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Original Article

Age validation of juvenile cod in the Western Baltic Sea

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The methods routinely used to estimate fish age are often un-validated and susceptible to errors and uncertainties. Despite numerous attempts, age determination of western Baltic cod (WBC, *Gadus morhua*) using otoliths is still unreliable, predominantly due to inconsistent interpretation of the first translucent zone (TZ). Length-frequencies of undersized (<38 cm) cod collected during 2013–2016 from pound nets near Fehmarn Island were analysed to understand TZ formation patterns. A clear minimum separated two cohorts within the length-frequency samples every year. The length-frequency information was combined with otolith edge analysis to observe the development of TZs in age-0 and age-1 cod otoliths, and to validate the timing of TZ formation, which was consistently completed between September and December. Mean TZ diameters of 4 917 juvenile cod otoliths varied between cohorts (mean diameters of the first TZ: 2.0 ± 0.5 mm; second TZ: 3.9 mm ± 0.5) and TZ diameter variation was found to be related to individual growth rate. The timing of formation of the first TZ was positively related to water temperature, and was confirmed as a "summer ring" rather than a "winter ring". TZ formation and shallow-water occupancy suggest an influence of peak summer water temperatures on WBC ecology. An age reading guide for juvenile WBC otoliths is provided.

Keywords: age determination, annuli, Baltic Sea, indirect validation, juvenile cod, otolith edge type

Introduction

Age determination is integral to the calculation of growth, mortality, and year-class strength in fish populations (Campana, 2001; Reeves, 2003). The age of an individual fish is most commonly determined by counting periodic growth increments within calcified structures such as scales, fin rays, vertebra, or otoliths (Campana and Thorrold, 2001). Otoliths are paired calcified structures located within the head of teleost fish and are used as the standard for aging many commercially important temperate marine fish stocks (Campana and Thorrold, 2001). Otoliths are ideally suited to this usage as they grow continuously throughout the life of the fish, and, unlike other calcified structures, are not subject to resorption (Campana and Thorrold, 2001).

Sagittal otoliths (hereafter referred to as otoliths) of cod (*Gadus morhua*) exhibit two distinct macrostructures, an opaque zone and a translucent zone (Høie *et al.*, 2009). Although the underlying mechanism remains unclear, the formation of these zones have been linked to patterns in fish growth (Høie *et al.*, 2009), food availability (Høie *et al.*, 2008), and environmental factors such as water temperature (Neat *et al.*, 2008; Millner *et al.*, 2011). The assumption that zones form on an annual basis allows aging of fish through counting of translucent zones (TZs, also known as annuli) (Williams and Bedford, 1974; Kalish *et al.*, 1995).

A major contention between age readers on the correct age determination of western Baltic cod [WBC, the cod stock which mainly inhabits ICES subdivisions (SD) 22–24, Figure 1] is the timing and periodicity of formation of the first and second TZs (Bingel, 1972; Rehberg-Haas *et al.*, 2012). In 1972, an investigation into the timing of otolith zone formation of WBC reported that they form one TZ per year, with the first TZ formed between October and November, and the second and third TZs formed over a more prolonged period, beginning in June or July and continuing until February or March (Bingel, 1972). More recently, Rehberg-Haas *et al.* (2012) counted daily otolith increments of WBC caught in 2008 and 2009 near the island of Fehmarn

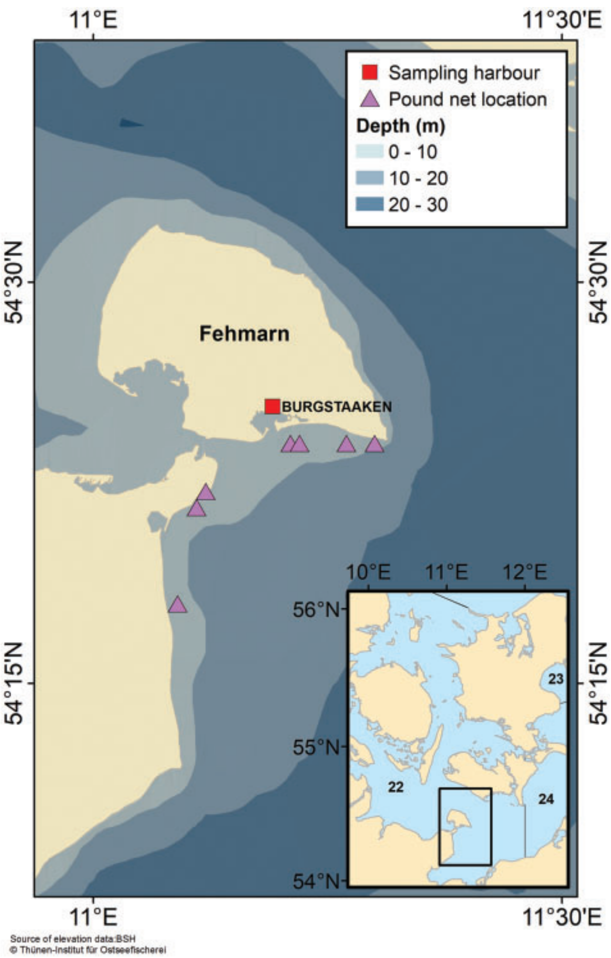

Figure 1. Location of commercial pound nets in the western Baltic Sea (SD 22) where cod samples originated. ICES subdivisions are delimited by black lines.

(Germany) and back-calculated the hatch date of juvenile cod. They determined that the first and second TZs can be formed within the same year, depending on hatch date, and suggested that individuals hatched early in the year form two TZs before December and those hatched later in the year form only one TZ (Rehberg-Haas *et al.*, 2012). The suggestion that WBC sometimes form one and sometimes two TZs during their first year of life complicates the age interpretation of WBC, and contributes to age-reading uncertainties.

Given these conflicting results, the sequence of TZ formation in WBC needs to be examined in more detail. As the decision on what constitutes a TZ is a subjective process (Williams and Bedford, 1974) a method to distinguish between false annuli, or settlement checks, and true annuli should be developed to reduce age reading error. Furthermore, the timing of TZ formation in WBC otoliths requires confirmation. Atlantic cod in the Skagerrak (Gjøsæter and Danielssen, 2011), Norway (Dannevig, 1956), and North Sea (Pilling *et al.*, 2007) form TZs over the summer, in contrast to the previous consensus that the TZ in WBC otoliths forms during winter.

Validation of the age-reading method is an essential prerequisite for the use of age information for any kind of analysis (stock assessment, growth estimation, etc.). However, even within commercially exploited stocks, the age-validation requirement is often ignored (Beamish and McFarlane, 1983; Campana, 2001). The periodicity of TZ formation within calcified structures should be validated for every age if the structures are to be used for age reading (Chilton and Beamish, 1982). One of the most reliable direct methods of validating the periodicity of otolith zone formation is mark–recapture of fish with chemically marked otoliths (Campana, 2001). When chemically marked individuals are recaptured, the number of TZs formed within otoliths between release and recapture can be directly related to time at liberty. This method of age-validation is currently being attempted with WBC (U. Krumme, unpublished data). However, it can be difficult or even impossible to tag small cod before the first TZ begins to form, as the individuals are so small that their catchability is low, and the tagging procedure is too stressful (U. Krumme, unpublished data). Alternative methods of age validation for fish include the release of known-age, marked fish, bomb radiocarbon analysis, radiochemical dating and the use of natural, date-specific markers. However, none of these methods is appropriate for very young, wild individuals which lack natural markers (Campana, 2001). Indirect methods of age validation can be more effective for fast growing, small individuals, such as tracking the progression of discrete length modes over time, and edge analysis to follow the development of annuli on the outer edges of otolith cross-sections (Campana, 2001).

The examination of high-resolution length-frequency data, combined with TZ and otolith edge analysis, was used to follow the TZ formation of juvenile WBC. Four cohorts of young-of-the-year (YOY) cod were examined, each for a period of 4–16 months. Otoliths (4 917) from the first and second age-classes present in the samples were categorized based on TZ number and edge type. The diameters of consecutive TZs were measured and compared between cohorts to provide a standard to facilitate the international age reading of WBC. Relationships between TZ formation and diameter and variables such as temperature and growth rate were explored to better understand the mechanisms involved in TZ formation.

Methods

Study population

Cod used in the analysis originated from the brackish waters of the western Baltic Sea (ICES SD 22, Figure 1), which contains the main spawning grounds of the WBC stock. This population spawns mainly during March–April in waters deeper than 20 m (Bleil *et al.*, 2009). The transition from the pelagic to the benthic lifestyle (settlement) occurs when juvenile cod attain a total length of 4–5 cm or within 2–3 months after fertilization (Hüssy *et al.*, 1997). Settlement takes place earlier in warmer water temperatures (Pepin *et al.*, 1997).

The sea surface temperature minimum in the western Baltic Sea (<3°C) usually occurs in February and the maximum in August, when temperatures can reach up to 20°C (Nausch *et al.*, 2016). The surface salinity can vary between 7 and 25, mainly due to wind-driven hydrodynamics linking the Baltic Sea and the Kattegat (Kullenberg and Jacobsen, 1981, Møller and Hansen, 1994). There is a strong annual oxygen cycle in the western Baltic Sea, with the greatest depletion in oxygen occurring in late summer/early autumn. In July 2015 low oxygen levels of 26% were observed, but the average saturation for the previous 6 years was >50%. In winter, the water column is usually completely vertically mixed, and oxygen saturation at the bottom layers reaches >90% (Nausch *et al.*, 2016).

Pound net samples

Two full-time, commercial pound net fishers, contributing up to 5 and 3 pound nets respectively, provided samples of cod between 2013 and 2016. The same fishers provided samples from the same area for a previous investigation into daily otolith increment formation of WBC (Rehberg-Haas *et al.*, 2012). The stationary uncovered pound nets were installed in shallow water (<5 m water depth) along the south coast of Fehmarn Island and the adjacent mainland (Figure 1). The nets were set perpendicularly to the coast line, in seagrass dominated substratum, and spanned the entire water column. Buoys at the head line and weights at the lead line forced fish to enter the catch chamber (bar-length mesh size 12 mm), located at the seaward end. The catch chamber was stretched by ropes attached to fixed pillars (Supplementary Figure S2) and was emptied every 1–17 days (mean 2.3 days) depending on weather conditions, during the fishing seasons (April–June and September–December). From 2014 onwards, efforts were made to collect samples year round, not only during the fishing seasons. The pound nets take advantage of the diel twilight migration of cod between daytime resting sites in deeper water and nightly feeding sites in structured shallow water habitats (Burrows *et al.*, 1994).

Unsorted samples (average sample weight: 3 kg) of small cod (<38 cm total length) were regularly collected and either frozen immediately (−20°C) after landing in the port, or measured, tagged, and re-released as part of an ongoing age validation study (U. Krumme, unpublished data). In addition to the unsorted catch sample of undersized cod, the fisher recorded the estimated total catch of undersized cod per pound net including the unsorted sample. For each sampling trip, a ratio between the total catch and sample weight was used to raise the length distribution of the sample to the total size of undersized cod in the catch. The estimated total number of undersized cod per length class was divided by the number of days soaking time, and the number of nets which contributed to the catch, to calculate an estimate of the average number of cod caught per length class, per net, per day. These results were then averaged for each month. The same standardized monthly length frequencies have additionally been used to estimate growth of juvenile cod in the western Baltic Sea (McQueen *et al.*, 2018).

Cod from the frozen samples were measured and processed at the Thünen Institute of Baltic Sea Fisheries (TI-OF), and the otoliths were extracted for further analysis.

Otolith analyses

Sectioning of otoliths

The whole right otolith from each sampled cod was embedded in GTS Polyester casting resin (Voss Chemie, 35–40% Styrol) with MEKP hardener. The left otolith was only used when the right otolith was damaged or crystallized. The otoliths were thin-sectioned (thickness: ca. 0.5 mm) through the core using an ATM Brilliant 250 bone saw. Images of each individual otolith were taken with transmitted light using a light microscope and the Zen Blue software (Carl Zeiss). Under these conditions, the opaque zones appeared darker and the TZ lighter than the surrounding material (Figure 2).

TZ diameter measurements

The outer edges of TZ within the images of the sliced otoliths were digitally marked using the software programme Image J (Rasband, 1997–2016). Age readers of cod usually start from the core and determine fish age by counting the number of completed TZs along the longest growth axis visible in the cross-section of the otolith. Unlike this standard approach, for this study the diameter of each TZ was measured. Each completed TZ was marked on the outer left and right edge of the digital image

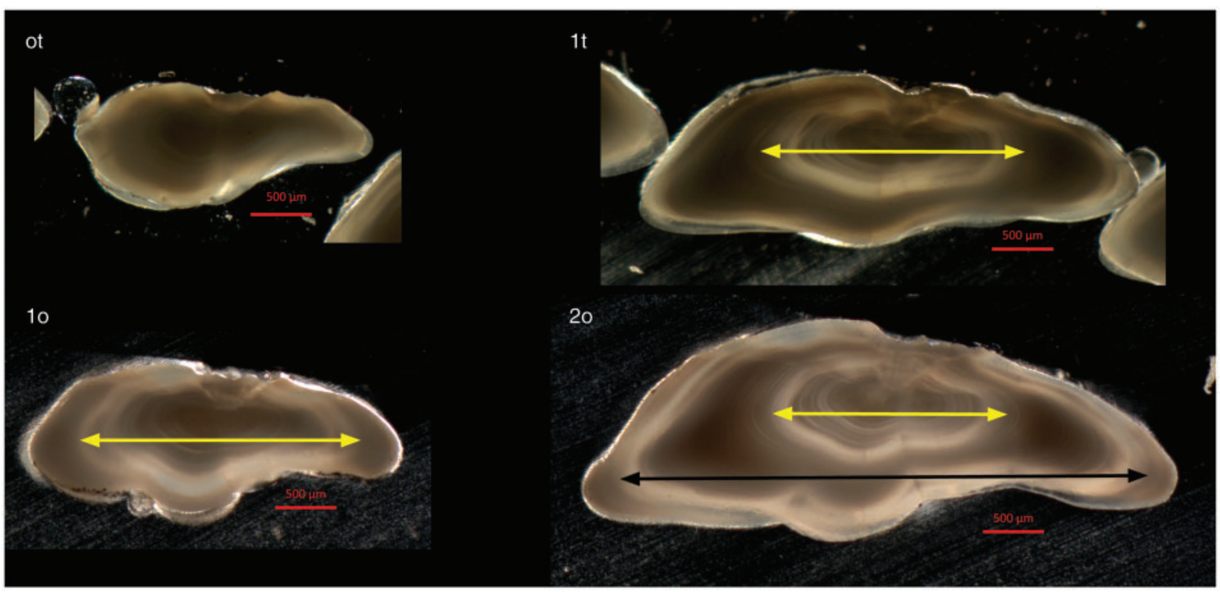

Figure 2. Cross sections of western Baltic cod otoliths. Otolith sections are viewed under transmitted light so TZs appear lighter than the darker opaque zones. Yellow (online version) arrows: diameter of the first TZ; black arrow: diameter of the second TZ. Top row: translucent edge type; bottom row: opaque edge types. Otoliths are from cod captured in pound nets in Fehmarn in 2015 and 2016 [0t: 28.09.2015, total length (TL) 13 cm, age 0; 1o: 12.01.2016, TL 17 cm, age 1; 1t: 14.10.15, TL: 21 cm, age 1; 2o: 12.01.2016, TL 28 cm, age 2]. Scale bar: 500 µm.

so that the distance measured was the maximum visible diameter of the annulus (Figure 2). However, the identification of the core region before sectioning is not precise, and the method of sectioning the otolith may result in sections which are not always sectioned precisely through the core. This will introduce some variability in subsequent TZ diameter measurements. The total number of completed TZs was recorded.

For the otoliths from 2015 to 2016, a scoring system was introduced to record the proportion of otoliths where the outer edges of the TZ could not be clearly distinguished. The TZ diameter of 12% of the otolith sections analysed from 2015 to 2016 were not included in diameter analysis, as the measurements were considered unreliable due to either diffuse or unclear translucent zones or broken otoliths. The exact numbers of otoliths excluded from the 2013 to 2014 samples were not recorded, but were similar or less than the 2015 and 2016 samples.

Edge analysis

Through examination of the otolith images, the developing zone present at the marginal edge (outer-zone) of the otolith was defined as either opaque or translucent (hereafter referred to as "edge type"). As the timing of initiation of zone formation was of interest here, edge types were classified as soon as the new zone could be detected on any part of the outer edge (Supplementary Figure S3). The same person performed all categorizations of edge type, to prevent between-reader bias. A small proportion (<2%) could not be confidently classified and were excluded. Information on fish length and date of catch were available to the otolith reader. To investigate whether this influenced edge type categorization, and to investigate the precision of this method, a random sample of 100 otoliths were re-analysed by the same reader without information on capture date and length (see Supplementary Material). Transmitted light was used throughout for otolith imaging, as this is the standard method currently used for age-reading of WBC in Germany. However, as previous authors have favoured the use of reflected light for examination of cod otolith edge types (Pilling *et al.*, 2007; Høie *et al.*, 2009) a sub-sample of 133 otoliths were additionally photographed under reflected light. The edge types of these otoliths were categorized without reference to the transmitted light otoliths, and the results were compared (see Supplementary Material).

Information on the number of completed TZs and edge type of individuals <38 cm were combined so that otoliths were categorized into "edge-zone categories" as follows: an otolith with 0 completed TZs and a translucent edge was classified as 0t, an otolith with 1 completed TZ and an opaque edge was classified as 1o and so on. In this study, the 6 most common edge-zone categories were: 0o, 0t, 1o, 1t, 2o, 2t. A very small proportion of otoliths were recorded as 3o or 3t. The proportions of each edge-zone category per month for each 1 cm length class was overlaid onto the standardized length frequencies to illustrate the development of translucent and opaque zones within cohorts over the course of the year. This analysis was used to investigate whether different length-frequency modes represent different cohorts. As the sample sizes from January to March were very small the edge-zone category of these individuals are displayed in Supplementary Figure S4, rather than including this data in the standardized length frequencies.

Cohort assignment

Based on the results of the length-frequency, edge type, and TZ analysis, an age-reading guide was prepared so that individuals could be assigned to a cohort based on month of capture and edge-zone category (see Results section). Cohort is defined in this study as all cod born in the same year.

All individuals <38 cm (except those excluded by the assumptions detailed in Results section) were assigned an age using this guide, and assigned to a cohort by subtracting their age from the year of catch. For example, a 1-year old fish caught in 2014 would be assigned to the 2013 cohort.

Water temperature

A HOBO Pro water temperature logger (Onset) recorded water temperature every 6 h (starting at midnight) from September 2013 until December 2016. The sensor was installed at a fixed pillar of a pound net about 2 m below the water surface. Data for every month were only available for 2016; gaps in other years were due to logger removal (e.g. for data download).

Statistical analysis

Length-frequency decomposition

The length-frequency data was pooled across all sampling years, and the average length-frequencies per month were estimated, in the same way as described above. The initial values (mean and standard deviations of the modes, and number of modes present in the sample) were selected through visual inspection of the length-frequency histograms. Parameter estimates for the overlapping normal distributions which best fit the length-frequencies were estimated using a combination of Newton-type method and EM algorithm, applied through the R package "mixdist" (McDonald, 2018). Plots of the distributions fitted distributions overlaid onto the histograms were inspected to ensure that the fits were reasonable.

The length-frequency distribution parameters were used to assign a "growth category" variable to each individual fish in the samples, given its assigned age. If total length of the fish was greater or less than one standard deviation from the mean length of cod in that month and mode, fish were classified as either "fast" or "slow" growing, respectively. Otherwise, they were classed as "medium" (Millner *et al.*, 2011).

Edge type and water temperature

The relationship between edge type and water temperature was analysed using binomial GLMs, with translucent edge types coded by a 1 and opaque edge types coded by a 0. To assess whether the relationship between temperature and edge type changed with length of fish, an interaction with length was included in the model. Length and age are collinear, so length was chosen to be included in the model rather than age as there is incomplete seasonal coverage of age classes-2 and -0 in this dataset. The significance of adding length and the interaction between the two explanatory variables to the model was examined using Chi-squared tests.

Variations in TZ diameter

To explore whether the first and second TZ diameters were stable between cohorts, age classes, and growth categories, analyses of variance (ANOVA) were carried out, with either first or second TZ as the response variable. The most complex model fitted included first TZ as response variable, growth category, cohort, and age as fixed effects and interactions between growth category and cohort, and growth category and age. The interaction terms were included to assess whether the relationship between growth category and TZ diameter varied between cohorts and ages, and in particular to test whether it was reasonable to model the growth category variable in relation to diameter of the first TZ, even for age-1 and age-2 cod.

The most complex models fit to the second TZ diameter data included growth category, cohort, and age as fixed effects. Models including interaction terms could not be applied to the second TZ diameter, as such models became rank deficient due to the smaller sample size. There were too few data on diameter of the third TZ to include these measurements in statistical analyses.

ANOVA tables were used to assess the significance of including each fixed effect and interaction term in the models. In addition, AIC values of models including all relevant variable combinations were compared to ensure that the best fitting models were selected. Linear model assumptions were checked through graphical exploration of residuals. All statistical analyses were carried out using R v3.5.0 (R Core Team, 2018).

Results

Age validation using length frequencies and edge-zone categories

The standardized length frequencies obtained from the pound net samples clearly depicted the growth progression of two cohorts of undersized cod present in the sampling site between September and December (Figure 3). One cohort can be tracked from April until the end of the year, during which time the average length increases from ca. 20–25 cm to 30–35 cm. A new cohort appears in the length-frequency in September. The average length of this cohort increases from ca. 12 cm to 18 cm by December. It can be assumed that the smaller cohort represents the YOY fish, spawned in spring and growing large enough to be retained in the pound nets by autumn, when they apparently start making use of the shallow water habitat where the pound nets were set. The larger cohort represents age-1 cod, spawned the previous year.

There was a 92% match between repeated categorization of a random sub-sample of otoliths. The agreement between categorization of edge type from transmitted and reflected light measurements was 87% (see Supplementary Material).

Overlaying the proportions of edge-zone category onto the length frequencies confirmed that the length-frequency modes represent two cohorts, and that in general, each cohort forms only one TZ per year (Figure 3). In April, most fish within the age-1 cohort (average length of ca. 20–25 cm) had 1 completed TZ and an opaque edge type. Also visible in the April samples was a second cohort, about 35 cm in length, mainly including fish which had 2 completed TZs and an opaque edge type. These are the age-2 cod. By June, fish in both cohorts started to develop a translucent edge type. By August, the age-2 cod were no longer present in the samples of undersized cod, and nearly all the age-1 cod had a translucent edge type. In August–September, the YOY cod began to enter the pound nets. Some had already completed their first TZ, exhibiting one TZ, and an opaque edge. The other individuals in the age-0 cohort were still forming their first TZ, and were recorded as having 0 TZs and a translucent edge type. Likewise, the age-1 cohort during this time contained a mixture of individuals which had either one completed TZ and a translucent edge, or two TZs and an opaque edge. By December, there was a very high proportion of fish (87%) from both cohorts which had completed the TZ, and exhibited an opaque edge. Very few individuals (13%) still exhibited a translucent edge. In general, this pattern of zone development was very consistent across the 4 study years. Only a very small number of individuals (<10%) diverged from the overall pattern (e.g. out of a total of 146 individual otoliths examined from the age-0 cohort in 2016, 4 individuals were recorded as 2o, and 7 as 1t, contrary to the general pattern, Figure 3).

Cohort assignment

An age-reading table was prepared so that individuals could be assigned to a cohort based on month of capture and edge-zone category (Supplementary Table S2). To conform to standard Baltic cod age reading methods, the birthday of all cod was set to 1st January.

Ages could only be assigned for months where clear length-frequency modes could be identified in the samples. Given the progression of translucent edge type, it was concluded that TZ formation generally began in June or July, and was completed by December at the latest. The small proportion of individuals (6%) with translucent edges outside of this period were considered outliers, and excluded from the classification scheme. August was judged to be a transitional month, during which the switch from translucent to opaque zone formation may start to occur in some individuals. As the first individuals of the YOY cohort also begin to appear in this month, there is opportunity for misclassification of individuals with a translucent edge. As such individuals were very rare in the August samples (5%), these individuals were classed "unclassifiable" (UC).

Water temperature data

The average monthly water temperature ranged from 2.6°C in January to 18.0°C in August. The temperature profile of each year followed a similar pattern (Figure 4), remaining at temperatures above 15°C between mid-June and mid-September. The summer of 2016 was particularly long, with average temperature of 18.0°C recorded in September, compared to the average September temperatures of 2013–2015 which ranged between 14.4 and 15.9°C.

Statistical analysis

Length-frequency decomposition and growth categorization

The best fitting mixture distributions for April–December, estimated by the MIX method and quality-checked by visual examination of the length frequencies overlaid with the fitted normal distributions (Supplementary Figure S5), indicated that two modes were present in the samples (representing ages-1 and -2 in April–June, and ages-0 and -1 in September–December), except for July where only one mode was visible (representing age-1).

Edge type and water temperature

There was a significant positive relationship between water temperature and the proportion of individuals with translucent zones (Figure 4, Table 1). Chi-squared tests indicated a significant improvement in fit when length of fish ($p < 0.001$) and interaction between temperature and length ($p < 0.001$) were included in the model.

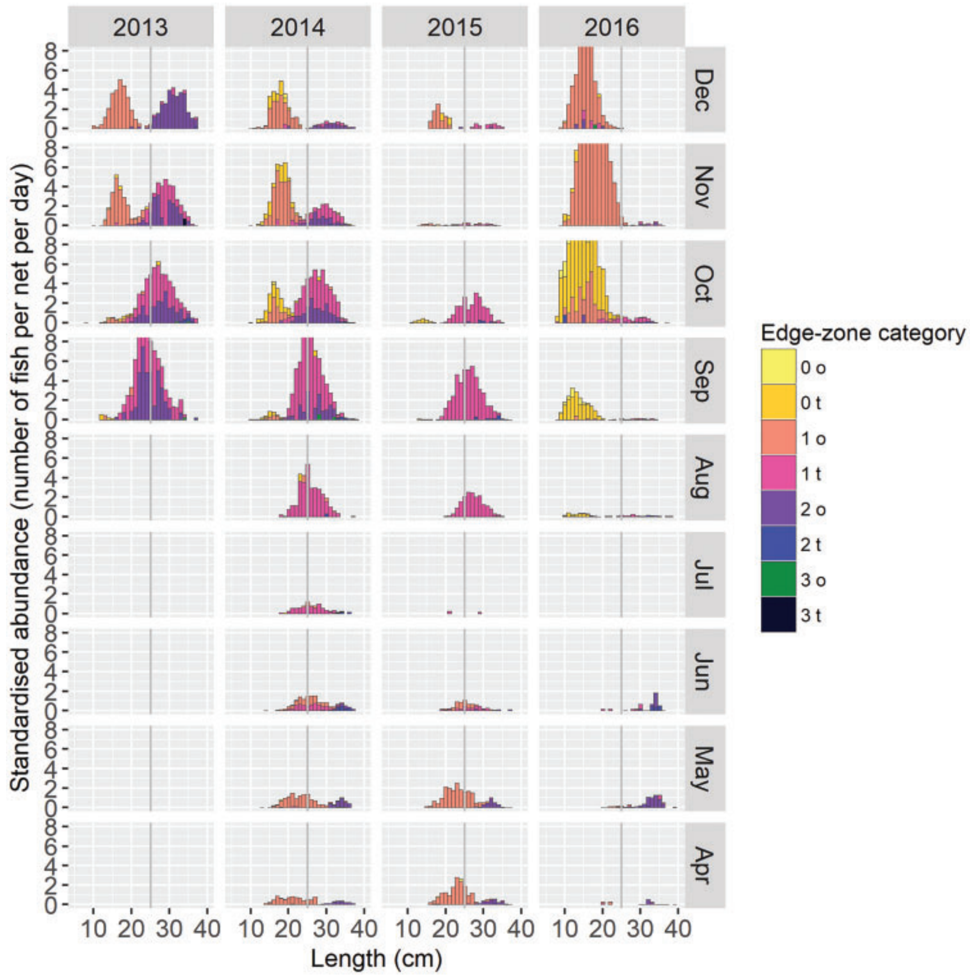

Figure 3. Standardized monthly length frequencies of cod <38 cm from commercial pound net samples from Fehmarn during the months April to December in 2013–2016. Overlaid onto these are the proportions of edge-zone category per cm length class. Otolith edge-zone categories are as follows: 0o = 0 translucent zones, opaque edge; 0t = 0 translucent zones, translucent edge; 1o = 1 translucent zone, opaque edge; 1t = 1 translucent zone, translucent edge; 2o = 2 translucent zones, opaque edge; 2t = 2 translucent zones, translucent edge. Due to large sample sizes in 2016, the *y*-axis has been truncated at 8 fish per net per day. No samples were collected between April and August in 2013.

Variations in TZ diameters

The fit of the model for first TZ was significantly improved by the sequential addition of the variables growth category ($F_{2,\ 3\ 855} = 264.85$, $p < 0.001$), cohort ($F_{4,\ 3\ 855} = 166.40$, $p < 0.001$), age ($F_{2,\ 3\ 855} = 5.46$, $p = 0.004$), and an interaction between growth category and cohort ($F_{2,\ 3\ 855} = 8.02$, $p < 0.001$). The addition of an interaction between growth category and age did not improve model fit ($F_{4,\ 3\ 855} = 0.51$, $p = 0.73$). Comparison of AIC values of models including the relevant combinations of these variables (Table 2) confirmed that the model including an interaction between growth category and cohort, with age included as a fixed effect (Model 6) had the best fit.

The non-significance of the interaction between growth category and age suggests that the growth category variable can reasonably be used as a predictor of fist TZ diameter for all age classes. Across almost all years, the slow growing individuals usually had a smaller first TZ diameter than the fast growing individuals (Table 3, Figure 5). The relationship between growth category and TZ diameter was apparently reversed in the 2015 cohort, though confidence in this conclusion is reduced due to the small sample size of fast growing individuals from this cohort (Figure 5).

The significance of the interaction between cohort and growth category indicates that there were inter-cohort variations in first TZ diameter which could not be explained by growth rate alone. There were significant variations in the average first TZ diameter between cohorts (Table 3), with average first TZ ranging from 2.4 mm in 2016 to 1.9 mm in 2015.

The effect of age on first TZ diameter was smaller and less significant than the effects of cohort and growth category, and indicated that the average diameter of the first TZ was slightly smaller in sampled age-1 and age-2 cod than sampled age-0 cod (Table 3).

The fit of the second TZ model was significantly improved by the addition of growth category ($F_{2,\ 537} = 81.5$, $p < 0.001$) and cohort ($F_{3,\ 537} = 29.5$, $p < 0.001$), but the influence of age on second TZ diameter was not significant ($F_{1,\ 537} = 0.17$, $p = 0.68$). Comparison of AIC values confirmed that the model including cohort and growth category fit best (Table 2, Model 11).

Similarly to the results for first TZ, the faster growing individuals generally had larger second TZs than the slower growing individuals (Table 4, Figure 5). There was significant variation in second TZ diameter between cohorts (Table 4), with the average

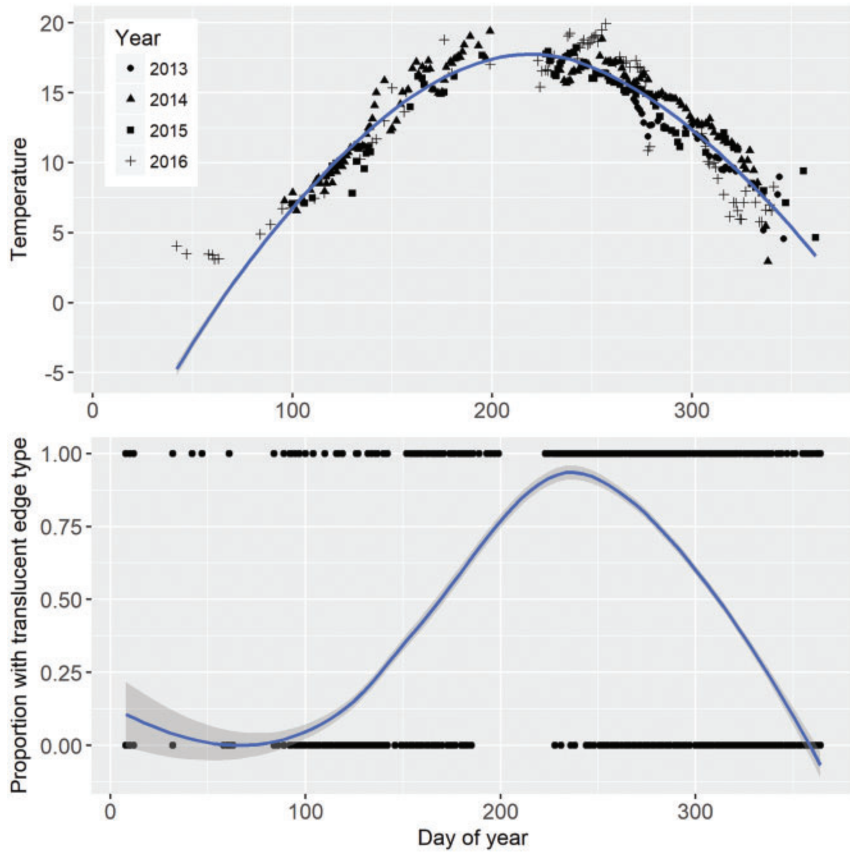

Figure 4. Top panel: water temperature recorded by a logger installed about 2 m below the water surface at a fixed pillar of a pound net, south of Fehmarn, where samples of undersized WBC were collected. Shape of points indicates year when data was collected. A LOESS smoother (with grey shaded area indicating 95% confidence interval) was added through all available data, to aid in visualization of the annual temperature trend. Bottom panel: the proportion of WBC otoliths from pound net samples with translucent edge types recorded throught the year, with data from 2013 to 2016 combined. Points indicate the spread of samples throughout the year. Edge types were classified as translucent (1) or opaque (0) and a LOESS smoother (with grey shaded area indicating 95% confidence interval) was included to visualize the trend.

Table 1. Parameter estimates (on the logit scale) of binomial GLM describing the proportion of WBC otoliths with a translucent edge type in relation to water temperature and fish total length.

Term	Parameter estimate	s.e.	z-Value	p-Value
Intercept	−8.34	0.66	−12.51	<0.001*
Temperature	0.59	0.050	11.85	<0.001*
Length	0.13	0.025	5.29	<0.001*
Temperature: length	−0.0082	0.0019	−4.29	<0.001*

The Wald statistic (z-value) is used to test whether the corresponding parameter estimate is significantly different from 0 ($p < 0.05$, indicated by *).

second TZ diameter ranging from 3.8 mm in 2012 to 4.3 mm in 2014, though the sample sizes for the 2014 and 2015 cohorts were very low (Figure 5).

Discussion

Age validation

Timing of zone formation

The method of combining monthly standardized length frequencies with edge-zone classification proved effective for indirectly validating the timing of TZ formation in juvenile cod in the western Baltic Sea. The timing of formation of the first TZ in juvenile cod (August–October) was similar to previous findings on timing of first TZ formation (September–December, Bingel, 1972), suggesting timing of TZ formation may not have changed drastically in the last 40 years. The timing of formation of the second TZ (June–December) corresponds to preliminary results from recaptures of chemically tagged WBC (U. Krumme, unpublished data).

Evidence for two cohorts

Previous analysis on juvenile WBC caught in pound nets in the area around Fehmarn suggested that fish from different spawning groups may be present in the same nursery grounds (Rehberg-Haas *et al.*, 2012). However, detailed examination of the standardized length frequencies supported an alternative hypothesis. In May, the only cohort present in the pound net samples had an average length of about 21 cm and already had one completed TZ. The growth of this cohort could be tracked over the summer months, in the years when sampling was undertaken during this season, until a second, smaller cohort appeared in the samples in September. This smaller cohort had an average length of about 15 cm, while the larger cohort was now about 27 cm. Given the

Table 2. Comparison of ANOVA model fits for the diameter of the TZ in relation to different combinations of explanatory variables.

Model no.	Variables included	df	AIC	*F*	*p*-Value	R^2
First TZ						
1	Cohort	6	5952	69.51	<0.001	0.07
2	Growth category	4	5739	253	<0.001	0.12
3	Age	4	6079	69.18	<0.001	0.03
4	Growth category *X* age	10	5637	79.43	<0.001	0.14
5	Growth category *X* cohort	16	5434	63.68	<0.001	0.19
6	Growth category *X* cohort + age	18	5430	56.28	<0.001	0.19
7	Growth category *X* cohort + growth category *X* age	22	5436	45.1	<0.001	0.19
Second TZ						
8	Cohort	4	545	31.31	<0.001	0.14
9	Growth category	5	566	58.57	<0.001	0.17
10	Age	3	614	16.19	<0.001	0.03
11	Cohort + growth category	7	429	50.35	<0.001	0.31

"*X*" indicates that both the fixed effects of the listed variables, and interactions between the variables are included in the model. "+" indicates that the following variable was included as a fixed effect without interaction. The AIC value, degrees of freedom (df), *F*-statistic, *p*-value, and adjusted R^2 of each model are provided to illustrate model fit. Models 1–7 were fit with diameter of the first TZ as the response variable ($n = 3867$); Models 8–11 were fit with diameter of the second TZ as the response variable ($n = 546$).

Table 3. Parameter estimates from best fitting model for explaining variation in diameter of the first TZ (Model 6, Table 2).

Term	Parameter estimate	s.e.	*t*-Value	*p*-Value
Intercept (2012 cohort: fast growth category + age 0)	2.27	0.05	45.4	<0.001*
Medium growth category	−0.28	0.05	−5.41	<0.001*
Slow growth category	−0.34	0.06	−5.38	<0.001*
2013 cohort	0.03	0.05	0.58	0.56
2014 cohort	0.11	0.05	2.19	0.029*
2015 cohort	−0.31	0.09	−3.30	<0.001*
2016 cohort	0.52	0.07	7.59	<0.001*
Age 1	−0.057	0.02	−2.33	0.019*
Age 2	−0.11	0.04	−2.52	0.011*
Medium growth category: 2013 cohort	−0.06	0.06	−1.05	0.29
Slow growth category: 2013 cohort	−0.24	0.07	−3.34	<0.001*
Medium growth category: 2014 cohort	0.080	0.06	1.28	0.20
Slow growth category: 2014 cohort	−0.14	0.08	−1.75	0.080
Medium growth category: 2015 cohort	0.23	0.11	2.09	0.037*
Slow growth category: 2015 cohort	0.62	0.17	3.68	<0.001*
Medium growth category: 2016 cohort	−0.17	0.08	−1.98	0.048*
Slow growth category: 2016 cohort	−0.47	0.10	−4.78	<0.001*

Parameter estimates significantly different from 0 at the 5% level ($p < 0.05$) are denoted by *.

apparent growth rates of these two groups (length-frequency modes shifted by about 1–2 cm per month), it seems more reasonable to assume that they belong to two different cohorts, rather than successive hatch groups of age-0 cod spawned during an extended spawning period or in different areas. In Rehberg-Haas *et al.* (2012), age was determined through counting daily increments in otoliths using scanning electron microscopy. As daily increments are harder to detect the older a fish is (Pannella, 1971), it is possible that the age of older individuals was underestimated in Rehberg-Haas *et al.* (2012). Individuals as large as 27.1 cm in 2008 and 32.5 cm in 2009 were used in their analysis, which by consulting the length frequencies from this region would mean that some individuals classified as age-0 in Rehberg-Haas *et al.* (2012) may have originated from the second, age-1 cohort. In this study, the majority of individuals within the first cohort formed only one TZ before the end of their first year, suggesting that if a small proportion of individuals do form more than one TZ during their first year, the proportion is so small that its contribution to the age-reading uncertainty for the stock should be negligible.

Edge type classification

The classification of edge type as a method of age validation is generally only appropriate for young, fast-growing fish (Campana, 2001). It can be difficult to classify an increment which is only partially completed, especially as otolith sections become thinner as the edge is approached. As the aim of this study was to investigate the timing of initiation of zone formation, efforts were made to detect a newly forming edge type even when the zone was only visible in a small portion of the edge of the otolith. This method becomes even more difficult for older individuals, when the width of zones decreases. Francis *et al.* (1992) stated that opaque zones in otoliths are often not visible until the subsequent zone in deposited, and that this effect is more pronounced in older fish. This problem was sometimes encountered even for very young cod otoliths in this study, as the method of using transmitted light to view an otolith slice embedded in resin caused the outer edge to sometimes appear bright, even when the outer edge was opaque. The comparison exercise with otolith sections viewed under reflected light did not produce identical results, but the proportion of edge classification matches

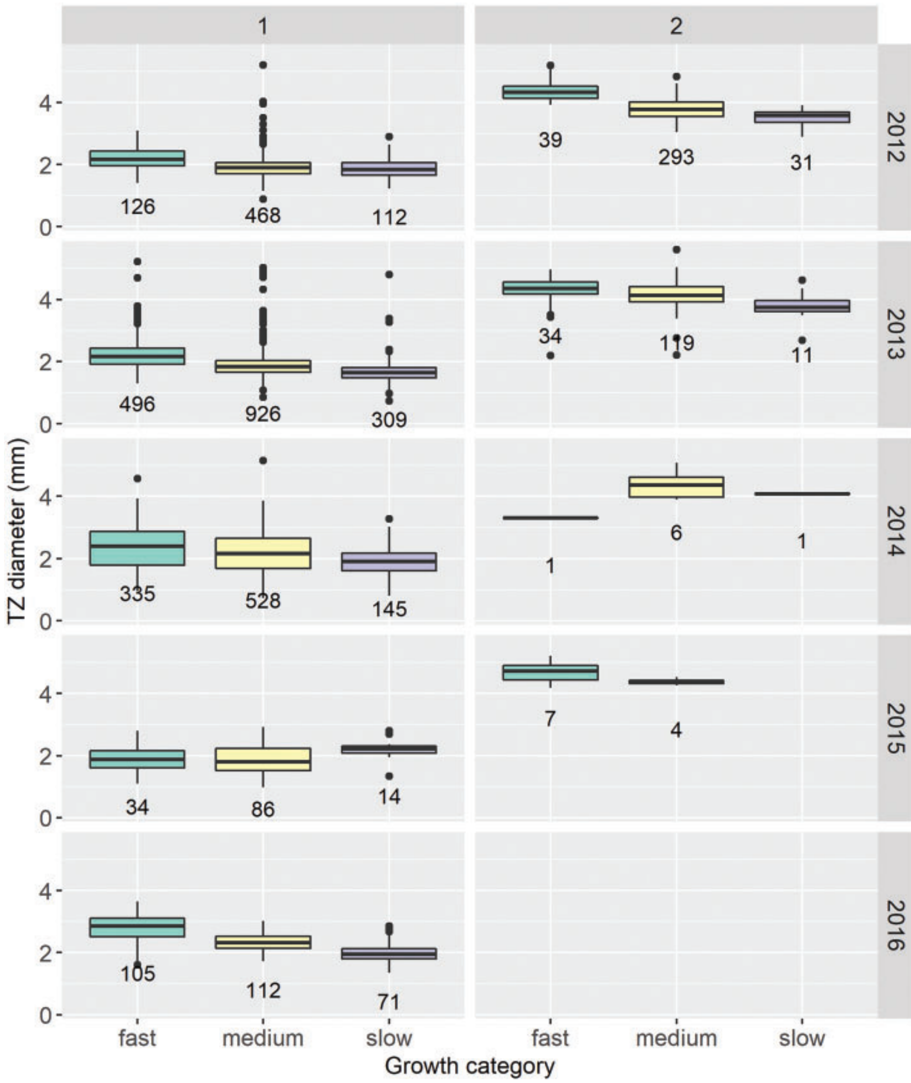

Figure 5. Diameters of first and second TZs, split into panels by back-calculated year of birth (rows) and TZ number (columns). The diameters of the first TZ are additionally split into growth category, which was used as an explanatory variable in Models 6 and 11 (Table 2). Solid horizontal black line: average diameter of the first and second TZs, coloured box: interquartile range, whiskers: 1.75× the interquartile range, outliers: single points. Numbers below boxes indicate sample size.

Table 4. Parameter estimates from best fitting model for explaining variation in second TZ (Model 11, Table 2).

Term	Parameter estimate	s.e.	*t*-Value	*p*-Value
Intercept (2012 cohort + fast growth category)	4.22	0.044	95.9	<0.001*
Medium growth category	−0.41	0.045	−9.0	<0.001*
Slow growth category	−0.71	0.069	−10.3	<0.001*
2013 cohort	0.27	0.038	7.99	<0.001*
2014 cohort	0.55	0.14	4.08	<0.001*
2015 cohort	0.50	0.11	4.44	<0.001*

Parameter estimates significantly different from 0 at the 5% level ($p < 0.05$) are denoted by *.

was high. Previous investigations which analysed edge type of sectioned otoliths have often used reflected light (Pilling *et al.*, 2007; Høie *et al.*, 2009; Millner *et al.*, 2011), though transmitted light is also sometimes used (Francis *et al.*, 1992). The main impediment to this approach was considered to be the subjectivity and imprecision of identifying the edge type, rather than the direction of light used.

The difficulty in detecting opaque outer edge types could account for previous findings which stated that the TZ in older fish is formed over a much more prolonged period than for YOY WBC (Bingel, 1972). However, as only juvenile cod otoliths were examined in this study, no conclusions can be drawn here about the timing of TZ formation in older WBC. Alternative age validation methods will need to be applied to confirm whether there is a shift in timing of TZ formation of WBC later in life. Despite the uncertainties associated with the edge analysis method, the classification of edge types of juvenile WBC proved useful in discriminating between individuals which had the same number of TZ, but belonged to different cohorts (Supplementary Table S2).

Age reading subjectivity and guidelines to reduce it

Otolith age reading continues to be a subjective process, even after validation techniques have been carried out (Campana *et al.*, 1995; Buckmeier, 2002), for example due to age-reader bias in deciding what constitutes an annual TZ (Williams and Bedford, 1974). The sources of subjectivity in this analysis included the decision on what constituted a TZ, the decision on what constituted the outer edge of a TZ, and the classification of the otolith edge type. Such sources of subjectivity are still largely unavoidable when using traditional age reading methods.

The major contention in age-reading of WBC which was addressed by this analysis was the identification of the first TZ (Bingel, 1972; Rehberg-Haas *et al.*, 2012). In the present analysis, by working with both modal progression information from a multi-year dataset of length frequencies and a large number of juvenile fish otoliths sampled from these modes, it was relatively straightforward to assign age to the fish while avoiding confusion between age-0 and age-1 individuals. Although some individuals apparently did not conform to the TZ formation pattern outlined, this proportion was very small (<10%), and an exception to the general rule.

The large sample size of juvenile fish used to estimate average TZ diameters should have resulted in relatively robust estimates, despite the possible sources of subjectivity and potential variation in the precision of the sections in relation to the otolith core. The overall average first TZ diameter estimate of 2 mm can be used as a guideline for future age reading for all ages of WBC, and could help to reduce some of the uncertainty in identification of the first TZ.

Mechanisms of TZ formation

The alternating pattern of opaque and TZ formation in otoliths of temperate and subpolar fish species has been used for decades as a method of aging fish, without any clear consensus as to the factors controlling the switch from one zone type to another. A review of 104 studies on the timing of opaque zone deposition reported that conflicting factors including periods of fast growth, periods of slow growth, periods of low temperature, and periods of high temperature have all been linked to the formation of opaque zones (Beckmann and Wilson, 1995). The conclusion of this review was that in most temperate fish species, the opaque zone forms during the spring and summer months. The TZ can therefore be considered a "winter ring" in these species, and has indeed been referred to as such in Baltic cod literature (Bingel, 1972; Hüssy, 2010; Rehberg-Haas *et al.*, 2012). However, this assumption is contradicted by the findings of studies on many other Atlantic cod populations where it has been reported that the TZ corresponds to the time of year when the warmest water temperatures are encountered [e.g. North Sea cod (Høie and Folkvord, 2006), Norwegian cod (Dannevig, 1956), Skagerrak cod (Otterbech, 1954; Gjøsæter and Danielssen, 2011), Gulf of Maine cod (Jensen, 1970), and Barents Sea cod (Høie *et al.*, 2009)].

There are several mechanisms which could explain why WBC form a TZ between summer and late autumn. Our results agree with previous findings that increasing temperatures correlate with decreasing opacity in cod otoliths, as we found a significant positive relationship between the proportion of individuals with translucent edge type and water temperature at the study site. Increasing temperature has also been correlated with decreasing cod otolith opacity in previous studies (Høie and Folkvord, 2006; Hüssy *et al.*, 2009), which may be explained by the temperature-dependent precipitation of aragonite (Fablet *et al.*, 2011).

Decreasing cod otolith opacity has also been connected to periods of starvation or reduced feeding (Hüssy and Mosegaard, 2004; Høie *et al.*, 2008) as otolith biomineralization can be assumed to be linked to fish metabolism (Fablet *et al.*, 2011). There is evidence to suggest that the effect of reduced feeding on TZ formation is secondary to increasing temperature (Høie *et al.*, 2008; Neat *et al.*, 2008), though the interaction between temperature and food availability has proved necessary to explain cod zone formation patterns in other regions (Fablet *et al.*, 2011).

In WBC, reduced feeding and high temperatures may be interlinked, and occur simultaneously. Freitas *et al.* (2015, 2016) found that under increased sea surface temperature conditions (>15°C) in a south Norwegian fjord, cod were absent from vegetated shallow habitats and selected instead non-vegetated rocky and sandy habitats in deeper, colder areas. A trade-off between food availability and unfavourable temperature conditions in shallow waters during peak summer months was suggested, where extended periods of surface waters >15°C during summer may deprive cod from productive shallow feeding areas. The catches of juvenile cod in the pound nets off the coast of Fehmarn were very low during June and July, indicating that the cod were not occupying this shallow water habitat during this time. The warm summer temperatures in the western Baltic Sea (with temperatures >15°C from June to October in shallow water, Figure 4) may also restrict cod's access to the productive shallow water habitats, thus limiting feeding opportunities (S. Funk, unpublished data). Cod were sometimes present in this area during the warmest months, especially from August onwards. High temperatures induce increased metabolic rates in fish (Claireaux *et al.*, 2000; Pauly, 2010) and the increased energy requirement may not be met by food availability. This potentially physiologically stressful period for WBC corresponds closely with the TZ formation period.

The TZ has also been associated with periods of slow growth in cod, and the opaque zone with periods of faster growth (Trout, 1954; Pilling *et al.*, 2007). In combination with the high temperatures and potential lower food availability, growth of cod in the western Baltic Sea is slowest during spring and summer, and growth rates increase at the beginning of autumn (McQueen *et al.*, 2018). The mechanism linking growth rates to zone formation could be due to a correlation between the incorporation of matrix protein into the otolith with whole body protein synthesis (Hüssy and Mosegaard, 2004) as the TZ in otoliths is mineral-rich and protein-poor (Panfili *et al.*, 2002), and increasing opacity within cod otoliths is related to increasing protein content (Hüssy *et al.*, 2004).

Variation in TZ diameters

Analysis of the variation in TZ diameter provided support to the hypothesis that larger TZ diameters were related to higher growth rates, as reported for cod in the eastern Baltic Sea (Baranova, 1992). The significant decrease in TZ width with increasing age of cod may be explained by this growth effect, as the pound nets only effectively sample small cod (<38 cm), so the largest, fastest growing individuals from the older age classes may not have been adequately represented within the dataset. There was still significant inter-cohort variation in the TZ diameters even when the assigned growth category of the individual was included in the model. There are several possible mechanisms which could result in these findings, including growth variability during the period of zone formation which was not captured by the length-at-age at capture. However, it is also possible that inter-annual variability in another biotic or abiotic factor played a role.

Possible factors which may influence TZ diameter, but which were not considered in this analysis, include hatch date and thermal experience. In an examination otoliths of *Gymnocypris selincuoensis*, Tao *et al.* (2018) reported that hatch date explained most of the variation in diameter of the first TZ, with larger TZ diameters indicative of earlier hatch dates, and temperature a significant secondary explanatory factor. The diameter of the TZ will result from a combination of the thickness of daily otolith increments, which can be partly related to growth rate (Campana and Neilson, 1985), and to the number of daily otolith increments, which is determined by hatch date. Without conducting additional daily increment counts, or biological growth intercept back calculations (Campana, 1990) to explore the variation in size of individuals at time of TZ formation, the relative effects of growth rate and hatch date on TZ diameter could not be differentiated.

Otolith accretion rate has been reported to increase with temperature, with daily increment width increasing with increasing temperatures (Neat *et al.*, 2008). This could potentially contribute to the differences in TZ diameter observed between years. In this study, the largest average first TZ diameter was found in the 2016 cohort, and the smallest in the 2015 cohort. Summer and early autumn temperatures were warmer in 2016 than 2015, which may have resulted in higher otolith accretion during the time of otolith development relevant to first TZ diameter in the 2016 cohort. In general, given the strength of the 2016 cohort compared to the 2015 cohort, it seems plausible that the 2016 cohort encountered better conditions for growth and survival which may have been reflected in the TZ diameters. However, with the data available from this analysis, it was not possible to disentangle the probably complex interaction of factors which resulted in the variation in TZ diameter between cohorts.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

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References

Baranova, T. 1992. On the growth of eastern Baltic cod. Baltic Fish Committee, ICES CM 1992/J: 29.

Beamish, R. J., and McFarlane, G. A. 1983. The forgotten requirement for age validation in fisheries biology. Transactions of the American Fisheries Society, 112: 735–743.

Beckmann, D. W., and Wilson, A. W. 1995. Seasonal timing of opaque zone formation in fish otoliths. *In* Recent Developments in Fish Otolith Research, pp. 27–43. Ed. by D. H. Secor, J. M. Dean, and S. E. Campana, University of South Carolina Press, Columbia, South Carolina.

Bingel, F. 1972. Zur Interpretation von Otolithenstrukturen des Dorsches (Gadus morhua L.). Thesis, Universität Kiel, Kiel. 42pp.

Bleil, M., Oeberst, R., and Urrutia, P. 2009. Seasonal maturity development of Baltic cod in different spawning areas: importance of the Arkona Sea for the summer spawning stock. Journal of Applied Ichthyology, 25: 10–17.

Buckmeier, D. L. 2002. Assessment of reader accuracy and recommendations to reduce subjectivity in age estimation. Fisheries, 27: 10–14.

Burrows, M. T., Gibson, R. N., Robb, L., and Comely, C. A. 1994. Temporal patterns of movement in juvenile flatfishes and their predators: underwater television observations. Journal of Experimental Marine Biology and Ecology, 177: 251–268.

Campana, S. E., and Neilson, J. D. 1985. Microstructure of fish otoliths. Canadian Journal of Fisheries and Aquatic Sciences, 42: 1014–1032.

Campana, S. E. 1990. How reliable are growth back-calculations based on otoliths? Canadian Journal of Fisheries and Aquatic Sciences, 47: 2219–2227.

Campana, S. E., Annand, M. C., and McMillan, J. I. 1995. Graphical and statistical methods for determining the consistency of age determinations. Transactions of the American Fisheries Society, 124: 131–138.

Campana, S. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology, 59: 197–242.

Campana, S. E., and Thorrold, S. R. 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? Canadian Journal of Fisheries and Aquatic Sciences, 58: 30–38.

Chilton, D. E., and Beamish, R. J. 1982. Age Determination Methods for Fishes Studied by the Groundfish Program at the Pacific Biological Station, Canadian Special Publication of Fisheries and Aquatic Sciences. 60: 102.

Claireaux, G., Webber, D. M., Lagardère, J. P., and Kerr, S. R. 2000. Influence of water temperature and oxygenation on the aerobic metabolic scope of Atlantic cod (Gadus morhua). Journal of Sea Research, 44: 257–265.

Dannevig, E. 1956. The influence of temperature on the formation of zones in scales and otoliths of young cod. Fiskeridirektoratets Skrifter Serie Havundersøkelser, 11: 3–16.

Fablet, R., Pecquerie, L., de Pontual, H., Høie, H., Millner, R., Mosegaard, H., and Kooijman, S. A. L. M. 2011. Shedding light on fish otolith biomineralization using a bioenergetic approach. PLoS One, 6: e27055.

Francis, R. I. C. C., Paul, L. J., and Mulligan, K. P. 1992. Ageing of adult snapper (*Pagrus auratus*) from otolith annual ring counts: validation by tagging and oxytetracycline injection. Australian Journal of Marine and Freshwater Research, 43: 1069–1089.

Freitas, C., Olsen, E. M., Moland, E., Ciannelli, L., and Knutsen, H. 2015. Behavioral responses of Atlantic cod to sea temperature changes. Ecology and Evolution, 5: 2070–2083.

Freitas, C., Olsen, E. M., Knutsen, H., Albretsen, J., and Moland, E. 2016. Temperature-associated habitat selection in a cold-water marine fish. Journal of Animal Ecology, 85: 628–637.

Gjøsæter, J., and Danielssen, D. S. 2011. Age, growth and otolith annulus formation of cod (*Gadus morhua*) in the Risør area on the Norwegian Skagerrak coast during 1986–1996. Marine Biology Research, 7: 281–288.

Høie, H., and Folkvord, A. 2006. Estimating the timing of growth rings in Atlantic cod otoliths using stable oxygen isotopes. Journal of Fish Biology, 68: 826–837.

Høie, H., Folkvord, A., Mosegaard, H., Li, L., Clausen, L. A. W., Norberg, B., and Geffen, A. J. 2008. Restricted fish feeding reduces cod otolith opacity. Journal of Applied Ichthyology, 24: 138–143.

Høie, H., Millner, R. S., McCully, S., Nedreaas, K. H., Pilling, G. M., and Skadal, J. 2009. Latitudinal differences in the timing of otolith growth: a comparison between the Barents Sea and southern North Sea. Fisheries Research, 96: 319–322.

Hüssy, K., St John, M. A., and Böttcher, U. 1997. Food resource utilization by juvenile Baltic cod *Gadus morhua*: a mechanism potentially influencing recruitment success at the demersal juvenile stage? Marine Ecology Progress Series, 155: 199–208.

Hüssy, K., and Mosegaard, H. 2004. Atlantic cod (*Gadus morhua*) growth and otolith accretion characteristics modelled in a bioenergetics context. Canadian Journal of Fisheries and Aquatic Sciences, 61: 1021–1031.

Hüssy, K., Mosegaard, H., and Jessen, F. 2004. Effect of age and temperature on amino acid composition and the content of different protein types of juvenile Atlantic cod (*Gadus morhua*) otoliths. Canadian Journal of Fisheries and Aquatic Sciences, 61: 1012–1020.

Hüssy, K., Nielsen, B., Mosegaard, H., and Clausen, L. 2009. Using data storage tags to link otolith macro- structure in Baltic cod *Gadus morhua* with environmental conditions. Marine Ecology Progress Series, 378: 161–170.

Hüssy, K. 2010. Why is age determination of Baltic cod (*Gadus morhua*) so difficult? ICES Journal of Marine Science, 67: 1198–1205.

Jensen, A. C. 1970. Validation of ages determined from otoliths of gulf of Maine cod. Transactions of the American Fisheries Society, 99: 359–362.

Kalish, J. M., Beamish, R. J., Brothers, E. B., Casselman, J. M., Francis, R. I. C. C., Mosegaard, H., Panfili, J. *et al.* 1995. Glossary for otolith studies 9, pp. 27–43. In: Recent developments in fish otolith research. Ed. by D. H. Secor, J. M. Dean and S. E. Campana. University of South Carolina Press, Columbia, South Carolina.

Kullenberg, G., and Jacobsen, T. S. 1981. The Baltic Sea: an outline of its physical oceanography. Marine Pollution Bulletin, 12: 183–186.

McDonald, P. 2018. mixdist: Finite Mixture Distribution Models. R package version 0.5-5. https://CRAN.R-project.org/package=mixdist (last accessed 1 August 2018).

McQueen, K., Eveson, J. P., Dolk, B., Lorenz, T., Mohr, T., Schade, F. M., and Krumme, U. 2018. Growth of cod (*Gadus morhua*) in the western Baltic Sea: estimating improved growth parameters from tag recapture data. Canadian Journal of Fisheries and Aquatic Sciences, https://doi.org/10.1139/cjfas-2018-0081

Millner, R. S., Pilling, G. M., McCully, S. R., and Høie, H. 2011. Changes in the timing of otolith zone formation in North Sea cod from otolith records: an early indicator of climate-induced temperature stress? Marine Biology, 158: 21–30.

Møller, J. S., and Hansen, I. S. 1994. Hydrographic processes and changes in the Baltic Sea. Dana. Charlottenlund, 10: 87–107.

Nausch, G., Naumann, M., Umlauf, L., Mohrholz, V., Siegel, H., and Schulz-Bull, D. E. 2016. Hydrographic-hydrochemical assessment of the Baltic Sea 2015. Meereswissenschaftliche Berichte No 101 2016—Mar. Sci. Rep, No 101 2016. doi:org/10.12754/msr-2016-0101.

Neat, F. C., Wright, P. J., and Fryer, R. J. 2008. Temperature effects on otolith pattern formation in Atlantic cod *Gadus morhua*. Journal of Fish Biology, 73: 2527–2541.

Panfili, J., Pontual, H.(de), Troadec, H., and Wright, P. J. (eds), 2002. Manual of fish sclerochronology. Ifremer-IRD coedition, Brest, France, 464p. ISBN: 2-7099-1490-5.

Pannella, G. 1971. Fish otoliths: daily growth layers and periodical patterns. Science, 173: 1124–1127.

Pauly, D. 2010. Gasping Fish and Panting Squids: Oxygen, Temperature and the Growth of Water-Breathing Animals, Excellence in Ecology. International Ecology Institute, Oldendorf/Luhe.

Pepin, P., Orr, D. C., and Anderson, J. T. 1997. Time to hatch and larval size in relation to temperature and egg size in Atlantic cod (Gadus morhua). Canadian Journal of Fisheries and Aquatic Sciences, 54: 2–10.

Pilling, G. M., Millner, R. S., Easey, M. W., Maxwell, D. L., and Tidd, A. N. 2007. Phenology and North Sea cod *Gadus morhua* L.: has climate change affected otolith annulus formation and growth? Journal of Fish Biology, 70: 584–599.

Otterbech, F. 1954. The cod population of the Oslofjord. Rapports et Procès-Verbaux des Rèunion du Conseil International pour l'Exploration de la Mer, 136: 15–21.

Rasband, W. S. 1997–2016. ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA. https://imagej.nih.gov/ij/ (last accessed 10 October 2016).

R Core Team. 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Reeves, S. A. 2003. A simulation study of the implications of age-reading errors for stock assessment and management advice. ICES Journal of Marine Science, 60: 314–328.

Rehberg-Haas, S., Hammer, C., Hillgruber, N., Hussy, K., and Temming, A. 2012. Otolith microstructure analysis to resolve seasonal patterns of hatching and settlement in western Baltic cod. ICES Journal of Marine Science, 69: 1347–1356.

Tao, J., He, D., Kennard, M. J., Ding, C., Bunn, S. E., Liu, C., Jia, Y. *et al.* 2018. Strong evidence for changing fish reproductive phenology under climate warming on the Tibetan Plateau. Global Change Biology, 24: 2093–2104.

Trout, G. C. 1954. Otolith growth of the Barents Sea cod. Rapp. Process-Verbeux Reun. Conseil Permanent International pour l'Exploration de la Mer, 136: 89–102.

Williams, T., and Bedford, B. C. 1974. The use of otoliths for age determination. *In* The Ageing of Fish. Ed. by T. B. Bagenal. Surrey, England, Unwin Brothers Ltd, The Gresham Press.

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