

Factors determining variations in otolith microincrement width of demersal juvenile Baltic cod *Gadus morhua*

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ABSTRACT: Pelagic and demersal juvenile Baltic cod *Gadus morhua* L. were collected on the slope and the top of Rønne bank in the Baltic Sea during 2 cruises in November and December 1998. The objective of this study was to evaluate distinct changes in otolith increment width observed in demersal juveniles by comparison with laboratory-reared individuals, and to investigate the factors determining variation in these increments. The different increment-width patterns were identified with a method based on the widths of consecutive increments. Otolith increment widths of juvenile cod were found to be highly variable within and between individuals, in both the experimental and the field samples. The first change in increment pattern observed in the field samples was related to settling. The formation periodicity of increments within the different pattern intervals was confirmed with a growth model based on otolith growth rates of juvenile cod reared in the laboratory under different conditions. In this model, otolith growth rate was expressed as a function of rearing temperature and fish dry weight. Otolith growth of the field samples was calculated using ambient temperatures obtained from a 3D-circulation model. The best fit to observed otolith growth rates was obtained under the assumption that fish on the slope performed daily vertical migrations between the warm surface layer and the cold bottom layer. The data suggested that fish stayed in the surface layer during the first increment-pattern interval, performed vertical migrations during the second interval, and stayed in association with the seafloor in the subsequent interval, corresponding to the time after the breakdown of the thermocline.

KEY WORDS: Otolith microstructure · Daily increments · Juvenile cod · Baltic Sea

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INTRODUCTION

In the Baltic Sea, newly settled Baltic cod *Gadus morhua* L. are predominantly found in nearshore areas down to a water depth of 60 to 80 m (Nielsen et al. 1997, Böttcher et al. 1998). These habitats may be very heterogeneous, particularly with respect to thermal stratification. The hydrography of the Baltic is governed by freshwater input from river runoff and salt-water inflows from the North Sea (Matthäus & Franck 1992), resulting in a permanent pycnocline at 60 to 80 m, with a temperature of ~4°C in the deep water (Gidhagen 1987). Above the pycnocline, a seasonal

thermocline develops in spring at around 30 m depth and remains stable until late autumn, with summer surface temperatures up to 18°C (Gidhagen 1987).

During their pelagic stage, juvenile cod make excursions to the seafloor, and following settling perform extensive vertical migrations into the water column (Böttcher & Oeberst 1996, Nielsen et al. 1997, Böttcher et al. 1998). The combined effects of stratified environment and vertical migration may influence the otolith microstructure of these juvenile fish.

After Pannella's (1971) discovery of otolith microincrements that appeared to be formed on a daily basis, great effort was directed towards the validation

of daily increment formation. A wide range of factors may affect increment formation. Constant light or darkness either has no effect on increment formation periodicity (Campana & Neilson 1982, Geffen 1982, Neilson & Geen 1982) or inhibits visible structure (Taubert & Coble 1977, Radtke & Dean 1982). However, changes in light intensity trigger the formation of increments (Tanaka et al. 1981), which promoted the hypothesis that the photoperiod does not affect increment formation periodicity but acts as a zeitgeber in an endogenously controlled circadian formation cycle (Campana & Neilson 1985). Food level does not appear to affect increment formation periodicity (Marshall & Parker 1982, Campana 1983a, Neilson & Geen 1985). A decrease in increment formation rate at low rations (Geffen 1982) may be an artefact caused by increment widths smaller than the resolution limit of the light microscope used (Campana et al. 1987). At constant temperatures, increments are formed daily, regardless of temperature (Neilson & Geen 1982, Radtke & Dean 1982), but only within the range of temperatures that allow positive somatic growth (Taubert & Coble 1977, Campana & Neilson 1982, Marshall & Parker 1982, Radtke & Dean 1982). Temperature effects on otolith increment-formation rate may also be an artefact caused by the resolution limit of the light microscope. Variations in temperature, on the other hand, result in a disruption of the formation period (Brothers 1981), and Neilson & Geen (1985) demonstrated the formation of 2 increments per 24 h at a temperature cycle of 12 h.

In homogenous water masses, temperature dependence of otolith increment formation does not pose a problem for the ageing of fish. However, in a heterogeneous environment like the settling habitat of Baltic cod, juvenile fish may experience large variations in temperature as a result of their daily vertical migrations. Previous studies have reported difficulties with age determination of juvenile Baltic cod (e.g. Steffensen 1980, Linkowsky & Kowalewska-Pahlke 1993, Böttcher & Oeberst 1996). Observed increments may not be daily and the resulting age estimates may be biased.

During the settling process on the slope of the Bornholm Basin, Denmark, fish are exposed to temperature changes when they migrate across the thermocline. Such temperature effects may overrule the formation of daily increments (Campana 1983b, Neilson & Geen 1985), resulting in pronounced subdaily increments. The main objective of this study was therefore to evaluate whether the increments observed in juvenile Baltic cod otoliths were formed on a daily basis. An otolith growth model was developed, where otolith weight-increase corresponding to individual increments was investigated in relation to different environ-

mental conditions. The parameters of this model were estimated in laboratory experiments with juvenile cod subjected to different rearing conditions after validation of daily increment formation in these fish. The hypothesis of daily increment formation in juvenile Baltic cod was addressed by comparison of otolith increment growth rates observed in field samples with model-derived estimates of these increments. Other objectives were to evaluate changes in increment pattern observed in field samples and the formation periodicity within the pelagic and demersal stages in relation to vertical migration behaviour.

MATERIALS AND METHODS

The validation of daily increment formation in field samples of Baltic cod is based on an otolith growth model developed from juvenile cod reared under different conditions in the laboratory. The daily formation of otolith increments in these samples was validated by comparison of observed increment numbers with known age. From these experimental samples, a model was developed, relating otolith weight increase to different variables. A method was developed to identify intervals with similar increment structure in otoliths of juvenile Baltic cod field samples. The daily periodicity of increment growth observed within these intervals was then validated using the model developed from the experimental samples.

Experimental samples. A total of 500 juvenile Atlantic cod, offspring of 2 females and 2 males, were obtained from the Isle of Man Marine Laboratory, University of Liverpool, British Isles, at an age of 60 d and approximately 20 ± 3.49 mm standard length (SL, average \pm SD). After 7 d of acclimatisation at 12°C at the Danish Institute for Fisheries Research, a temperature mark was induced in the otoliths of the fish by alternating the water temperature between 6 and 12°C, with 3 periods of 3 h exposure to 12°C followed by 1 h exposure to 6°C. After 12 h at 12°C, this temperature cycle was repeated, resulting in a clearly visible increment pattern (Fig. 1).

The fish were measured and weighed to the nearest 0.5 mm and 0.01 g. They were kept in 32‰ seawater at full aeration and a light cycle of 16:8 h light:dark, and fed a diet of dry food pellets (Perla Marine). Three temperature treatments were maintained, 4.9 ± 0.4 , 10.1 ± 1.2 and 15.1 ± 0.6 °C, and monitored daily by a Tinytag temperature logger. Two food levels were provided: ad libitum feeding (approximately 4.0, 5.5 and 7.5% of fish dry weight per day for 5, 10 and 15°C respectively) and approximately 65% of ad libitum level. These 2 feed levels are referred to as high and low, respectively. Food was provided continually by automatic

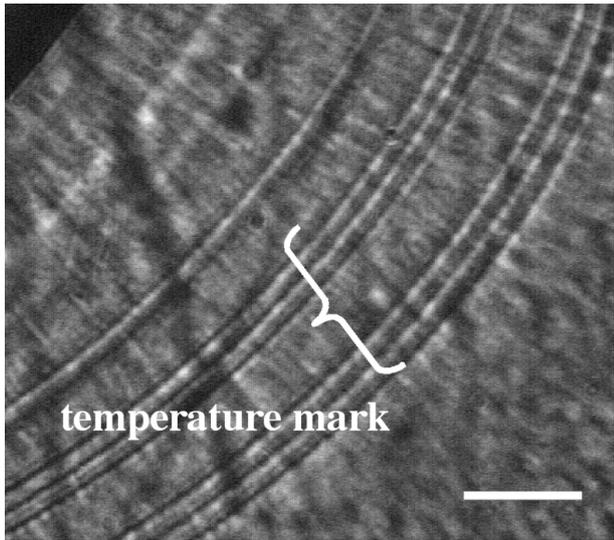


Fig. 1. *Gadus morhua*. Otolith of experimental fish with temperature mark (scale bar = 15 μm)

feeders during daylight hours. At Days 0, 10, 20, 30 and 40 all fish were anaesthetised with MS 222, measured and weighed, and a random subsample of 10 fish from each treatment was killed. The remaining 150 fish were used in other experiments. No other mortalities occurred during the experiment. Sagittal otoliths, hereafter referred to as otoliths, were extracted, rinsed in water and stored in labelled plastic bags.

Otolith treatment. All otoliths were weighed to the nearest μg (otolith weight: OW), mounted on microscope slides with thermoplastic cement (Büehler) with the proximal side facing up, ground to the central plane on a rotating disc covered with grinding paper (grit 1200; M3), and polished on grinding paper (3 μm) and with aluminium oxide paste (Büehler, 0.05 μm). They were then turned, ground to a uniform thickness of about 80 μm and polished again.

For the analysis of otolith microstructure, an image analysis system (IMAGE PRO, Version 4) was used with a microscope coupled to a monitor screen by a CCD camera and a frame grabber. Images were digitised and saved as TIFF-files in black and white. Total otolith length (OL) was measured along the longest distance from the nucleus to the tip of the rostrum. Increment widths (IW_i), counting from the temperature mark, were measured along a profile of grey values (from 0 = black to 255 = pure white, band width = 50 μm) with the 'caliper' tool of Image Pro at a magnification corresponding to 2.2 $\mu\text{m pixel}^{-1}$. The start of an increment was defined as a rising point of inflection calculated from the divergence of the individual pixel's grey value from the running average. Measurements taken at a slightly different angle were scaled to OL.

The number of increments found with this method were tested against the known number of days in the time intervals used.

Otolith growth model. The relationship between OL and OW of the pooled experimental samples is described by the model:

$$\ln(\text{OW}) = a_{\text{OL}} + b_{\text{OL}} \cdot \ln(\text{OL}) + \varepsilon_{\text{OW}} \quad (1)$$

where OW = otolith weight in mg, OL = otolith length in μm , ε_{OW} = error of the individual, and a and b are model parameters.

For each increment i , otolith length (OL_i) was calculated and the corresponding OW estimated as:

$$\text{OW}_i = \exp\{a_{\text{OL}} + [\ln(\text{OL}) - a_{\text{OL}}] \cdot [\ln(\text{OL}_i - \text{OL})]\} \quad (2)$$

where OW_i = otolith weight at increment i , OL = otolith length at death, and OL_i = otolith length at increment i . OW change per day was calculated as $\Delta\text{OW}/\Delta t_i = \text{OW}_i - \text{OW}_{i-1}$.

Explaining variation in fish size by size-dependent growth alone has been shown to fail (Imsland et al. 1998). For the back-calculation of fish dry weight at previous time steps, this parameter was therefore not considered. For all treatments, fish dry weight at previous time steps was analysed using general linear models (GLMs) and was best described by the model:

$$\ln(\text{DW}_i) = a_{\text{DW}} + b_{\text{DW}} \cdot \ln(T_i) + c_{\text{DW}} \cdot \text{day} + d_{\text{DW}} \cdot \text{ration} + \varepsilon_{\text{ind}} \quad (3)$$

where DW_d = fish dry weight in g on Day d , T = actual temperature in $^{\circ}\text{C}$ on Day d , day = day of the experiment, ration = food level, where ad libitum was assigned a value of 100 and low ration a value of 65, and ε_{ind} = model error. Dry weights of all fish were estimated for each day of the experiment using Model (3).

The effect of otolith size and temperature on otolith growth over the experimental period was then also tested using a GLM. Since all cod juveniles and otoliths had roughly the same start size, these variables were not included. The model that described the data best was:

$$\ln(\Delta\text{OW}_i/\Delta t_i) = a_{\text{OW}} + b_{\text{OW}} \cdot \ln(\text{DW}_i) + c_{\text{OW}} \cdot \ln(T_i) + \varepsilon_{\text{mod}} \quad (4)$$

Field samples. A total of 82 pelagic juvenile Baltic cod were collected with RV 'Solea' on Days of Year 305 to 307 (from 3 to 5 November) with an Isaac-Kidd mid-water trawl towed at a speed of 4 knots (Fig. 2). A subsample of 20 fish was selected. Demersal juvenile Baltic cod were collected with RV 'Dana' in 2 main areas of interest in the same region: on 8 December, 169 fish were caught in a shallow area on Oder bank (54° 41' 30" N, 14° 31' 30" E, bottom depth 27 m) and 105 fish were caught in a deeper area on the slope of the Bornholm Basin (54° 33' 30" N, 15° 22' 80" E, bottom depth 47 m) in

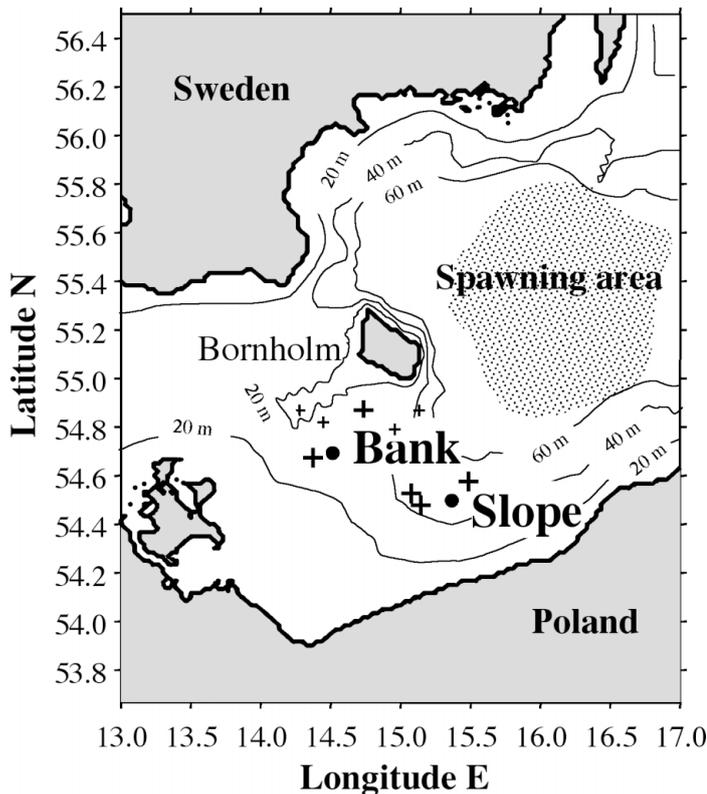


Fig. 2. Map of the Baltic Sea, with sampling stations from the 2 cruises. •: December RV 'Dana' cruise marked as 'Slope' and 'Bank'; +: November RV 'Solea' cruise, where large symbols signify >2 fish, small symbols = 2 fish. Axis units in decimal numbers

a Grande Overture (GOV) bottom trawl towed at a speed of 4 knots (Fig. 2). For analysis, a random sub-sample of 50 fish was selected from each area.

After capture, the fish were immediately frozen at -20°C . In the laboratory fish were thawed, SL measured to the nearest 0.5 mm, dried at 60°C for 48 h and weighed (DW) to the nearest 0.01 g. Otoliths were treated like the experimental samples, and IW_i counted and measured from the accessory primordia to the edge of the otolith.

Increment structure in the field samples. In the field samples, drastic changes in IW_i were observed, particularly in otoliths from the slope. Examples of IW_i patterns representative for fish from the slope and the bank are shown in Fig. 3, with interval patterns indicated by vertical lines. To identify the timing of these changes in increment pattern, as well as the magnitude of the change objectively, we developed a method based on the width of consecutive IW_i .

The difference between the running average of the 10 previous and the 10 following increments was calculated for every increment: D_i = absolute value of

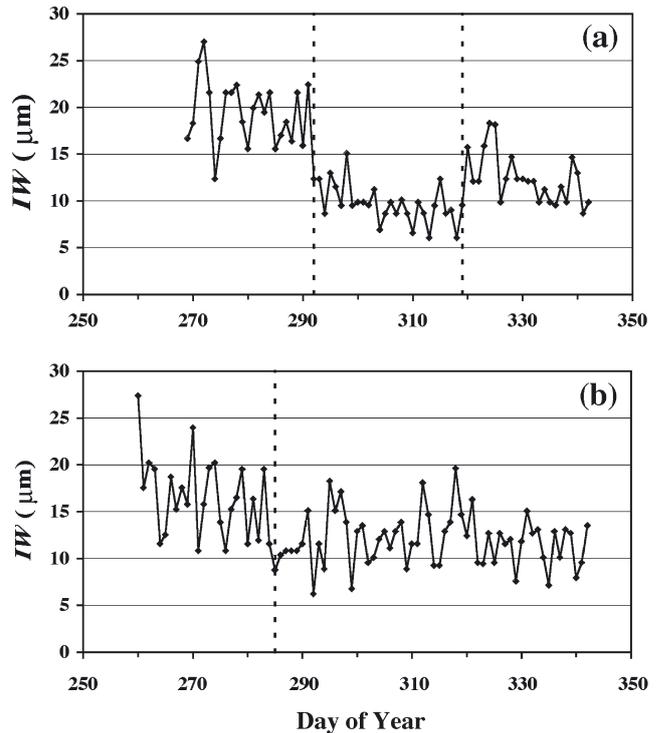


Fig. 3. *Gadus morhua*. Examples of otolith increment width (IW) development over time of juvenile cod caught on (a) slope and (b) bank. Vertical broken lines indicate change in increment width pattern

average(IW_{i-10} to IW_i) – average(IW_i to IW_{i+10}). For the 5 to 10 first and last increments, values were calculated by reducing the number of increments included in the running average. The 5 first and last increments were thus excluded from the analysis. $D_{\max j}$ were defined as the largest D_j s where both D_{i-1} and D_{i+1} had smaller values. These $D_{\max j}$ were achieved when increment widths change from one pattern to another and matched the visual observations well. $D_{\max j}$ were numbered from the accessory primordia outward, and the corresponding otolith lengths (OL_j) were recorded. The corresponding otolith weights (OW_j) were calculated using Model (2). Intervals of similar microstructure patterns (I_j) were numbered from the accessory primordia in the rostral axis and outward, so that $D_{\max j}$ is the j th maximum observed at OL_j , the otolith length at the end of interval I_j (see overview of these terms in Fig. 4). Numbers of increments observed within the different pattern intervals (n_j) were counted. Otolith length and weight at accessory primordia were called OL_0 and OW_0 .

Baltic Sea temperature data. Temperature is a key component in the models used to estimate otolith growth. Daily temperature profiles at the 2 localities were therefore estimated for each day using the 3D

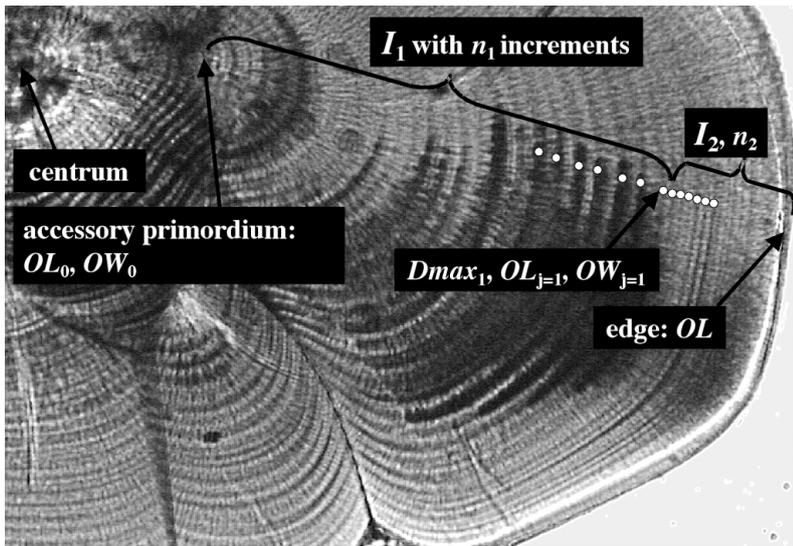


Fig. 4. *Gadus morhua*. Overview of measurements recorded and their abbreviations. centrum = nucleus of the otolith; OL_0 , OW_0 = otolith length and weight at formation of accessory primordia; D_{max1} = maximum difference in average(IW_{i-10} to IW_i) – average(IW_i to IW_{i+10}) denoting change in increment pattern; I_1 , I_2 = increment pattern intervals 1 and 2, with corresponding number of increments n_1 , n_2 ; and corresponding otolith length and weight OL_1 and OW_1 . The 6 increments before and after the change in increment width pattern are indicated with white points

eddy-resolving baroclinic model of the Baltic Sea (Lehmann 1995). This model was developed at the Institute for Marine Research in Kiel, Germany, and is based on the free-surface Bryan-Cox-Semtner model, a modified version of the Cox numerical general ocean circulation model (Bryan 1969, Semtner 1974, Cox 1984). The model covers the whole Baltic, from the Kattegat and Skagerrak to the Gulfs of Riga and Bothnia. It has a horizontal resolution of 5 km with 41 vertical levels and a time-step of 5 min. The model is forced by actual atmospheric data (wind, air temperature, humidity, etc.), provided by the Swedish Meteorological and Hydrological Institute and river runoff taken from a mean runoff database (Bergström & Carlsson 1994), as well as field data obtained during research cruises. Model output values were averaged to obtain a 24 h resolution.

On the slope, a strong thermocline developed around Day of Year 120 (where Day 1 = January 1) at a depth of 35 m, with 14°C at the surface and 5°C at the bottom and persisted approximately until Day of Year 290. After Day of Year 320, an inverse thermocline, with cold water above warmer bottom water, developed and persisted to the day of capture (Fig. 5a). On the bank, the water column was mixed virtually

throughout the year, with temperatures decreasing from approximately 14 to 4.5°C in the investigated time period (Fig. 5b). Data were not available from Day of Year 334 onward, but were extrapolated linearly to measured values from the December cruise.

Validation of daily increment formation in the field samples. For every increment-pattern interval of the field sample, an observed otolith growth rate was calculated as: $\Delta OW/Dt_j = (OW_{j+1} - OW_j)/n_j$. The experiment started 15 d after metamorphosis and the formation of accessory primordia, and Model (4) may thus not be valid for the period from metamorphosis to 15 d post metamorphosis. The increments of the field samples, on the other hand, were analysed back to the accessory primordia. Otolith growth rates of the field sample were therefore not estimated for the first 15 d after formation of the accessory primordia.

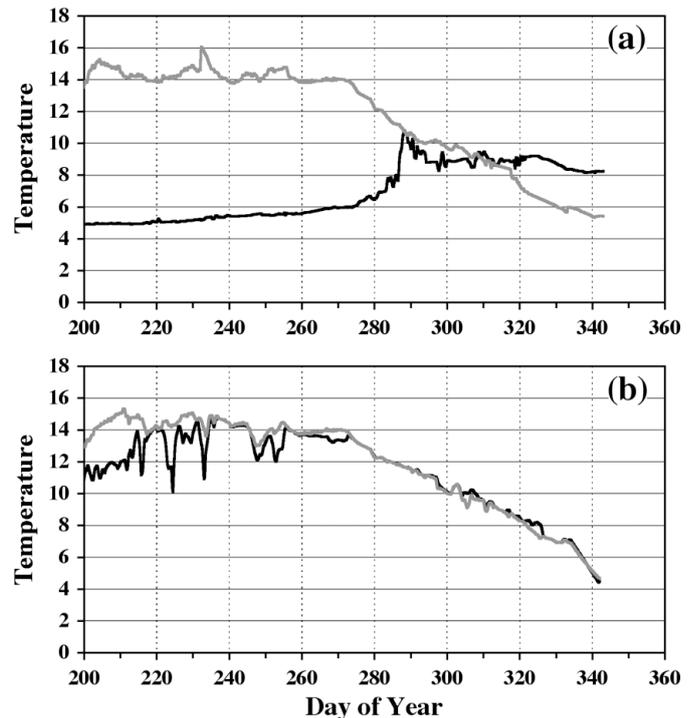


Fig. 5. Temperature in the surface layer and near the bottom of 2 localities on Rønne Bank, Baltic Sea: (a) slope and (b) bank. Black lines: 0.5 m above bottom, grey lines: 1.5 m below surface. In (b) temperatures after Day of Year 334 extrapolated to values observed on Day of Year 342

For the same pattern intervals, expected otolith growth rates were calculated using Model (4). The DW-OW relationship of the pooled November and December samples was best described by the model: $\ln(\text{DW}) = a + b \cdot \ln(\text{OW}) + c \cdot \ln(\text{OW})^2$ ($r^2 = 0.96$, $n = 120$, where $a = -2.025$, $b = 0.924$, $c = 0.094$). Assuming a proportional relationship between fish- and otolith-size over time, the DW variable of the field sample used in Model (4) was thus estimated as:

$$\ln(\text{DW}_i) = \frac{\ln(\text{DW}_{\text{catch}}) \times a + b \cdot \ln(\text{OW}_i) + c \cdot \ln(\text{OW}_i)^2}{a + b \cdot \ln(\text{OW}_{\text{catch}}) + c \cdot \ln(\text{OW}_{\text{catch}})^2} \quad (5)$$

Newly settled juvenile cod are known to spend the day in close association with the seafloor, and migrate up into the water column at night (Perry & Neilson 1988, Lough & Potter 1993). The temperature input used in Model (4) was therefore modelled under 3 different scenarios: (1) juvenile cod stay above the thermocline, (2) juvenile cod stay close to the bottom throughout the day and night and (3) juvenile cod stay close to the bottom during the day and move up into the water column at night. The temperature experienced over 24 h by migrating fish was modelled as:

$$T = [h_{\text{daylight}} \cdot T_{\text{bottom}} + (24 - h_{\text{daylight}}) \cdot T_{\text{surface}}]/24 \quad (6)$$

where h_{daylight} = hours of day light from sunrise to sunset. Surface temperatures over time were very similar at the 2 localities and assumed to be representative of the whole surface mixed layer in which the fish drift from the spawning area in the Bornholm Basin to the capture site (see e.g. Lehmann 1995). Horizontal migrations were assumed not to occur.

Within the pattern intervals I_j , the observed OL_j s were used as starting points and transformed to otolith weight OW_j using Model (2). Otolith growth was estimated using Model (4) for as many days as the number of observed increments, n_j , in I_j . The estimated OW at the end of each interval $\text{OW}_{\text{est } j+1}$ was recorded and otolith growth rates calculated as: $\Delta\text{OW}_{\text{est}}/\Delta t_j = (\text{OW}_{\text{est } j+1} - \text{OW}_{\text{est } j})/n_j$.

RESULTS

Validation of daily increment formation in the experimental samples

For all sampling dates, temperatures and ration levels individually, the slopes of the known age-observed increment-number regressions did not differ significantly from 1 (all $p > 0.05$, all $n = 10$) and the intercepts did not differ significantly from zero (all $p > 0.05$, all $n = 10$). Neither slope nor intercept of the known age-observed increment regression were affected by tem-

perature (ANCOVA, $p > 0.05$, $n = 350$) and ration (ANCOVA, $p > 0.05$, $n = 350$). Increments were therefore assumed to be formed daily in the experimental samples.

Otolith growth model

The parameters of Models (1) to (4) were estimated from the experimental samples after validation of daily increment formation in these samples.

Models (1) and (2)

In the experimental samples, the parameters of Model (1) were estimated as $a_{\text{OL}} = -16.309$ (0.240) and $b_{\text{OL}} = 2.4197$ (0.035), where values in brackets are SEs of the estimate ($p < 0.05$, $r^2 = 0.95$, $n = 350$). The model error ϵ_{OW} was distributed normally with homogenous variance ($s^2 = 0.15$). Neither temperature, ration level nor interaction between the 2 variables were found to have an effect on the OL-OW relationship (ANCOVA, all $p > 0.05$, $n = 350$). There was also no significant difference in the OL-OW relationship between experimental and field samples (ANCOVA, $p > 0.05$, $n = 470$). Therefore, Model (2) was used for all estimations of OW.

Model (3)

The constants of Model (3) were estimated as $a_{\text{DW}} = -4.439$ (0.147), $b_{\text{DW}} = 0.866$ (0.046), $c_{\text{DW}} = 0.041$ (0.002) and $d_{\text{DW}} = 0.003$ (0.001), where values in brackets are SEs of the estimate. All variables were significantly different from zero (GLM, all $p < 0.05$, $r^2 = 0.89$, $\text{df} = 261$), but none of the interactions were significant (GLM, all $p > 0.05$, $\text{df} = 261$). The model error ϵ_{ind} was distributed normally with homogenous variance ($s^2 = 0.11$).

Model (4)

The parameters of Model (4) were estimated to be $a_{\text{OW}} = -3.659$ (0.062), $b_{\text{OW}} = 0.544$ (0.012) and $c_{\text{OW}} = 0.490$ (0.022), where values in brackets are SEs of the estimate (GLM, all $p < 0.001$, $r^2 = 0.74$, $\text{df} = 2253$). The DW term was derived from Model (3). The $\ln(\text{DW}) \times \ln(T)$ interaction was not significant ($p > 0.05$). The residuals were normally distributed with homogenous variance ($s^2 = 0.35$). Substituting the DW term of Model (4) with the working equation of Model (3), otolith growth of the experimental samples is described by the model:

$$\ln(\Delta\text{OW}_i/\Delta t_i) = -6.074 + 0.961 \cdot \ln(T_i) + 0.022 \cdot \ln(\text{day}) + 0.002 \cdot \ln(\text{ration}) \quad (7)$$

Increment structure in the field samples

No changes in increment pattern were found in the pelagic juveniles from the November sample. For the demersal juveniles of the December cruise, an overview of the values of the 3 pattern changes in relation to Day of Year is shown in Fig. 6. For all otoliths with more than one change in increment pattern, the first increment pattern change ($D_{\max 1}$) was found to be significantly larger than the subsequent $D_{\max 2}$ (paired t -test, $p < 0.001$). The secondary changes were predominantly associated with the time of thermocline turnover, while there was no relation between $D_{\max 1}$ and date. The corresponding average increment widths decreased significantly between intervals I_1 , I_2 and I_3 (paired t -tests, $p < 0.001$). Thus, the first change in increment pattern is very distinct from the subsequent, presumably temperature-related, change.

For comparison with published results, the average fish size at OL_1 was estimated using the otolith length–fish length regression of the pooled field samples: $\ln(SL) = a + b \cdot \ln(OL) + c \cdot \ln(OL)^2$, where $a = 0.182$, $b = -1.776$ and $c = 7.550$ ($df = 119$ and $r^2 = 0.97$). This yielded a presumed settling size of 43.4 ± 9.4 mm for the slope and 45.5 ± 6.1 mm for the bank. Accordingly, fish size corresponding to OL_2 and OL_3 were calculated to 59.9 ± 19.2 and 97.2 ± 14.2 mm for the slope and 60.7 ± 9.3 and 79.4 ± 16.0 mm for the bank, respectively.

Validation of daily increment formation in the field samples

On the bank, where fish did not have the option to migrate across a thermocline, and thereby induce a

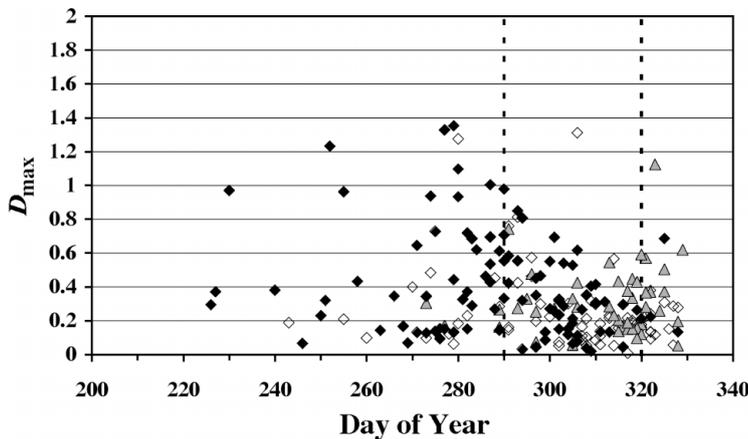


Fig. 6. *Gadus morhua*. Magnitude of change in increment width, calculated as $D_{\max j} = \text{absolute value of average}(IW_{i-10} \text{ to } IW_i) - \text{average}(IW_i \text{ to } IW_{i+10})$. Vertical broken lines indicate time of thermocline turnover. ◆: transition from pelagic to demersal; ◇: first pattern change in demersal stage; ▲: second pattern change in demersal stage

Table 1. *Gadus morhua*. Overview of the comparison of otolith growth rates in the different growth-increment pattern intervals I_j . Behaviour patterns are the 3 scenarios with which the model was run: (1) fish stay above thermocline (Surface), (2) fish stay close to seabed (Bottom), and (3) fish undertake daily migrations (Migration). For fish on the bank a non-migratory behaviour in association with the bottom was assumed (No migration). a = proportionality constant of the $\Delta OW_{\text{est}}/\Delta t_j = a \cdot \Delta OW/\Delta t_j$ relationship (see ‘Materials and methods’ for details), r^2 = correlation coefficient of this relationship, n = number of observations and p = significance (* = 0.05, ** = 0.01, *** = 0.001) level of comparison between observed and estimated growth rates (paired t -test)

Locality	I_j	Behaviour pattern	a	n	r^2	p
Bank	1	No migration	1.108	50	0.74	ns
	2	No migration	0.858	30	0.94	*
	3	No migration	0.741	14	0.57	***
Slope	1	Surface	1.080	50	0.63	ns
	2	Surface	0.869	39	0.59	ns
	3	Surface	0.721	22	0.46	**
	1	Migration	1.114	50	0.52	*
	2	Migration	1.010	39	0.61	ns
	3	Migration	0.626	22	0.45	***
	1	Bottom	1.230	50	0.58	**
	2	Bottom	0.953	39	0.61	ns
	3	Bottom	0.848	22	0.85	ns

subdaily increment structure, observed ($\Delta OW/Dt_j$) and estimated ($\Delta OW_{\text{est}}/\Delta t_j$) growth rates did not differ significantly (paired t -test, $df = 49$, $p > 0.05$) in the first increment interval I_1 , whereas the proportionality constant a of the $\Delta OW_{\text{est}}/\Delta t_j = a \cdot \Delta OW/\Delta t_j$ relationship decreases in I_2 and I_3 (see Table 1). In all intervals, residuals were evenly scattered around zero, with constant variance. On the slope, no significant differences between observed and expected growth rates were found under the assumptions that juvenile cod stay above the thermocline during I_1 , undertake daily vertical migration in I_2 and stay close to the bottom in I_3 (paired t -test, $df = 49$ in I_1 , 38 in I_2 , 21 in I_3 , all $p > 0.05$). Under all 3 assumptions the proportionality constant a decreased from I_1 to I_3 (see Table 1). The residuals were evenly scattered around zero, with constant variance.

DISCUSSION

In this study we showed that it is possible to distinguish between different increment patterns in otoliths of juvenile Baltic cod with an objective method based on the difference between the running means of consecutive

increment widths. Such pattern changes are known to be induced by metamorphosis in a wide range of fish species, with respect to both morphology, e.g. formation of accessory primordia, and suddenly changing growth rates and visual appearance (Lee & Byun 1996, Modin et al. 1996, Arai et al. 1997). Similar changes in otolith growth have been associated with the transition from pelagic to demersal habitat utilisation (Brothers & McFarland 1981, Victor 1982), but have mainly been determined by visual census (e.g. Wilson & McCormick 1997, 1999, see however Victor 1986). In the pelagic juveniles of this study, no change in increment pattern was observed, while the first pattern change in demersal-caught juvenile Baltic cod occurred within a few days at a fish size of 40 to 50 mm, corresponding to the settling size determined by 24 h trawling studies (Böttcher & Oeberst 1996, Nielsen et al. 1997) and stomach content analysis (Hüssy et al. 1997, Nielsen et al. 1997). Thus, the first change in increment pattern, from wide to narrow increments, seems to be correlated with settling.

The periodicity of increment formation has traditionally been validated by laboratory experiments. In larval fish this has predominantly been achieved by comparing increment counts with the known age of the fish (e.g. Geffen 1982, Folkvord et al. 1997). In juvenile fish, marking of the otolith prior to release in the wild or rearing of the fish for a known period of time has been the preferred procedure. Such marks may be induced either with chemical reagents, such as oxytetracycline and alizarin complexone (e.g. review in Geffen 1992) or by periodical variation in water temperatures (e.g. Mosegaard & Titus 1987, Volk et al. 1990, 1994, this study). Neither of these approaches is applicable to the evaluation of increment structures observed in unmarked field samples. For this purpose, an otolith growth model was developed, based on data from laboratory-reared fish, where the hypothesis of daily increment formation was tested and accepted.

The best fit between observed and estimated growth rates indicated that juvenile cod spend most of their time above the thermocline during the pelagic stage (I_1), undertake vertical migrations during the early demersal stage (I_2) and remain associated with the bottom to a higher degree in the late demersal stage (I_3). These results correspond well with previous observations from 24 h trawl studies on the vertical distribution of juvenile Baltic cod (Böttcher et al. 1998).

Due to the high correlation between observed and estimated otolith growth rates, it was not possible to reject the hypothesis that the observed increments in juvenile Baltic cod otoliths were formed daily. The estimated number of days within the different increment pattern intervals was therefore accepted to be representative of true fish age.

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