Two- and three-dimensional maturation reaction norms for the eastern Baltic cod, *Gadus morhua*

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Industrial fisheries have caused decreases in the size and age at maturation in several stocks of cod (*Gadus morhua*). Although earlier maturation can be a phenotypic response to improved growth conditions, estimation of probabilistic maturation reaction norms (PMRNs) can remove most impacts of growth and demography from maturation schedules and has often revealed a residual, potentially genetic, trend. In this study, size- and condition-based PMRNs were estimated for eastern Baltic cod cohorts from 1987 to 2003. The PMRNs suggested that both length and condition at 50% probability of maturing (Lp_{50} and Cp_{50}) had decreased by 15–20%, more notably in females. Simultaneously with changes in maturation schedules, the length-at-age of mature fish decreased for most combinations of age and sex. Decrease in growth may be partly explained by advanced maturation, but temporal fluctuations in PMRNs suggest also a residual environmental impact on both growth and maturation. The results may indicate a genetic change in the eastern Baltic cod stock, adding pressure to reduce fishing mortality on it.

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Introduction

Exploitation may threaten genetic structures in fish and game populations both directly and by favouring alterations that enhance adaptation to changed mortality patterns (Law, 2000; Coltman *et al.*, 2003; Hutchinson *et al.*, 2003). Increased mortality caused by harvesting decreases the chances for survival and reproduction later in life and therefore favours early age at maturation that ensures reproduction before being captured (Law and Grey, 1989). In addition, size- or state-selective fishing may favour decreased growth rate, increased reproductive effort, and altered feeding and mating behaviour (Conover *et al.*, 2005; Reznick and Ghalambor, 2005; Walsh *et al.*, 2006). Aquaculture studies demonstrate that age-at-maturity and growth rate provide additive genetic variance that converts to a high heritability under controlled conditions (Kolstad *et al.*, 2006). Therefore, strong selective pressures imposed by fisheries potentially affect these traits also in exploited populations.

As a plastic response to harvesting, individual growth rates may increase, so age-at-maturity might decrease through an earlier attainment of maturation size (Trippel, 1995). However, when selection continues for decades, size-at-maturity may also decrease, and there may be an underlying genetic change in maturation schedules (Olsen *et al.*, 2004; Grift *et al.*, 2007; selectively reviewed by Jørgensen *et al.*, 2007). A major concern is that genetic changes towards earlier maturity or slower growth rate can reduce yields through reduced adult body size (Law and Grey, 1989; Conover and Munch, 2002), and possibly hamper recruitment through an increase in the proportion of small spawners (Marshall *et al.*, 2006). Fishing-induced evolutionary change might even affect population viability (Walsh *et al.*, 2006) and eventual recovery from a collapse (Hutchings, 2005).

The probabilistic maturation reaction norm (PMRN) offers a statistical concept that can help to disentangle genetic effects from environmentally driven phenotypic variation in maturation schedules (Heino *et al.*, 2002). A PMRN describes the population-level probability of an average individual maturing as a function of age and predominantly environmentally driven variables, such as length and condition. The ability of PMRNs to reflect a genetic property depends on the validity of the variables included (reviewed by Dieckmann and Heino, 2007). Most often, PMRNs are constructed using information on age and length at the time of or before spawning. However, body energy reserves may contribute extensively to the maturation of cod (Marshall and McAdam, 2007), suggesting that some measure of body condition, e.g. relative body mass index or relative liver weight, should be used in the estimation of PMRNs for cod. In contrast, not all studies agree that condition is key in the maturation of cod (Morgan and Lilly, 2006). However, body condition index has been included in the estimation of PMRNs for North Sea plaice (*Pleuronectes platessa*; Grift *et al.*, 2007) and sole (*Solea solea*; Mollet *et al.*, 2007), and in those cases its inclusion only strengthened the results obtained using age- and size-based PMRNs. In this study, relative body condition index (the condition factor) is included in the analysis to compare the results obtained using age- and size-based, and age-, size-, and condition-based PMRNs.

The Baltic Sea supports western and eastern stocks of Atlantic cod (*Gadus morhua*) that spawn at discrete locations and times, and which have genetically diverged from other North Atlantic stocks of cod (Nielsen *et al.*, 2001). Age groups 2−5 are currently the most important for fisheries (ICES, 2006a). However, as a consequence of access to sufficient data of suitable quality, our focus is solely on the eastern stock (from now on called Baltic cod), which spawns mainly in the Bornholm basin (Kraus *et al.*, 2002) and is distributed in ICES Subdivisions 25−28. Through years of increases in cod abundance, fishing effort increased which then, together with years of poor recruitment, led to massive depletion of the stock by the 1990s (Köster *et al.*, 2005). Despite the present low level of the stock, fishing for cod in the Baltic Sea has remained at an unsustainably high level (ICES, 2006a).

Cod in the Baltic Sea mature unusually young, generally between ages 2 and 4 years, males a year earlier than females (Rutkowicz, 1963). In accordance with life-history theory, Andersen *et al.* (2007) predicted that intense fishing of cod in the Baltic Sea would lead to an evolutionary response towards maturation at smaller size. Indeed, the proportions of cod that mature very young have increased during the past two decades simultaneously with decreasing masses-at-age and population size (Cardinale and Modin, 1999; ICES, 2006a). Assuming that slow growth rate relates to delayed maturation (Grover, 2005),

the decrease in mean age-at-maturity, despite the declining growth rates and condition, suggests that the change must include a genetic component (Trippel, 1995; Dieckmann and Heino, 2007). In this study, age- and length-based and age-, length-, and condition-based PMRNs are estimated for the eastern Baltic cod stock to examine whether the changes in maturation patterns could have taken place independently of mortality and growth, whether such changes differed between sexes, and whether the inclusion of condition factor could change the results of age- and size-based PMRNs.

Material and methods
Data

Cod were collected in the first quarter of the year between 1989 and 2007 in ICES Subdivisions 25−28 ($n = 7204$, 1113, 1626, 1833 per subdivision, respectively, Figure 1) as part of the Baltic International Bottom Trawl Survey by the Swedish RV "Argos". The known problems in age determination of Baltic cod between countries (ICES, 2006b) prevented consistent analyses of the international dataset despite country-specific exclusion of data (results not shown). For consistency, therefore, exclusively Swedish data that, in terms of trends in weight-at-age and other parameters, are comparable with international data (ICES, 2006a) were used. Part of this dataset was used in the analysis of

Figure 1. Map of the study area with ICES Subdivisions (SDs).

age- and length-based maturity ogives by Cardinale and Modin (1999).

The analysis focused on ages 2–4 years, because Baltic cod have matured in this age range for at least 50 years (Rutkowicz, 1963; ICES, 2005). Data from the cohorts spawned in the years 1987–2003 were used for the analyses of two- and three-dimensional PMRNs (age-, length-, and condition-based PMRNs, respectively), length (precision 1 cm), mass (precision 1–5 g), and Fulton's condition factor derived from sex-specific mass/length regressions at age ($n = 5228$ males and 6112 females, on average 348 fish per cohort per sex). The final age-specific sample sizes fulfil the minimum recommended sample size (100) derived from simulation analysis by Barot *et al.* (2004). Juvenile fish were well represented in the samples (43.7% of all cod 2–4 years old).

Before 2001, trawls were conducted systematically in space, but they have since been random, though relatively constant by subdivision during the whole period. The subsampling of trawled fish for determination of age, length, mass, and maturity was size stratified (in 1-cm classes), targeting five fish for every location and size class sampled. The maturity status (4–8 classes of gonad states, reclassified to mature/immature) was determined by macroscopic examination (Tomkiewicz *et al.*, 2003), and age was determined from otoliths by experienced personnel according to standardized methods. Skipped spawning was observed, but was extremely rare (frequency 0.6%). All cod exhibiting skipped spawning were classified as mature.

Estimation of PMRNs

Because the first-time spawners could not be separated from repeat-spawners, the PMRNs were estimated using the demographic estimation method of Barot *et al.* (2004). All mature 2-year-old cod were assumed to be mature for the first time (in the data, there was one mature 1-year-old fish out of 1191 fish). The most important requirement of the estimation method is that the proportions of mature fish within each age and length are unbiased (Barot *et al.*, 2004). This assumption is likely met when sampling has been size stratified. However, potential spatial changes in fishing pressure could not be controlled for and could have altered the proportions of mature and immature fish even within size classes over time.

Maturation reaction norms

The age- and length-based (two-dimensional) [Equation (1)] and age-, length-, and condition-based (three-dimensional) [Equation (2)] maturation reaction norms were constructed for sexes and cohorts separately according to Barot *et al.* (2004) as

$$m(a, s) = \frac{o(a, s) - o(a - 1, s - \Delta s)}{1 - o(a - 1, s - \Delta s)} \tag{1}$$

$$m(a, s, c) = \frac{o(a, s, c) - o(a - 1, s - \Delta s, c - \Delta c)}{1 - o(a - 1, s - \Delta s, c - \Delta c)}, \tag{2}$$

where maturation ogives $o(a, s)$ and $o(a, s, c)$ are defined as a function of age (a), size (length; s), and condition (c). Δs refers to the annual increase in the length, and Δc to the annual change in condition of the fish. The term $o(a - 1, s - \Delta s, c - \Delta c)$ becomes zero for 2-year-old fish.

The probabilities of being mature as a function of age and size, i.e. the maturity ogives, were estimated using the following simple logistic regression model, chosen based on biological relevance and because it had good explanatory power for the probability of being mature:

$$Logit[o(a, s)] \sim \alpha_0 + \alpha_1 a + \alpha_2 s, \tag{3}$$

where a refers to age, s to length, and the various values of α are the estimated parameters. This model restricts the contours of the PMRN to be linear, and its width to be independent of age (Heino *et al.*, 2002). For three-dimensional PMRNs, a logistic regression with a stepwise backward model-selection procedure based on log-likelihood ratio tests between two alternative models, starting from a model including all main effects and second-order interactions, was used to examine which statistical model could best predict the probability of being mature as a function of age, length, and condition. However, the final sex-specific models were further customized to obtain just one model for both sexes (see the "Results" for the details of model selection).

As individual-based growth estimates were not available, and the length-at-age data were not random but came from length-stratified samples, lengths-at-age were estimated by fitting von Bertalanffy's growth curve on individual age-at-length data, then reversing the function to output the mean lengths-at-age using the following pair of functions:

$$\begin{cases} t(s) = -\dfrac{1}{k}\log\left(\dfrac{L_\infty - s}{L_\infty - L_0}\right) \\ s(t) = L_\infty - ((L_\infty - L_0)\exp(-kt)), \end{cases} \tag{4}$$

where $t(s)$ refers to age-at-length, $s(t)$ to length-at-age, k the growth rate, L_∞ the asymptotic length, and L_0 the length at hatching. The annual length increments [Equations (1) and (2)] were then derived from the growth model. To examine the sensitivity of PMRNs on estimated population-level length increments, the two-dimensional PMRNs were calculated using lengths-at-age derived using data from all cod, or from juvenile cod only (Dieckmann and Heino, 2007).

By definition, the relative body condition index (here defined as the adjusted Fulton's condition factor, K) is independent of length (Pearson's correlation between the sex-specific K and length, $p > 0.05$, and for the common K, $R = -0.026$, $p = 0.005$). Therefore, for three-dimensional PMRNs, the population-level sex-specific mean condition factors at age were used to derive annual changes in condition. The adjusted Fulton's condition factor, K, was calculated for each cod using the equation, $K = 100\ \text{g}^{-1}\ \text{cm}^{b}\ W\ (\text{g}) \times (L_\text{t}\ (\text{cm}))^{-b}$, where W is the total body mass, L_t the total body length, and b the slope of a regression of $\ln(W)$ on $\ln(L_\text{t})$ of the population (Jones *et al.*, 1999). In the reaction norm analyses and Figures, $b = 3.156$ for males and $b = 3.131$ for females. The exponent 3.141 was used in other analyses to allow comparison between sexes. PMRN midpoints (i.e. length at 50% probability of maturation, Lp_{50}) for the three-dimensional reaction norms were calculated at fixed condition 0.6, and the Cp_{50} (condition at 50% probability of maturing) correspondingly at a fixed length of 35 cm, because these values represented the condition and length when maturation generally occurred. If variation in condition reflects feeding conditions, the annual changes in condition factor should capture the growth variation lost by

forcing the interannual growth to follow the von Bertalanffy growth curve. The age and size combinations yielding the expected probability of maturing, and the probabilities of maturing at fixed length or at mean lengths and condition were calculated for each age from Equations (1) and (2).

Estimation of error

As it is beyond realistic options to derive analytical estimates of the error in maturation reaction norms (Barot *et al.*, 2004), the whole estimation procedure including growth estimations was repeated 1000 times by bootstrapping (with replacement) the original data using the original sample size and stratification for age. Values for the 95% confidence intervals were derived using the first percentile technique, i.e. picking the 25th and 975th values from the sorted dataset of 1000 values. If the bootstrap sample was unsuitable for determining the maturity ogive (i.e. the logistic regression procedure failed), the bootstrap sample was discarded and a sample number <1000 was used to estimate the reaction norm midpoint and its variation. The average success rate, however, was >95%. The squared inverse value of the standard deviation of individual bootstrapped $Lp_{50}s$ was used to weight the least squares in linear regressions for the trend over cohorts.

Statistics

All fitting of von Bertalanffy growth models, estimation of maturation reaction norms and length-at-age calculations were performed with customized procedures included in AV Bio-Statistics 4.8 software (freely available from the first author). SPSS (SPSS 14.0.1, SPSS Inc., USA) was used for stepwise model selection for logistic regressions, and for weighted linear regressions to study trends in estimated cohort-wise condition factors, lengths-at-age, Lp_{50} and Cp_{50} values, and age-specific maturation probabilities. Pearson's correlation analysis was used to study the correlation between age, length, and condition factor. Fully factorial (including all possible main effects and their interactions) ANCOVA, performed in SPSS, was used to examine factors (cohort, subdivision, age, sex, maturity) affecting the variation in condition. Condition factor was log-transformed before ANCOVA analysis to meet the assumptions of the test. Otherwise, no transformations were carried out before analysis.

Results

Variation in condition factor

The condition factor decreased over time between the 1987 and 2003 cohorts, for both sexes (Table 1, Figure 2). Cohort explained more of the variation in condition than age (which corresponds to catch year, because cohort = catch year − age; Table 1), suggesting the existence of a long-term trend. In addition, 1% of the total variation was explained by variation between subdivisions (Figure 1), the difference being that fish in the southwestern Baltic Sea (Subdivision 25) were in slightly but statistically significantly worse condition than fish in the other subdivisions (pairwise Bonferroni comparisons against Subdivision 25; $p < 0.001$). The difference between the sexes was marginal, but males were on average in better condition than females (Table 1, Figure 2). However, mature fish were in better condition than immature fish (Table 1; 0.60 vs. 0.58). The mean estimated condition in juvenile males was 0.582, in juvenile females 0.584, in mature males 0.603, and in mature females 0.598.

Maturity ogive models

The best single explanatory variable to correctly assign fish to maturity class was body mass (Table 2) which, however, includes gonad mass and is therefore an apparent indicator of maturity, but not an appropriate predictor of maturation. For the two-dimensional (age- and length-based) analysis of PMRNs, a maturity ogive model including a constant and the main effects of age and length was chosen (Table 2). In that model, the odds ratios for age (years) and length (cm) were, respectively, 1.20 and 1.28 for males, and 2.32 and 1.35 for females, indicating that length was a more important determinant of being mature than age. All model variables except the effect of age in males (cohort as a factor, $p = 0.059$) were statistically significant ($p < 0.001$).

The statistically optimal three-dimensional (age, length, condition) maturity ogive models appeared to be different for males than for females (Table 2). In males, only the main effect of length was included, but all the second-order interaction terms appeared statistically significant. In females, a more complex model including the main effects of age, length, and condition, and the interaction terms between age and length, and condition factor and length, proved best in terms of explanatory power. In that model, however, length was not statistically significant ($p = 0.071$). To be able to compare the results between sexes,

Table 1. Factors explaining the variation in condition factor between cohorts 1987 and 2003 according to a fully factorial ANCOVA.

Source of variation	Type III sum of squares	d.f.	F	Significance	η^2
Age	2.8	1.0	261.9	<0.001	0.023
Cohort	15.9	1.0	1 475.4	<0.001	0.115
Subdivision	1.3	3.0	38.8	<0.001	0.010
Sex	0.0	1.0	0.1	0.773	<0.001
Maturity	1.0	1.0	91.6	<0.001	0.008
Area × sex	0.1	3.0	2.8	0.039	0.001
Area × maturity	0.2	3.0	7.3	<0.001	0.002
Sex × maturity	0.1	1.0	6.6	0.010	0.001
Area × sex × maturity	0.0	3.0	0.4	0.743	<0.001
Error	121.7	11 315.0			
Total	3 332.7	11 333.0			

Values of η^2 indicate effects, i.e. the variation explained by the focal variable.
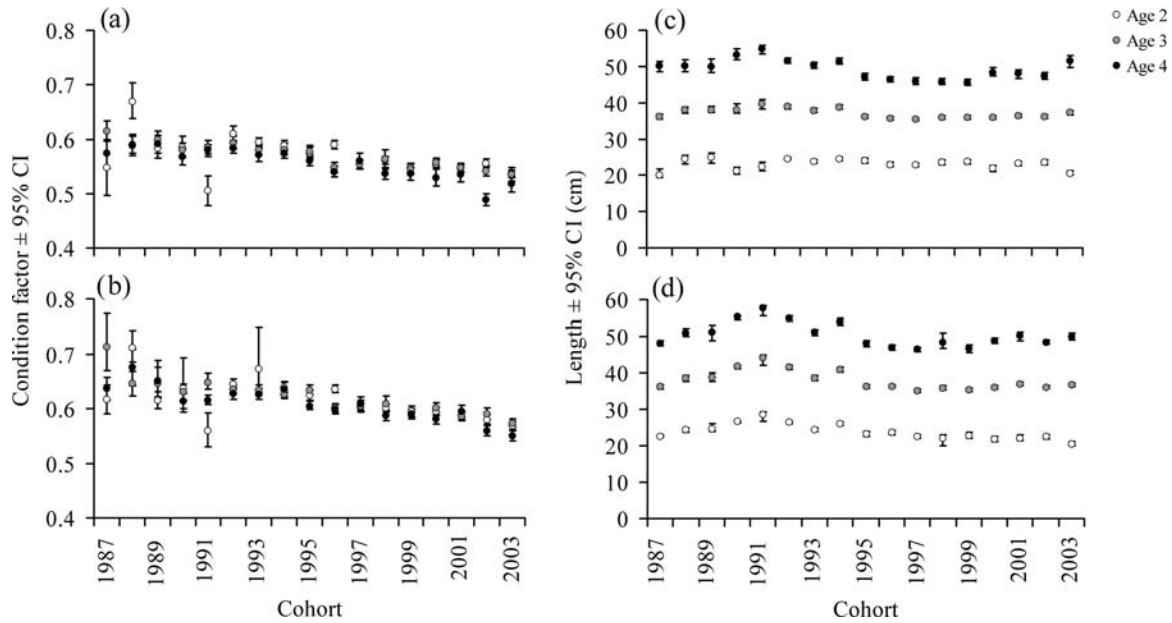
Figure 2. Changes in (a and b) the condition factor and (c and d) the length-at-age of eastern Baltic cod over cohorts 1987–2003 in 2–4-year-old (a and c) males and (b and d) females. For details of significance levels, refer to Table 1.

Table 2. Comparison of the factors explaining the probability of being mature (ogive models) for 2–4-year-old cod of the cohorts 1987 and 2003.

Model (cohort and constant +)	Males		Females	
	Nagelkerke's R^2	% Correct	Nagelkerke's R^2	% Correct
Only cohort (as factor)	0.07	63.9	0.06	57.1
Age	0.45	78.2	0.68	86.0
Length	0.57	81.6	0.77	89.6
Mass	0.58	83.0	0.77	89.8
Condition	0.07	63.6	0.06	57.5
Age, length	0.57	81.8	0.78	90.1
Age, mass	0.58	83.0	0.78	90.1
Age, length, age^2	0.58	81.8	0.78	90.1
Age, length, age × length	0.58	81.9	0.78	90.0
Age (as factor), length, age × length	0.58	81.9	0.78	90.0
L, A × C, C × L, A × L (best for males)	0.60	83.2	0.79	90.3
A, L, C, A × L, C × L (best for females)	0.60	83.4	0.79	90.3
Length, age × length, age × condition	0.59	82.4	0.79	90.3

The last model was chosen for the three-dimensional PMRN analysis for both sexes. All logistic regression models included cohort as a factor and a constant in addition to the listed variables. Capital letters refer to terms: A, Age; L, Length; C, Condition.

one model was customized for both. The final model, including the effects of length, age × length, and age × condition, had very good predictive capacity compared with other possible ogive models (Table 2), all terms were statistically highly significant for both sexes ($p < 0.001$), and the odds ratios for non-constant continuous variables (age in years, length in centimetres, condition without an unit) in males were 1.37, 0.97, and 12.33, and in females, 1.42, 0.99, 7.81, respectively. That model also assigned slightly more fish to the correct maturity class than the two-dimensional model (Table 2), and the average realized classification performance in the cohort-wise analyses was 86.8%.

Trends in growth and PMRNs based on age and length

Linear regression analysis indicated that lengths-at-age modelled using an inverse von Bertalanffy growth equation showed a decreasing trend over cohorts 1987 and 2003 in males at ages 3 and 4 years, and in females at ages 2 and 3 years (Table 3, Figure 2). The growth rate of juvenile fish did not show any linear trends over time (Table 3).

Females had a probabilistic tendency to mature on average 1 year later and ∼5 cm longer than males (Table 3, see also Figure 3). The cohort of 1987 was excluded from the analyses of temporal trends because it had anomalously high values of Lp_{50} (Figure 3). Values of Lp_{50} decreased statistically significantly in

Table 3. Variation in lengths-at-age and midpoints of the two-dimensional maturation reaction norm analysis (Lp_{50}) over the cohorts 1988 and 2003.

Age	Sex	Growth	Length-at-age (cm)				Two-dimensional reaction norm midpoint (Lp_{50})				
			1988	2003	Percentage change	Significance	1988	2003	Percentage change	Significance	Rate[*]
2	Males	All	24.2	22.7	−5.8	0.252	28.9	30.0	3.7	0.694	n.s.
2	Males	Juveniles	23.8	23.4	−1.3	0.698	28.9	30.0	3.7	0.699	n.s.
3	Males	All	37.9	35.8	−5.6	0.047	34.6	29.2	−16	0.074	n.s.
3	Males	Juveniles	37.1	36.9	−0.5	0.823	34.5	29.1	−16	0.079	n.s.
4	Males	All	52.6	45.6	−13	0.006	38.9	26.5	−32	0.017	25.6
4	Males	Juveniles	48.7	49.0	0.7	0.739	38.9	26.3	−32	0.026	26.1
2	Females	All	25.6	22.2	−13	0.025	39.5	34.2	−13	0.097	n.s.
2	Females	Juveniles	24.2	23.1	−4.8	0.231	40.0	33.6	−16	0.043	11.6
3	Females	All	39.5	35.5	−10	0.027	38.7	33.5	−14	0.014	9.6
3	Females	Juveniles	36.6	38.0	3.8	0.174	38.8	33.5	−14	0.013	9.8
4	Females	All	52.7	47.7	−9.6	0.063	40.6	31.7	−22	0.015	16.5
4	Females	Juveniles	47.8	51.1	6.8	0.050	40.4	31.8	−21	0.019	16.0

All estimates of change are based on linear regressions on bootstrapped means using the inverse of standard deviation of bootstrapped means as weighting for the least squares ($n = 16$ cohorts). The column headed "Growth" indicates whether the growth estimates were based on all fish or on just juvenile fish. n.s., not significant.
[*]The evolutionary rate for statistically significant trends is in kdarwins (for calculation, see Jørgensen *et al.*, 2007).
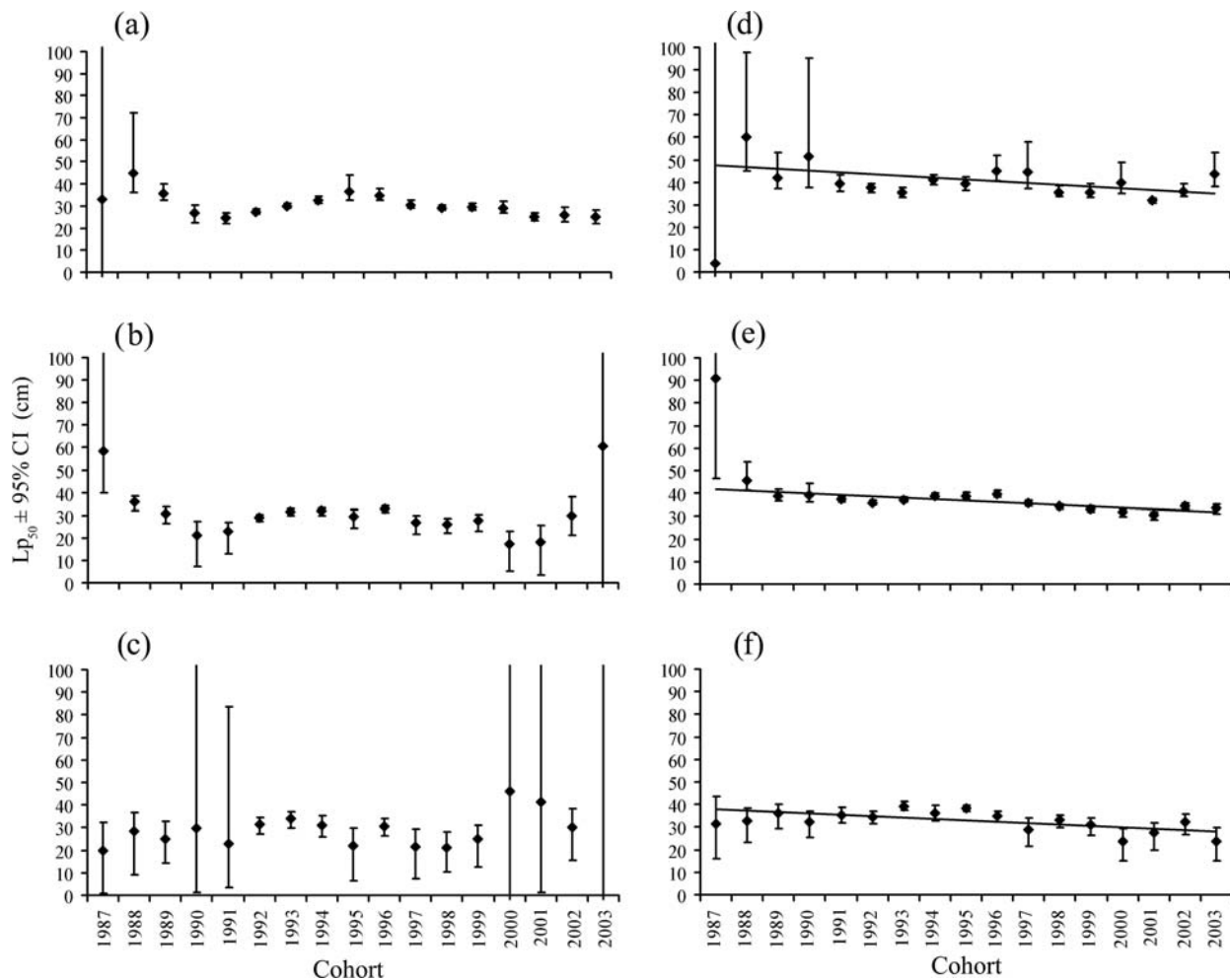

Figure 3. Lp_{50} estimates at fixed condition 0.6 for males (a) 2, (b) 3, and (c) 4 years old, and for females (d) 2, (e) 3, and (f) 4 years old for the 1987–2003 cohorts. The error bars represent bootstrapped ($n \leq 1000$) 95% confidence intervals (CIs), and the lines statistically significant linear regression lines for cohorts 1988–2003. For details of significance levels, refer to Table 4.

Table 4. Probabilities of cod maturing at a fixed length of 35 cm over the cohorts 1988 and 2003, according to the two-dimensional PMRN analysis.

Age	Sex	Growth	1988	2003	% Change	Significance
2	Males	All	0.86	0.77	−11.4	0.469
2	Males	Juveniles	0.87	0.76	−12.6	0.436
3	Males	All	0.74	0.85	14.6	0.409
3	Males	Juveniles	0.69	0.86	24.1	0.206
4	Males	All	0.77	0.95	23.5	0.113
4	Males	Juveniles	0.74	0.95	27.4	0.088
2	Females	All	0.05	0.22	309	0.164
2	Females	Juveniles	0.05	0.23	336	0.146
3	Females	All	0.21	0.57	173	0.019
3	Females	Juveniles	0.20	0.57	180	0.019
4	Females	All	0.18	1.06	481	0.002
4	Females	Juveniles	0.16	1.05	552	0.002

All estimates of change are based on linear regressions on bootstrapped means using the inverse of standard deviation of bootstrapped means as weight for the least squares ($n = 16$ cohorts). The column headed "Growth" indicates whether the growth estimates were based on all fish or just juvenile fish.

Table 5. Results of the three-dimensional PMRN analysis over cohorts 1988 and 2003.

Age	Sex	1988	2003	% Change	Significance	Rate[*]
Cp_{50} at length 35 cm						
2	Males	0.55	0.44	−20.2	0.173	n.s.
3	Males	0.54	0.37	−32.2	<0.001	25.2
4	Males	0.66	0.34	−48.5	<0.001	44.2
2	Females	0.90	0.51	−43.0	0.097	n.s.
3	Females	0.70	0.53	−24.0	0.030	18.5
4	Females	0.66	0.52	−21.8	0.009	15.9
Lp_{50} at condition 0.6						
2	Males	30.3	28.0	−7.5	0.444	n.s.
3	Males	32.9	27.7	−15.9	0.145	n.s.
4	Males	34.1	25.9	−24.0	0.144	n.s.
2	Females	41.2	32.2	−21.8	0.004	16.4
3	Females	39.9	32.5	−18.7	0.002	13.7
4	Females	40.3	30.6	−24.0	0.031	18.4

All estimates are based on linear regressions on bootstrapped means ($n = 16$ cohorts). The inverse value of bootstrap variance was used to weight the least squares in the regression analysis. n.s., not significant.
[*]The evolutionary rate for statistically significant trends is in kdarwins.

males at age 4 years and in females at ages 3 and 4 years, independently of the maturity status of the cod used for length-at-age estimation (Table 3). If the growth increments were estimated only from juvenile cod, the decrease was also significant in females at age 2 years (Table 3). The proportional decreases in values of Lp_{50} were in general larger than those in lengths-at-age (Table 3), demonstrating that decreased size at maturation (attributable to slower growth) could not fully explain the shifts in reaction norms, but that maturation also shifted towards earlier age (the level of Lp_{50} values scales with the growth rate).

The probability of maturing at age and at fixed length (35 cm) followed a slightly different pattern, because the probability of maturing at 35 cm increased statistically significantly only in females at ages 3 and 4 years (Table 4). The mismatch between the values of Lp_{50} and the probabilities of maturing at fixed size depended on the non-linear form of the reaction norm with respect to size and the growth increment. Therefore, the age-specific values of Lp_{50} provide the best measures of change in maturation schedules.

PMRNs based on age, size, and condition
The inclusion of condition factor in the PMRN analysis strengthened the conclusion on sex-differentiated temporal trends. Values of Lp_{50} at a fixed condition of 0.6 decreased in females at all ages, but not in males (Table 5, Figure 3). The condition at 50% probability of maturing (Cp_{50}) at a fixed length of 35 cm decreased in both males and females at ages 3 and 4 years (Table 5).

Discussion
The analysis of length- and age-based PMRNs suggested that the length-at-50%-probability of maturing decreased between cohorts 1988 and 2003 in females at ages 2 and 4 years, and in males at age 4 years, so confirming the theoretical predictions of fishing-induced evolution in the Baltic cod (Andersen et al., 2007). The inclusion of condition factor in the analysis strengthened the results obtained using two-dimensional maturation reaction norms in females, but explained the variation in maturity

ogives in males so that no trends remained in condition-controlled values of male Lp_{50}. However, the values of Cp_{50} decreased not only in females (at ages 3 and 4 years) but also in males at ages 3 and 4 years, indicating increased probability of maturing at low condition. These results are in line with previously documented changes in age- and length-based maturation ogives for the Baltic cod (Cardinale and Modin, 1999), but they suggest that demographic effects or changes in growth or mortality alone cannot explain the observed shift in the timing of the maturation. The rate of evolution of PMRN midpoints observed in this study (9.6–25.6 kdarwins; Tables 3 and 5) lies within the range of earlier observations on different cod stocks, but it is faster than in flatfish species such as plaice and sole (Jørgensen et al., 2007).

Variation in growth and its implications for the PMRN analysis
The mean length-at-age of all cod decreased simultaneously with the decrease in values of Lp_{50} in almost all age groups, as has been observed in northwestern Atlantic cod by Olsen et al. (2005). This opposes the expectation that fishing should relax the density-dependence in growth usually prevalent in fish populations, and increase phenotypic growth rates (Lorenzen and Enberg, 2002). However, there were no temporal trends among juvenile cod, so the decrease in growth rate was likely caused by advanced maturation via an individual-level trade-off between growth and reproduction (Lester et al., 2004). In addition to the energetic costs induced by advanced maturation, there could be several potential explanations for the decreased growth rate of Baltic cod during this relatively short period. Gislason (1999), for example, used single and multispecies VPA to examine the development of mass-at-age over time in Baltic cod and showed an increase between years (not cohorts) from 1977 to the late 1980s. Thereafter, however (for ages and catch years corresponding to cod of the 1989 cohort), mass-at-age levelled off or even decreased (Gislason, 1999). Although only extending until catch year 1996, this corresponds to our results on decreasing

length- and condition-at-age. In combination, Gislason's (1999) and our results could indicate that the current decrease in growth may represent natural periodic variation in growth of Baltic cod and be related to shifts in the relative abundances of clupeids and cod (Köster *et al.*, 2005; Österblom *et al.*, 2006; ICES, 2007). Still, direct effects of fisheries selection on the frequency of (especially old and therefore also mature) fish exhibiting high growth rates (Kristiansen and Svåsand, 1998) cannot be excluded, because they have been documented as significant for other cod stocks (Law and Rowell, 1993; Sinclair *et al.*, 2002; Swain *et al.*, 2007). However, because the growth rate of juvenile cod remained constant, the decreased growth rate among mature cod was most likely caused by earlier maturation and/or was an artefact of size-selective removal of the largest cod in the cohort.

Although the demographic estimation method of PMRNs has been claimed to be robust to variation in length increments (Barot *et al.*, 2004), the violation of equal growth rates between mature and immature cod did make a difference, because the estimated trends differed depending on the subset of individuals (immature vs. all) used to estimate growth increments. Optimally, PMRN estimations should be based on juvenile growth rates (Dieckmann and Heino, 2007), because diversion of resources to reproduction is not likely to affect them. However, using growth data from juvenile fish and fitting the maturity ogive to a mixture of mature and immature fish leads to a mismatch of the sizes-at-age used for the maturity ogive and the PMRN [Equation (1); Barot *et al.*, 2004]. Our results show that the observed trends in values of Lp_{50} did not depend qualitatively on the type of growth data used, but that the exclusion of mature fish from length-at-age estimations strengthened the significance levels of observed trends in the values of Lp_{50}.

Three- vs. two-dimensional PMRNs

Additional environmental variation can be captured in PMRNs by including the effect of condition (Heino *et al.*, 2002; Grift *et al.*, 2007; Mollet *et al.*, 2007). Fulton's morphometric condition factor correlates well with energy reserves in the liver and muscle tissues in cod during the seasonal cycle (Lambert and Dutil, 1997), and condition factor can predict the post-spawning survival of cod (Dutil and Lambert, 2000). However, condition measured just before spawning, i.e. here in the first quarter of the year, may not be a good predictor of maturation, because a decision to mature takes place much earlier (Wright, 2007), and gonad mass may contribute to condition. However, assuming that relative differences in condition among the cod remain more or less constant within a year, measures obtained at the time of sampling should reflect the condition that affected the decision to mature (cf. Lambert and Dutil, 2000). As a conclusion, this study supports the earlier findings that variation in condition cannot fully account for trends in two-dimensional PMRN midpoints and therefore suggests that the estimation of traditional age- and size-based PMRNs is satisfactory usually (Grift *et al.*, 2007; Mollet *et al.*, 2007).

Additional potential explanatory variables

At best, proximate factors such as age, length, and condition correlate tightly with the ultimate processes that determine the timing of maturation (Marshall and McAdam, 2007; Wright, 2007). Other environmental factors affecting maturation include the social environment, the nutrient composition of food, salinity, and temperature (Sohn, 1977; Kraak, 2007). These factors may explain some of the remaining variation in the current PMRN estimates. There are also two potential sources of error. First, a potential error source relates to the timing of spawning within a year, because the PMRN can be defined only at annual time-steps. A shift in the timing of spawning in Baltic cod towards later in summer (Wieland *et al.*, 2000) has been suggested to explain a smaller size at maturity (Cardinale and Modin, 1999). On the other hand, a shift in the timing of spawning itself has been suggested to be an indication of altered energy allocation patterns (Bleil and Oeberst, 2005). Second, the basic estimation method described by Barot *et al.* (2004) assumes that reproduction does not impose any mortality cost and that mature and immature fish are harvested with equal probability. This is an issue to be solved by future work, because reproduction is physiologically highly stressful for fish (Dutil and Lambert, 2000). Moreover, the recent implementation of spawning reserves for Baltic cod may, at least temporarily, increase the relative proportion of mature fish in each size class in the population, and consequently falsely decrease PMRN midpoints estimated from survey data.

Sexual dimorphism in maturation trends

Eastern Baltic cod exhibited clear sexual dimorphism in growth and maturation pattern; males grew more slowly and matured about a year earlier and ∼5 cm smaller than females (see also Cardinale and Modin, 1999). A similar sex difference in growth and maturity ogives has been reported for many other cod stocks, including those around Labrador and Newfoundland, and in the Barents Sea (Ajiad *et al.*, 1999; Olsen *et al.*, 2005; Marshall *et al.*, 2006). Further, the trends in maturation reaction norms differed between the sexes: only females showed a temporal trend towards maturing at earlier ages and sizes, whereas both sexes started to mature at lower condition. Additional to being an issue related to an ability to detect evolutionary trends in a short time-series, this may indicate that female cod are more vulnerable to the selection pressures imposed by current fishing practices in the Baltic Sea. If immature female cod were exposed to fishing more often than immature males, owing to their faster growth and later maturation, this would make them more vulnerable to evolutionary change than males (Heino, 1998). In comparison, Olsen *et al.* (2005) did not report a notable difference in response between the sexes. Therefore, further comparative analyses of the prevalence and importance of sex-differentiated selection pressures from fishing and sex-specific adaptive responses to fishing need to be conducted.

Conclusions

As the Baltic cod has matured between ages 2 and 4 years for at least five decades (Rutkowicz, 1963), the absolute age window for maturation has not changed. However, more fish mature currently at early age, at lower condition, and at smaller size than in the past. The changes in PMRNs might depend on some residual phenotypic components driven by large-scale environmental change in the Baltic Sea, as well as on genetic components driven by environmental changes rather than fishing. Nevertheless, because depressed maturation sizes and ages potentially decrease the recruitment capacity of a stock (Hutchings, 2005), rapid management action needs to be taken to stop or reverse the trends in the maturation schedules of Baltic cod.

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