Supplementary Material

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

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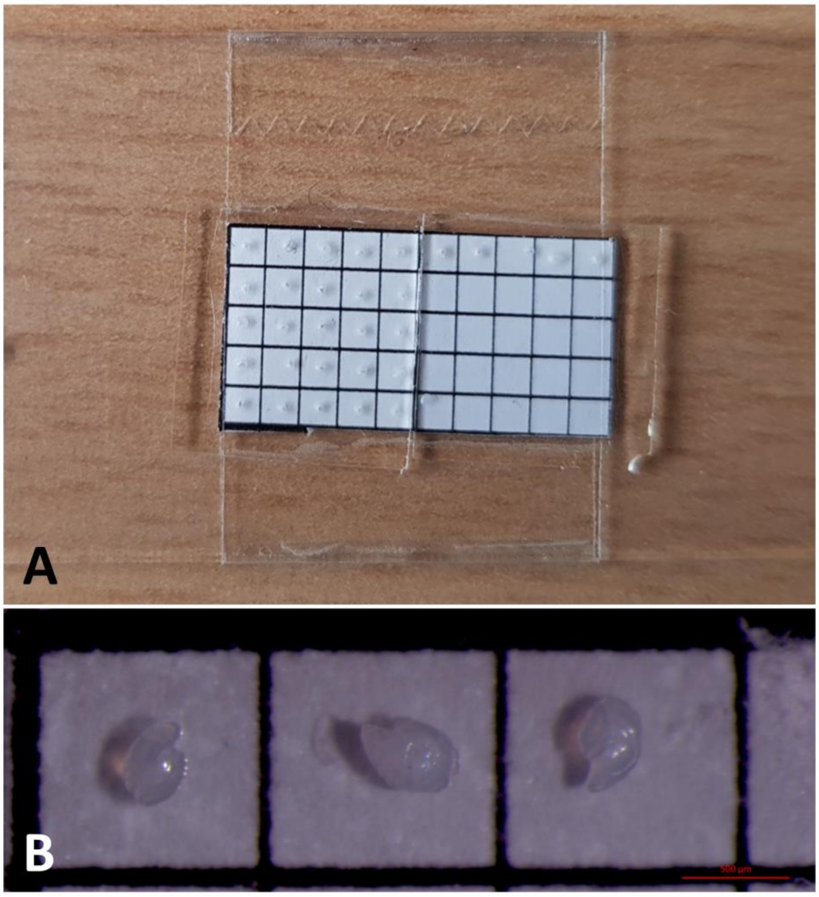
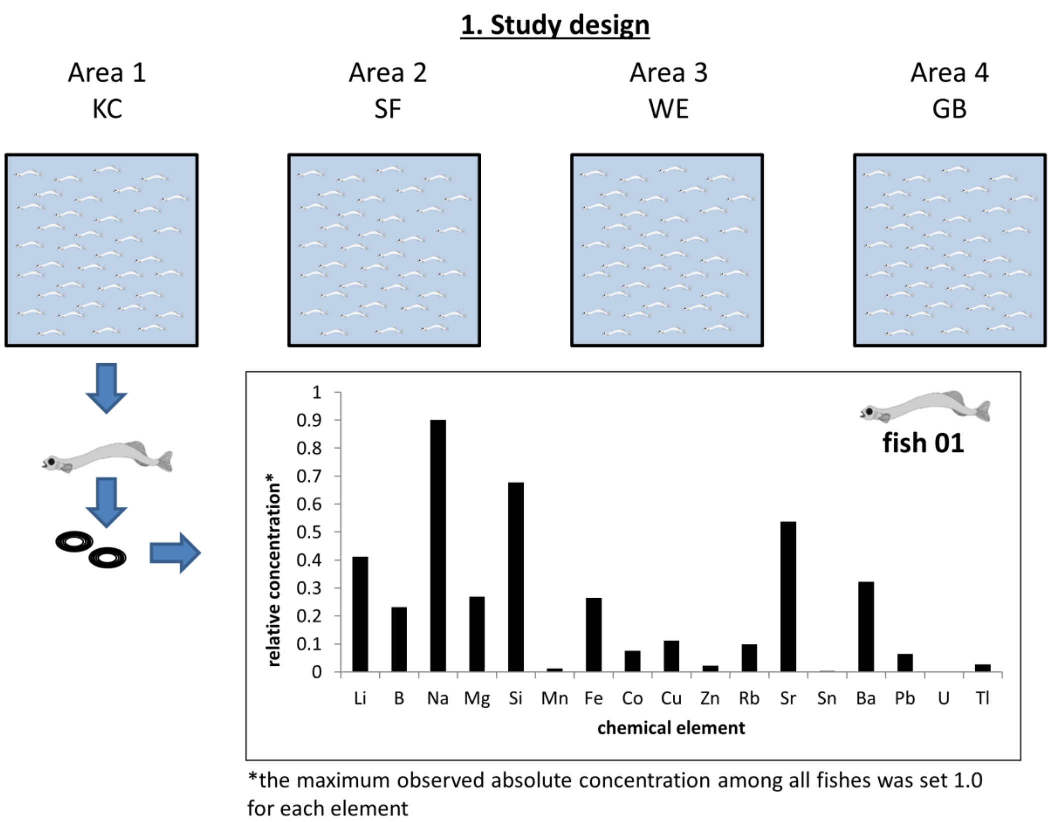
Supplementary Figures

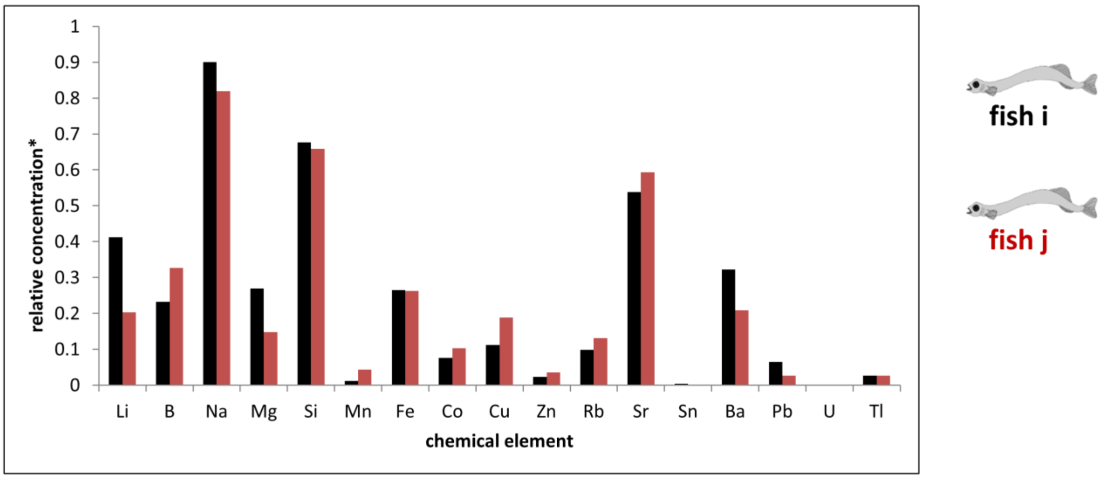


Supplementary Figure 1. Otolith fixing procedure, scale paper, microscope slides and transparent double-sided adhesive pads (UHU® on LDPE-basis (polyethylene)) were used for otolith fixation (**A**). Fixing material was measured prior to sample ablation for potential chemical components. Glass slides were washed in 2% nitric acid and were rinsed several times with ultrapure water and were dried under a flow cabin. Scale paper was mounted on glass slides via sticky tape and adhesive glue pads were mounted on top. Each age-0 herring otolith was then mounted on one square of the scale paper (**B**) to relocate the samples under the microscope, during the LA-ICP-MS measurements.



Supplementary Figure 2. Additional information on the study design. At each of the 4 study sites (Kiel Canal (KC), Schlei Fjord (SF), Warnow Estuary (WE) and Greifswald Bay (GB)) 30 individuals were sampled and analyzed separately (GB: 15 individuals). Otolith microchemistry measurements were conducted on each individual otolith core region, measuring the concentration of 17 different elements. Bar cart indicates the relative concentrations of 17 different elements.

2. Comparing two individuals using the elemental fingerprinting index (EFI)



*the maximum observed absolute concentration among all fishes was set 1.0 for each element

$$EFI_{i,j} = \frac{2 \times \sum_{x=1}^{17} \min(conc\_x_i\,;conc\_x_j)}{\sum_{x=1}^{17} (conc\_x_i + conc\_x_j)}$$

0 ≤ EFI ≤ 1

→ For the example given above:

$$EFI_{i,j} = \frac{2 \times (Li + B + Na + Mg + Si + Mn + Fe + Co + Cu + Zn + Rb + Sr + Sn + Ba + Pb + U + Tl)}{Li + Li + B + B + Na + Na + Mg + Mg + Si + Si + Mn + Mn + Fe + Fe + Co + Co + Cu + Cu + Zn + Zn + Rb + Rb + Sr + Sr + Sn + Sn + Ba + Ba + Pb + Pb + U + U + Tl + Tl}$$

Supplementary Figure 3. Detailed information on the elemental fingerprinting index (*EFI*) calculations. The *EFI* expresses the similarity between two individuals considering all 17 elements based on the principles of ecological spatial overlap indices.

Supplementary Tables

Supplementary Table 1. Technical information about the laser ablation system and operating parameters of the ICP-MS, used for trace element analysis of juvenile otoliths (Jochum et al., 2007).

New Wave laser ablation system	
Wave length/nm	213
Pulse length/ns	5
Energy density/ J cm^{-2}	7-10
Irradiance at sample/GW cm^{-2}	1.4-2.0
Spot size/µm	5-160
Pulse repetition rate/Hz	1-20
Operating parameters	
rf power/W	1270
Cool gas flow rate/l min^{-1}	15
Auxiliary gas flow rate/l min^{-1}	1
Carrier gas (Ar) flow rate/l min^{-1}	0.8
Carrier gas (He) flow rate/l min^{-1}	0.7

References

Jochum, K.P., Stoll, B., Herwig, K., and Willbold, M. (2007). Validation of LA-ICP-MS trace element analysis of geological glasses using a new solid-state 193 nm Nd:YAG laser and matrix-matched calibration. *J. Anal. At. Spectrom.* 22**,** 112-121. doi: 10.1039/B609547J.

Supplementary Table 2. Mean otolith element concentrations (µg g^{-1}) of 17 elements, found in age-0 herring otoliths from four different spawning areas (Schlei Fjord (SF), Kiel Canal (KC), Warnow Estuary (WE), Greifswald Bay (GB)) with standard deviation (± SD), median and number of analyzed individuals (n).

	SF				KC				WE				GB			
Elements	**Mean**	**± SD**	**Median**	**n**	**Mean**	**± SD**	**Median**	**n**	**Mean**	**± SD**	**Median**	**n**	**Mean**	**± SD**	**Median**	**n**
Li	0.037	0.02	0.040	30	0.055	0.02	0.053	30	0.132	0.09	0.125	29	0.086	0.05	0.073	15
B	1.66	0.18	1.63	30	2.62	0.40	2.52	30	3.64	1.13	3.49	30	1.88	0.29	1.81	15
Na	2611.1	141.1	2593.9	30	2841.8	95.3	2841.7	30	3011.4	263.7	2981.8	30	2919.6	179.6	2933.4	15
Mg	57.7	28.9	48.9	30	135.9	108.7	87.1	30	249.8	109.7	257.1	30	173.4	65.69	179.7	15
Si	313.7	25.9	313.7	30	303.8	37.6	300.9	30	269.9	55.5	268.7	30	211.9	41.91	197.3	15
Mn	21.3	22.0	10.6	30	4.38	1.74	4.00	30	6.50	4.67	6.4	30	3.79	2.71	3.3	15
Fe	48.8	2.9	48.6	30	72.3	8.9	71.5	30	60.4	31.2	51.1	30	50.5	4.4	52.9	15
Co	0.035	0.01	0.034	30	0.108	0.06	0.113	28	0.090	0.11	0.048	30	0.034	0.01	0.032	15
Cu	0.060	0.02	0.056	30	0.079	0.04	0.068	30	0.234	0.21	0.130	30	0.173	0.09	0.144	15
Zn	1.29	0.79	1.073	30	2.68	2.38	2.306	30	8.19	7.66	6.29	30	13.5	17.67	8.6	15
Rb	0.094	0.02	0.092	30	0.158	0.05	0.145	30	0.208	0.23	0.122	30	0.168	0.08	0.150	15
Sr	640.4	74.7	640.4	30	651.9	78.9	655.6	30	817.6	149.5	825.9	30	726.8	102.0	732.7	15
Sn	0.028	0.01	0.028	30	0.239	1.13	0.026	30	0.100	0.11	0.050	30	0.029	0.02	0.023	14
Ba	6.9	3.1	6.49	30	12.7	5.1	11.8	30	6.49	2.45	6.46	30	8.93	2.98	9.25	15
Pb	0.003	0.00	0.002	29	0.018	0.04	0.009	30	0.183	0.20	0.098	28	0.043	0.05	0.027	15
Tl	0.005	0.00	0.004	30	0.019	0.01	0.019	30	0.036	0.07	0.003	28	0.010	0.00	0.009	15
U	0.000	0.00	0.000	28	0.001	0.00	0.000	30	0.027	0.07	0.006	29	0.004	0.00	0.002	15

Supplementary Table 3. Statistical results for two-group comparisons and post-hoc results for calculated *EFI*-values, with degrees of freedom (*df1* and *df2*), *F*-value and significance level (*p*) for the four different sampling areas Schlei Fjord (SF), Kiel Canal (KC), Warnow Estuary (WE) and Greifswald Bay (GB).

Comparison between groups	Robust tests of equality of means (Welch test)	post-hoc results (Games Howell test) for calculated *EFI*s	
GB & KC	$df1 = 2$ $df2 = 288.7$ $F = 253.2$ $p < 0.001$	EFI_{GB} & EFI_{GB_KC}	$p < 0.001$
		EFI_{GB} & EFI_{KC}	$p = 0.031$
		EFI_{KC} & EFI_{GB_KC}	$p < 0.001$
GB & WE	$df1 = 2$ $df2 = 345.1$ $F = 144.8$ $p < 0.001$	EFI_{GB} & EFI_{GB_WE}	$p < 0.001$
		EFI_{GB} & EFI_{WE}	$p < 0.001$
		EFI_{WE} & EFI_{GB_WE}	$p = 0.640$
GB & SF	$df1 = 2$ $df2 = 289.1$ $F = 410.8$ $p < 0.001$	EFI_{GB} & EFI_{GB_SF}	$p < 0.001$
		EFI_{GB} & EFI_{SF}	$p = 0.001$
		EFI_{SF} & EFI_{GB_SF}	$p < 0.001$
WE & KC	$df1 = 2$ $df2 = 1767$ $F = 959.5$ $p < 0.001$	EFI_{WE} & EFI_{WE_KC}	$p < 0.001$
		EFI_{WE} & EFI_{KC}	$p = 0.001$
		EFI_{KC} & EFI_{WE_KC}	$p < 0.001$
WE & SF	$df1 = 2$ $df2 = 1013.4$ $F = 1413.6$ $p < 0.001$	EFI_{WE} & EFI_{WE_SF}	$p < 0.001$
		EFI_{WE} & EFI_{SF}	$p = 0.001$
		EFI_{SF} & EFI_{WE_SF}	$p < 0.001$
SF & KC	$df1 = 2$ $df2 = 975.5$ $F = 563.2$ $p < 0.001$	EFI_{SF} & EFI_{SF_KC}	$p < 0.001$
		EFI_{SF} & EFI_{KC}	$p < 0.001$
		EFI_{SF_KC} & EFI_{KC}	$p < 0.001$