https://doi.org/10.5194/egusphere-2023-512
Preprint. Discussion started: 31 March 2023
© Author(s) 2023. CC BY 4.0 License.

Marked recent declines in boron in Baltic Sea cod otoliths – a bellwether of incipient acidification in a vast hypoxic system?

Karin E. Limburg[1,2], Yvette Heimbrand[2], Karol Kuliński[3]

[1]Department of Environmental Biology, State University of New York College of Environmental Science and Forestry, Syracuse, NY USA

[2]Department of Aquatic Resources, Swedish University of Aquatic Sciences, Uppsala, Sweden

[3]Department of Marine Chemistry and Biochemistry, Institute of Oceanology of the Polish Academy of Sciences, Sopot, Poland

Correspondence to: Karin E. Limburg (klimburg@esf.edu)

Abstract: Ocean acidification is spreading globally as a result of anthropogenic CO_2 emissions, but the Baltic Sea has until recently been thought to be relatively well-buffered by terrigenous inputs of alkalinity from its watershed. We discovered a 3- to 5-fold decline in boron (as B:Ca) in otoliths of eastern Baltic Sea cod (EBC) between the late 1990s and 2021. B:Ca is positively proportional to pH in carbonates, as B in the form of borate is taken up in the $CaCO_3$ matrix. Examining a time series of EBC otoliths, we found varying levels of B:Ca since the 1980s, with the most recent years at an all-time low during this period. This trend correlates with declines in pH and dissolved oxygen, but not with changes in salinity. We examined possible physiological influences on B:Ca by including a collection of healthy Icelandic cod as an out-group. Icelandic cod otoliths showed strongly positive correlations of B:Ca with physiologically regulated P:Ca; this was not the case for EBC. Finally, B:Ca in EBC otoliths is anti-correlated to some extent with Mn:Mg, a proposed proxy for hypoxia exposure. This negative relationship is hypothesized to reflect the dual phenomena of hypoxia and acidification as a result of decomposition of large algal blooms. Taken together, the otolith biomarkers Mn:Mg and B:Ca suggest a general increase in both hypoxia and acidification within the Baltic intermediate and deep waters in the last decade reflected in cod otoliths.

Keywords: Baltic Sea cod, otolith chemistry, boron, pH, hypoxia

https://doi.org/10.5194/egusphere-2023-512
Preprint. Discussion started: 31 March 2023
© Author(s) 2023. CC BY 4.0 License.

1 Introduction

Ocean acidification, a known result of greenhouse gas emissions of CO_2 loading into the oceans, is highly variable in its distribution in the world's oceans (Doney et al., 2009). This is true in shallow coastal seas such as the brackish Baltic Sea, where in some locations surface pCO_2 values in winter greatly exceed other coastal regions (Reusch et al., 2018), but buffering can occur from river inputs bearing proton acceptors weathered from rock and soil (Müller et al., 2016; Kuliński et al., 2017). Alkalinity, defined as the sum of acid neutralizing compounds, is a complicated mix in the Baltic Sea (Kuliński et al., 2022). In the northern Baltic, i.e., the Bothnian Sea, total alkalinity (A_T) is low as it is largely driven by A_T-poor riverine inputs from the Scandinavian Peninsula that is built mostly of granite. In the southern and central Baltic, A_T is higher due to salt inputs from the North Sea, influence of A_T-rich continental rivers, and an additional contribution from internal cycling of carbon as algal blooms decompose at anoxic conditions (Kuliński et al., 2022). Despite buffering from alkalinity sources, pH is highly variable in space and time with its recent tendency to decline at greater depths in many parts of the Baltic Sea, which is associated with the ongoing eutrophication and higher vertical export of organic matter that leads also to worsening deoxygenation (Kuliński et al., 2022).

The population of the Eastern Baltic cod (*Gadus morhua*, hereafter referred to as EBC) has been severely impacted by a number of factors that include past overfishing, hypoxia, parasite infections, and seal predation (Eero et al., 2020), leading to reduced growth and age at maturity (Eero et al., 2016; ICES, 2019). Cod exposed to hypoxia were shown to have lower growth rates and worsened condition (Limburg & Casini, 2018, 2019). Hypoxia exposure was quantified by means of a proxy developed from analyzing manganese concentrations in otoliths (ear-stones) of fish (Limburg et al., 2015), in ratio to magnesium which corrects to some extent for growth influences on Mn uptake (Limburg et al., 2018; Limburg & Casini, 2018). Briefly, otoliths, the calcified structures that form part of the hearing/balance system in teleost fishes, are sectioned and then analyzed by ablating micro-transects along the major growth axis and analyzing by mass spectrometry (see Methods). The resulting elemental data are lifetime concentration histories, being incorporated at the time of exposure. The use of otolith manganese to track hypoxia exposure is one of the emerging biomarkers in fisheries ecology (Reis-Santos et al., 2022).

https://doi.org/10.5194/egusphere-2023-512
Preprint. Discussion started: 31 March 2023
© Author(s) 2023. CC BY 4.0 License.

Recently, with acquisition of more sensitive instrumentation, we began to experiment with quantifying elements having sub-ppm concentrations in cod otoliths, including the trace element boron. Boron in seawater generally correlates with salinity (Kuliński et al., 2017) predominantly in the form of weak boric acid (H_3BO_3) at standard seawater salinity (35 PSU) and pH of 8. However, the dissociated anion borate $B(OH)^-_4$ is positively, nonlinearly correlated with pH (Yu et al., 2007). Borate is the dominant form incorporated into carbonates such as limestones, foraminifera, and corals in proportion to pH (Yu et al., 2007; Hönisch et al., 2012; Levin et al., 2015) and is often confirmed by $\delta^{11}B$ analysis, since it has a large, negative fractionation compared to boric acid. However, even bulk boron, as B:Ca ratios, has been found to correlate positively (and linearly) with pH in foraminifera (Yu et al., 2007), albeit in a species-specific manner.

We measured sub-ppm to low-ppm concentrations of B (ca. 0.5 – 5 ppm, see Methods section) in otoliths of EBC captured in 2000 that showed what appeared to be seasonal variations

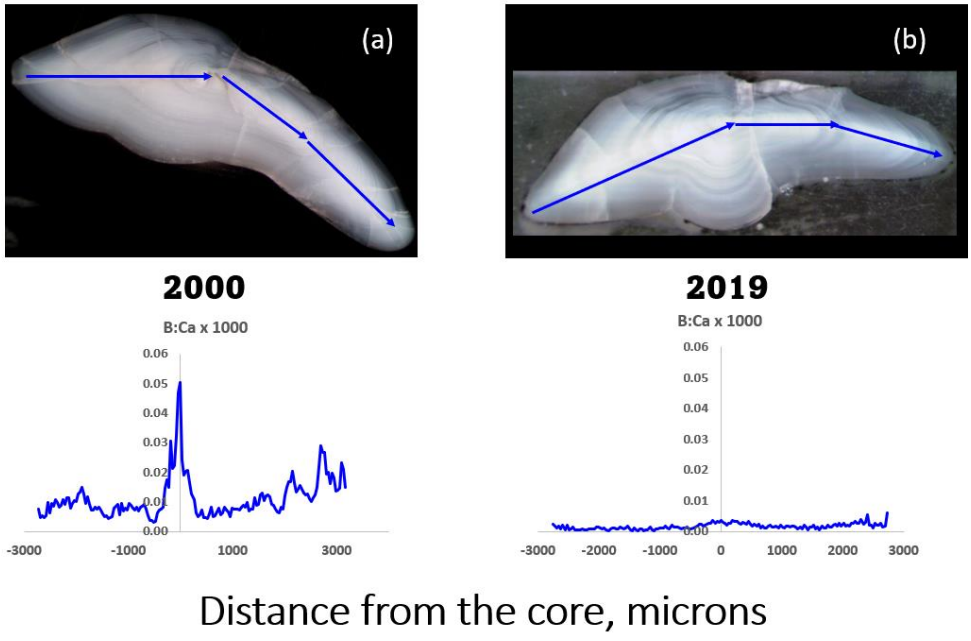

Figure 1. Comparison of transect analyses of boron (in ratio to calcium, mass basis) in sectioned Baltic cod otoliths. Transects were ablated from the ventral edge, through the core, and out to the dorsal edge (arrows on photos). (a) Fish captured in March 2000, Age 3, length 46 cm; mean [B] = 3.56 ppm ± 0.156 (s.e.). (b) Fish captured in November 2019, Age 2, length 40 cm, mean [B] = 0.791 ppm ± 0.033 (s.e.). Both came from the same part of the Baltic Sea (ICES subdivision SD25, see Figure 2).

https://doi.org/10.5194/egusphere-2023-512
Preprint. Discussion started: 31 March 2023
© Author(s) 2023. CC BY 4.0 License.

(example shown in Figure 1(a)), albeit not in synchrony with other elements that have proven useful to age cod (Heimbrand et al., 2020). It was therefore surprising when we began to analyze otoliths of EBC captured in 2019 and discovered greatly reduced concentrations (Figure 1(b)). The measurements were repeated for verification.

With such differences suggestive of change, we returned to an archive of otoliths used for another project (Limburg and Casini, 2018), as well as adding previously un-analyzed otoliths, to develop a time series of otolith B:Ca to document any temporal trends that could be correlated with pH, salinity, or other environmental factors. We posed the following questions:

1. What are the temporal trends in otolith boron (as B:Ca ratios)?
2. How do B:Ca ratios correlate to trends in water chemistry, particularly salinity and pH?
3. If pH declines are associated with hypoxia, do we see a correlation of B:Ca with our hypoxia proxy, manganese:magnesium (Mn:Mg)?
4. If there are any physiological influences, do we see a correlation of B:Ca with elemental ratios known to be under physiological control, e.g., phosphorus:calcium (P:Ca)?

2 Materials and Methods

2.1 Otolith collections and microchemical analysis

https://doi.org/10.5194/egusphere-2023-512
Preprint. Discussion started: 31 March 2023
© Author(s) 2023. CC BY 4.0 License.

Otoliths of Baltic cod (N = 156) were obtained from both fishery-independent and fishery-dependent surveys conducted by the Swedish Fisheries Board and its successor, the Department of Aquatic Resources, Swedish University of Agricultural Sciences, collected from ICES sub-divisions (SD) 24, 25, 27, and 28 (Figure 2). These were measured, weighed, and subsequently embedded in epoxy (Struers EpoFix) and sectioned transversely to expose the core. They were smoothed with a succession of lapping films to 3 microns.

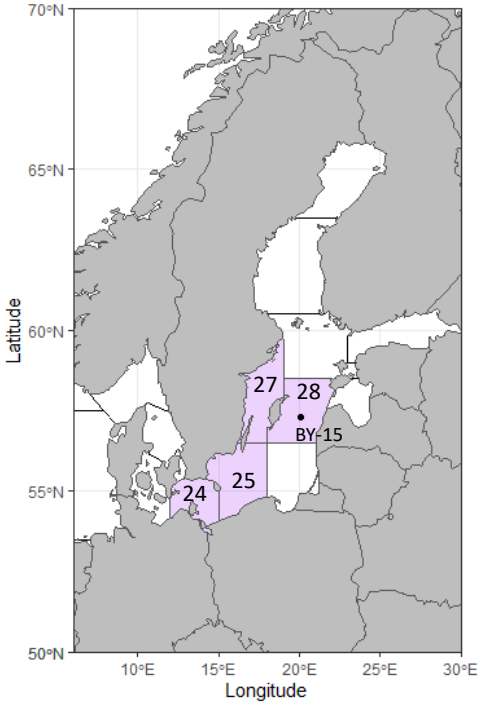

Figure 2. Map of the Baltic Sea region, showing the ICES subdivisions from where otoliths were sampled in purple. Black dot marks water monitoring station BY-15, located in the Gotland Deep.

In addition, cod otoliths (N = 14) were obtained from fisheries-independent surveys from Iceland (ICES SD 5a), as an out-group for studies of possible physiological correlations. This part of the North Atlantic does not have hypoxia or acidification issues in the deep waters. The Icelandic otoliths were prepared and analyzed in similar fashion.

Chemical analyses were performed by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) at the Analytical and Technical Services group at SUNY ESF. Otoliths were ablated with a 192-nm laser ablation unit (Teledyne Cetac Excite 2) along transects as illustrated in Figure 1; the ablated material was transported via an Ar-He carrier gas mixture into a Thermo iCAP TQ plasma mass spectrometer where isotopes were quantified (^{11}B, ^{25}Mg, ^{31}P, ^{43}Ca, ^{55}Mn, ^{63}Cu, ^{66}Zn, ^{88}Sr, ^{127}I, ^{138}Ba, and ^{208}Pb). Daily performance of the system was optimized with NIST 612 glass standard and U.S. Geological Survey standards MACS-3,

https://doi.org/10.5194/egusphere-2023-512
Preprint. Discussion started: 31 March 2023
© Author(s) 2023. CC BY 4.0 License.

MAPS-4, and MAPS-5 were used as standards for calibration and drift correction. The MAPS (abbreviation for Micro-Analytical Phosphate Standard) standards, while having good matching to the otolith matrix, were slightly softer and were therefore ablated at a higher energy (25% power, 2.03 J/cm^2 fluence) than the otoliths (20% power, 1.62 J/cm^2) to achieve an otolith calcium value around 38 weight percent. Transects were made with 110 µm-diameter ablation spots at a speed of 7 µm/sec. Once data were worked up, annual mean values were parsed by the age determination technique in Heimbrand et al. (2020), i.e., averages within annual growth rings were calculated. These were matched to corresponding calendar years.

2.2 Water data

Water properties data (temperature (T, °C), salinity (S, psu), dissolved oxygen (DO, mL/L), pH, and total alkalinity (A_T, mmol/L)) were downloaded from the Swedish Meteorological and Hydrological Institute's database, SHARKWeb (https://sharkweb.smhi.se/). Station BY-15, Gotland Deep, was selected as being representative of the central Baltic (Figure 2) and having one of the longest and most detailed timeseries. We selected depths that corresponded to where cod had been found in fishery-independent surveys (Casini et al., 2021). Between 1985-1995, we averaged water data from 30-60 meters, and from 1996 onward, between 40-75 meters. Annual mean water values were computed and matched to corresponding otolith chemistry data (annual means of Element:Ca data parsed to calendar years as described above).

2.3 Analysis

Visualization and statistical analyses were performed in Excel, R (R Core Team 2022), and JMP version 17.0 (JMP 2022). Statistical analyses were separated into examination of relationships of B:Ca values to potential environmental drivers (primarily pH and S, but also A_T, DO, and T) and to the internal variables P:Ca and Mn:Mg. P:Ca was found to correlate highly with B:Ca in Icelandic cod otoliths (Figure 3(a)). Otolith P:Ca is known to be under physiological influence (Thomas et al., 2017; Heimbrand et al., 2020). In cod, higher values of P:Ca occur during the growing season and are thus hypothesized to be associated with growth and other activities. Otolith Mn:Mg, on the other hand, is our proxy for hypoxia exposure (Limburg and Casini, 2018), and often correlated negatively with B:Ca (Figure 3(b)) - consistent with co-occurrence of reduced conditions in water (and presence of bioavailable ionic Mn^{2+}) and

https://doi.org/10.5194/egusphere-2023-512
Preprint. Discussion started: 31 March 2023
© Author(s) 2023. CC BY 4.0 License.

high pCO_2 and thus low pH, both being the effect of enhanced organic matter remineralization and/or poor ventilation of the water layers occupied by cod. For the environmental drivers, graphical analysis was performed followed by mixed linear models and visualizations, with each fish's age (nested within fish ID) being treated as a random effect. For the internal variables, the P:Ca and Mn:Mg transect data were regressed on corresponding B:Ca for each fish, and the

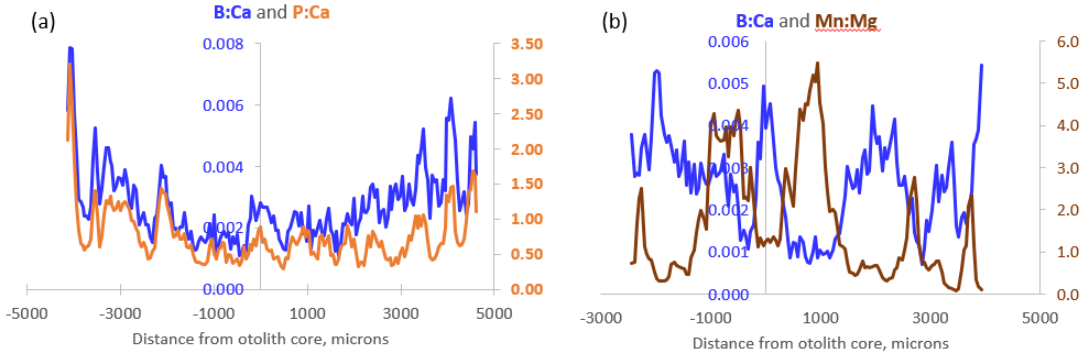

Figure 3. (a) Transects of B:Ca and P:Ca from an 8-year-old Icelandic cod captured in 2020. Note the close resemblance of the dynamics. (b) Inverse transects of B:Ca and Mn:Mg from a 4-year-old Baltic cod captured in 2014.

slopes, intercepts and R^2 values were calculated. These were examined for trends by decade (1980s, 1990s, 2000s, and 2010s) as well as compared with the Icelandic out-group. Positive correlations with P:Ca would suggest a physiological influence on B:Ca incorporation, whereas negative correlations with Mn:Mg would suggest a linkage between B:Ca as an indicator of pH and our indicator of hypoxia exposure (cf. Cavole, 2021).

3 Results

3.1. Trends in water variables

A principal components analysis of the water variables showed three groupings: (1) DO and pH that were opposite to (2) salinity and A_T, and (3) temperature was on a separate axis (Figure S1). Examination of the long-term time series of data from Station BY-15 shows that while temperature generally increased (Figure S2(a)), the other water variables showed very nonlinear patterns (Figures S2(b-e). Alkalinity is in part a function of salinity; dividing A_T by S produces a time series showing a dramatic state change around 1990 (Figure S2(f)), particularly

https://doi.org/10.5194/egusphere-2023-512
Preprint. Discussion started: 31 March 2023
© Author(s) 2023. CC BY 4.0 License.

in the water layers occupied by cod. Despite the increasing A_T, pH has declined more or less monotonically at midwater depths (40-75 m, Figure S2(c)).

3.2 Trends in otolith B:Ca with salinity and pH

Over the period 1985-2019, box plots of B:Ca by year show a pattern of increase toward a maximum, albeit with great variation, in the late 1990s, followed by gradual decline (Figure 4). Visual comparisons with salinity and pH show that neither explain all the variation in B:Ca, but since 2000, pH and B:Ca both trend downward whereas salinity has increased. The increase in B:Ca in 2021 is from age-0, which is elevated relative to older ages (cf. Figure 1(a)).

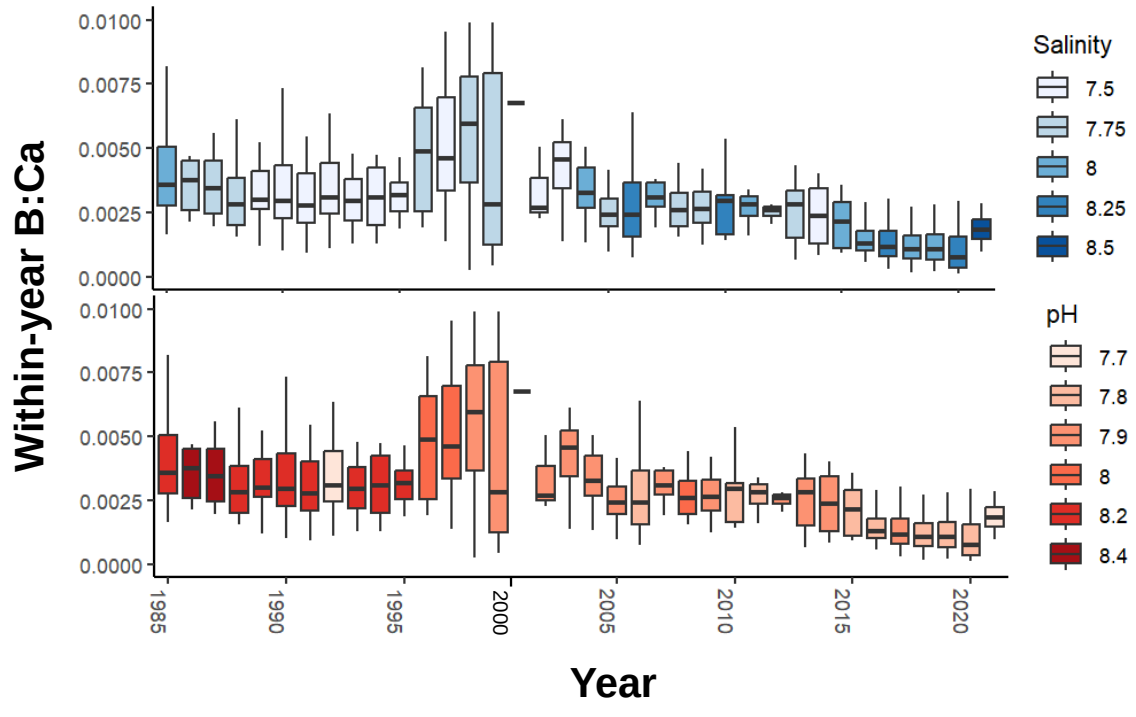

Figure 4. Box plots of annual B:Ca in Baltic cod otoliths, colored by salinity (top) and pH (bottom). Salinity has been increasing and pH declining. Since the late 1990s, there has been a general decline in B:Ca.

https://doi.org/10.5194/egusphere-2023-512
Preprint. Discussion started: 31 March 2023
© Author(s) 2023. CC BY 4.0 License.

3.3. Effects of water parameters on otolith B:Ca

3.3.1. Univariate effects of water parameters on otolith B:Ca

Repeated measures regressions of otolith B:Ca on each of the five water variables was conducted to examine effect sizes and significance. Repeated measures regressions showed significant effects of each of the five water variables on otolith B:Ca (Table 1). A_T had the largest effect and was negative (Table 1). Dissolved oxygen and pH both had positive slopes, and temperature and salinity both were negative. All univariate analyses were highly significant. Ages of individual fish were not significant as measured by Wald's p-value in restricted maximum likelihood (REML) analysis.

Table 1. Repeated measures univariate regressions of otolith B:Ca on water variables, ordered by goodness of fit (-2 log likelihood criterion; lowest is best).

Variable	Parameter slope	R^2	df	F ratio	p	-2 Log Likelihood
Total Alkalinity	-9.42E-03	0.24	647	158.8	< 0.0001	-6358
Dissolved Oxygen	1.00E-03	0.21	615	142.2	< 0.0001	-6341
Salinity	-3.00E-03	0.22	565	133.5	< 0.0001	-6336
Temperature	-8.30E-04	0.13	639	58.7	< 0.0001	-6269
pH	5.10E-03	0.15	633	109.4	< 0.0001	-6189

3.3.2. Two-factor tests of water parameters on otolith B:Ca

Because of our hypothesized positive relationships of salinity and pH on B:Ca, as well as the large effect size of A_T, we examined pairwise models of these independent variables on otolith B:Ca (Table 2). Aside from an overall model, these were filtered by decade to examine finer scale trends.

Table 2. Repeated measures 2-factor models of effects on otolith B:Ca, by decade. Numbers are estimated coefficients. Red = significant at p < 0.01. Blue = significant at p < 0.05. Negative 2 Log Likelihood is a metric of goodness-of-fit, with smaller numbers indicating better fit.

1. pH and Alkalinity:

Parameter	1980s	1990s	2000s	2010s	Overall
pH	0.00128	-0.00774	-0.00375	-0.00121	-0.00385

https://doi.org/10.5194/egusphere-2023-512
Preprint. Discussion started: 31 March 2023
© Author(s) 2023. CC BY 4.0 License.

Alkalinity	0.00873	-0.00538	-0.01840	-0.02585	-0.01585
-2 Log Lik.	-399.2	-1231.8	-896	-3979.4	-6225.5
2. pH and Salinity:					
Parameter	**1980s**	**1990s**	**2000s**	**2010s**	**Overall**
pH	0.00151	-0.00226	-0.00983	0.00378	0.00225
Salinity	0.00322	0.00365	-0.00347	-0.00049	-0.00208
-2 Log Lik.	397.9	-1231.6	-894.8	-3948.2	-6201.6
3. Alkalinity and Salinity:					
Parameter	**1980s**	**1990s**	**2000s**	**2010s**	**Overall**
Alkalinity	-0.007269	0.002042	-0.01253	-0.027822	-0.006591
Salinity	0.003768	0.004802	-0.000171	0.000478	-0.001274
-2 Log Lik.	-401.4	-1357.9	-891.9	-3977.6	-6354.8

In both pairwise tests with pH, this parameter had positive slopes in the 1980s and 2010s but negative slopes in the 1990s and 2000s. Alkalinity had mostly a negative relation to otolith B:Ca. Salinity had a positive effect in the 1980s and 1990s but became negative in the 2000s and 2010s. Salinity overall had a negative effect on B:Ca, in contrast to expectations. We note the strong, negative effect of pH in the 1990s when paired with A_T, and the strong, positive effect of pH in the 2010s when paired with salinity. Age, when treated as a random variable nested within individual fish (by fish ID), was again not significant. However, in a separate analysis (Figure S3), age (not treated as a random variable) and otolith B:Ca were inversely related in the 1980s, 1990s, and 2000s, and had a weakly positive trend in the 2010s.

3.4. Internal parameters and otolith B:Ca

3.4.1. P:Ca as a proxy for physiological status

For this analysis, the Icelandic "out-group" of cod living in normoxic water were included. Annual average P:Ca was highly correlated with B:Ca in Icelandic cod otoliths, but inversely correlated in Baltic cod with a great deal of scatter (Figure 5). Although the overall trend for Baltic cod is negative, this hides trends that become more visible at a finer temporal scale. Thus, we examined the slopes and R^2 values of individual fish regressions of P:Ca on B:Ca

https://doi.org/10.5194/egusphere-2023-512
Preprint. Discussion started: 31 March 2023
© Author(s) 2023. CC BY 4.0 License.

(Figure S4). The 2010s showed the strongest trends when slopes were plotted on R^2 values, with Icelandic cod and the 2000s also trending positively, and weaker trends in the 1980s and 1990s (Figure S4(a)). Box plots of R^2 values by decade and out-group showed wide variation (Figure S4(b)), and an analysis of variance found no statistical difference among Baltic fish by decade, but Icelandic otoliths had significantly higher R^2 values ($p < 0.001$).

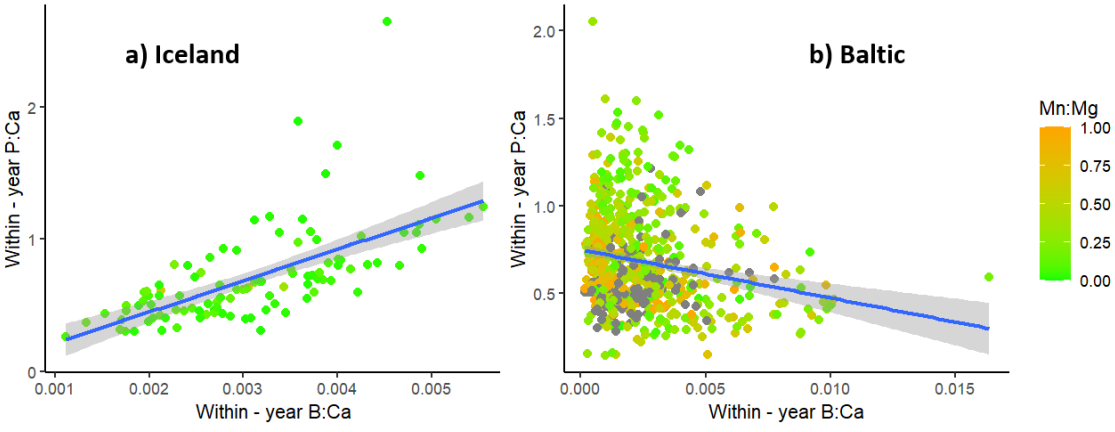

Figure 5. Comparison of relationships of otolith boron:calcium to phosphorus:calcium in (a) healthy Icelandic cod vs. (b) Baltic Sea cod. Color bar shows the corresponding average Mn:Mg values. Points represent annual observations, and most fish have multiple observations. (Dark points on Baltic graph have Mn:Mg values > 1.) Outlier point on right-hand graph actually decreases the slope; its removal improves the goodness of fit (R^2).

3.4.2. Mn:Mg as a proxy for hypoxia exposure

We tested the hypothesis that hypoxia (indexed by increased otolith Mn:Mg) was coupled to acidification (indexed by declining B:Ca). We used a similar analysis of individual fish regressions of Mn:Mg, our hypoxia exposure proxy, on B:Ca, as was performed with P:Ca. This analysis led to somewhat inconclusive results (Figure S5). The decades of the 1980s, 1990s, and 2000s all had mostly positive slopes of Mn:Mg with B:Ca, and slopes increased with increasing R^2 values. Only the 2010s displayed a pattern consistent with the hypothesis. Iceland showed no relationship of slopes to goodness of fit. Further examination of the data included plotting trends

https://doi.org/10.5194/egusphere-2023-512
Preprint. Discussion started: 31 March 2023
© Author(s) 2023. CC BY 4.0 License.

of these slopes against fish age (Figure S6). This revealed that younger EBC otoliths tended to have positive slopes, with slopes becoming more negative with age, for the decades of the 1990s-2010s; no trend was observed in the 1980s, and Icelandic cod had increasingly positive slopes as they aged (Figure S6). For the 2010s, it suggests that as Baltic cod age they encounter more hypoxia perhaps as they use deeper habitats, and with it, a decline in B:Ca that may indicate lower environmental pH. Overall, this analysis suggests that the decade of the 2010s may have had evidence of acidification driven by the same mechanisms that produced hypoxia.

4. Discussion

This study was initiated because of finding a large discrepancy in otolith B:Ca ratios between fish captured in 2000 and others captured in 2019 – 2021. Archived otoliths from previous analyses were used to create a time series of data from 1985-2021. Otolith B:Ca ratios were analyzed in relation to long-term records of water chemistry in a central location in the Baltic Sea to represent trends in environmental conditions. Ratios were also examined against an elemental ratio (P:Ca) determined in other studies to be controlled physiologically, as well as a ratio (Mn:Mg) that serves as a proxy for hypoxia exposure (Limburg and Casini, 2018).

Boron is an essential micronutrient for plants, and is found in cyanobacteria and macroalgae (Howe, 1998). Its toxicity to fishes appears to be less than for invertebrates (Taylor et al., 1985; Howe, 1998) and is considered to be non-toxic in most aquatic systems.

Boron has been included in a number of otolith chemistry studies, mostly as part of a multivariate suite of tracers to determine provenance. Martino et al. (2017) studied boron as part of a suite of trace elements that might be affected by experimentally elevated pCO_2, but found no effect. Cavole (2021) performed the first studies of otolith B:Ca as a potential proxy for ocean acidification, using wild fishes across a natural pH gradient (7.4 to 8.1). Cavole et al. (2023), examining potential tracers of warming, hypoxia, and acidification in deep-sea fishes, found wide variation in B:Ca but no consistent trends.

In this study, univariate regressions of otolith B:Ca on five water variables all were highly significant (Table 1). These variables are interrelated through ecological processes: warming temperatures and salinity in combination with nutrients produces massive algal blooms

https://doi.org/10.5194/egusphere-2023-512
Preprint. Discussion started: 31 March 2023
© Author(s) 2023. CC BY 4.0 License.

in the Baltic Sea, which as they die off and decay, consume oxygen and change the pH and alkalinity (Kuliński et al., 2022). In that analysis, total alkalinity (A_T) showed the strongest effect, followed by dissolved oxygen and salinity.

Further pairwise analysis of A_T, pH, and salinity by decade showed varying patterns of effect sizes and significance. Importantly, when testing pH and salinity together, pH had a significant, positive effect on B:Ca and salinity a significant, negative effect. Kuliński et al. (2018), building on earlier studies, showed that dissolved boron is tightly coupled to salinity in the Baltic Sea, with an intercept > 0 indicating watershed sources of B input. The decoupling of otolith B:Ca from salinity and strong relationship to pH and alkalinity in the 2010s suggests that environmental conditions moved into a different regime from the previous three decades.

Among the analyzed environmental variables (Fig. S2), temperature shows a clear, positive trend starting from the 1980s. This is consistent with analyses performed by Lehmann et al. (2022) who found warming of 0.3-0.5 °C per decade in the whole water column in the central Baltic (station BY15) between 1979 and 2018. For other variables, trends are not that straightforward. For salinity, the variability reveals a stratification pattern typical for the central Baltic, with significantly higher S in the deep waters and a brackish surface water layer. However, in the 1980s and early 1990s there was a clear S drop in the deep waters being the result of a lower frequency of so-called Major Baltic Inflows that bring dense, saline water from the North Sea to the Baltic (Meier et al. 2022). This relaxed haline stratification in the central Baltic, which in turn led to better ventilation of deep waters and significantly raised O_2 availability (Fig. S2 (b) and (d); Lehmann et al., 2022) increased water volume for cod spawning and feeding. However, the 1980s decade was also the time when the maximum nutrient loads from land occurred, propelling eutrophication in the central Baltic. High organic matter production (and its export to bottom waters) co-occurred with the re-enhancement of water column stratification at the turn of the 20th and 21st centuries (increase in bottom S, Fig. S2 (d)). Both these factors led to a significant drop in O_2 in deeper waters (Fig. S2 (b); Kuliński et al. (2022)), which limited the optimal water volume for cod expansion – a negative factor, that also coincided with high fishing exploitation.

Furthermore, all these changes together with a rising pressure from atmospheric CO_2 increase made fundamental changes in the marine CO_2 system and seawater pH. First, the

https://doi.org/10.5194/egusphere-2023-512
Preprint. Discussion started: 31 March 2023
© Author(s) 2023. CC BY 4.0 License.

relatively low pH in the 1960s was increasing by the 1990s as an initial effect of eutrophication (Fig. S2 (c)). Surprisingly, this trend was paralleled with a drop in A_T (Fig. S2 (e)), which is a counterintuitive phenomenon in marine CO_2 system studies. However, the relatively constant A_T/S ratio (Fig. S2 (f)) clearly suggests that this initial A_T decrease was an effect of salinity decrease. Already in the 1990s we can observe a regime shift and a clear decoupling of A_T from S as revealed by the marked A_T/S increase, suggesting A_T increase being higher than observed S changes at that time (Fig. S2 (d,e,f)). While in surface waters this A_T increase was likely due to climate-related (or CO_2-induced) enhancement of weathering on land and higher riverine A_T loads, the A_T increase observed in deep waters was probably an effect of hypoxic/anoxic A_T generation (Müller et al., 2016, Neumann et al., 2022). The latter is consistent with a large and sudden expansion of the hypoxic/anoxic areas in the central Baltic that occurred in the late 1990s (Meier et al., 2022) – a phenomenon that is also believed to be a root cause for a biogeochemical regime shift to the so-called vicious cycle – a self-supporting eutrophication mechanism (Kuliński et al., 2022). Due to these effects, pH after reaching its maximum in the late 1980s (effect of eutrophication), dropped somewhat and remained relatively constant in the 21st century expressing in that way the mutually cancelling effects of ocean acidification (effect of rising atmospheric CO_2) and hypoxia/anoxia/weathering-driven alkalinization.

As is the case for some other trace elements (Hüssy et al., 2021), in this study boron uptake into otoliths appears to have some unexplained physiological regulation. This conclusion is based on close correspondence between B:Ca and P:Ca in otoliths from Icelandic cod that are not stressed by hypoxia (Figure 3(a), Figure 5(a)). In contrast to the Icelandic cod, the relationship of P:Ca to B:Ca in Baltic cod otoliths was highly variable, with a negative trend in the aggregate (Figure 5(b)). Regressions of P:Ca on B:Ca conducted on individual fish revealed mostly positive slopes, with highest slopes observed in the 2010s (Figure S4(a)), but lower, more variable R^2 values (Figure S4(b)). Although not statistically different, R^2 values were lowest in the 1980s and next-lowest in the 2010s. Compared to the Icelandic cod, all Baltic otoliths showed less coupling between P:Ca and B:Ca.

The CO_2^- and A_T-induced pH changes have definitely modified borates' availability in water and likely the B:Ca ratio in the Baltic cod's otoliths (Fig. 4). However, as shown by the strong correlation of B:Ca and the physiologically regulated P:Ca in the healthy, normoxic

https://doi.org/10.5194/egusphere-2023-512
Preprint. Discussion started: 31 March 2023
© Author(s) 2023. CC BY 4.0 License.

Icelandic cod's otoliths, the B:Ca ratio may also contain built-in information about the overall physiological condition of fish (and not only the pH-dependency). This suggests that a complex picture of B:Ca development in the EBC otoliths over the recent decades may be affected by multiple factors, not only by pH changes but also physiological stress caused by overall changes that have recently occurred in the Baltic (Eero et al., 2020). These include expansion of hypoxia/anoxia, eutrophication, and biogeochemical regime shift. Together with overfishing, these factors led to an overall collapse of the EBC population in the Baltic.

Examination of B:Ca in relation to an otolith index of hypoxia exposure, Mn:Mg, revealed mostly positive slopes of individual fish regressions, with the notable exceptions of Baltic cod from the 2010s and Icelandic cod (Figure S5). Limburg and Casini (2018) adopted the ratio Mn:Mg as a hypoxia exposure proxy rather than Mn:Ca in part to correct for growth influences on Mn (see Limburg et al., 2015) as otolith Mg is related to metabolism and growth (Limburg et al., 2018). The positive relationships of Mn:Mg to B:Ca cannot be fully explained, but the inverse relationships observed in the 2010s is consistent with the hypothesis that cod were exposed to both hypoxia and lower pH (and also higher alkalinity). Some of the positive slopes were due to young ages of fish; we observed more coupling of Mn:Mg and B:Ca in young fish, and we hypothesize that at younger ages the growth effect on otolith Mn is not fully removed by dividing by Mg. Baltic cod had increasingly negative slopes of the Mn:Mg vs. B:Ca regressions as they aged (Figure S6), with the 2010s displaying the most negative slopes.

5. Conclusions

Baltic cod showed variable patterns of otolith B:Ca over a 35-year period, with maximum values in the late 1990s, and a gradual decline in B:Ca thereafter, to an all-time low in 2019-20. These trends are imperfectly related to water chemistry data, but showed strong correlations that varied by decade. Otolith B:Ca ratios and relationships in the 2010s were suggestive of environmental changes in pH and total alkalinity that could be coupled to deoxygenation. Physiological influences observed in a healthy Icelandic population were diminished in Baltic cod.

https://doi.org/10.5194/egusphere-2023-512
Preprint. Discussion started: 31 March 2023
© Author(s) 2023. CC BY 4.0 License.

Undoubtedly, some of the relationships found could be due to the choice of samples, and a more focused study might select otoliths from areas where trends in acidification are clear. Future work could also examine otoliths from other species, as well as determine, if possible, the form of boron (borate or boric acid) taken up by otoliths. Complex interactions notwithstanding, we suggest that B:Ca in cod's otoliths can be a prospective variable in the palette of important and available tools to look into the environmental changes through the lens of otolith chemistry.

https://doi.org/10.5194/egusphere-2023-512
Preprint. Discussion started: 31 March 2023
© Author(s) 2023. CC BY 4.0 License.

Acknowledgements.

We thank S. Campana, M. Casini, and I. Jonsdottir for providing otoliths; Deb Driscoll for assistance with LA-ICP-MS analysis; B. Hönisch for discussions of $\delta^{11}B$; C. Humborg and B. Gustafsson for discussions of Baltic acidification; L. Levin and L. Cavole for reading and commenting on an earlier version of this work, and xx anonymous reviewers. This work was supported by grant OCE-1923965 from the US National Science Foundation. Through the input of Karol Kuliński this work contributes to Baltic Earth - Earth System Science for the Baltic Sea Region (www.baltic.earth).

Literature cited.

Casini, M., Hansson, M., Orio, A. and Limburg, K.: Changes in population depth distribution and oxygen stratification are involved in the current low condition of the eastern Baltic Sea cod (*Gadus morhua*). Biogeosciences, 18, 1321-1331, https://doi.org/10.5194/bg-18-1321-2021, 2021.

Cavole, L. M.: Fish otoliths and fisher knowledge as mobile monitors of environmental conditions: an integrated approach, Ph.D. dissertation, University of California at San Diego. ProQuest ID: Cavole_ucsd_0033D_20228. Merritt ID: ark:/13030/m5868htx. Retrieved from https://escholarship.org/uc/item/3531j2t0, 2021.

Cavole, L. M., Limburg, K. E., Gallo, N. D., Salvanes, A. G. V., Ramirez-Valdez, A., Levin, L. A., Oropeza, O. A., Hertwig, A., Liu, M.-C., and McKeegan, K. D.: Otoliths of marine fishes record evidence of low oxygen, temperature and pH conditions of deep Oxygen Minimum Zones, Deep-Sea Res. Part 1: Oceanogr. Res. Papers, 103941, 2022.

Doney, S. C., Fabry, V. J., Feely, R. A., and Kleypas, J. A.: Ocean acidification: the other CO_2 problem, Ann. Rev. Mar. Sci., 1, 169-192, https://doi.org/10.1146/annurev.marine.010908.163834, 2009.

Eero, M., Hjelm, J., Behrens, J., Buchmann, K., Cardinale, M., Casini, M., Gasyukov, P., Holmgren, N., Horbowy, J., Hüssy, K., Kirkegaard, E., Kornilovs, G., Krumme, U., Köster, F. W., Oeberst, R., Plikshs, M., Radtke, K., Raid, T., Schmidt, J., Tomczak, M. T., Vinther, M., Zimmermann, C., and Storr-Paulsen, M.: Eastern Baltic cod in distress: biological changes and challenges for stock assessment. ICES J. Mar. Sci., 72, 2180-2186, 2015.

Eero, M., Cardinale, M., and Storr-Paulsen, M.: Emerging challenges for resource management under ecosystem change: Example of cod in the Baltic Sea, Ocean Coast. Manage., 198, 105314, 2020.

Heimbrand, Y., Limburg, K. E., Hüssy, K., Casini, M., Sjöberg, R., Palmén Bratt, A. M., Levinsky, S. E., Karpushevskaia, A., Radtke, K. and Öhlund, J.: Seeking the true time: Exploring otolith chemistry as an age-determination tool, J. Fish Biol., 97, 552-565, 2020.

https://doi.org/10.5194/egusphere-2023-512
Preprint. Discussion started: 31 March 2023
© Author(s) 2023. CC BY 4.0 License.

Hönisch, B., Ridgwell, A., Schmidt, D. N., Thomas, E., Gibbs, S. J., Sluijs, A., Zeebe, R., Kump, L., Martindale, R. C., Greene, S. E. Kiessling, W., Ries, J., Zachos, J. C., Royer, D. L., Barker, S., Marchitto Jr., T. M., Moyer, R., Pelejero, C., Ziveri, P, Foster, G. L., and Williams, B.: The geological record of ocean acidification, Science, 335(6072), 1058-1063, 2012.

Howe, P. D., A review of boron effects in the environment, Biol. Trace Elem. Res., 66, 153-166, 1998.

Hüssy, K., Limburg, K. E., de Pontual, H., Thomas, O. R., Cook, P. K., Heimbrand, Y., Blass, M. and Sturrock, A. M.: Trace element patterns in otoliths: the role of biomineralization, Rev. Fish. Sci. Aquacult., 29, 445-477, 2021.

ICES (International Council for the Exploration of the Sea): Benchmark Workshop on Baltic Cod Stocks (WKBALTCOD2), ICES Scientific Reports, 1:9, 1-310, http://doi.org/10.17895/ices.pub.4984, 2019.

JMP®, Version 17.0. SAS Institute Inc., Cary, NC, 1989–2022.

Kuliński, K., Schneider, B., Szymczycha, B., and Stokowski, M.: Structure and functioning of the acid-base system in the Baltic Sea, Earth Syst. Dynam., 8, 1107-1120, 2017.

Kuliński, K., Szymczycha, B., Koziorowska, K., Hammer, K., and Schneider, B.: Anomaly of total boron concentrations in the brackish waters of the Baltic Sea and its consequence for the CO_2 system calculations. Mar. Chem., 204, 11–19, https://doi.org/10.1016/j.marchem.2018.05.007, 2018.

Kuliński, K., Rehder, G., Asmala, E., Bartosova, A., Carstensen, J., Gustafsson, B., Hall, P. O., Humborg, C., Jilbert, T., Jürgens, K. Meier, H. E. M., Müller-Karulis, B., Naumann, M., Olesen, J. E., Savchuk, O., Schramm, A., Slomp, C. P., Sofiev, M., Sobek, A., Szymczycha, B., and Undemann, E.: Biogeochemical functioning of the Baltic Sea. Earth Syst. Dynam., 13, 633-685, https://doi.org/10.5194/esd-13-633-2022, 2022.

Lehmann, A., Myrberg, K., Post, P., Chubarenko, I., Dailidiene, I., Hinrichsen, H.-H., Hüssy, K., Liblik, T., Meier, H. E. M., Lips, U., and Bukanova, T.: Salinity dynamics of the Baltic Sea, Earth Syst. Dynam., 13, 373–392, https://doi.org/10.5194/esd-13-373-2022, 2022.

Levin, L. A., Hönisch, B., & Frieder, C. A.: Geochemical proxies for estimating faunal exposure to ocean acidification. Oceanography, 28, 62-73, https://doi.org/10.5670/oceanog.2015.32, 2015.

Limburg, K.E., Walther, B. D., Lu, Z., Jackman, G., Mohan, J., Walther, Y., Nissling, A., Weber, P. K., and Schmitt, A. K.: In search of the dead zone: use of otoliths for tracking fish exposure to hypoxia, J. Mar. Sys., 141, 167-178, 2015.

Limburg, K.E. and Casini, M.: Effect of marine hypoxia on Baltic Sea cod *Gadus morhua*: evidence from otolith chemical proxies. Front. Mar. Sci., 5, 482, 2018.

Limburg, K. E. and Casini, M.: Otolith chemistry indicates recent worsened Baltic cod condition is linked to hypoxia exposure. Biol. Lett., 15, 20190352, 2019.

Limburg, K. E., Wuenschel, M. J., Hüssy, K., Heimbrand, Y., and Samson, M.: Making the otolith magnesium chemical calendar-clock tick: plausible mechanism and empirical evidence, Rev. Fish. Sci. Aquacult., 26, 479-493, 2018.

https://doi.org/10.5194/egusphere-2023-512
Preprint. Discussion started: 31 March 2023
© Author(s) 2023. CC BY 4.0 License.

435 Martino, J., Doubleday, Z. A., Woodcock, S. H., and Gillanders, B. M.: Elevated carbon dioxide
436 and temperature affects otolith development, but not chemistry, in a diadromous fish, J. Exp.
437 Mar. Biol. Ecol., 495, 57-64, 2017.

438 Meier, H. E. M., Kniebusch, M., Dieterich, C., Gröger, M., Zorita, E., Elmgren, R., Myrberg, K.,
439 Ahola, M. P., Bartosova, A., Bonsdorff, E., Börgel, F., Capell, R., Carlén, I., Carlund, T.,
440 Carstensen, J., Christensen, O. B., Dierschke, V., Frauen, C., Frederiksen, M., Gaget, E.,
441 Galatius, A., Haapala, J. J., Halkka, A., Hugelius, G., Hünicke, B., Jaagus, J., Jüssi, M.,
442 Käyhkö, J., Kirchner, N., Kjellström, E., Kulinski, K., Lehmann, A., Lindström, G., May,
443 W., Miller, P. A., Mohrholz, V., Müller-Karulis, B., Pavón-Jordán, D., Quante, M.,
444 Reckermann, M., Rutgersson, A., Savchuk, O. P., Stendel, M., Tuomi, L., Viitasalo, M.,
445 Weisse, R., and Zhang, W.: Climate change in the Baltic Sea region: a summary, Earth Syst.
446 Dynam., 13, 457–593, https://doi.org/10.5194/esd-13-457-2022, 2022.

447 Müller, J. D., Schneider, B., and Rehder, G.: Long-term alkalinity trends in the Baltic Sea and
448 their implications for CO_2-induced acidification, Limnol. Oceanogr., 71. 1983-2002, 2016.

449 Neumann T., Radtke H., Cahill B., Schmidt M., Rehder G. 2022. Non-Redfieldian carbon model
450 for the Baltic Sea (ERGOM version 1.2) – implementation and budget estimates, Geosci.
451 Model Dev., 15, 8473–8540, https://doi.org/10.5194/gmd-15-8473-2022.

452 Reis-Santos, P., Gillanders, B. M., Sturrock, A. M., Izzo, C., Oxman, D. S., Lueders-Dumont, J.
453 A., Hüssy, K, Tanner, S. E., Rogers, T, Doubleday, Z. A., Andrews, A. H., Trueman, C.,
454 Brophy, D., Thiem, J. D., Baumgartner, L. J., Wilmes, M., Chung, M.-T., Johnson R. C.,
455 Heimbrand, Y., Limburg, K. E, & Walther, B. D.: Reading the biomineralized book of life:
456 expanding otolith biogeochemical research and applications for fisheries and ecosystem-
457 based management, Reviews in Fish Biology and Fisheries, 1-39,
458 https://doi.org/10.1007/s11160-022-09720-z, 2022.

459 R Core Team: R: A language and environment for statistical computing, R Foundation for
460 Statistical Computing, Vienna, Austria, 2022. (https://www.R-project.org/)

461 Reusch, T.B., Dierking, J., Andersson, H. C., Bonsdorff, E., Carstensen, J., Casini, M.,
462 Czajkowski, M., Hasler, B., Hinsby, K., Hyytiäinen, K., Johannesson K., Jomaa, S.,
463 Jormalainen, V., Kuosa, H., Kurland, S., Laikre, L., MacKenzie, B.,R., Margonski, P.,
464 Melzner, F., Oesterwind, D., Ojaveer, H., Refsgaard, J. C., Sandström, A., Schwarz, G.,
465 Tonderski, K., Winder, M., and Zandersen, M.: The Baltic Sea as a time machine for the
466 future coastal ocean. Sci. Adv. 4, eaar8195, DOI: 10.1126/sciadv.aar8195, 2018.

467 Thomas, O.R.B., Ganio, K., Roberts, B.R., and Swearer, S.E.: Trace element-protein interactions
468 from the inner ear of fish: implications for environmental reconstructions using fish otolith
469 chemistry, Metallomics, 9, 239-249, 2017.

470 Yu, J., & Elderfield, H.: Benthic foraminiferal B/Ca ratios reflect deep water carbonate
471 saturation state, Earth Planet. Sci. Lett., 258, 73-86, 2007.