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

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11 **Abstract**: Ocean acidification is spreading globally as a result of anthropogenic CO_2 emissions,
12 but the Baltic Sea has until recently been thought to be relatively well-buffered by terrigenous
13 inputs of alkalinity from its watershed. We discovered a 3- to 5-fold decline in boron (as B:Ca)
14 in otoliths of eastern Baltic Sea cod (EBC) between the late 1990s and 2021. B:~~Ca is positively~~
15 ~~proportional to pH in carbonates, as B in the form of borate is taken up in the CaCO₂ matrix.~~
16 Examining a time series of EBC otoliths, we found varying levels of B:Ca since the 1980s, with
17 the most recent years at an all-time low during this period. This trend correlates with declines in
18 pH and dissolved oxygen, but not with changes in salinity. We examined possible physiological
19 influences on B:Ca by including a collection of ~~healthy~~ Icelandic cod as an out-group. Icelandic
20 cod otoliths showed strongly positive correlations of B:Ca with physiologically regulated P:Ca;
21 this was not the case for EBC. Finally, B:Ca in EBC otoliths is ~~anti~~ negatively correlated to some
22 extent with Mn:Mg, a proposed proxy for hypoxia exposure. This negative relationship is
23 hypothesized to reflect the dual phenomena of hypoxia and acidification as a result of
24 decomposition of large algal blooms. Taken together, the otolith biomarkers Mn:Mg and B:Ca
25 suggest a general increase in both hypoxia and acidification within the Baltic intermediate and
26 deep waters in the last decade reflected in cod otoliths.

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

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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~~,~~ (Kulinski et al., 2022). ~~which is~~This decline is associated with the ongoing eutrophication and higher vertical export of organic matter that leads also to worsening deoxygenation (Kuliński et al., 2022).
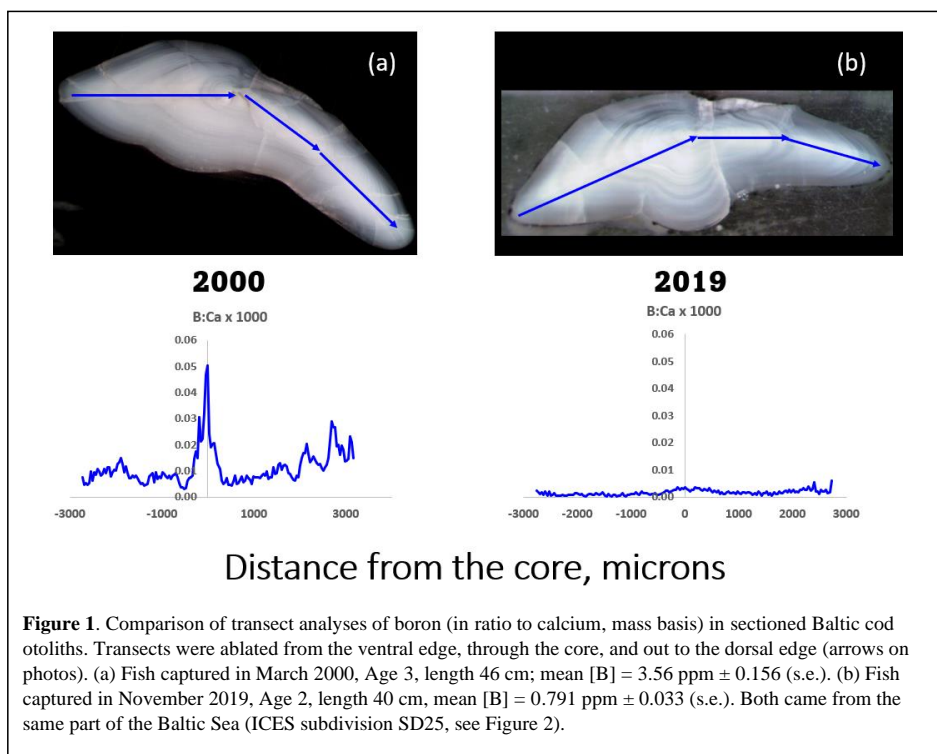
The population of the Eastern Baltic cod (*Gadus morhua*, hereafter referred to as EBC) has been severely impacted by a number of factors that include past overfishing, hypoxia, parasite infections, and seal predation (Eero et al., 2020), leading to reduced growth and age at maturity (Eero et al., 2016; ICES, 2019). Cod exposed to hypoxia were shown to have lower growth rates and worsened body condition ~~(, quantified by the Fulton K index of weight:length ratio (~~~~Limburg & Casini, 2018, 2019~~Casini et al., 2016; Limburg & Casini, 2018).

~~Hypoxia~~ Recent studies that reported links between hypoxia exposure and body condition have relied on the measurement of trace elements in fish otoliths (ear-stones). ~~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

60 mass spectrometry (see Methods). Hypoxia exposure was quantified by means of a proxy

61 developed from analyzing manganese concentrations in otoliths (ear-stones) of fish (Limburg et

62 al., 2015), in ratio to magnesium which corrects to some extent for growth influences on Mn

63 uptake, since both are affected by growth but only manganese is affected directly by hypoxic

64 conditions (Limburg et al., 2018; Limburg & Casini, 2018). The resulting elemental data are

65 lifetime concentration histories, being incorporated at the time of exposure. The use of otolith

66 manganese to track hypoxia exposure is one of the emerging biomarkers in fisheries ecology

67 (Reis-Santos et al., 2022).

68   Recently, with acquisition of more sensitive instrumentation, we began to experiment

69 with quantifying elements having sub-ppm concentrations in cod otoliths, including the trace

70 element boron. Interestingly, preliminary results from this work showed elevated levels of boron

71 in 2000 but not in 2019. Boron is noteworthy because it is an indicator of salinity. Specifically,

72 Boron in seawater generally correlates with salinity (Kuliński et al., 2017) predominantly in the

73 form of weak boric acid (H_3BO_3) at standard seawater salinity (35 PSU) and pH of 8. However,

74 the dissociated anion borate $B(OH)_4^-$ is positively, nonlinearly correlated with pH (Yu et al.,

75 2007). Borate is the dominant form incorporated into carbonates such as limestones,

76 foraminifera, and corals in proportion to pH (Yu et al., 2007; Hönisch et al., 2012; Levin et al.,

77 2015) and is often confirmed by $\delta^{11}B$ analysis, since it has a large, negative fractionation

78 compared to boric acid. However, even bulk boron, as B:Ca ratios, has been found to correlate

79 positively (and linearly) with pH in foraminifera (Yu et al., 2007), albeit in a species-specific

80 manner.

3

81   We measured sub-ppm to low-ppm concentrations of B (ca. 0.5 – 5 ppm, see Methods

82   section) in otoliths of EBC captured in 2000 that showed what appeared to be seasonal variations

83   (example shown in Figure 1(a)), albeit not in synchrony with other elements that have proven

84   useful to age cod (Heimbrand et al., 2020). It was therefore surprising when we began to analyze

85   otoliths of EBC captured in 2019 and discovered greatly reduced concentrations (Figure 1(b)).

86   The measurements were repeated for verification.

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).

87   With such differences suggestive of change, we returned to an archive of otoliths used for

88   another project (Limburg and Casini, 2018), as well as adding previously un-analyzed otoliths, to

89   develop a time series of otolith B:Ca.

90   In this study, we explored the extent to which otolith B:Ca varied through time and

91   whether its values were correlated with pH, salinity, or other otolith derived values that are

92   proxies for environmental or physiological factors. In particular, otolith P:Ca is known to be

93 under physiological influence (Thomas et al., 2017; Heimbrand et al., 2020). In cod, higher

94 values of P:Ca occur during the growing season and are thus hypothesized to be associated with

95 growth and other activities. We selected this as well as our proxy for hypoxia exposure, otolith

96 Mn:Mg, and to document any temporal trends that could be correlated with pH, salinity, or other

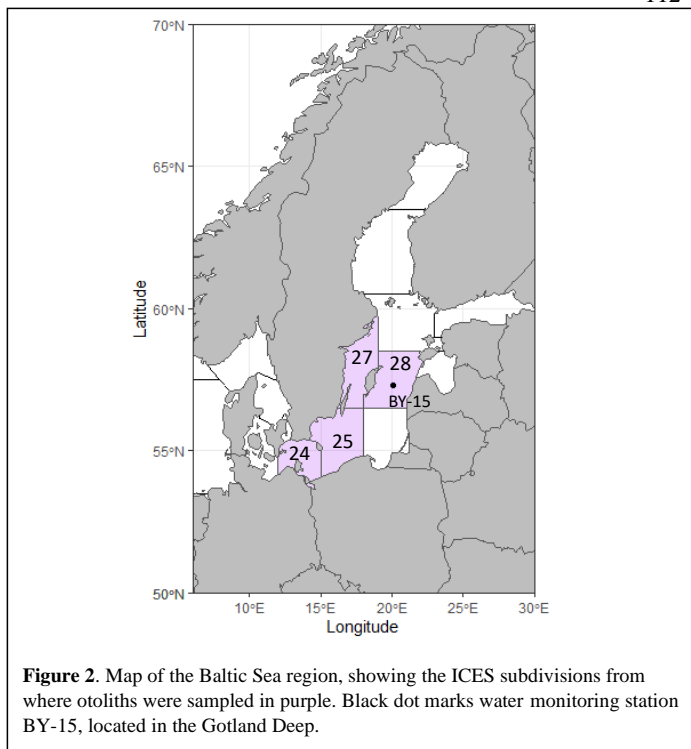97 environmental factors. We posed the following questions:

98

99 1. What are the temporal trends in otolith boron (as B:Ca ratios)?

100 2. How do B:Ca ratios correlate to trends in water chemistry, particularly salinity and pH?

101 3. If pH declines are associated with hypoxia, do we see a correlation of B:Ca with our hypoxia

102 proxy, manganese:magnesium (Mn:Mg)?

103 4. If there are any physiological influences, do we see a correlation of B:Ca with elemental ratios

104 known to be under physiological control, e.g., phosphorus:calcium (P:Ca)?

105

106 **2 Materials and Methods**

107 **2.1 Otolith collections and microchemical analysis**

5

108   Otoliths of Baltic cod (N = 156) were obtained from both fishery-independent and
109   fishery-dependent surveys conducted by the Swedish Fisheries Board and its successor, the
110   Department of Aquatic Resources, Swedish University of Agricultural Sciences,~~.~~. Fish were
111   collected from ICES sub-divisions (SD) 24, 25, 27, and 28 (Figure 2), spanning the period 1988-

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.

112   2021. To construct the time series, we selected previously analyzed (but not for B:Ca) otoliths that were comparable in age range to those collected in 2019-2021. ~~These~~ All otoliths 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.

       In addition, cod otoliths (N = 14) were obtained from fisheries-

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

134   Baltic cod were aged by examining seasonal patterns in otolith Mg:Ca and P:Ca,
135   following Heimbrand et al. (2020). Icelandic cod were also aged by otolith chemistry, although
136   several of them had been previously aged visually by age readers in Iceland. For the latter
137   otoliths, the seasonal chemical patterns produced age estimations that matched exactly with the
138   visual estimates.

6

139   Chemical analyses were performed by laser ablation inductively coupled plasma mass

140   spectrometry (LA-ICP-MS) at the Analytical and Technical Services group at SUNY ESF.

141   Otoliths were ablated with a 192-nm laser ablation unit (Teledyne Cetac Excite 2) along transects

142   as illustrated in Figure 1; the ablated material was transported via an Ar-He carrier gas mixture

143   into a Thermo iCAP TQ plasma mass spectrometer where isotopes were quantified (^{11}B, ^{25}Mg,

144   ^{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

145   optimized with NIST 612 glass standard and U.S. Geological Survey standards MACS-3,

146   MAPS-4, and MAPS-5 were used as standards for calibration and drift correction. The MAPS

147   (abbreviation for Micro-Analytical Phosphate Standard) standards, while having good matching

148   to the otolith matrix, were slightly softer and were therefore ablated at a higher energy (25%

149   power, 2.03 J/cm^2 fluence) than the otoliths (20% power, 1.62 J/cm^2) to achieve an otolith

150   calcium value around 38 weight percent. Transects were made with 110 μm-diameter ablation

151   spots at a speed of 7 μm/sec. Although often edge-to-edge laser transects were made (to check

152   for symmetry), only data collected on the dorsal (longer) axis were used in our analysis due to

153   greater spatial resolution. Once data were worked up, annual mean values were parsed by the age

154   determination technique in Heimbrand et al. (2020), i.e., averages within annual growth rings

155   were calculated. These were matched to corresponding calendar years.

156   **2.2 Water data**

157   Water properties data (temperature (T, °C), salinity (S, psu), dissolved oxygen (DO,

158   mL/L), pH, and total alkalinity (A_T, mmol/L)) were downloaded from the Swedish

159   Meteorological and Hydrological Institute's database, SHARKWeb (https://sharkweb.smhi.se/).

160   Station BY-15, Gotland Deep, was selected as being representative of the central Baltic (Figure

161   2) and having one of the longest and most detailed timeseries. We selected depths that

162   corresponded to where cod had been found in fishery-independent surveys (Casini et al., 2021).

163   Between 1985-1995, we averaged water data from 30-60 meters, and from 1996 onward,

164   between 40-75 meters. Annual mean water values were computed and matched to corresponding

165   otolith chemistry data (annual means of Element:Ca data parsed to calendar years as described

166   above).

167

168   **2.3 Analysis**

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169 Visualization (graphs) and statistical analyses were performed in Excel, R (base R (R

170 Core Team 2022) and ggplot2 (Wickham, 2016)), and JMP version 17.0 (JMP 2022). Statistical

171 analyses were separated into examination of relationships of B:Ca values to potential

172 environmental drivers (primarily pH and S, but also A_T, DO, and T) and to the internal variables

173 P:Ca and Mn:Mg. ~~P:Ca was found to correlate highly with B:Ca in Icelandic cod otoliths~~

174 ~~(Figure 3(a)). Otolith P:Ca is known to be under physiological influence (Thomas et al., 2017;~~

175 ~~Heimbrand et al., 2020). In cod, higher values of P:Ca occur during the growing season and are~~

176 ~~thus hypothesized to be associated with growth and other activities. Otolith Mn:Mg, on the other~~

177 ~~hand, is our proxy for hypoxia exposure (Limburg and Casini, 2018), and often correlated~~

178 ~~negatively with B:Ca (Figure 3(b))—consistent with co-occurrence of reduced conditions in~~

179 ~~water (and presence of bioavailable ionic Mn^{2+}) and high pCO_2 and thus low pH, both being the~~

180 ~~effect of enhanced organic matter remineralization and/or poor ventilation of the water layers~~

181 ~~occupied by cod.~~ For the environmental drivers, ~~graphical~~ a PCA analysis was first performed to

182 examine relationships among them. Thereafter, B:Ca was regressed on the first principal

183 component eigenvector to test for its relationship to the main drivers. ~~performed~~ This was

184 followed by mixed linear models of environmental drivers on B:Ca ~~and visualizations~~, with each

185 fish's age (nested within fish ID) being treated as a random effect. This analysis took account of

186 repeated measures within the same individual, and was intended to examine more closely the

187 effect of individual drivers.

188 For the internal variables, the P:Ca and Mn:Mg transect data were regressed on

189 corresponding B:Ca for each fish, and the slopes, intercepts and R^2 values were calculated. These

190 were examined for trends by decade (1980s, 1990s, 2000s, and 2010s) as well as compared with

191 the Icelandic out-group. Positive correlations with P:Ca would suggest a physiological influence

192 on B:Ca incorporation, whereas negative correlations with Mn:Mg would suggest a linkage

193 between B:Ca as an indicator of pH and our indicator of hypoxia exposure (cf. Cavole, 2021).

194 These were tested using ANOVA with slopes as the dependent variable and decades (for Baltic

195 Sea) and Iceland as grouping variables.

196

197 **3 Results**

198 **3.1. Trends in water variables**

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199   A principal components analysis of the water variables found that the first two
200   components explained 92.9% of the variance (72.4% by PC1 and 20.5% by PC2). The analysis
201   showed three groupings: (1) DO and pH that were opposite to (2) salinity and A_T, respectively;
202   and (3) temperature was on a separate axis (Figure S1). Linear regression of B:Ca on the first
203   principal component yielded a negative relation (B:Ca = 0.000485(PC1) + 0.00246, $R^2 = 0.21$, p
204   < 0.0001).

205   Examination of the long-term time series of data from Station BY-15 shows that while
206   temperature generally increased (Figure S23(a)), the other water variables showed very nonlinear
207   patterns (Figures S23(b-e). Alkalinity is in part a function of salinity; dividing A_T by S produces
208   a time series showing a dramatic state change around 1990 (Figure S23(f)), particularly in the
209   water layers occupied by cod. Despite the increasing A_T, pH has declined more or less
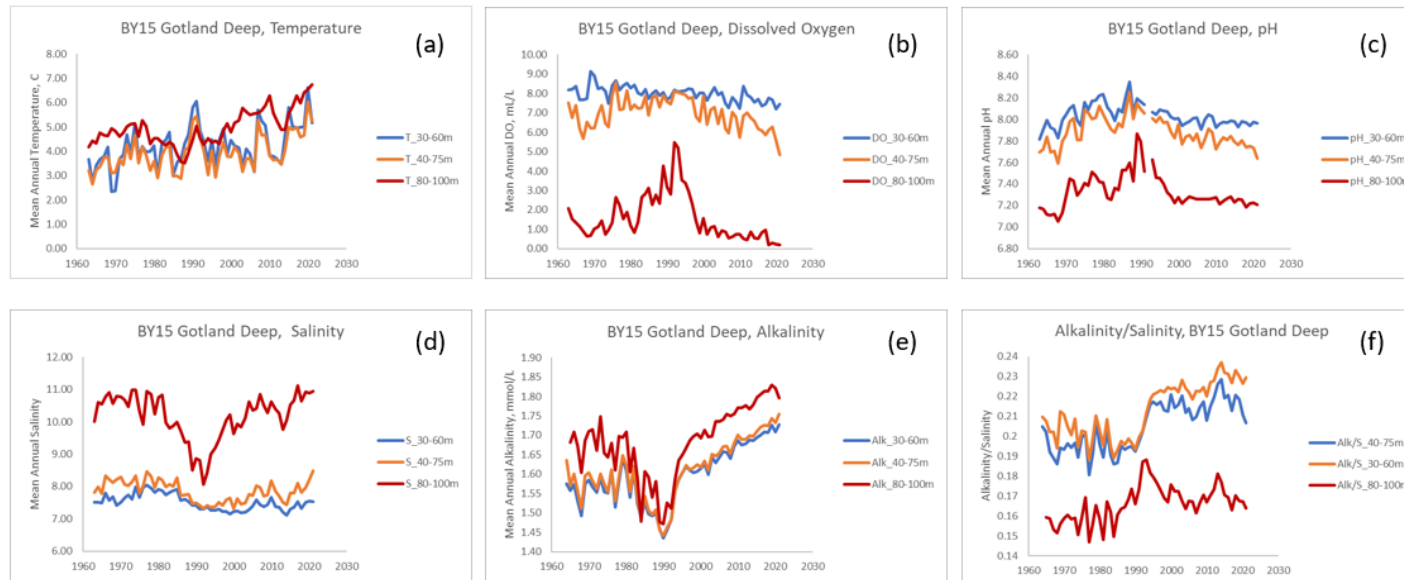210   monotonically at midwater depths (40-75 m, Figure S23(c)).

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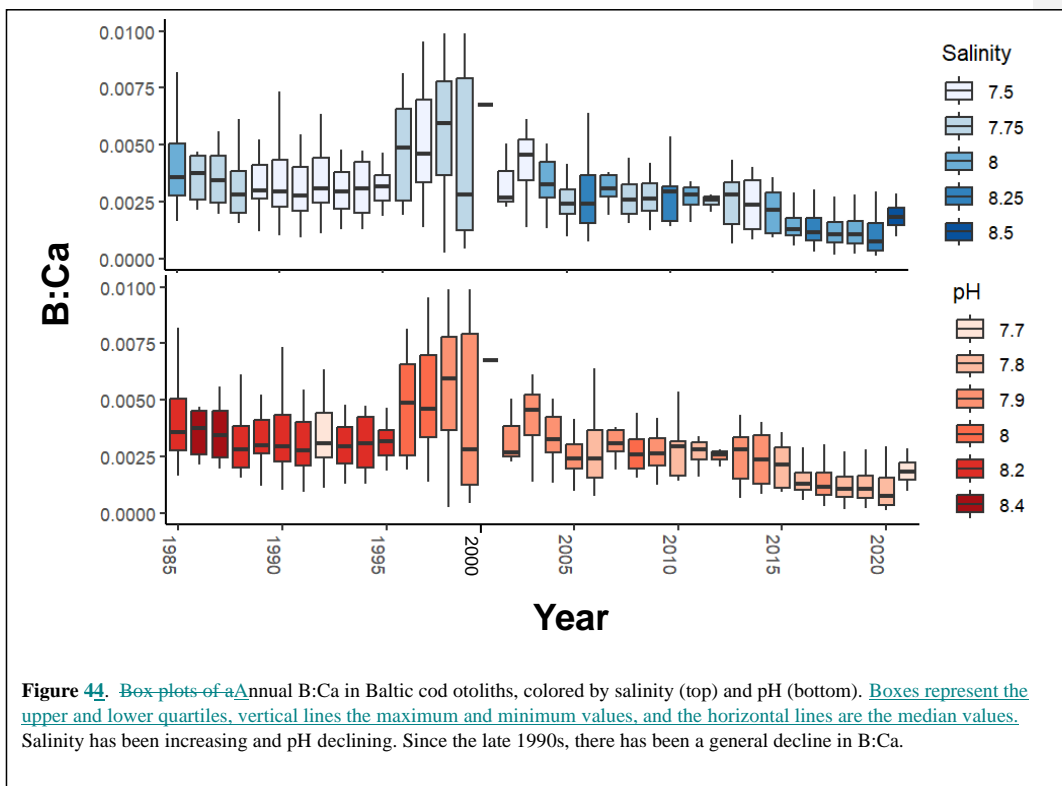
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Station BY-15, Gotlandsdjupet 1963-2021

Figure 3. Time series of water data for three depth zones, Gotland Deep Station BY-15. (a) Temperature. (b) Dissolved oxygen. (c) pH. (d) Salinity. (e) Total alkalinity. (f) Alkalinity normalized to salinity.

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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 44). 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)).

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Figure 44. ~~Box plots of a~~Annual B:Ca in Baltic cod otoliths, colored by salinity (top) and pH (bottom). Boxes represent the upper and lower quartiles, vertical lines the maximum and minimum values, and the horizontal lines are the median values. Salinity has been increasing and pH declining. Since the late 1990s, there has been a general decline in B:Ca.

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223

224 **3.3. Effects of water parameters on otolith B:Ca**

225 **3.3.1. Univariate effects of water parameters on otolith B:Ca**

226 ~~Repeated~~ Following the PCA, repeated measures regressions of otolith B:Ca on each of

227 the five water variables was conducted to examine effect sizes and significance each variable

228 alone. ~~Repeated measures~~These regressions showed significant effects of each of the five water

229 variables on otolith B:Ca (Table 1). A_T had the largest effect and was negative (Table 1).

230 Dissolved oxygen and pH both had positive slopes, and temperature and salinity both were

231 negative. All univariate analyses were highly significant. Ages of individual fish were not

232 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

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236 **3.3.2. Two-factor tests of water parameters on otolith B:Ca**

237 Because of ~~our~~ hypothesized positive relationships of salinity and pH on B:Ca, as well as

238 the large effect size of A_T, we examined pairwise (two-factor) models of these independent

239 variables on otolith B:Ca (Table 2). This allowed us to examine the relative strength and

240 direction of trends (slope parameters, Table 2) on B:Ca when testing two independent variables

241 together. Aside from an overall model using all the data, these were filtered by decade to

242 examine finer scale trends.

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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
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

243

244   In both pairwise tests with pH, this parameter had positive slopes in the 1980s and 2010s
245 but negative slopes in the 1990s and 2000s. Alkalinity had mostly a negative relation to otolith
246 B:Ca. Salinity had a positive effect in the 1980s and 1990s but became negative in the 2000s and
247 2010s. Salinity overall had a negative effect on B:Ca, in contrast to expectations. We note the
248 strong, negative effect of pH in the 1990s when paired with A_T, and the strong, positive effect of
249 pH in the 2010s when paired with salinity. Age, when treated as a random variable nested within
250 individual fish (by fish ID), was again not significant. However, in a separate analysis (Figure
251 S3S2), age (not treated as a random variable) and otolith B:Ca were inversely related in the
252 1980s, 1990s, and 2000s, and had a weakly positive trend in the 2010s.

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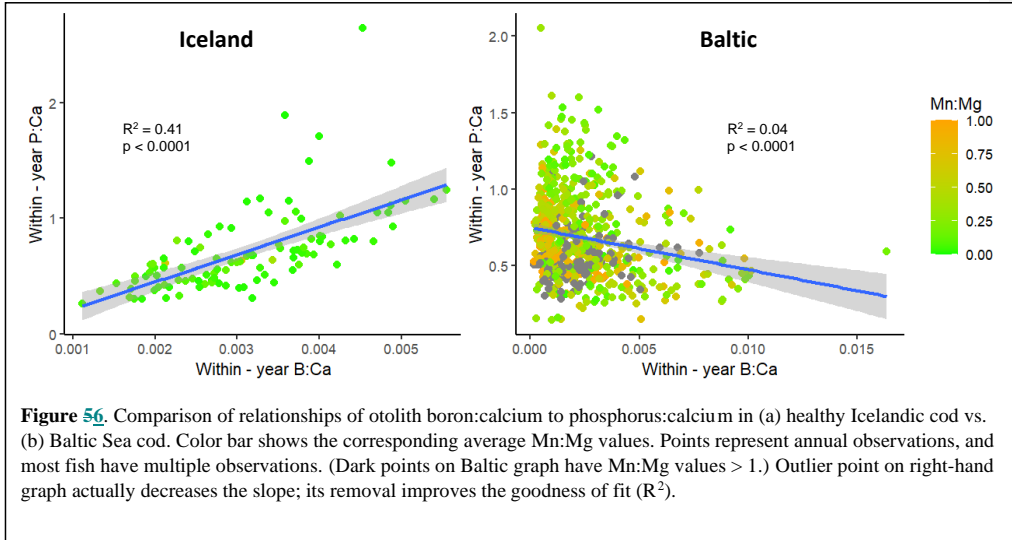254

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3.4. Internal parameters and otolith B:Ca

During exploratory examination of the data, P:Ca was found to correlate highly with B:Ca in Icelandic cod otoliths (Figure 5(a)). 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 5(b)). This is consistent with co-occurrence of reduced conditions in water (and presence of bioavailable ionic Mn^{2+}) and 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.
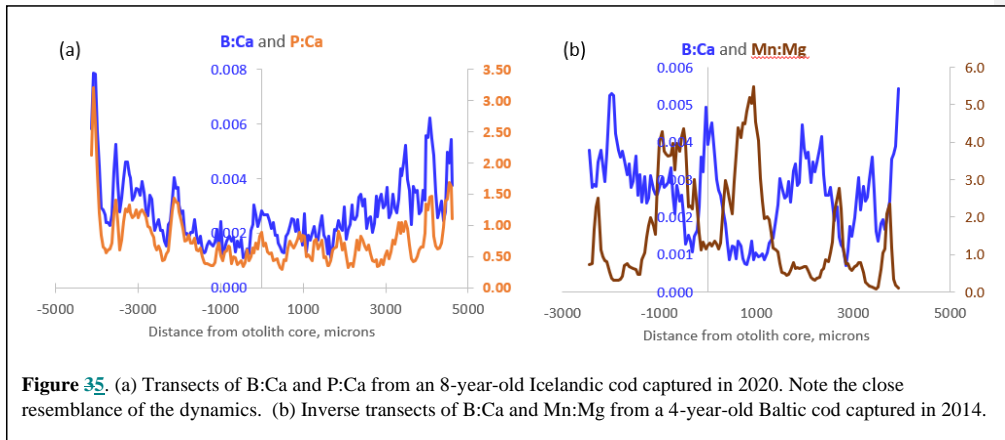
Figure 35. (a) Transects of B:Ca and P:Ca from an 8-year-old Icelandic cod captured in 2020. Note the close resemblance of the dynamics. (b) Inverse transects of B:Ca and Mn:Mg from a 4-year-old Baltic cod captured in 2014.

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 56). 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 (Figure S4S3). 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 S4S3(a)). Box plots of R^2 values by decade and out-group showed wide variation (Figure S4S3(b)), and an analysis of variance found no statistical difference among Baltic fish by decade, but Icelandic otoliths had significantly higher R^2 values ($p < 0.001$).

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Figure 56. 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).

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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 (see Methods). This analysis led to somewhat inconclusive results (Figure S5S4). 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 of these slopes against fish age (Figure S6S5). 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

291 decline in B:Ca that may indicate lower environmental pH. Overall, this analysis suggests that

292 the decade of the 2010s may have had evidence of acidification driven by the same mechanisms

293 that produced hypoxia.

294

295 **4. Discussion**

296   This study was initiated because of finding a large discrepancy in otolith B:Ca ratios

297 between fish captured in 2000 and others captured in 2019 – 2021. Archived otoliths from

298 previous analyses were then used to create a time series of data from 1985-2021. Otolith B:Ca

299 ratios were analyzed in relation to long-term records of water chemistry in a central location in

300 the Baltic Sea to represent trends in environmental conditions. Ratios were also examined against

301 an elemental ratio (P:Ca) determined in other studies to be controlled physiologically, as well as

302 a ratio (Mn:Mg) that serves as a proxy for hypoxia exposure (Limburg and Casini, 2018).

303   Boron is an essential micronutrient for plants, and is found in cyanobacteria and

304 macroalgae (Howe, 1998). Its toxicity to fishes appears to be less than for invertebrates (Taylor

305 et al., 1985; Howe, 1998) and is considered to be non-toxic in most aquatic systems.

306   Boron has been included in a number of otolith chemistry studies, mostly as part of a

307 multivariate suite of tracers to determine provenance. Martino et al. (2017) studied boron as part

308 of a suite of trace elements that might be affected by experimentally elevated pCO_2, but found no

309 effect. Cavole (2021) performed the first studies of otolith B:Ca as a potential proxy for ocean

310 acidification, using wild fishes across a natural pH gradient (7.4 to 8.1). Cavole et al. (2023),

311 examining potential tracers of warming, hypoxia, and acidification in deep-sea fishes, found

312 wide variation in B:Ca but no consistent trends.

313   In this study, univariate regressions of otolith B:Ca on five water variables all were

314 highly significant, with dissolved oxygen and pH showing positive relationships while negative

315 relationships were observed with alkinity, temperature, and salinity (Table 1). These variables

316 are interrelated through ecological processes: warming temperatures and salinity in combination

317 with nutrients produces massive algal blooms in the Baltic Sea, which as they die off and decay,

318 consume oxygen and change the pH and alkalinity (Kuliński et al., 2022). In that analysis, total

319 alkalinity (A_T) showed the strongest effect, followed by dissolved oxygen and salinity.

16

320   Further pairwise analysis of A_T, pH, and salinity by decade showed varying patterns of

321   effect sizes and significance. Importantly, when testing pH and salinity together, pH had a

322   significant, positive effect on B:Ca and salinity a significant, negative effect. Kuliński et al.

323   (2018), building on earlier studies, showed that dissolved boron is tightly coupled to salinity in

324   the Baltic Sea, with an intercept > 0 indicating watershed sources of B input. The decoupling of

325   otolith B:Ca from salinity and strong relationship to pH and alkalinity in the 2010s suggests that

326   environmental conditions moved into a different regime from the previous three decades.

327   Among the analyzed environmental variables (~~Fig. S2~~Figure 3), temperature shows a

328   clear, positive trend starting from the 1980s. This is consistent with analyses performed by

329   Lehmann et al. (2022) who found warming of 0.3-0.5 °C per decade in the whole water column

330   in the central Baltic (station BY15) between 1979 and 2018. For other variables, trends are not

331   that straightforward. For salinity, the variability reveals a stratification pattern typical for the

332   central Baltic, with significantly higher S in the deep waters and a brackish surface water layer.

333   However, in the 1980s and early 1990s there was a clear S drop in the deep waters being the

334   result of a lower frequency of so-called Major Baltic Inflows that bring dense, saline water from

335   the North Sea to the Baltic (Meier et al. 2022). This relaxed haline stratification in the central

336   Baltic, which in turn led to better ventilation of deep waters and significantly raised O_2

337   availability (~~Fig. S2~~Figure 3 (b) and (d); Lehmann et al., 2022) increased water volume for cod

338   spawning and feeding. However, the 1980s decade was also the time when the maximum

339   nutrient loads from land occurred, propelling eutrophication in the central Baltic. High organic

340   matter production (and its export to bottom waters) co-occurred with the re-enhancement of

341   water column stratification at the turn of the 20th and 21st centuries (increase in bottom S, ~~Fig.~~

342   ~~S2~~Figure 3 (d)). Both these factors led to a significant drop in O_2 in deeper waters (~~Fig. S2~~Figure

343   3 (b); Kuliński et al. (2022)), which limited the optimal water volume for cod expansion – a

344   negative factor, that also coincided with high fishing exploitation.

345   Furthermore, all these changes together with a rising pressure from atmospheric CO_2

346   increase made fundamental changes in the marine CO_2 system and seawater pH. First, the

347   relatively low pH in the 1960s was increasing by the 1990s as an initial effect of eutrophication

348   (~~Fig. S2~~Figure 3 (c)). Surprisingly, this trend was paralleled with a drop in A_T (~~Fig. S2~~Figure 3

349   (e)), which is a counterintuitive phenomenon in marine CO_2 system studies. However, the

17

350 relatively constant A_T/S ratio (Fig. S2Figure 3 (f)) clearly suggests that this initial A_T decrease

351 was an effect of salinity decrease. Already in the 1990s we can observe a regime shiftchange in

352 trends and a clear decoupling of A_T from S as revealed by the marked A_T/S increase, suggesting

353 A_T increase being higher than observed S changes at that time (Fig. S2Figure 3 (d,e,f)). While in

354 surface waters this A_T increase was likely due to climate-related (or CO_2-induced) enhancement

355 of weathering on land and higher riverine A_T loads, the A_T increase observed in deep waters was

356 probably an effect of hypoxic/anoxic A_T generation (Müller et al., 2016, Neumann et al., 2022).

357 The latter is consistent with a large and sudden expansion of the hypoxic/anoxic areas in the

358 central Baltic that occurred in the late 1990s (Meier et al., 2022) – a phenomenon that is also

359 believed to be a root cause for a biogeochemical regime shift to the so-called vicious cycle – a

360 self-supporting eutrophication mechanism (Kuliński et al., 2022). Due to these effects, pH after

361 reaching its maximum in the late 1980s (effect of eutrophication), dropped somewhat and

362 remained relatively constant in the 21st century expressing in that way the mutually cancelling

363 effects of ocean acidification (effect of rising atmospheric CO_2) and hypoxia/anoxia/weathering-

364 driven alkalinization.

365   As is the case for some other trace elements (Hüssy et al., 2021), in this study boron

366 uptake into otoliths appears to have some unexplained physiological regulation. This conclusion

367 is based on close correspondence between B:Ca and P:Ca in otoliths from Icelandic cod that are

368 not stressed by hypoxia (Figure 34(a), Figure 56(a)). In contrast to the Icelandic cod, the

369 relationship of P:Ca to B:Ca in Baltic cod otoliths was highly variable, with a negative trend in

370 the aggregate (Figure 56(b)). Regressions of P:Ca on B:Ca conducted on individual fish revealed

371 mostly positive slopes, with highest slopes observed in the 2010s (Figure S4S3(a)), but lower,

372 more variable R^2 values (Figure S4S3(b)). Although not statistically different, R^2 values were

373 lowest in the 1980s and next-lowest in the 2010s. Compared to the Icelandic cod, all Baltic

374 otoliths showed less coupling between P:Ca and B:Ca.

375   The CO_2^- and A_T-induced pH changes have definitely modified borates' availability in

376 water and likely the B:Ca ratio in the Baltic cod's otoliths (Fig. 45). However, as shown by the

377 strong correlation of B:Ca and the physiologically regulated P:Ca in the healthy, normoxic

378 Icelandic cod's otoliths, the B:Ca ratio may also contain built-in information about the overall

379 physiological condition of fish (and not only the pH-dependency). This suggests that a complex

380 picture of B:Ca development in the EBC otoliths over the recent decades may be affected by
381 multiple factors, not only by pH changes but also physiological stress caused by overall changes
382 that have recently occurred in the Baltic (Eero et al., 2020). These include expansion of
383 hypoxia/anoxia, eutrophication, and biogeochemical regime shift. Together with overfishing,
384 these factors led to an overall collapse of the EBC population in the Baltic.

385   Examination of B:Ca in relation to an otolith index of hypoxia exposure, Mn:Mg,
386 revealed mostly positive slopes of individual fish regressions, with the notable exceptions of
387 Baltic cod from the 2010s and Icelandic cod (Figure S5S4). Limburg and Casini (2018) adopted
388 the ratio Mn:Mg as a hypoxia exposure proxy rather than Mn:Ca in part to correct for growth
389 influences on Mn (see Limburg et al., 2015) as otolith Mg is related to metabolism and growth
390 (Limburg et al., 2018). The positive relationships of Mn:Mg to B:Ca cannot be fully explained,
391 but the inverse relationships observed in the 2010s is consistent with the hypothesis that cod
392 were exposed to both hypoxia and lower pH (and also higher alkalinity). Some of the positive
393 slopes were due to young ages of fish; we observed more coupling of Mn:Mg and B:Ca in young
394 fish, and we hypothesize that at younger ages the growth effect on otolith Mn is not fully
395 removed by dividing by Mg. Baltic cod had increasingly negative slopes of the Mn:Mg vs. B:Ca
396 regressions as they aged (Figure S6S5), with the 2010s displaying the most negative slopes.

397

398 **5. Conclusions**

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

406   Undoubtedly, some of the relationships found could be due to the choice of samples, and
407 a more focused study might select otoliths from areas where trends in acidification are clear.
408 Future work could also examine otoliths from other species, as well as determine, if possible, the

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409 form of boron (borate or boric acid) taken up by otoliths. Complex interactions notwithstanding,
410 we suggest that B:Ca in cod's otoliths can be a prospective variable in the palette of important
411 and available tools to look into the environmental changes through the lens of otolith chemistry.

412

413 *Code/Data availability.* The codes and data are available upon request to the corresponding
414 author.

415 *Author contributions:* KL designed and carried out the study. YH contributed data and figures.
416 All authors contributed text.

417 *Competing interests.* The authors declare that they have no conflict of interest.

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