated manually, due to individual otolith size and thickness. Further technical information of the New Wave laser ablation system and the operating parameters for the ELEMENT2 ICP-MS (further information is given in Supplementary Table 1).

Data Analysis
Values near detection limits were excluded and only elements with no more than two missing values were considered for the analysis. In total, the following 17 elements were used: lithium (Li), boron (B), sodium (Na), magnesium (Mg), silicon

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**TABLE 1** | Description of each inshore sampling area [Schlei Fjord (SF), Kiel Canal (KC), Warnow Estuary (WE), Greifswald Bay (GB)] with respective water body type, prevailing salinity ranges in PSU (Messner and von Certzen, 1990; Feibicke, 1997; Kafemann et al., 2000), abiotic parameters at sampling location (SST and salinity), number of analyzed individuals and mean total length TL [mm] of caught age-0 herring with standard deviation.

<table>
<thead>
<tr>
<th>Location</th>
<th>Water body type</th>
<th>Salinity range</th>
<th>SST °C</th>
<th>Salinity PSU</th>
<th>No. of individuals</th>
<th>Mean TL mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF</td>
<td>Fjord 42 km</td>
<td>Mesohalin</td>
<td>20.8</td>
<td>4.03</td>
<td>30</td>
<td>37.2 (±2.9)</td>
</tr>
<tr>
<td>KC</td>
<td>Artificial water way 97 km</td>
<td>Mesohalin</td>
<td>20.0</td>
<td>7.05</td>
<td>30</td>
<td>38.0 (±1.8)</td>
</tr>
<tr>
<td>WE</td>
<td>Estuary 12.6 km²</td>
<td>Mesohalin</td>
<td>20.1</td>
<td>10.7</td>
<td>30</td>
<td>23.2 (±3.5)</td>
</tr>
<tr>
<td>GB</td>
<td>Lagoon 514 km²</td>
<td>Mesohalin</td>
<td>018.3</td>
<td>07.8</td>
<td>15</td>
<td>22.7 (±2.2)</td>
</tr>
</tbody>
</table>

The SST and salinity for GB are averaged due to different pooled stations.
(Si), manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), rubidium (Rb), strontium (Sr), tin (Sn), barium (Ba), lead (Pb), thallium (Tl) and uranium (U). Potential chemical differences between different sampling locations were visualized by multidimensional scaling (MDS-plot), using the PRIMER Software (Version 6.1.13) and the dissimilarity matrix of calculating the Euclidean distances. Elemental concentrations were log(x+1)-transformed for standardization. Statistical tests were performed with the software SPSS, statistical significance (significance level of \( p < 0.05 \)) was tested by using one-way analysis of variance (ANOVA). Data sets were tested for variance homoscedasticity by Levene's test. Due to variance heteroscedasticity and unequal sample sizes, statistical significances were tested with ANOVA including Welch correction (Welch, 1938) which also compensates for non-normal distributions and post-hoc test for inhomogeneous variances and sample sizes (Games Howell test). EFI values were tested for significant differences within and between sampling areas using Welch Test and Games Howell post-hoc test to investigate significant differences between the four sampling sites.

**Elemental Fingerprinting Index (EFI)**

We visualized chemical otolith signatures for each spawning area by radar plots, based on the relative mean proportion of each element to the highest concentration found among the total of 105 individuals included in this study. We modified a well-established ecological overlap index (Schoener index, Schoener, 1974) to create the EFI, a novel measure of similarity between 2 fish individuals considering numerous chemical signatures in their otoliths. The EFI\( _{ij} \) describes the comparison of two fish individuals that includes 17 elements and was calculated according to the following equation:

\[
\text{EFI}_{ij} = \frac{2 \times \sum_{x=1}^{17} \min(\text{conc}_{x_i}; \text{conc}_{x_j})}{\sum_{x=1}^{17} (\text{conc}_{x_i} + \text{conc}_{x_j})}
\]

Where \( \text{conc}_{x_i} \) and \( \text{conc}_{x_j} \) represent the concentrations of element \( x \) in fish \( i \) and fish \( j \), respectively, while \( \min(\text{conc}_{x_i}; \text{conc}_{x_j}) \) represents the lower value of these two concentrations (for detailed information, see Supplementary Figures 2, 3). The EFI ranges from 0 to 1, where a value of 0 indicates that two compared individuals are most different in otolith elemental composition, whereas a value of 1 indicating the highest similarity in elemental composition of two comparing individuals. We conducted elemental concentration comparisons for all individuals within one sampling area resulting in \( \binom{30}{2} = 435 \) pairs for each sampling area (except for GB: \( \binom{15}{2} = 105 \) pairs).

And comparisons of all individuals between the different sampling areas:

\[
\binom{60}{2} - 2 \times \binom{30}{2} = 900 \text{ pairs for each comparison (GB: } \binom{45}{2} - (\binom{30}{2} + \binom{15}{2}) = 450) .
\]

Subsequently, we tested whether the variance among the EFIs derived from comparisons within the areas differed significantly from the EFIs obtained from comparisons of individuals from differing areas.

**RESULTS**

**Chemical Signatures in Herring Otoliths**

In total, 39 elements could be determined in young-of-the-year otolith samples from different inshore spawning areas. Altogether, 17 elements were used to distinguish the chemical otolith signatures from different sampling sites. The mean elemental concentrations are given in **Supplementary Table 2**. The element concentrations found in all 105 individuals were included in an MDS-plot for visualizing the differences in chemical signatures for each sampling area (Figure 2). Otolith signals from the western sampling areas Warnow Estuary (WE) and Greifswald Bay (GB) with some intermingling signals from KC, GB and WE. Elemental otolith compositions from WE showed a higher variability in individual element concentration. However, a clear separation between the western and eastern spawning areas is discernible (Figure 2).

**Significant Differences in Elemental Compositions**

We found continuously high significant differences (\( p \leq 0.001 \)) in element concentrations between all four sampling areas and significant differences (\( p = 0.008 \)) for tin (Sn) (Table 2). Based on these results we performed post-hoc tests for single elements to identify habitat-specific significant differences between single sampling areas. Detailed information on the results for each element is given in Table 3. We found 13 elements to be significantly different between SF-WE comparison. The comparisons SF-KC and SF-GB revealed significant differences of 11 elements with uranium (U), being significantly different between SF-GB. Ten elements were significantly different within the KC-WE comparison. Furthermore, 7 elements were significantly different between KC-GB. The comparison WE-GB revealed significant differences in 6 elements (Table 3).

**Elemental Fingerprinting Indices (EFI)**

The entire chemical otolith inventory for each inshore coastal spawning area revealed 4 habitat-specific fingerprint plots with similarities in the generally dominance of single elements, but clear differences in their relative element ratio (Figure 3). We conducted multi-elemental concentration pairwise comparisons for individuals within one sampling site and pairwise comparisons of individuals between different sampling areas.
sites, using EFI to identify similarities in element concentrations within one group and differences between groups. Elemental concentrations of individuals within each of the SF, KC and GB sample groups were rather stable, whereas WE samples were slightly more variable in their multi-element composition (lower mean EFI). Comparisons of individual fingerprints between two sampling groups revealed clear differences between WE and SE, whereas comparisons between SF-KC and KC-GB were more similar in their element signatures (Table 4). EFI values for comparisons of SF-GB, WE-KC and WE-GB were smaller than EFI values of within-group comparisons, indicating that elemental concentrations between all four groups are more different than within groups (Table 4). Regarding statistical differences between two group comparisons, the EFI values showed highly significant differences for all comparisons (GB-KC: $F_{(2,288.7)} = 253.2$, $p < 0.001$; GB-WE: $F_{(2,345.1)} = 144.8$, $p < 0.001$; GB-SF: $F_{(2,289.1)} = 410.8$, $p < 0.001$; WE-KC: $F_{(2,1767)} = 959.5$, $p < 0.001$; WE-SF: $F_{(2,1013.4)} = 1413.6$, $p < 0.001$; SF-KC: $F_{(2,975.5)} = 563.2$, $p < 0.001$). Post-hoc test results are shown in Supplementary Table 3.

### DISCUSSION

The successful outcome of otolith microchemistry studies strongly depends on spatial variability of elemental concentrations between investigated habitats. There is abundant evidence that fish otoliths from different basins and even sites within basins show spatial variability in their elemental composition (De Pontual et al., 1999; Rooker et al., 2001; Yamane et al., 2010). Our approach included the entire inventory of otolith-core elements summarized by a unique EFI, based on 17 elements. To our best knowledge, this approach allows comparing multi-elemental chemical composition of otoliths

![MDS-Plot](image-url)
The incorporation of elements into the growing surface of fish otoliths is related to environmental variables, such as water temperatures, salinity and the availability of specific elements in the water (Tzeng et al., 1997; Elsdon and Gillanders, 2003). In addition, endogenous factors (such as physiology, genetics and growth effects) control incorporation processes (Fowler et al., 1995; Martin and Thorrold, 2005). The concentrations levels found in YOY herring otoliths corresponded to levels for single elements (Mg, Zn, Sr, Ba, Pb) from a study by Brophy et al. (2003), except for the two elements Sr and Ba. Those in particular are marker elements for an origin relative to certain salinity conditions, with higher Sr concentrations in marine habitats and higher Ba concentrations in freshwater habitats (Tzeng and Tsai, 1994; Elsdon and Gillanders, 2005). The concentrations found in otolith samples from inshore juvenile areas were generally approach, including also heavy metals, we assume that observed chemical patterns are more related to habitat-specific differences than affected by endogenous factors. However, more research is required to understand the impact and interaction of different factors on elemental incorporation in herring otoliths.

Potential temporal variability of element concentrations is one important aspect to be considered in future connectivity studies (Milton et al., 1997; Hamer et al., 2003; Swearer et al., 2003; Arkhipkin et al., 2009). Although some studies showed high temporal variability in elemental concentrations, other studies revealed inter-annual consistency of elements (Patterson et al., 1999; Rooker et al., 2001), indicating that variability might be habitat-specific. Gillanders (2002b) stated to build up a library of elemental fingerprints over several years for each study area to increase the interpretation success of fingerprint patterns from juvenile fish, regarding contribution estimates of single reproduction areas to the overall fish population. A multi-year sampling should be considered in the future to investigate temporal consistency of the found habitat-specific signals.

### Environmental Factors

The incorporation of elements into the growing surface of fish otoliths is related to environmental variables, such as water temperatures, salinity and the availability of specific elements in the water (Tzeng et al., 1997; Elsdon and Gillanders, 2003). In addition, endogenous factors (such as physiology, genetics and growth effects) control incorporation processes (Fowler et al., 1995; Martin and Thorrold, 2005; Barnes and Gillanders, 2003). Elemental uptake by prey digestion might be marginal, at least for some species (Farrell and Campana, 1996; Milton and Chenery, 2001; Buckel et al., 2004). The impact of environmental variables on element incorporation is sufficiently studied, but results are rather contradictory (Kalish, 1989; Radtke et al., 1990; Townsend et al., 1992; Hoff and Fuiman, 1995), indicating that interactions between environmental and endogenous factors might be species-specific. However, it is stated that non-physiologically regulated elements (such as Sr, Ba, Mn) are usable for life history studies (Elsdon and Gillanders, 2003). Woodcock et al. (2012) indicated that Mg, an element often used in microchemistry studies, might also be physiologically regulated in fish otoliths. Hence, validation experiments for the species of interest are highly recommended to verify interpretations (Kalish, 1989).

Whereas studies on the elemental uptake are almost entirely focused on single elements (see review in Elsdon and Gillanders, 2003), multi-element investigations are rather scarce (Fowler et al., 1995; Elsdon and Gillanders, 2002). With our multi-element approach, including also heavy metals, we assume that observed chemical patterns are more related to habitat-specific differences than affected by endogenous factors. However, more research is required to understand the impact and interaction of different factors on elemental incorporation in herring otoliths.

### Element Concentrations in Herring Otoliths

The concentrations levels found in YOY herring otoliths corresponded to levels for single elements (Mg, Zn, Sr, Ba, Pb) from a study by Brophy et al. (2003), except for the two elements Sr and Ba. Those in particular are marker elements for an origin relative to certain salinity conditions, with higher Sr concentrations in marine habitats and higher Ba concentrations in freshwater habitats (Tzeng and Tsai, 1994; Elsdon and Gillanders, 2005). The concentrations found in otolith samples from inshore juvenile areas were generally

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**TABLE 3 |** Results of Games Howell post-hoc test to identify site-specific significant differences for each element with respective significant levels (p ≤ 0.05), including sample sizes of n = 30 for Schlei Fjord (SF), Kiel Canal (KC) and Warnow Estuary (WE) and n = 15 for Greifswald Bay (GB).

<table>
<thead>
<tr>
<th>Element</th>
<th>SF-KC</th>
<th>SF-WE</th>
<th>SF-GB</th>
<th>KC-WE</th>
<th>KC-GB</th>
<th>WE-GB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>0.006</td>
<td>&lt;0.001</td>
<td>0.107</td>
<td>0.110</td>
</tr>
<tr>
<td>B</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.070</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Na</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.011</td>
<td>0.419</td>
<td>0.523</td>
</tr>
<tr>
<td>Mg</td>
<td>0.003</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.486</td>
<td>0.028</td>
</tr>
<tr>
<td>Si</td>
<td>0.634</td>
<td>0.002</td>
<td>&lt;0.001</td>
<td>0.038</td>
<td>&lt;0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>Mn</td>
<td>0.001</td>
<td>0.006</td>
<td>0.001</td>
<td>0.108</td>
<td>0.873</td>
<td>0.083</td>
</tr>
<tr>
<td>Fe</td>
<td>&lt;0.001</td>
<td>0.202</td>
<td>0.547</td>
<td>0.207</td>
<td>&lt;0.001</td>
<td>0.337</td>
</tr>
<tr>
<td>Co</td>
<td>&lt;0.001</td>
<td>0.045</td>
<td>0.990</td>
<td>0.824</td>
<td>&lt;0.001</td>
<td>0.040</td>
</tr>
<tr>
<td>Cu</td>
<td>0.118</td>
<td>0.001</td>
<td>0.001</td>
<td>0.003</td>
<td>0.005</td>
<td>0.536</td>
</tr>
<tr>
<td>Zn</td>
<td>0.023</td>
<td>&lt;0.001</td>
<td>0.077</td>
<td>0.003</td>
<td>0.192</td>
<td>0.691</td>
</tr>
<tr>
<td>Pb</td>
<td>&lt;0.001</td>
<td>0.046</td>
<td>0.012</td>
<td>0.632</td>
<td>0.968</td>
<td>0.810</td>
</tr>
<tr>
<td>Sr</td>
<td>0.938</td>
<td>&lt;0.001</td>
<td>0.038</td>
<td>&lt;0.001</td>
<td>0.088</td>
<td>0.095</td>
</tr>
<tr>
<td>Sn</td>
<td>0.736</td>
<td>0.005</td>
<td>1.000</td>
<td>0.907</td>
<td>0.738</td>
<td>0.007</td>
</tr>
<tr>
<td>Ba</td>
<td>&lt;0.001</td>
<td>0.898</td>
<td>0.203</td>
<td>&lt;0.001</td>
<td>0.017</td>
<td>0.052</td>
</tr>
<tr>
<td>Pb</td>
<td>0.234</td>
<td>&lt;0.001</td>
<td>0.047</td>
<td>0.001</td>
<td>0.370</td>
<td>0.009</td>
</tr>
<tr>
<td>Th</td>
<td>&lt;0.001</td>
<td>0.152</td>
<td>&lt;0.001</td>
<td>0.645</td>
<td>&lt;0.001</td>
<td>0.282</td>
</tr>
<tr>
<td>U</td>
<td>0.158</td>
<td>0.175</td>
<td>0.016</td>
<td>0.189</td>
<td>0.051</td>
<td>0.280</td>
</tr>
</tbody>
</table>

Significant p-values are given in bold type.
lower in Sr and higher in Ba, compared to herring otoliths from the North Sea (Brophy et al., 2003), reflecting the brackish Baltic Sea ecosystem, with high salinity gradients (from rather marine salinities of ∼30 PSU) in adult herring feeding areas up North in the Kattegat and Skagerrak to more brackish conditions within the Western Baltic Sea. In addition to the overall Baltic Sea salinity gradient from Southwest to Northeast, there are multiple local inshore-offshore salinity gradients composed by river estuaries or other transitional waters, making it difficult to interpret results solely based on these salinity signatures. Hence, we focused on the multiple-element approach to find differences in elemental composition of herring otolith from different coastal spawning grounds.

Sampling in four historically known juvenile areas was performed in summer 2016. Due to sampling site-specific time of spawning and larval growth, we were not able to sample YOY herring otoliths of the same size. However, high concentration variations between different otolith sizes could not be observed during our measurements. Using the LA-ICP-MS approach for unpolished herring otoliths within a specific size-range was applicable, at least for the Western Baltic Sea, since all herring samples were collected within their inshore coastal reproduction areas. Moreover, the approach of solution-based ICP-MS is an approved method, where whole otoliths are dissolved in nitric acid to analyze chemical signatures and habitat-specific patterns (Patterson et al., 1999; Rooker et al., 2003). However, the choice of sample processing and analytical methods is depending on single fish species and their particular habitat utilization.

**Multi-Element Approach**

Although we were able to detect concentration values of 39 elements in total with the LA-ICP-MS approach, we had to exclude all rare earth metals and some heavy metals

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**FIGURE 3 |** Mean standardized concentrations of each single element for each sampling area are shown in a defined order, revealing radar plots with site-specific elemental fingerprints (blue radar plots in horizontal rows). Data were standardized to deal with different concentration units. Each site-specific radar plot was compared with the respective other three site-specific radar plots (shown by column in red) to visualize similarity or differences in elemental concentrations of western locations [Schlei Fjord (SF), Kiel Canal (KC)] and eastern locations [Warnow Estuary (WE) and Greifswald Bay (GB)].
To their traditional spawning areas. If important nursery grounds are affected by human-induced habitat degradation and environmental conditions of important nurseries become unfavorable for successful herring reproduction, but herring continue their migrations to these specific anthropogenic-stressed habitats, this might have severe impacts for the population persistence. This unknown habitat dependency demonstrates the relevance of our results, which set the baseline for future estimates on habitat productivity, which could lead to a more directed coastal zone management of important spawning grounds and coastal nursery areas in the future.

### AUTHOR CONTRIBUTIONS

DM, PK, LvN, and PP conceptualized the study. DM wrote the manuscript, performed the sampling and was responsible for laboratory processing and sample preparation. KJ and DM performed the measurements with the LA-ICP-MS. DM and PK analyzed the data. PK contributed the EFI calculations and EFI statistical analyses. PK, LvN, PP, and KJ revised the manuscript.

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### SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2019.00191/full#supplementary-material
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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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