



Compensatory Feeding in Eastern Baltic Cod (*Gadus morhua*): Recent Shifts in Otolith Growth and Nitrogen Content Suggest Unprecedented Metabolic Changes

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***Correspondence:**

Henrik Svedäng
henrik.svedang@su.se

†ORCID:

Henrik Svedäng
orcid.org/0000-0002-3639-7105
Sofia A. Wikström
orcid.org/0000-0003-3508-2109
Martin J. Whitehouse
orcid.org/0000-0003-2227-577X

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Henrik Svedäng^{1*†}, Viktor Thunell^{1,2}, Ale Pålsson^{1,3}, Sofia A. Wikström^{1†} and Martin J. Whitehouse^{4†}

¹ Baltic Sea Centre, Stockholm University, Stockholm, Sweden, ² Department of Aquatic Resources, Institute of Coastal Research, Swedish University of Agricultural Sciences, Öregrund, Sweden, ³ Department of History, Uppsala University, Uppsala, Sweden, ⁴ NordSIM Laboratory, Swedish Museum of Natural History, Stockholm, Sweden

The productivity of the Eastern Baltic cod (*EBC*) has been severely reduced over the last 25 years, for reasons that remain unclear. The size distribution of *EBC* has become increasingly truncated, condition and health status have deteriorated, and sexual maturation has started to occur at increasingly smaller sizes. Despite an increasing trend in recruitment during this period, reduced growth or increased mortality rates after the recruitment phase have resulted in decreasing landing levels and low profitability in the cod fishery, whereas the scientific community has difficulties in disentangling the causes of the decline of *EBC*. We studied changes in metabolic status in *EBC* between the capture years of 1995 and 2015, by investigating two aspects of fish metabolism that can be extracted retrospectively from otolith (earstone) morphometry and nitrogen content. Changes in relative otolith size to fish size are related to the metabolic history of the individual fish, and the otolith nitrogen content reveals the level of protein synthesis and feeding rate. Because otoliths accrue continuously on their surface and are biological stable (inert), the chemical content of the otolith trajectory reflects the timeline of the fish. We measured the N/Ca ratio as a proxy for protein content in *EBC* otolith along distal radius traverses from the core to the edge of the otolith by using secondary ion mass spectrometry (SIMS). Here we show that the otoliths were similar or larger at a given fish size, and the ratio of N/Ca has increased over the studied period. These proxies reveal significant metabolic changes during the same period as the condition, and stock productivity has declined. We discuss potential mechanisms behind the metabolic changes, including elevated temperature and compensatory feeding due to nutrient deficiencies. Such changes in food quality may, in turn, relate to still unrecognized but on-going ecosystem shifts, where climate change could be the ultimate driver.

Keywords: Baltic cod, ecosystem change, food quality, growth, otolith microchemistry, protein uptake

INTRODUCTION

The Baltic Sea is a large, and in geological terms, young (about 9 KY) brackish watershed (**Figure 1**). The restricted number of animal and plant species, often with unique adaptations (Johannesson et al., 2011), reflects its challenging environment. On an intermediate time-scale, the hydrographic development of the Baltic Sea shows large-scale cyclic variation, influenced by the frequency of inflows of saline water from the North Sea (Mohrholz, 2018). Regime shifts due to such climate-induced alternations in the frequency of saltwater inflows from the Atlantic in combination with eutrophication (Savchuk, 2018), may have long-lasting effects on ecosystem structure and function (Österblom et al., 2007; Möllmann et al., 2015). The inflow of salt leads to a stratification of the water column, which may also promote the development of hypoxic conditions in the deepwater, as a consequence of organic matter originating from the surface waters sedimenting to the deeper parts of the sea (Fonselius, 1972). Eutrophication has raised primary productivity of phytoplankton in the Baltic Sea over the last century, leading to more widespread hypoxia (Carstensen et al., 2014; Andersen et al., 2017).

Eastern Baltic cod (*Gadus morhua*; *EBC*) is a former commercially and ecologically important fish stock (e.g., Hammer et al., 2008; Lindegren et al., 2009). *EBC* shows adaptations necessary for reproducing and thriving in the low-saline Baltic Sea environment (Westin and Nissling, 1991; Berg et al., 2015), and is genetically well-separated from the western Baltic cod stocks (Poćwierz-Kotus et al., 2015). The stock is suggested to have benefited from the eutrophication of the Baltic Sea during the 20th century, as its food base was enlarged (Eero et al., 2011). *EBC* biomass increased massively from the 1940s to the beginning of the 1980s. This development was followed by shrinkage of recruitment and biomass due to the loss of spawning sites as the Baltic deepwater became increasingly hypoxic (MacKenzie et al., 2000), leading eventually to lower landings (Eero et al., 2007, 2011). The continued high fishing pressure in the 1990s further reduced the biomass at the remaining spawning area around the island of Bornholm (Hammer et al., 2008).

Improved recruitment and lower fishing effort resulted in a remarkable recovery in abundance in the late 2000s (Köster et al., 2017). However, the enhanced recruitment was not, transformed into higher biomass, as landings continued to wane through the 2000s and 2010s. Body condition and hepatosomatic index (*HSI*) started to decline in the mid-1990s (Eero et al., 2012), while the size distribution of *EBC* became increasingly truncated (Svedäng and Hornborg, 2017) and the fish mature at progressively smaller sizes (ICES, 2019a). Due to the deteriorated status of *EBC*, a fishing ban was eventually introduced in 2019.

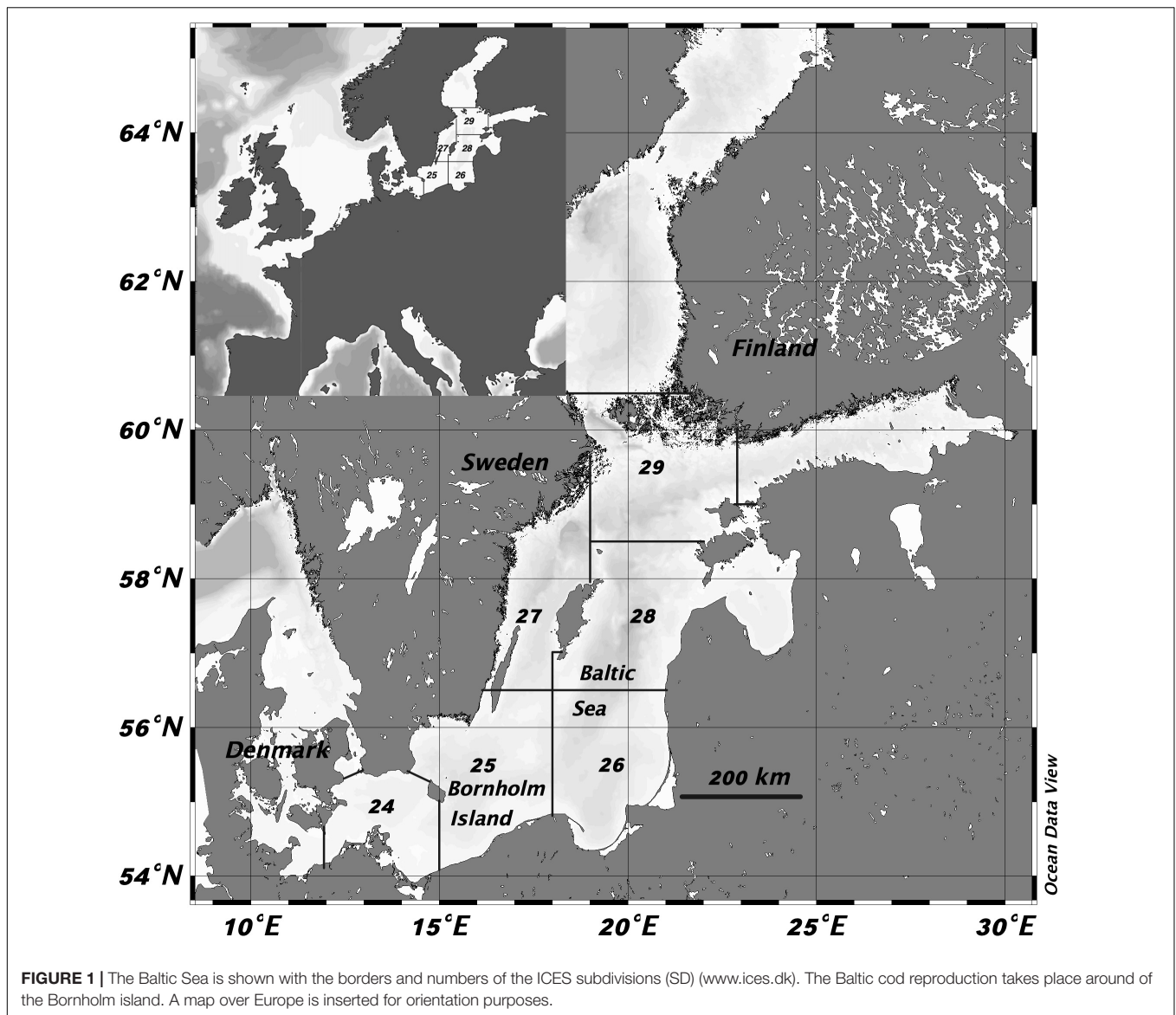
There is no common understanding on why and how the productivity of *EBC* has declined (Eero et al., 2015). Several hypotheses have been put forward connecting the decreased productivity to a reduced feeding rate, which could stem from (a) regionally unbalanced fishing (Eero et al., 2012), (b) density-dependent feeding competition due to selective fishing (Svedäng and Hornborg, 2014, 2017), (c) the spread of hypoxic seafloor

leading to density-dependent growth and lower condition (Casini et al., 2016), and substantially higher natural mortality (Neuenfeldt et al., 2019), or (d) that spread of hypoxic or low oxygen-saturated water may affect metabolism and thereby reducing the rate of digestion and hence food consumption in *EBC* (Limburg and Casini, 2018, 2019; Brander, 2020). Other hypotheses instead point at the increase of seal parasites, impairing growth and health status (e.g., Horbowy et al., 2016), or at the occurrence of thiamin deficiency affecting *EBC* among other Baltic animal populations (Engelhardt et al., 2020).

Reduced individual growth may be one of the proximate causes behind declining landings and truncated size distribution, including decreases in the length class diversity and the mean length in the survey catches between 1995 and 2015 (Eero et al., 2012; Svedäng and Hornborg, 2014, 2017). However, increased opacity and less distinct annuli (similar to the combination of one dark and light growth band observed in cross-section of a tree) have made *EBC* cod otoliths difficult to read (Hüsey et al., 2016). Age readings are, therefore, considered as unreliable by ICES (2015). Consequently, it is still unknown whether growth has declined or not, or if just condition (e.g., Casini et al., 2016) and health status (Statens veterinärmedicinska anstalt [SVA], 2016) has become weaker. Moreover, recent modeling work by ICES (2019b) suggests that exceptional increases in natural mortality might also result in truncated size distributions, assuming in the modeling that individual growth and survival rates are inversely related to each other. Because high, or normal, growth must be weighed against lower survival rates and *vice versa* in the modeling, it is unknown to what degree the truncated size distribution is related to reduced growth or higher natural mortality, or at which stage of the life cycle these processes may have been altered (ICES, 2019a; Neuenfeldt et al., 2019). As most of the proposed factors behind lower productivity in *EBC* suggest metabolic changes, it is crucial to determine when and how such metabolic alterations may have occurred in the stock.

Fisheries biologists take advantage of the morphometric and chemical properties of otoliths (earstones), in particular, the sagitta, which in *Gadus morhua* is the largest of three pairs located in the inner ear of fish (Campana, 1999). Analysis and interpretation of the chemistry of otoliths can inform us about origin and movements, thermal regimes, and salinities faced by fish over their lifetimes (e.g., Elfman et al., 2015). Further, otolith growth/size and opacity patterns can help us to extract records on experienced fish growth, metabolism, and protein uptake (Campana, 1983; Fablet et al., 2011; Pecquerie et al., 2012).

Otoliths are composed of crystalline calcium carbonate (CaCO_3) in aragonite form which is enzymatically precipitated on a protein matrix that controls the shape and growth of the otolith (Söllner et al., 2003). The protein content accounts for 0.45–2.3% of otolith weight (Hüsey et al., 2004). Because otoliths accrue continuously on their surface and are biologically stable (inert), the chemical content of the otolith reflects the timeline as they have grown (Campana, 1999). This accretionary process is a three-dimensional succession of concentric layers of translucent and opaque materials, whose physicochemical properties vary from a daily to a seasonal basis due to endogenous and exogenous factors (Høie et al., 2008 and the references therein). Opaque



zones absorb more light than translucent zones. Opaque zones, therefore, appear dark when viewed with transmitted light and bright when viewed with reflected light, and *vice versa* for translucent zones.

Aragonitic calcium crystals dominate translucent zones, while opaque structures contain a higher amount of protein (Mugiya, 1965; Campana, 1999). Both temperature and metabolism are hypothesized to affect the formation of opaque and translucent zones (Fablet et al., 2011). It has been suggested that the secretion of protein into the endolymphatic sac of the inner ear, and hence the incorporation into the otolith, is proportional to the overall body [global] protein synthesis (Hüssy and Mosegaard, 2004). Rearing experiments on cod have shown that opacity is linked to the feeding level at constant temperature, while at higher temperature the otoliths become more translucent (Høie et al., 2008). In other words, otolith opacity is likely to mirror the level of protein content, but is also affected by changes in thermal

regimes where both increases and decreases lead to less opacity. Translucent structures are formed at low feeding conditions or during seasonal changes in temperature regimes, as a result of the associated decrease of the global protein synthesis and incorporation to otolith (Fablet et al., 2011).

While a relationship between otolith size and fish size is often evoked, for instance, in back-calculation practices, otolith growth is, in fact, seldom proportional to body growth (somatic growth) over time (Mosegaard et al., 1988 and the references therein). Slow, or null somatic growth leads to a decoupling between somatic and otolith growth; even if body growth has ceased due to low/high water temperature or starvation, otolith growth may still occur (Mosegaard et al., 1988; Hüssy and Mosegaard, 2004; Høie et al., 2008). This lack of correspondence is because otolith growth is better ascribed to metabolic activity than somatic growth alone (Mosegaard et al., 1988; Hüssy and Mosegaard, 2004; Fablet et al., 2011; Pecquerie et al., 2012). Otolith growth

could thus be seen as an integral of fish metabolism, which, in turn, are dependent on food intake and the surrounding water temperature (Hüsey and Mosegaard, 2004; Fablet et al., 2011).

Otoliths thus may become bigger in older fish compared to younger at similar body sizes, as they integrate the total metabolism over a more extended period (Mosegaard et al., 1988; Hüsey and Mosegaard, 2004). It means that slow-growing fish have larger otoliths than fast-growing fish *at the same body size*, even though fast-growing fish have a higher otolith growth than slow-growing fish due to a higher instantaneous rate of metabolism (Mosegaard et al., 1988; Campana, 1990). On the other hand, at different temperature regimes but at similar somatic growth rates, otoliths may differ in size between fish of the same body size and age, due to the differences in experienced metabolic history (Mosegaard et al., 1988). However, additional factors may be at play as it seems not possible to describe the relationship between otolith growth and total metabolism as a simple proportionality (Grønkjær, 2016, and the references therein).

Nevertheless, the relationship between otolith and fish size may inform us about the experienced growth and metabolic history considering that there is a close correlation between otolith growth and O₂-consumption (Wright et al., 2001). Fish of similar body size but different otolith size and age have experienced different growth rates. In contrast, fish of the same size and age but with different otolith size may have experienced different metabolic history due to differences in temperature or another factor.

Here, we aim at retrospectively reconstructing the metabolic status of *EBC* by estimating otolith size to fish size and otolith protein content, i.e., how fish growth and global protein synthesis in *EBC* may have changed between the capture years of 1995 and 2015, as the productivity of *EBC* deteriorated. We thereby assume that the otolith N-content reflects the protein feeding rate (Hüsey and Mosegaard, 2004). We also intend to investigate whether the observed increased opacity in *EBC* otoliths is due to increased protein content. Thus, instead of using the indirect proxy on otolith protein content by estimating opacity, we chose to measure the ratio between nitrogen (N) and calcium (Ca) as a better and a more direct proxy on otolith protein content.

By obtaining new insights into *EBC* metabolism and feeding rates over the period between the capture years of 1995 and 2015, we may confront hypotheses that relate the reduced productivity of *EBC* to lower feeding rates (Eero et al., 2012; Svedäng and Hornborg, 2014, 2017; Casini et al., 2016; Limburg and Casini, 2018, 2019; Karlson et al., 2019; Neuenfeldt et al., 2019; Orio et al., 2019; Brander, 2020). Finally, by finding *when* a shift in feeding rates has occurred, it is also possible to evaluate if the spread of seal parasites is a potential driver behind changed metabolism in *EBC* (e.g., Horbowy et al., 2016).

MATERIALS AND METHODS

The Study Material

This project studied fish metabolism in *EBC* retrospectively by exploring stored sagittal otoliths, through analyzing the nitrogen

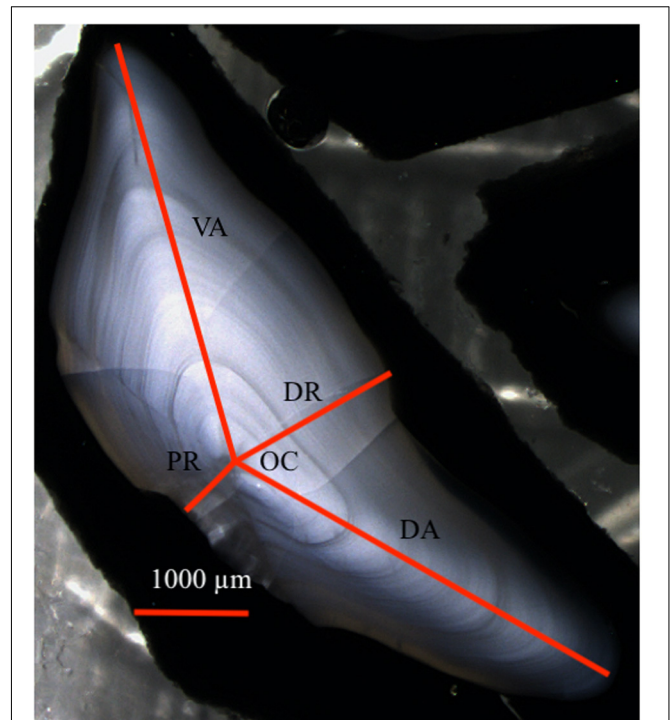


FIGURE 2 | The measured morphometry of the otolith. OC – otolith core, DA – dorsal axis, VA – ventral axis, DR – distal radius, PR – proximal radius.

(N) content and measuring otolith size relative to fish size. It thereby allowed us to follow both individual growth in relative terms and protein synthesis, which we assumed to be proportional to feeding rate over the studied period (Hüsey and Mosegaard, 2004). We used otoliths from the regular Swedish part of Baltic International Trawl Survey (BITS) in ICES subdivision (SD) 25 in the southern Baltic Sea (**Figure 1**), saved for age determination purposes (ICES, 2012). Information on date of capture, depth, fish length, weight, gonad developmental status, estimated age was obtained from the Swedish BITS (**Supplementary Table S1**). Since we wanted to study changes in feeding rates, we tried to avoid unnecessary variance in growth by choosing otoliths from fish that were similar at size-at-age. In total, we selected 100 otoliths from fish divided into 20 otoliths per the following capture years: 1995, 2000, 2005, 2010, and 2015 at the nominal age groups 2–5, i.e., five per age group and capture year (**Supplementary Table S2** and **Supplementary Figure S1**). However, age was only treated as a nominal factor in the analyses, since age readings are no longer considered to be valid for *EBC* (ICES, 2015). All otoliths were sectioned in the transverse plane by micro-saw. The cut through the core to the edge represents the timeline of the fish; its first part forms during embryonic stage before hatching and the otolith then grows continuously until death. The study is thus investigating the relative otolith N-content retrospectively up to 5 years before capture.

The otolith sections were polished, cast in groups of four into 25 mm epoxy pucks, polished once more, and coated with a 30 nm layer of gold (Au) to prevent charging during the

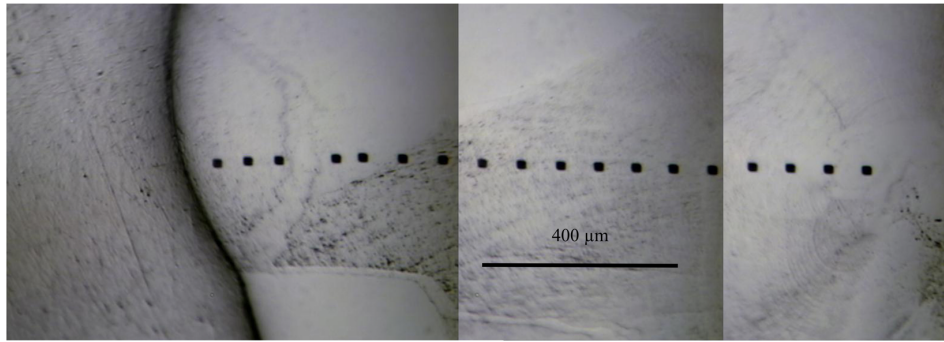


FIGURE 3 | A merged photo of an otolith showing the spots taken on a transverse from the core along the distal radius toward the edge.

analysis process. The morphometrics of the prepared otoliths were measured (**Figure 2**). The width of the otolith (*OW*), i.e., the sum of the dorsal axis from the core to edge (*DA*), and the ventral axis in the opposite direction (*VA*) is a consistent way of measuring otolith size (Mosegaard et al., 1988). The ratio between otolith and fish length (*OW/FL*) was used to normalizing otolith size relative to fish size and thereby to compare otolith growth between individuals and year classes.

The Otolith Chemistry Analysis

We carried out the otolith N/Ca analysis at the Vega Centre, the Swedish Museum of Natural History, using a large geometry CAMECA ims1280 secondary ion mass spectrometer (SIMS) at the NordSIMS Laboratory, Stockholm. A critically focused, *circa* 0.6 nA Cs⁺ primary beam with 20 kV impact energy was utilized to sputter the otoliths, with a 10 μm raster applied to homogenize the beam density over the analyzed area. A low energy, normal incidence electron flooding gun was used to counteract charge build-up on the sample surface. Secondary ions were admitted to the mass spectrometer using a high-transmission transfer lens configuration and analyzed at a mass resolution ($M/\Delta M$) of 8000. The two ionic species $^{12}\text{C}^{14}\text{N}^-$ and $^{40}\text{Ca}^-$ were measured in a single, low noise ion counting electron multiplier, by peak switching between the two masses, with integration times of 2 and 4 s, respectively. Analyses were performed in automated sequences comprising a 20 μm pre-sputter to remove the gold coating, centering of the secondary ion beam in the field aperture, and optimization of the mass calibration, followed by 12 cycles of data acquisition.

We determined the $^{12}\text{C}^{14}\text{N}^-/^{40}\text{Ca}^-$ ratios along a traverse at the distal radius (*DR*) from the otolith core to the edge of each studied otolith, i.e., perpendicular to the transversal axis and to the opaque and translucent ring structures (**Figure 3**). The distance was measured in μm, starting at the core while ending at the last spot along the transect. Deviations from *DR* occurred due to cracks and irregularities in the otolith, which have been accounted for. In total, 55 otolith were analyzed for the $^{12}\text{C}^{14}\text{N}^-/^{40}\text{Ca}^-$ ratios (**Table 1**). The otoliths were selected to obtain an even coverage of all age groups by year of capture within the constraints of casting the otoliths together on the epoxy pucks.

The small-scale of the analyzed area (ca. 10 μm in diameter), allowed us to measure ~15 spots along each traverse (**Table 1**). Assuming a relatively constant C and Ca content in the otoliths, given that these are crystallographic matrix elements, the $^{12}\text{C}^{14}\text{N}^-/^{40}\text{Ca}^-$ ratio acts as a proxy for relative N concentration. The precision on the measured $^{12}\text{C}^{14}\text{N}^-/^{40}\text{Ca}^-$ ratio was generally better than 5% (1s), typically averaging around 1%, which translates directly to the precision in relative N concentration. The absence of a calibrated reference material for determining N concentration in CaCO₃, however, precludes converting this ratio to an accurate N concentration in the otolith targets. Still, the relative variations both between analyses along each traverse and between otoliths will be valid. For this reason, we henceforth use the term “N-content,” to discuss our results, either as N-content per spot (*NPS*) or as mean value per otolith for one or more ontogenic stages (see below).

Ontogenic Stages

Since we wanted to study changes in protein uptake over time, it is important to discount for the effects of ontogenic changes from year effects, because opacity is usually highest at the core and then declines slowly (Pecquerie et al., 2012). The spots measurements were assigned into four different ontogenic stages (egg, larval, juvenile, and adult) according to the distances from the core since *DR* represents an otolith timeline. The egg stage was defined according to the radius of the sagitta at hatching, i.e., <15 μm (e.g., Geffen, 1995). *OW* at settling in Baltic cod has been estimated to ~1000 μm (Fey and Linkowski, 2006). The relative proportion of *DR* to *OW* was established, using on the studied otolith material (mean ± s.d.: 22 ± 2.7%; $n = 98$). The larval stage or pelagic phase until settling was thus defined by the distance from core between 16 and 220 μm. The division between juvenile and adult stages was arbitrarily set at 900 μm (data not shown) since *DR* of studied cod at ages 2 and 3 fluctuated around this size, which is the age at maturity in *EBC* (ICES, 2019b), i.e., the size intervals of the juvenile and adult stages were set at 221–899 and ≥900 μm, respectively. Since we assumed that the otolith accretion was similar over time, while the relative otolith size to fish size found to become smaller, this procedure may have exaggerated the juvenile stage for latter years of capture.

We noted that *NPS* varied strongly for the ontogenetic stages 1 and 2 (egg and larval), displaying high and low values (Table 2). The low values may be due to the transverse cut that had not gone through the structure or that the first spot may have been put outside the nucleus. Because of these methodological issues connected with the ontogenic stages 1 and 2, we excluded them from the mixed linear analysis.

Condition Factor

We estimated changes in fish body condition by analyzing the development in Fulton’s condition factor *CF* (Ricker, 1975):

$$CF = W/L^3 \times 100 \tag{1}$$

where *W* is the weight of the fish in gram, and *L* is the length in cm.

Statistical Analysis

To analyze changes in the N-content per spot (*NPS*) over time, we applied linear mixed models to account for the hierarchical structure in our study. Because the N-content was measured repeatedly on the same otoliths, *NPS* was nested within otoliths. Since the distances from the otolith core were used to assign individual *NPS* to an ontogenetic stage, the otoliths were also nested within the ontogenetic stage. Only two ontogenetic stages were included: 3 (juvenile) and 4 (adult). We, therefore, chose to test four models, which assumed either (a) random intercept with fixed mean (i.e., models 1 and 3), (b) intercept varying among the grouping factor 1 (ontogenetic stage) and grouping factor 2 (otolith) within grouping factor 1 (i.e., models 2 and 4). *NPS* were logarithmically transformed to attain better approximation to normality. Also, we included models with and without covariates, including interaction terms. The following formulations were thus tested by using “lme4” package for R (Bates et al., 2015):

1. $NPS \sim Year + (1 | Otolith)$
2. $NPS \sim Year + (1 | Ontogenic\ stage/Otolith)$
3. $NPS \sim Year + Ontogenic\ stage + (1 | Otolith)$
4. $NPS \sim Year + Ontogenic\ stage + (1 | Ontogenic\ stage/Otolith)$

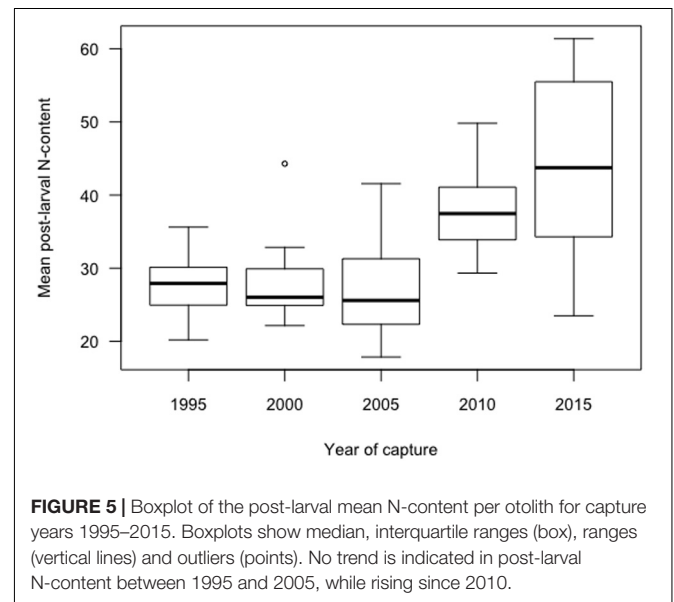
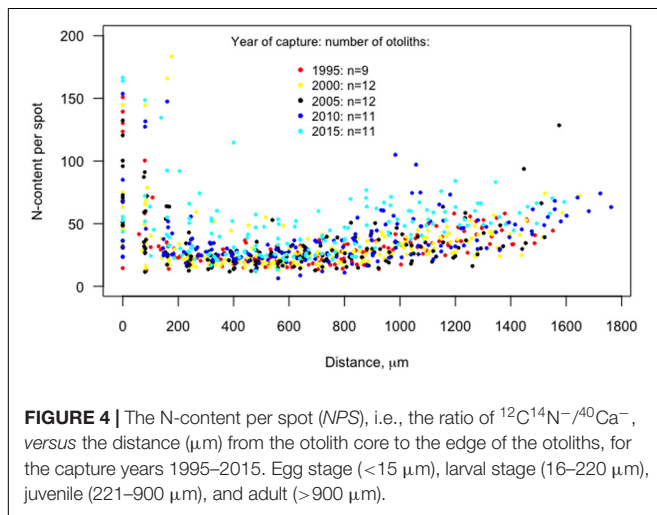
where *Year* is the year of capture, which was considered as a fixed factor. *Ontogenic stage* is life history stage (juvenile or adult) defined by the distance from the otolith core to the spot measured was considered as both covariate (treated as a continuous variable) and grouping factor, and *Otolith* was the grouping variable representing different otoliths. It should be observed that the “lme4” package for R provides no *p*-value in ANOVA tests (Bates et al., 2015), but are indicative regarding the relative importance of different sources of variation. To test for differences between individual years while taking the repeated measurements into account, we used the Tukey HSD *post hoc* test after applying sequential Bonferroni correction, using the “multcomp” package for R (Hothorn et al., 2008). The information criteria AIC and BIC were used to select the best models (Wang and Liu, 2006 and the references therein). It should also be observed that the relatively small sample

TABLE 1 | Number of otoliths, mean number (± s.d.) of spots per otolith, by the year of capture and nominal age group.

Year	Age 2			Age 3			Age 4			Age 5		
	No otoliths	Mean number of spots ± s.d.	No otoliths	Mean number of spots ± s.d.	No otoliths	Mean number of spots ± s.d.	No otoliths	Mean number of spots ± s.d.	No otoliths	Mean number of spots ± s.d.	No otoliths	Mean number of spots ± s.d.
1995	1	14.0 ± 0	4	18.0 ± 1.6	3	17.3 ± 1.2	1	15.0 ± 0	9	15.0 ± 0	1	15.0 ± 0
2000	1	12.0 ± 0	5	15.8 ± 2.3	2	16.5 ± 2.1	4	18.8 ± 4.2	12	18.8 ± 4.2	4	18.8 ± 4.2
2005	4	12.8 ± 2.6	2	14 ± 0	2	15.5 ± 0.7	4	18.3 ± 1.7	12	18.3 ± 1.7	4	18.3 ± 1.7
2010	2	14 ± 2.8	4	9 ± 1.2	2	17.0 ± 4.2	3	20.3 ± 3.1	11	20.3 ± 3.1	3	20.3 ± 3.1
2015	3	10.7 ± 1.5	1	13.0 ± 0	4	13.0 ± 3.6	3	19.0 ± 1.7	11	19.0 ± 1.7	3	19.0 ± 1.7
Total no/mean s.d.	11	12.7 ± 1.4	16	14.0 ± 3.4	13	15.9 ± 1.7	16	18.3 ± 2.0	55	18.3 ± 2.0	16	18.3 ± 2.0

TABLE 2 | The mean (\pm s.d.), minimum and maximum values, and c.v. of *NPS* per year of capture and ontogenic stage.

Ontogenic stage	Year of capture				
	1995	2000	2005	2010	2015
(1) Egg stage: mean \pm s.d.	94.0 \pm 55.4	65.8 \pm 37.1	72.9 \pm 32.9	60.3 \pm 47.4	96.4 \pm 63.0
Min-max	14.5–150	34.9–145	30.7–132	23.4–154	43.5–166
c.v.	59%	56%	45%	79%	65%
(2) Larval stage: mean \pm s.d.	35.8 \pm 21.4	50.7 \pm 48.7	42.1 \pm 22.7	54.4 \pm 43.1	58.0 \pm 37.1
min-max	12.5–100	13.3–183	11.5–91.1	24.6–148	22.6–149
c.v.	60%	96%	54%	79%	64%
(3) Juvenile stage: mean \pm s.d.	22.7 \pm 5.6	24.4 \pm 8.9	23.6 \pm 8.1	27.6 \pm 9.0	37.3 \pm 17.1
min-max	13.5–37.1	12.6–59.4	11.7–52.9	6.4–55.6	12.2–115
c.v.	24%	37%	34%	33%	46%
(4) Adult stage: mean \pm s.d.	38.4 \pm 10.2	36.9 \pm 10.6	37.2 \pm 20.1	47.4 \pm 18.3	55.9 \pm 11.8
min-max	13.8–58.1	22.3–73.9	14.6–128	19.9–105	30.6–84.1
c.v.	27%	29%	54%	39%	21%



size in this study may lead to type 2 error, i.e., the power to detect a change is weak, leading to the non-rejection of a false null hypothesis.

For analysis of the otolith N-content in relation to *FL* and *CF*, we applied linear regression modeling by using the “stats v3.6.2” package for R¹. As a dependent variable, the arithmetic mean value of the post-larval N-content of all spots per otolith was used, i.e., except for the egg and larval stages. These spots were excluded due to the large variability in the estimated relative N-content (Table 2 and Figure 4).

To analyze the changes in ratio *OW/FL* over time, we applied an ANOVA model with fixed effects for *Year* (model 1) by using the “stats v3.6.2” package for R (see text footnote 1). The response variable was logarithmically transformed, to attain better approximation to normality. To test for differences between individual years, we used the Tukey HSD *post hoc* test after applying sequential Bonferroni correction.

¹<https://www.R-project.org>

RESULTS

The Otolith N-Content

The accuracy of our estimations of the $^{12}\text{C}^{14}\text{N}^-/^{40}\text{Ca}^-$ ratios was supported by the fact that the Ca-content over the transverse was stable with a mean coefficient of variation (c.v.) per otolith of $2.7\% \pm 3.0$ (Supplementary Table S3 and Supplementary Figure S2), while the N-estimation showed more than 20 times higher variability (mean c.v. = $51\% \pm 23$). The N-content per spot (*NPS*) varied systematically between ontogenic stages over time (Figure 4 and Table 2). *NPS* varied strongly for the ontogenic stages 1 and 2 (egg and larval), displaying the highest recorded *NPS* (the maximum value of 166 for stage 1 in 2015) but also low values (e.g., the minimum value of 14.5 for stage 1 in 1995, and 11.5 for stage 2 in 2005). Because of methodological issues connected with the ontogenic stages 1 and 2 that may have given

TABLE 3 | The comparison between different mixed linear regression models with post-larval *NPS* as the dependent variable and *Year* and *Ontogenic stage* as fixed factors and *Otolith* and *Ontogenic stage* as grouping variables.

	DF	AIC	BIC	logLik	deviance	Chisquare	Df	Pr(> Chisq)
Model 1	7	648.89	680.88	-317.45	634.89			
Model 2	8	250.08	286.63	-117.04	234.08	400.818	1	<2.2e-16 ***
Model 3	8	294.27	330.83	-139.14	278.27	0.000	0	1
Model 4	9	242.17	283.29	-112.08	224.17	54.107	1	1.899e-13 ***

Models 1 and 3 assume a random intercept with fixed mean, whereas models 2 and 4 assume intercepts varying among the grouping factor 1 (*Ontogenic stage*) and grouping factor 2 (*Otolith*) within grouping factor 1. Models tested: (1) $NPS \sim Year + (1 | Otolith)$ (2) $NPS \sim Year + (1 | Ontogenic\ stage/Otolith)$ (3) $NPS \sim Year + Ontogenic\ stage + (1 | Otolith)$ (4) $NPS \sim Year + Ontogenic\ stage + (1 | Ontogenic\ stage/Otolith)$ Significance codes: "****" 0.001 "." 0.1 " 1.

rise to this variation, we excluded them from the mixed linear analysis.

For ontogenic stage 3 (juvenile) and, in particular, stage 4 (adult), the mean value of *NPS* (increasing over the study period from 22.7 to 37.3 for stage 3, and from 38.4 to 55.8 for stage 4) showed an increasing trend since the capture year of 2010, i.e., the increase in N-content started before 2010 (Figure 4 and Table 2). The median of the mean post-larval N-content per cod otolith per year of capture were scattered below 30 before 2010, while increased in 2010 to around 38 (Figure 5). In the following capture year, 2015, the variability in mean post-larval N-content, was also high.

The comparison between different mixed linear regression models with *NPS* as the dependent variable showed the best fit for model 4, where the intercepts are assumed to be varying among the grouping factor 1 (*Ontogenic stage*) and grouping factor 2 (*Otolith*) within grouping factor 1 (Table 3). This model indicated that the *Ontogenic stage* as a grouping factor was the dominating source of variation for the random effects, while the fixed effects of *Year* were significantly more important than *Ontogenic stage* (Table 4). Pairwise comparisons (Tukey multiple comparisons of means) between years, confirmed that *NPS* was lowest at the start of the time series in 1995 (mean: 22.7 and 38.4 for stages 3 and 4, respectively) and highest in 2015 (mean: 37.3 and 55.9 for stages 3 and 4, respectively), and that a shift in N-content occurred between the capture years of 2005 and 2010 (from 37.2 to 47.4 for stage 4, Figure 5 and Table 2).

There was no trend in the mean post-larval N-content with increasing fish length for the period 1995–2005, while the mean N-content was positively related to fish length for the capture years 2010–2015 (Figure 6). The mean post-larval N-content showed no correlation with the condition factor for neither the whole time series nor the separate periods of 1995–2005 (mean \pm s.d.: 28.4 ± 5.9) and 2010–2015 (mean \pm s.d.: 40.2 ± 9.2 , Figure 7).

Morphometrics

The otoliths tended to become bigger over time, i.e., the ratios between otolith width (*OW*) and fish length (*FL*) increased marginally between the start and the end of the study period (Table 5, Figure 8). This pattern was more marked in younger age groups. All otoliths exhibited a similar relationship between *OW* and *FL* (Figure 9). ANOVA results

and pairwise comparisons (Tukey multiple comparisons of means) indicated that *OW/FL* increased over time, as the ratios were significantly larger in 2010 compared to 1995 (Table 6).

The two proxies used to study metabolic changes, *OW/FL* and mean post-larval N-content, were found not to be related (linear regression, $p = 0.34$, adjusted $r^2 = 0.02$, Supplementary Figure S3).

DISCUSSION

This study on the metabolic status of *EBC* shows that the N-content per spot (*NPS*) has increased since the end of the 2000s and that the otolith sizes relative to fish size (*OW/FL*) became smaller since the mid-2000s. These observations show that significant metabolic changes occurred during the same period as when the productivity of *EBC* declined dramatically (ICES, 2019a). Given that the otolith N-content is a reliable measure of the otolith protein content (Mugiya, 1965; Campana, 1999), our results suggest a marked increase in protein content between 2005 and 2010. This observation may explain why *EBC* otoliths have become opaquer and hence less readable around 2010 (Hüssy et al., 2016) since protein-rich otoliths are opaquer than protein-poorer ones (c.f. Fablet et al., 2011, and references therein). Such change in otolith protein content indicates a shift in internal global protein synthesis, which depends on individual feeding rates (Hüssy and Mosegaard, 2004).

We also found for the period between 1995 and 2005 that the *NPS* was highest at the core, and then, after a sharp decline, slowly increased through ontogeny. This observation is partly similar to what is commonly observed in the development of otolith opacity over the ontogeny (Pecquerie et al., 2012), where opacity is highest at the core and then declines slowly. However, here we found that the pattern in *NPS* over the life-time of the fish has become much more erratic since the end of the 2000s, which signals metabolic disturbances.

Records on in this study suggests that *OW/FL* in *EBC* have been rather constant or increased marginally. However, otoliths could also become smaller due to decreased metabolic rates even though they are expressing identical somatic growth rates (Mosegaard et al., 1988). Experimental studies on Atlantic cod and Atlantic salmon (*Salmo salar*) suggest that proportionality between otolith size and somatic size may not always be affected

TABLE 4 | Summary of linear mixed regression model for logarithmically transformed post-larval NPS with year of sampling (Year) and Ontogenic stage as fixed factors.

Linear mixed model fit by REML:

Groups	Name	Variance	Std. Dev.
Random effects:			
Otolith: Ontogenic stage	(Intercept)	0.05061	0.2250
Ontogenic stage	(Intercept)	15.81817	3.9772
Residual		0.06189	0.2488

	Estimate	Std. Error	t-value
Fixed effects:			
(Intercept)	1.66210	19.88684	0.084
Year_2000	-0.00967	0.07720	-0.125
Year_2005	-0.04752	0.07825	-0.607
Year_2010	0.23593	0.07922	2.978
Year_2015	0.40992	0.08023	5.109
Ontogenic stage	0.48321	5.62483	0.086

	Df	Sum Sq	Mean Sq	F value
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Analysis of Variance:

Year	4	3.2635	0.81587	13.1816
Ontogenic stage	1	0.0005	0.00046	0.0074

Tukey multiple comparisons of means (Tukey HSD, test = adjusted with Bonferroni correction for the linear mixed regression model:

$$NPS \sim Year + Ontogenic\ stage + (1 | Ontogenic\ stage/Otolith).$$

	Estimate	Std. Error	z value	Pr(> z)
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Linear Hypotheses:

2000-1995 == 0	-0.00968	0.07720	-0.125	1.00000
2005-1995 == 0	-0.04752	0.07825	-0.607	1.00000
2010-1995 == 0	0.23593	0.07922	2.978	0.02898*
2015-1995 == 0	0.40992	0.08023	5.109	3.23e-06***
2005-2000 == 0	-0.037848	0.07329	-0.516	1.00000
2010-2000 == 0	0.24560	0.07435	3.303	0.00955**
2015-2000 == 0	0.41959	0.07540	5.565	2.63e-07***
2010-2005 == 0	0.28345	0.07542	3.759	0.00171**
2015-2005 == 0	0.45744	0.07642	5.986	2.15e-08***
2015-2010 == 0	0.17399	0.07747	2.246	0.24714

Number of observations: 713, groups: Otolith: Ontogenic stage, 106; Ontogenic stage, 2. Significance codes: 0 '***' 0.001; '**' 0.01; '*' 0.05; '.' 0.1; ' ' 1. The intercepts are assumed to be varying among the grouping factor 1 (Ontogenic stage) and grouping factor 2 (Otolith) within grouping factor 1, according to the model: $NPS \sim Year + Ontogenic\ stage + (1 | Ontogenic\ stage/Otolith)$. p-values are not given for fixed effects, nor for the ANOVA results, t-values and F-values are, therefore, to be seen as indicative (Bates et al., 2015). Pairwise comparisons between means of Years (Tukey HSD, the test is adjusted with Bonferroni correction for the linear mixed regression model) are presented with p-values.

by experienced differential energy allocation patterns (Aubin-Horth and Dodson, 2002; Høie et al., 2003).

Since we have not chosen fish in proportion to their frequency in the catch size distribution, it is not possible to tell whether individual growth on a population level has been stable or not. On the other hand, estimates based on accurately aged material comprising ages 1–3 as well as length-frequency analysis, support

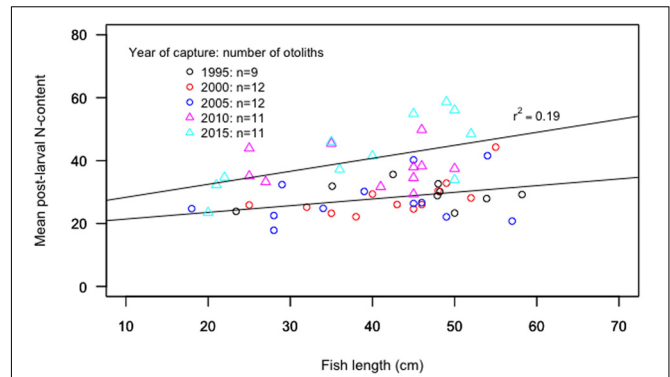


FIGURE 6 | The mean value of the post-larval N-content per otolith in relation to fish length (cm) for all sampled cod. There is a significant increase in mean value of N-content in relation to fish length for the period 2010–2015 ($p < 0.05$, $r^2 = 0.19$), while not for the previous period 1995–2005.

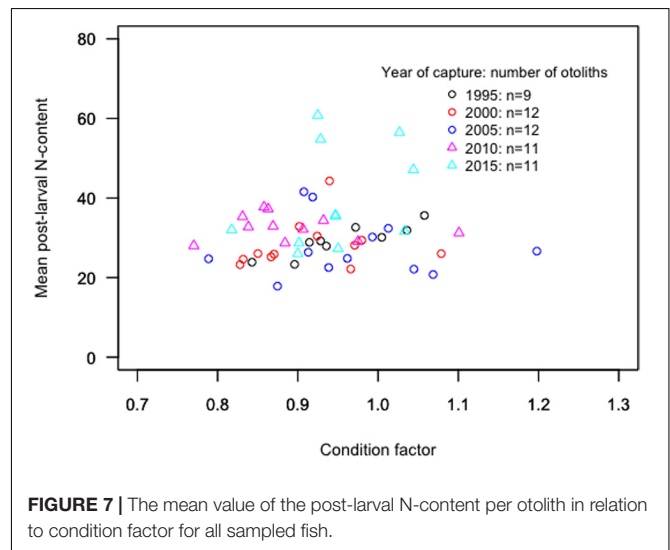


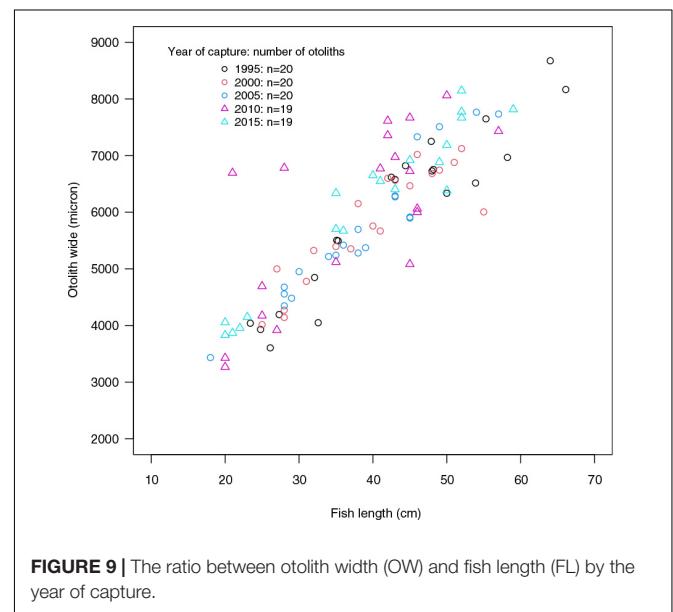
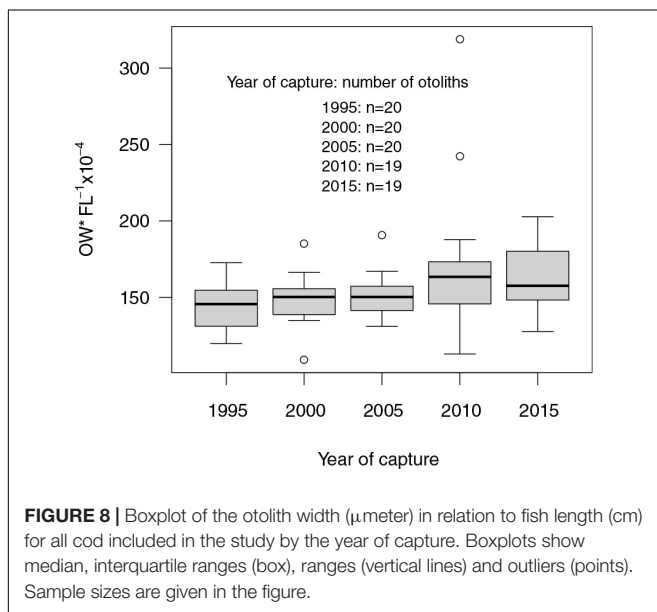
FIGURE 7 | The mean value of the post-larval N-content per otolith in relation to condition factor for all sampled fish.

the view of moderately reduced individual growth in EBC over the last two decades (Hüssy et al., 2018). Also, the continued decline in the length class diversity and the mean length in the survey catches between 1995 and 2015 indicates lower individual growth (Svedäng and Hornborg, 2017) or increased natural mortality for fish above 30 cm (c.f. ICES, 2019a). Therefore, the present observations indicate reduced individual growth rates rather than higher metabolic rates.

Even though most observations support a decreased rather than increased individual growth in EBC, the changes in the maturation pattern indicate the opposite. Over the last 30 years, EBC has started to mature at increasingly smaller sizes; the maturity ogive, i.e., the size at which 50% of the stock attains maturity, has decreased from 40 to 20 cm (ICES, 2019a). Other studies have shown that faster growth, or an increased energetic surplus (Rowe and Thorpe, 1990), may lead to earlier maturation both in the wild and in reared cod (Hüssy et al., 2018). It is hence possible that the observed early maturation in EBC has been induced by growth retardation in size groups around 20 cm,

TABLE 5 | The mean (\pm s.d.), minimum, and maximum values of the ratio between otolith width and fish length (*OW/FL*) are shown by year of capture.

Age	Year of capture				
	1995	2000	2005	2010	2015
Age 2: mean \pm s.d.	149.4 \pm 18.7	163.1 \pm 15.1	166.1 \pm 14.7	201.7 \pm 66.1	187.7 \pm 9.6
Min-max	124–172	153–185	154–191	163–319	180–203
Age 3: mean \pm s.d.	143.6 \pm 14.7	154.0 \pm 10.3	149.0 \pm 11.2	155.7 \pm 50.2	157.3 \pm 6.0
Min-max	121–157	144–166	138–165	113–242	149–163
Age 4: mean \pm s.d.	147.7 \pm 7.9	149.1 \pm 7.5	141.8 \pm 9.8	162.9 \pm 19.7	157.0 \pm 16.4
Min-max	138–156	138–157	131–151	130–181	140–181
Age 5: mean \pm s.d.	126.3 \pm 8.2	131.6 \pm 12.6	147.6 \pm 9.1	152.9 \pm 17.3	142.9 \pm 12.7
Min-max	120–136	109–139	136–159	130–170	128–157



i.e., in size groups whose condition has remained stable (Casini et al., 2016).

Our results give new insights to the decreasing productivity in *EBC* in the whole southern distribution area in the Baltic Sea, manifesting as lower body condition (Casini et al., 2016), an increasing truncated size distribution (Svedäng and Hornborg, 2017), poor health status (Statens veterinärmedicinska anstalt [SVA], 2016), and dwindling landings (ICES, 2019a). We found that the N-content was higher in the capture years from 2010 than up to 2005 for both the juvenile and adult stages, at the same time as *EBC* became increasingly unproductive (Eero et al., 2012; Svedäng and Hornborg, 2014; ICES, 2019a). From 2010 the mean post-larval N-content tended to increase with fish size and was higher in the adult compared to the juvenile stage. If the increased N-content is indicative of an increased feeding rate, it is clear that the feeding rate has increased mostly in larger and older fish. Thus, the results do not support the hypothesis that the lower productivity of *EBC* is related to food limitations (e.g., Eero et al., 2012; Svedäng and Hornborg, 2014, 2017; Casini et al., 2016; Karlson et al., 2019; Neuenfeldt et al., 2019; Orio et al., 2019).

Neither does our study corroborate the conjectures of lower metabolic rates due to physiological stress, as a consequence of encounter hypoxic or low oxygen-saturated water (Limburg and Casini, 2018, 2019), reducing the rate of digestion and hence food consumption in *EBC* on a systemic level (Brander, 2020).

Another possible mechanism behind the change in metabolism is changed in food quality rather than quantity. Analyses of cod stomach data suggest substantial changes in dietary composition of *EBC* during the time period from 1990 to 2013, including an increased proportion of sprat (*Sprattus sprattus*) and slightly decreased proportion of herring (*Clupea harengus*; Kulatska et al., 2019). Because the sprat abundance increased over the period while the herring stock declined, Kulatska et al. suggested that *EBC* showed a strong preference for herring over sprat. Moreover, according to the “food-quality-limitation hypothesis” (Litzow et al., 2006), a climate-induced shift in prey for pelagic fish may have occurred in the Baltic Sea. Food quality changes are one of the few agents to affect *EBC* in a systemic way, which is in line with the observed change throughout the area (c.f. Gorokhova, 2019, and the references

TABLE 6 | Summary of ANOVA results for logarithmically transformed *OW/FL*-values with year of sampling (*Year*) as fixed factor.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
<i>Year</i>	4	0.2640	0.0660	3.37	0.0123
Residuals	93	3.404	0.0366		
Fixed effects (<i>Year</i>)					
Factor	1995	2000	2005	2010	2015
	-0.068	-0.032	-0.015	0.074	0.048
repetition	20	20	20	19	19

Table of Tukey multiple comparisons of means (Tukey HSD, test = adjusted (type = "Bonferroni")) for ANOVA model: $\log(OW/FL) \sim Year$.

Year	Difference	Lower C.I.	Upper C.I.	p-level adjusted
2000–1995	0.03581827	-0.087341857	0.1589784	0.9272270
2005–1995	0.05337794	-0.069782182	0.1765381	0.7480666
2010–1995	0.14180742	0.017037289	0.2665775	0.0176232
2015–1995	0.11560078	-0.009169351	0.2403709	0.0829408
2005–2000	0.01755968	-0.105600450	0.1407198	0.9946908
2010–2000	0.10598915	-0.018780979	0.2307593	0.1347923
2015–2000	0.07978251	-0.044987618	0.2045526	0.3919744
2010–2005	0.08842948	-0.036340654	0.2131996	0.2879126
2015–2005	0.06222284	-0.062547294	0.1869930	0.6372285
2015–2010	-0.02620664	-0.152566262	0.1001530	0.9781359

Pairwise comparisons between means of *Year* are tested with Tukey HSD (adjusted with Bonferroni correction for the ANOVA model). Significance codes: 0 '****' 0.001 '***' 0.01 '**' 0.05 '*' 0.1 '.' 1.2 observations deleted due to missingness. 95% confidence level of is shown.

therein). Such changes in the food chain are most likely related to large-scale ecosystem changes, where primary producers are likely to play a critical role as producers of essential bio-molecules (Sylvander et al., 2013). Variability in phytoplankton production, such as increased cyanobacteria blooms, could hence be a crucial factor (Kahrúa et al., 2020). Alternations in phytoplankton production may then affect the transfer of essential biomolecules in the food-chain (Sylvander et al., 2013; Snoeijs and Häubner, 2014; Fridolfsson et al., 2018). The eutrophic status of the Baltic Sea may be a prerequisite for the present intense cyanobacteria blooms (Andersen et al., 2017). However, increased summer temperatures due to climate warming could be the actual driver (Kahrúa et al., 2020).

Engelhardt et al. (2020) proposed that the declining condition and health status in *EBC* are due to thiamin deficiency, as the thiamin content declined with fish size in a recent investigation on *EBC*. However, to avoid circularity, it remains to be shown whether the thiamin content in *EBC* deviates from a regular pattern, or whether the lower thiamin content could be due to some other perturbation, i.e., rather the effect than the cause of the *EBC* impaired condition. It is nonetheless reasonable to suggest that *EBC* could be suffering from lower food quality than previously encountered, i.e., it is limited by a complex molecule which it cannot produce on its own. The individual fish could then be trying to compensate for such a limiting

nutrient by increasing feeding rates (c.f. Soengas, 2014). Higher feeding rates would hence lead to initially higher growth rates if the essential nutrient requirements are fulfilled by enhanced feeding, or higher energetic status, but could eventually also lead to increased natural mortality. The increasingly truncated size distribution would then be due to increased natural mortality (c.f. Neuenfeldt et al., 2019), in size groups above 30 cm, inflicted by malnutrition.

Changes in thermal regime may affect otolith growth even at similar individual [somatic] growth rates (Mosegaard et al., 1988). Otoliths may become relatively larger at both low and high suboptimal temperatures in comparison to more optimal temperatures (Hüssy and Mosegaard, 2004; Fablet et al., 2011). The progressively higher water temperature in the Baltic Sea since the mid-1990s due to climate change (Kniebusch et al., 2019) may have led to a thermal habitat with potential to affect *OW/FL*. However, metabolic changes may not always result in changed proportionality between otolith and somatic growth (Aubin-Horth and Dodson, 2002).

In conclusion, since we cannot find that N-content has declined, nor that condition is related to the N-content, this study gives no evidence that lower feeding rates is the prevailing driver behind the decline in *EBC* productivity, either by food limitation (Eero et al., 2012; Svedäng and Hornborg, 2014, 2017; Casini et al., 2016; Orio et al., 2019) or physiological stress (Limburg and Casini, 2018, 2019; Brander, 2020). Furthermore, because these metabolic changes occurred well before the prevalence of seal parasites became higher (e.g., Horbowy et al., 2016), then these changes in metabolism in the *EBC* cannot be attributed to higher abundance of gray seals (*Halichoerus grypus*) in the southern Baltic Sea. Further studies are needed to understand better the driver of the higher feeding rate and how it is connected to the decreased productivity of the *EBC*. The study raises several questions on N-content and *OW/FL* relationships in fish stocks where thermal and nutritional regimes have changed, which may be interesting to explore in other fish stocks.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The sampling of fish presented in this study followed the Swedish legislation and the regulations laid down by the Swedish Board of Agriculture. Ethical permit was given by the Gothenburg Animal Ethical Committee (license number 126-2015).

AUTHOR CONTRIBUTIONS

HS coordinated the study, designed the research, and analyzed the data. HS, VT, and AP prepared the otolith material. MW designed and conducted the otolith chemistry analysis. HS, SW,

and MW wrote the manuscript. All authors contributed to the article and approved the submitted version.

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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.2020.00565/full#supplementary-material>

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Conflict of Interest: 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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