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Three-Dimensional Maturation Reaction Norms for North Sea Plaice

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THREE-DIMENSIONAL MATURATION REACTION NORMS FOR NORTH SEA PLAICE

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ABSTRACT

Probabilistic maturation reaction norms (PMRNs) with up to three explanatory dimensions were estimated for female North Sea plaice. The three-dimensional PMRNs reported here (1) are the first ones to be obtained for any organism, (2) reveal the differential capacity of alternative life-history state variables to predict the onset of reproduction, (3) document consistent temporal trends in maturation, and (4) help disentangle the contributions of genetic and plastic effects to these trends. We first show that PMRNs based on age and weight provide slightly more accurate approximations of maturation probabilities than PMRNs based on age and length. At the same time, weight-based PMRNs imply a much wider spread of maturation probabilities than length-based PMRNs. We then demonstrate that including condition as a third explanatory variable improves predictions of maturation probability. The resultant three-dimensional PMRNs for age-length-condition or age-weight-condition not only show how, at given size and age, maturation probability increases with condition, but also expose how this impact of condition decreases with age and has changed over time. Our analysis reveals several interesting temporal trends. First, it is demonstrated that, even after removing plastic effects on maturation captured by age, length, weight, and condition, residual trends towards maturation at younger ages and smaller lengths remain. Second, we find that the width of both length- and weight-based PMRNs decreased significantly over time. Third, age and condition are nowadays affecting maturation probabilities less than they did decades ago. We think that plaice is currently maturing at a very low age, size and body condition, and think that the narrow and steep reaction norms do not allow a strong continuation of the observed trends. All obtained findings are in good agreement with predictions from life-history theory based on the hypothesis of evolutionary change caused by heavy exploitation.

KEYWORDS: fisheries-induced evolution, body condition, probabilistic maturation reaction norms, phenotypic plasticity, maturation process.

INTRODUCTION

Combinations of ages and lengths at maturation strongly influence an individual's expected reproductive success, and thus a stock's reproductive potential. Since the allocation of energy to reproduction decreases somatic growth (e.g., Reznick 1983), the trade-off between reproduction and growth implies a trade-off between current and future reproduction which depends on the level of mortality at different life stages (Bell 1976, 1980, Heino & Kaitala 1999). Maturation is a complex physiological process influenced by bioenergetic factors such as resource availability and body reserves, which, in turn, are affected by the local environmental and individual experiences. The age or length at which most species mature is therefore not fixed, but is described by a reaction norm that can be characterized either by a switch curve deterministically relating maturation age to maturation length (Stearns & Koella 1986, Heino et al. 2002a), or rather more realistically, by curves of of age- and size-dependent maturation probabilities (Heino et al. 2002a). Sets of such maturation probabilities are known as probabilistic maturation reaction norms, or PMRNs.

It is helpful to highlight that maturation reaction norms are bivariate reaction norms and thus fundamentally differ from the more widely familiar univariate reaction norms describing how a single phenotypic character varies with a single environmental variable. Maturation reaction norms, by contrast, describe how two phenotypic characters – age and length at maturation – are jointly affected by a single environmental variable – the average growth rate of individuals before maturation. Environmental effects are here manifested in the variation of length at age, i.e., in the slopes of growth trajectories. The latter may in turn depend on many other environmental variables, including temperature and the abundances of food items or of competitors: in this way, growth rates or lengths at age conveniently integrate a multitude of factors of the physical and biotic environment that are eminently relevant for the studied organism. This physiological integration naturally accounts for the differential impacts of - and potentially complex interactions between- these factors, which as such would typically be very difficult to pry apart empirically. Harnessing individual-level explanatory variables beyond age and length for describing maturation probabilities more accurately is conceptually straightforward (Heino et al. 2002a, Van Dooren et al. 2005) but has not been attempted before.

The selection pressures on, and resulting evolution of, maturation reaction norms are determined by environmental conditions such as size-dependent mortality rates (Heino & Kaitala 1999) and resource availability (Siems & Sikes 1998). In general, ecological settings with low survival and slow growth among potentially reproducing individuals evolutionarily favour high reproductive effort at early ages (Reznick et al. 1990, Hutchings 1993, Reznick et al. 1997). In particular, high fishing mortality imposed on reproducing fish may cause evolutionary changes in maturation reaction norms by selecting for genotypes that effectively produce more offspring under conditions of heavy fishing (Borisov 1978, Law 2000, Heino & Godø 2002). Superimposed on these evolutionary effects of fishing, other changes in the physical and biotic environment occur, such as temperature fluctuations and changes in food conditions, which are also bound to influence the processes of growth and maturation (Law 2000). Disentangling these effects of phenotypic plasticity from any underlying genetic changes in maturation probabilities thus becomes an important challenge (Rijnsdorp 1993).

During the 20th century, maturation in the heavily exploited North Sea plaice *Pleuronectes platessa* L. has shifted towards younger ages and smaller lengths at 50% maturity (Rijnsdorp 1993, Grift et al. 2003). Statistical analyses show that improved food conditions caused increased growth rates during the second part of the 20th century (Rijnsdorp & Van Leeuwen 1992, 1996), resulting in earlier maturation. Above and beyond this effect of phenotypic plasticity, evidence suggests that the maturation schedule of North Sea plaice has also undergone evolutionary changes (Rijnsdorp 1993, Grift et al. 2003). More specifically, the analysis of PMRNs for age and size at maturation (Rijnsdorp 1993, Grift et al. 2003) supports the hypothesis of fisheries-induced evolution towards lower ages and lengths at maturation and suggests a picture in which a persistent long-term trend resulting from genetic and plastic responses to faster growth are superimposed on short-term fluctuations originating from residual plastic responses. Grift et al. (2003) showed that, while length was an important cue for maturation, other factors, such as water temperature and food conditions, may also play important roles in the maturation process of North Sea plaice.

In this article we estimate PMRNs based on different combinations of age, length, weight, and condition, in order to take best advantage of all information available for understanding the maturation schedules of female North Sea plaice, and for disentangling genetic and plastic changes of these schedules. We thus focus on two aspects of the maturation process: first we describe the roles of weight and condition in the maturation process, either in place of or in combination with length and second, we use the results to investigate if the maturation process has changed due to fisheries induced change. When modelling a population’s maturity status, length is most often used as a measure of size, and only few studies (Cook et al. 1999, Bromley 2003) have used weight. Weight may be expected, however, to provide a more accurate cue for maturation than length, because it more directly reflects the physiological status and body reserves of fish. Reflecting the importance of bioenergetics for maturation, condition – often measured by morphometric condition indices such as Fulton’s condition factor *K* (weight per cubed length) – can have a positive influence on the fraction of mature fish, as was shown for salmonids (Bohlin et al. 1990, Rowe & Thorpe 1991, Simpson 1992, Bohlin et al. 1994), walleye *Sander vitreus* (Henderson & Morgan 2002), cod *Gadus morhua* (Marteinsdottir & Begg 2002), and American plaice *Hippoglossoides platessoides* (Morgan 2004). We estimate maturation probabilities in a three-dimensional space, in which the effects of body size, age, and condition are considered simultaneously. Several studies estimated the probability of being mature at a given age, length and condition also (Henderson & Morgan 2002, Morgan 2004) but whereas earlier studies focused on probabilities of *being* mature, the PMRN approach helps to investigate probabilities of *becoming* mature. This means that in our analyses confounding effects influencing maturation via growth and survival can be separated from those effects that influence maturation directly. The PMRNs with three explanatory dimensions presented here are the first ones obtained for any organism.

MATERIAL AND METHODS

North Sea plaice

Plaice is a sexual dimorphic iteroparous broadcast spawner following a capital spawning strategy (Rijnsdorp 1989, Rijnsdorp & Witthames 2005). It has been a target species of the mixed demersal fisheries in the North Sea since the start of the industrial revolution in the second half of the 19th century (Rijnsdorp & Millner 1996). Over the study period, mortality rates imposed by fishing have been high, exceeding the instantaneous natural mortality rate (of about 0.1 year^{-1}) by a factor of two to four. From the 1950s until 1980, fishing mortality rate (ages 2-10) increased from 0.2 year^{-1} to 0.4 year^{-1} after which it stabilized around this level. The exploitation pattern has been dome shaped with a peak in fishing mortality rate at age 5 (Grift et al. 2003). Superimposed on changes in fishing mortality, food availability has increased, leading to accelerated growth of plaice smaller than 30 cm (Rijnsdorp & Van Leeuwen 1992, 1996).

Data collection

We use data on female plaice from the Dutch sampling programme for landings by the fishing fleet, carried out since 1957: a detailed description of this data is provided in (Grift et al. 2003). In addition to the date of landing and the geographical position of the catch, length (mm), total weight (g), sex, maturity stage (1: immature; 2: ripening; 3, 4, 5: spawning; 6: nearly spent; 7: spent; with stages 2-7 thus referring to mature individuals; (Rijnsdorp 1989), and age (years, using January 1 as the nominal birthday) were recorded. The age of fish was determined from the pattern of growth zones in the otoliths under the assumption that each zone corresponds to one year. A stratified random subset of otoliths were used to calculate yearly length increments of individual females by using otolith back-calculations. The age determination and otolith back-calculation methods have been validated (Rijnsdorp et al. 1990).

Data selection

Only data of cohorts from 1955 onwards were used, since this cohort was the first that occurred in the sampling programme from age 2 onwards. Only data collected in the first quarter of each year were selected, because during this period adult fish return to their spawning grounds in the southern-eastern North Sea. Rijnsdorp (1989) showed that samples from commercial landings during the spawning season allow for a reliable estimate of the maturity-length and maturity-age relationships. Sample locations were restricted to the south-eastern North Sea (51°–56° N, east of 2° E, and 51°–53.5° N, 1°–2° E. Data of females of ages 2–6 years were used, because younger female plaice are not landed and because at age 7 all females were mature. We only used data from female plaice, because males mature at a size well below the minimum landing size of 27 cm (Rijnsdorp 1989). In total, data on the length, weight, age, and maturity status of 18,416 females were available for analysis. For the analysis of annual length increments, back-calculated lengths of 2,429 females were used from an updated dataset from Rijnsdorp and van Leeuwen (1996).

Normalization of observed weights

Weights of mature females strongly and systematically vary within the spawning season, due to the seasonal cessation of feeding and the spawning of eggs (Figure 1). The observed weights of mature females of different maturity stages were therefore adjusted to account for these changes. The dependence of weight (W in g) on length (L in mm) and year (Y) for the different maturity stages (M = 2 to 7) were analysed by a linear model, using log-transformed weights and lengths,

$$\log W \sim \log L + F_M + Y + (\log L \times F_M) + (\log L \times Y) + (F_M \times Y), \qquad (1)$$

where weight, length, and year are continuous variables, and the maturity stage (F_M) of mature females is a class variable. This model describes weights well ($R^2 = 0.97$, 21 df) and was thus used to normalize the observed weights of mature females, as if they had all been collected when in maturity stage 2 (ripening). In other words, the normalized weight is obtained by adding to the observed weight the difference between observed weight and weight predicted for an individual with the same length but with maturity stage 2 in the same year. Weights of immature fish were not corrected. In our subsequent analysis, we have thus controlled for otherwise unaccounted egg mass and for the metabolic loss of body weight that occurs because plaice do not feed during the spawning season (Rijnsdorp & Ibelings 1989).

Estimation of Maturity ogives

The fraction of mature fish of a particular age or size is customarily described by maturity ogives. In our analysis, these ogives were estimated using logistic regression, with cohort, age, weight, length, and condition as independent variables. Assuming a binomial error structure, the fraction of mature females (O) is logit transformed, logit(O)= log[O/(O–1)]. Ogives were modelled using the GENMOD procedure of the SAS software system in which the log-likelihood functions with respect to the regression parameters are maximized (Allison 1999). Four ogive models were used, and their performance to explain the fraction of mature females compared, to comprehensively investigate the effects of cohort, age, weight, length, and condition,

$$\mathrm{logit}(O) \sim L + A + F_C + (L \times A) + (L \times F_C) + (F_C \times A), \quad (2)$$

$$\mathrm{logit}(O) \sim W + A + F_C + (W \times A) + (W \times F_C) + (F_C \times A), \quad (3)$$

$$\mathrm{logit}(O) \sim L + K + A + F_C + (L \times K) + (L \times A) + (L \times F_C) + (K \times A) + (K \times F_C) + (A \times F_C), \quad (4)$$

$$\mathrm{logit}(O) \sim W + K + A + F_C + (W \times K) + (W \times A) + (W \times F_C) + (K \times A) + (K \times F_C) + (A \times F_C) \quad (5)$$

where length (L), weight (W), condition factor ($K = W/L^3$), and age (A) are continuous variables, and cohort (F_C) is a class variable. The additional value of including condition in models (4) and (5) was statistically tested by computing a likelihood-ratio test statistic and the Akaike Information Criterion (AIC) in which model (4) was compared to model (2), and model (5) was compared to model (3). By using Fulton's condition factor we thus assumed a cubic relationship between length and weight. This assumption is reasonable because the exponent in the length-weight relationship is 3.2 for female plaice (linear model for all data, 18416 observations, $P<0.0001$, $R^2=0.96$). To check the validity of the assumption, all analyses were also executed with the length independent condition factor ($K = W/L^{3.22}$) but this did not lead to different results or conclusions.

Whether these models were linear on the logit-scale was evaluated by testing the effects of logarithmic and square-root transformations of length and weight and by using the technique of fractional polynomials (Royston & Altman 1994, Hosmer & Lemeshow 2000). Logarithmic and square root transformations did not lead to an increase in the fraction of deviance explained (R^2). Fractional polynomials resulted in a significant ($P < 0.0001$) but very small absolute increase of R^2 (less than 0.01). As the parameter estimates of such a model had large standard errors (more than 50% of the parameter estimate), we chose to use the generalized linear models specified above.

Estimation of probabilistic maturation reaction norms

In view of the data available for plaice, the probability of maturation at a certain age and size needs to be estimated with a method based on maturity ogives and annual size increments. Here size can be given by either length or weight, $S = L, W$. Based on the probability O of being mature at age A and size S, given by the maturity ogive $O(A, S)$, the probability P of maturation at age A and size S is given by (Barot et al. 2004a):

$$P(A, S) = [O(A, S) - O(A-1, S-\delta S)] / [1- O(A-1, S-\delta S)], \quad (6)$$

where (A-1) indicates the age previous to the one for which we estimate the probability to mature and (S-δS) being the size increment between age (A-1) and age (A). The method assumes iteroparity which is the case for North Sea plaice. We used length and weight as proxies for measures of size. We also considered a model where maturation probability depends on age, size (s, length or weight) and condition (K):

$$\mathrm{P(A, S, K) = [O(A, S, K) - O(A-1, S-\delta S, K-\delta K)] / [1- O(A-1, S-\delta S, K-\delta K)]} \quad (7)$$

Estimation of the maturation probabilities for each cohort and age comprised three steps (Grift et al. 2003, Barot et al. 2004a): (A) estimation of maturity ogives; (B) estimation of annual increments in length, weight, and condition; and (C) estimation of maturation probabilities and thus of PMRNs. Two further steps then consisted of (D) estimating confidence limits around the estimated PMRN midpoints using a bootstrap method, and (E) testing the significance of trends in maturation probabilities. The general rationale and assumptions underlying this procedure are described in Barot et al. (2004a,b; see also Grift *et al.*, 2003). Our procedure's five steps are described in more detail below.

(A) *Maturity ogives*. Whereas models (2), (3), (4), and (5) were used to investigate the effects of cohort, length, age, weight, and/or condition on the maturation process, simpler sub-models were used for estimating maturity ogives and maturation probabilities. The model selection was based on the significance of variables ($P < 0.05$) and on the standard errors of their parameter estimates (Table 1). Whereas models for age and size were constructed with cohort (F_C) as a class variable, models with age, size and condition were constructed with cohort (C) as a continuous variable, to reliably reveal trends in the resultant three-dimensional PMRNs.

(B) *Annual increments.* Annual length increments δL were estimated as the differences in mean length between two consecutive ages of a cohort. The mean length at each age was calculated for each cohort from the back-calculated lengths. Annual weight increments δW and annual changes δK in condition factor between age groups of cohorts were estimated based on the otolith data combined with length-weight relationships estimated from the maturity data. From the otolith data, lengths at all ages for all individual fish for which otoliths had been measured were retrieved. Next, length-weight relationships were estimated for each year using a linear model with log-transformed weights and lengths. Fish below age 4 were not representative for the length-weight relationship in the population, because the fishery selects for the larger individuals of these age groups. Relationships were thus based on ages 4–10. There was a significant ($P < 0.0001$) but small (absolute increase of R^2 by 0.06) effect of age on the length-weight relationship. We thus chose to omit this small effect of age and extrapolated length-weight relationships to ages 1–3. With these relationships, length at age from otolith readings was transformed to weight at age and condition at age for ages 1–6 for each individual fish. Then, the average weight and condition of each age of each cohort was calculated and the annual weight increments δW and changes δK in condition factor were estimated as the differences in mean weight and condition, respectively, between two consecutive ages of a cohort. We assumed that increments were similar for all immature and mature individuals within an age group of a particular cohort. Obviously this assumption is not accurately met in a natural population, but Barot et al. (2004a) showed that the method is not sensitive to violation of the assumption.

(C) *Maturation probabilities.* Equations (6) and (7) were used to estimate the probabilities of maturation for each cohort, age, and size class, with a resolution of 1 cm for length and of 1 g for weight. Linear interpolation was used, when necessary, to estimate the combinations of explanatory variables that yield a specific maturation probability (e.g., 10%, 25%, 50%, 75%, 90% denoted below by subscripts P10, P25, P50, P75, and P90 respectively). In particular, the term ‘reaction norm midpoint’ applies to the combinations of length, weight, and/or condition that yield a maturation probability of 50% at a certain age.

(D) *Confidence limits.* In this step, a bootstrap analysis was carried out. A new dataset was created by choosing observations of individual fish chosen randomly from the maturity and otolith data with replacement. This selection was stratified by age and cohort such that the new dataset had the same number of samples per age and cohort. With the new dataset, the reaction norm midpoints were calculated for each age and cohort. This procedure was repeated 1000 times and the confidence limits of the reaction norm midpoints were approximated as the 2.5 and 97.5 percentiles of the distribution of the 1000 midpoints of each age and cohort (Manly 1997).

(E) *Trends in maturation probabilities.* The effect of cohort on reaction norm midpoints (W_{P50}) of the two-dimensional PMRNs for age and weight was analysed per age group using a linear model with cohort as a continuous variable. In this model, the estimated reaction norm midpoints were weighted with the inverse of the variance of each midpoint, with the variance estimates being obtained from the bootstrap analysis. Because three-dimensional PMRNs were constructed with cohort as a continuous variable, and hence all parameters in these ogive models were continuous parameters, we could demonstrate trends by only showing resulting PMRNs from the beginning and end of our study period. We chose to show results from cohorts 1960 and 1994 because from these cohorts, sufficient fish were sampled to illustrate how observed sizes and conditions fit in the estimated PMRNs.

RESULTS

Our analysis shows that individual weight is a slightly better variable than length for explaining the fraction of mature female plaice (Table 1). The maturity ogive model with cohort, age, and length (model 2, 123 df) explained 44% of the deviance, whereas the analogous model with weight instead of length (model 3, 123 df) explained 48% of the deviance.

Including condition in addition to length or weight resulted in small increases of predictive power. The model with cohort, age, length, and condition (model 4, 165 df) explained 51% of the deviance, whereas the analogous model with weight instead of length explained 52% of the deviance (model 5, 165 df). Table 1 shows that when length was used as the first component in the build-up of model (4), inclusion of a single term for condition explained a statistically significant extra 6.1% of the deviance. Analogously, when weight was used as the first component in the build-up of model (5), inclusion of a single term for condition explained an additional 1.1% of the deviance. Models in which condition was included (models 4 and 5) performed significantly better than models without (models 2 and 3; lower AIC values, significance based on chi-square test for likelihood-ratio test statistic, 165 df, $P < 0.0001$).

All estimated PMRNs turned out to have negative slopes, such that the weight (and length) at which plaice attains a certain probability of maturation decreases with age: in other words, at the same weight (or length), older females have a higher maturation probability than younger ones (Figure 2).

PMRNs for weight and age are much wider than PMRNs for length and age (Table 2). The difference between $W_{\mathrm{P}90}$ and $W_{\mathrm{P}10}$ (averaged across all cohorts and expressed relative to $W_{\mathrm{P}50}$ on a logarithmic scale) ranges between 1.34 and 2.26 for the different age groups, whereas the corresponding difference between $L_{\mathrm{P}90}$ and $L_{\mathrm{P}10}$ only ranges between 0.41 and 0.47 (see Table 2 for details). The wider PMRNs for weight are to be expected given the curvi-linearity in the weight-length relationship. This can explain the difference in age group 3 and perhaps 4, but not in the older age groups. The ratio of the width of the PMRNs for weight and length was close to the slope of the weight-length relationship (3.2) in age group 3 and 4 (2.9 and 3.4, respectively), but exceeded this slope in age group 5 and 6 (4.3 and 5.2, respectively). Moreover, the width of PMRNs for weight and age increases sharply with age, whereas the width of PMRNs for length and age is almost constant across all ages.

Three-dimensional PMRNs show the additional effect of condition, on top of size and age, on the probability to mature. Results are shown for two selected cohorts only (Figure 3). The isoprobability surfaces of this PMRN are tilted along the condition axis, resulting in a lower $S_{\mathrm{P}50}$ at higher condition. This corroborates the expectation that female plaice of a certain length or weight and age will have a higher probability of maturation if they are in better condition. Interestingly, this facilitating effect of good condition on maturation probability decreases with age. This can be seen more clearly when the three-dimensional PMRNs are projected on the length-condition plane (Figure 4): in both of the shown cohorts, the PMRN gets steeper with increasing age, indicating a weaker effect of condition on the probability of maturation. PMRNs based on weight give analogous results (Figure 4).

Our analysis also revealed three interesting temporal trends, in the form of significant cohort effects. First, over the whole period and for all ages, the weight (and length) at which fish had a certain maturation probability decreased significantly (Figure 6; $R^2 = 0.37$ to 0.54; $P < 0.0001$ for all ages). Second, the width of both length- and weight-based PMRNs decreased significantly over time (Figures 5 and 7; $P < 0.01$ for all ages, except for age 2 where $P = 0.07$ for trend in $W_{P75}–W_{P25}$). Third, the influence of both age and condition on maturation probability has decreased. The decreased contribution of age can be inferred from the fact that in both length-based and weight-based three-dimensional PMRNs the midpoints of all ages have converged (Figure 4). The decreased contribution of condition can be inferred from the fact that both length-based and weight-based PMRNs have become steeper (Figures 4 and 5). There was a significant ($P < 0.0001$) but very weak (R^2=0.15 %) and small (0.038 g / cm^{-3} per cohort) temporal trend in the average condition. On average, the condition factor increased with 1.5 % over the 41 cohorts studied.

DISCUSSION

Weight versus length as a measure of size

Weight as a measure of size for predicting maturation probabilities offers one advantage over length, in that ogive models for age and weight performed slightly but significantly better than ogive models for age and length. Although using weight as a measure of size thus yielded models that explained more variation based on the same degrees of freedom, this approach suffers from at least two drawbacks.

First, weight can vary much more significantly than length. The resulting fluctuations may reflect, for example, food conditions, reproductive stage, and/or diseases. While these changes may well be caused by factors that are relevant for the onset of maturation (such as the level of body reserves), they may equally well be caused by factors that are completely irrelevant in that regard (such as current stomach content or the accumulation and release of eggs). In the analysis reported here, measured weights had to be normalized. While the need for this normalization seems incontrovertible, having to take decisions about how to accomplish it in practice is inconvenient, and the uncertainty thus introduced is practically impossible to account for in the subsequent analyses. Length, in contrast, provides a more robust measure of size, as it is less sensitive to environmental fluctuations.

Second, length can only increase during the life of a fish, while weight can readily decrease. Since the state of maturity can be reached via diverse growth trajectories (Bernardo 1993), and since maturation probabilities average across all of them, the extra fluctuations in weight are bound to increase uncertainty in the estimation of maturation probabilities. Only few studies of maturation processes actually use the weight of fish as a measure of size (e.g., (Cook et al. 1999, Bromley 2003), but these do not account for negative slopes in the growth trajectory. In accordance with these considerations, our results show that the relative widths of PMRNs for age 5 and 6 are much higher when based on weight instead of on length. We also found that the widths of weight-based PMRNs increase sharply with age, which, for example, makes the extrapolation and interpolation of maturation probabilities across ages more problematic than it is for length-based PMRNs.

Considering these extra difficulties, and balancing them against the only slightly elevated predictive power afforded by weight-based PMRNs in female plaice, in our opinion strengthens the case for length-based PMRNs.

The impact of condition on maturation

The three-dimensional reaction norms confirm that good condition has a significant and positive effect on maturation, in line with earlier research (Rowe & Thorpe 1991, Bohlin et al.

1994, Morgan 2004). While this is just as expected based on standard bioenergetic considerations, it has to be pointed out that in the present study an entirely different explanation based on the timing of measurements might also apply, as is explained below.

In the selected data, maturation stage was measured during the spawning season, from January to March, rather than at the time at which plaice actually mature or initiate their maturation process: maturation starts in summer, when oocytes become vitellogenic (Rijnsdorp & Witthames 2005). Both maturing and non-maturing plaice will still grow considerably thereafter, with maturing fish partitioning their surplus energy between somatic growth and energy reserves for reproduction, while the latter investment naturally is absent in non-maturing fish. Since investment into reproduction increases the weight, but not the length, of fish at the time of spawning, it results in a higher condition factor K. Indeed, mature female plaice had a significantly higher condition factor than immature ones (comparison of means, $P < 0.0001$, $K = 0.0102$ and 0.0094 g cm^{-3} respectively). This effect may contribute to the observed positive effect of condition on the probability of maturation. In this alternative interpretation, the higher condition factor is not the cause but rather the consequence of maturation. Data on other measures of condition, such as liver weight, are not available for North Sea plaice. In flatfish, body condition is, however, a better indicator for the condition than liver weight because energy reserves are mainly stored in the soma (Dawson & Grimm 1980, Morgan 2004), in contrast to for instance gadoids (Lambert & Dutil 1997, Marshall et al. 1999).

Our finding that the facilitating effect of better condition on maturation decreases with age means that particularly young female plaice mature at earlier ages and smaller lengths when they have above-average condition, while older females in good and bad condition instead mature similarly. This agrees with observations on walleye, for which a good condition was found to increase the probability of maturation for younger females, while older females matured even if they were in poor condition (Henderson & Morgan 2002). Similarly, turbot *Scophthalmus maximus* had a higher probability to mature when they were fed well before maturation (Bromley et al. 2000). These findings can be interpreted by supposing that all these fish can mature at low age and small length only if they have ample surplus energy stores, whereas at higher ages they mature anyway, even if they suffer from relatively low energy reserves and even though the extra costs of reproduction may further decrease their survival rate. An understanding of these patterns is likely to relate to the so-called ‘desperado’ effect (Grafen 1987), through which an individual chooses a risky strategy due to a lack of alternative options.

Interpretation of maturation trends

Trends in the maturation process of North Sea plaice suggest that the trade-off between current and future reproduction of female plaice may have shifted to increased current reproduction by maturing at a very low age, size, and body condition, thus decreasing the potential for future reproduction. This conclusion is based on three clear temporal trends: for all ages, the weight and length at which fish had a certain maturation probability decreased significantly, the width of both length- and weight-based PMRNs decreased significantly over time and the influence of both age and condition on the maturation probability has decreased. Although the trend in the weight at maturation (Fig. 6) was not corrected for any trend in condition factor, we think that the small increase in average condition could only explain a minor part of the decreased W_{P50}. Fig. 4 shows that the 1.5 % by which the condition factor increased over 41 cohorts would lead to a decrease in W_{P50} of 3-4 % whereas over all cohorts, W_{P50} decreased by 32-37 %. The observed trends are in good agreement with a general prediction from life-history theory: if fish are faced with high adult mortality, such as in the North Sea plaice population, investment into future reproduction may not pay and, consequently, selection will not only favour earlier reproduction but also higher reproductive effort at age, at the expense of body growth and/or survival (Heino & Kaitala 1999). We do not know if plaice is currently maturing at the lowest possible age, size and body condition, but we think that the narrow and steep reaction norms do not allow a strong continuation of the observed trends.

In a study based on the same dataset as was used here, an analysis trying to elucidate explicit fisheries-induced changes in reproductive investment in North Sea plaice remained inconclusive (Rijnsdorp et al. 2005). A review of fecundity and ovary weight data of female plaice from the literature, however, tentatively suggested that an increase in reproductive investment occurred since the late 1940s. This finding is consistent with expectations based on the hypothesis of fisheries-induced evolutionary change (Rijnsdorp et al. 2005).

The question is to what extent the observed changes in the maturation process are due to phenotypic plasticity or to genetic evolution. Our method has dealt with the phenotypically plastic response to varied growth and to variations in body condition, revealing a residual trend that, in the absence of alternative equally plausible explanations, suggests evolutionary changes in maturation. Possible factors that may influence the process of maturation other than through their effects on growth and condition, are temperature (Grift et al. 2003, Dhillon & Fox 2004, Dembski et al. 2006) and social factors (Sohn 1977, Hobbs et al. 2004).

No observational study can exclude – as a matter of principle – the possibility that uncontrolled, and potentially as yet unimagined, factors might fully account, through phenotypic plasticity, for the observed phenotypic trends in maturation. A conclusive proof of genetic evolution would require explicit data on changes in the sequences of genes together with functional insights into how such genetic changes affect maturation. Similarly, no observational study can ever unambiguously demonstrate that fisheries-induced selection is the cause of maturation trends, since observational studies can achieve no more than the establishment of suggestive correlations. Yet the present analysis, in conjunction with the consistency of patterns found in many studies based on other exploited species and on different parts of the oceans (e.g., (Heino et al. 2002b, Barot et al. 2004b, Olsen et al. 2004, Barot et al. 2005, Olsen et al. 2005), leads us to suggest that fisheries-induced evolution indeed offers the most parsimonious explanation of the residual trends reported here.

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REFERENCES

Allison PD (1999) Logistic regression using the SAS system; theory and application, Vol. SAS institute inc., Cary, NC

Barot S, Heino M, Morgan MJ, Dieckmann U (2005) Maturation of Newfoundland American plaice (Hippoglossoides platessoides): long-term trends in maturation reaction norms despite low fishing mortality? ICES Journal of Marine Science 62:56-64

Barot S, Heino M, O'Brien L, Dieckmann U (2004a) Estimating reaction norms for age and size at maturation when age at first reproduction is unknown. Evolutionary Ecology Research 6:659-678

Barot S, Heino M, O'Brien L, Dieckmann U (2004b) Long-term trend in the maturation reaction norm of two cod stocks. Ecological Applications 14:1257-1271

Bell G (1976) On Breeding More Than Once. American Naturalist 110:57-77

Bell G (1980) The costs of reproduction and their consequences. American Naturalist 116:45-76

Bernardo J (1993) Determinants of maturation in animals. Trends in Ecology & Evolution 8:166-173

Bohlin T, Dellefors C, Faremo U (1990) Large or small at maturity - theories on the choice of alternative male strategies in anadromous salmonids. Annales Zoologici Fennici 27:139-147

Bohlin T, Dellefors C, Faremo U (1994) Probability of first sexual maturation of male parr in wild sea-run brown trout (*Salmo trutta*) depends on condition factor 1 yr in advance. Canadian Journal of Fisheries and Aquatic Sciences 51:1920-1926

Borisov VM (1978) The selective effect of fishing on the population structure of species with long life cycle. Journal of Ichthyology 18:896-904

Bromley PJ (2003) The use of market sampling to generate maturity ogives and to investigate growth, sexual dimorphism and reproductive strategy in central and south-western North Sea sole (Solea solea L.). ICES Journal of Marine Science 60:52-65

Bromley PJ, Ravier C, Witthames PR (2000) The influence of feeding regime on sexual maturation, fecundity and atresia in first-time spawning turbot. Journal of Fish Biology 56:264-278

Cook RM, Kunzlik PA, Hislop JRG, Poulding D (1999) Models of growth and maturity for North Sea Cod. Journal of Northwest Atlantic Fishery Science 25:91-99

Dawson AS, Grimm AS (1980) Quantitative changes in the protein, lipid and energy content of the carcass, ovaries and liver of adult female plaice, *Pleuronectes platessa* L. Journal of Fish Biology 16:493-504

Dembski S, Masson G, Monnier D, Wagner P, Pihan JC (2006) Consequences of elevated temperatures on life-history traits of an introduced fish, pumpkinseed *Lepomis gibbosus*. Journal of Fish Biology 69:331-346

Dhillon RS, Fox MG (2004) Growth-independent effects of temperature on age and size at maturity in Japanese Medaka (*Oryzias latipes*). Copeia 2004:37-45

Grafen A (1987) The Logic of Divisively Asymmetric Contests - Respect for Ownership and the Desperado Effect. Animal Behaviour 35:462-467

Grift RE, Rijnsdorp AD, Barot S, Heino M, Dieckmann U (2003) Fisheries-induced trends in reaction norms for maturation in North Sea plaice. Marine Ecology Progress Series:247-257

Heino M, Dieckmann U, Godø OR (2002a) Measuring probabilistic reaction norms for age and size at maturation. Evolution 56:669-678

Heino M, Dieckmann U, Godø OR (2002b) Reaction norm analysis of fisheries-induced adaptive change and the case of

the Northeast Arctic cod. ICES CM 2002/Y:14:1-14

Heino M, Godø OR (2002) Fisheries-induced selection pressures in the context of sustainable fisheries. Bulletin of Marine Science 70:639-656

Heino M, Kaitala V (1999) Evolution of resource allocation between growth and reproduction in animals with indeterminate growth. Journal of Evolutionary Biology 12:423-429

Henderson BA, Morgan GE (2002) Maturation of walleye by age, size and surplus energy. Journal of Fish Biology 61:999-1011

Hobbs JPA, Munday PL, Jones GP (2004) Social induction of maturation and sex determination in a coral reef fish. Proceedings Of The Royal Society Of London Series B-Biological Sciences 271:2109-2114

Hosmer DW, Lemeshow S (2000) Applied logistic regression, Vol. John Wiley and sons, New York

Hutchings JA (1993) Adaptive life histories effected by age-specific survival and growth rate. Ecology 74:673-684

Lambert Y, Dutil JD (1997) Can simple condition indices be used to monitor and quantify seasonal changes in the energy reserves of Atlantic cod (*Gadus morhua*)? Canadian Journal of Fisheries and Aquatic Sciences 54:104-112

Law R (2000) Fishing, selection, and phenotypic evolution. ICES Journal of Marine Science 57:659-668

Manly BFJ (1997) Randomization, bootstrap and monte carlo methods in biology, Vol. Chapman & Hall, London

Marshall CT, Yaragina NA, Lambert Y, Kjesbu OS (1999) Total lipid energy as a proxy for total egg production by fish stocks. Nature 402:288-290

Marteinsdottir G, Begg GA (2002) Essential relationships incorporating the influence of age, size and condition on variables required for estimation of reproductive potential in Atlantic cod *Gadus morhua*. Marine Ecology Progress Series 235:235-256

Morgan MJ (2004) The relationship between condition and the probability of being mature in American plaice (*Hippoglossoides platessoides*). ICES Journal of Marine Science 61:64-70

Olsen EM, Heino M, Lilly GR, Morgan MJ, Brattey J, Ernande B, Dieckmann U (2004) Maturation trends indicative of rapid evolution preceded the collapse of northern cod. Nature 428:932-935

Olsen EM, Lilly GR, Heino M, Morgan MJ, Brattey J, Dieckmann U (2005) Assessing changes in age and size at maturation in collapsing populations of Atlantic cod (*Gadus morhua*). Canadian Journal of Fisheries and Aquatic Sciences 62:811-823

Reznick DN, Bryga H, Endler JA (1990) Experimentally induced life-history evolution in a natural population. Nature 346:357-359

Reznick DN, Shaw FH, Rodd FH, Shaw RG (1997) Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). Science 275:1934-1936

Rijnsdorp AD (1989) Maturation of male and female North Sea plaice (*Pleuronectes platessa* L.). Journal de la Conservation International pour la Exploration de la Mer 46:35-51

Rijnsdorp AD (1993) Fisheries as a large-scale experiment on life-history evolution: disentangling phenotypic and genetic effects in changes in maturation and reproduction of North Sea plaice, *Pleuronectes platessa* L. Oecologia 96:391-401

Rijnsdorp AD, Grift RE, Kraak SBM (2005) Fisheries-induced adaptive change in reproductive investment in North Sea plaice (*Pleuronectes platessa*)? Canadian Journal of Fisheries and Aquatic Sciences 62:833-843

Rijnsdorp AD, Ibelings B (1989) Sexual dimorphism in the energetics of reproduction and growth of North Sea plaice, *Pleuronectes platessa* L. Journal of Fish Biology 35:401-415

Rijnsdorp AD, Millner RS (1996) Trends in population dynamics and exploitation of North Sea plaice (*Pleuronectes platessa* L.) since the late 1800s. ICES Journal of Marine Science 53:1170-1184

Rijnsdorp AD, Van Leeuwen PI (1992) Density-dependent and independent changes in somatic growth of female North Sea plaice (*Pleuronectes platessa* L.) between 1930-1985 as revealed by back-calculations of otoliths. Marine Ecology Progress Series 88:19-32

Rijnsdorp AD, Van Leeuwen PI (1996) Changes in growth of North Sea plaice since 1950 in relation to density, eutrophication, beam-trawl effort, and temperature. ICES Journal of Marine Science 53:1199-1213

Rijnsdorp AD, Van Leeuwen PI, Visser TAM (1990) On the validity and precision of back-calculation of growth from otoliths of the plaice (*Pleuronectes platessa* L.). Fisheries Research 9:97-117

Rijnsdorp AD, Witthames PR (2005) Ecology of reproduction. In: Gibson RN (ed) The biology of flatfish. Blackwell Scientific press, London, p 68-93

Rowe DK, Thorpe JE (1991) Role of fat stores in the maturation of male Atlantic salmon (*Salmo salar*) parr. Canadian Journal of Fisheries and Aquatic Sciences 48:405-413

Royston P, Altman DG (1994) Regression using fractional polynomials of continuous covariates: parsimonious parametric modelling. Applied Statistics 43:429-467

Siems DP, Sikes RS (1998) Tradeoffs between growth and reproduction in response to temporal variation in food supply. Environmental Biology of Fishes 53:319-329

Simpson AL (1992) Differences in body size and lipid reserves between maturing and nonmaturing atlantic salmon parr, *Salmo salar* L. Canadian Journal Of Zoology 70:1737-1742

Sohn JJ (1977) Socially induced inhibition of genetically determined maturation in the platyfish, *Xiphophorus maculatus*. Science 195:1999-1200

Stearns SC, Koella JC (1986) The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at maturity. Evolution 40:893-913

Van Dooren TJM, Tully T, Ferriere R (2005) The analysis of reaction norms for age and size at maturity using maturation rate models. Evolution 59:500-506

Tables

Table 1. Results of maturity ogive models (2), (3), (4), and (5). The columns labelled R^2 show the deviance additionally explained as terms in these models are successively introduced from one row to the next (*L*: length; *A*: age; *C*: cohort; *W*: weight; *K*: condition factor). All terms where highly significant ($P < 0.001$), except those in italics ($P > 0.10$). Terms in bold were selected for the maturity ogive models used in the estimation of PMRNs where models (4) and (5) were adapted such that cohort was treated as a continuous variable. The lower rows show the R^2 of the complete model (Total) and of the model with selected terms only (Total selected), and the value of the Akaike information criterion (AIC).

Model (2)	R^2 (%)	Model (3)	R^2 (%)	Model (4)	R^2 (%)	Model (5)	R^2 (%)
L	38.5	**W**	43.7	**L**	38.5	**W**	43.7
A	1.4	**A**	1.1	**K**	6.1	**K**	1.1
C	2.2	C	2.2	**A**	2.3	**A**	2.3
L x A	0.2	*W x A*	0.0	**C**	2.7	**C**	2.6
L x C	0.8	**W x C**	0.7	*L x K*	0.0	W x K	0.0
A x C	0.6	A x C	0.7	**L x A**	0.1	**W x A**	0.0
				L x C	0.6	**W x C**	0.6
				K x A	0.0	*K x A*	0.0
				K x C	0.0	K x C	0.4
				A x C	0.7	**A x C**	0.7
Total	44		48		51		52
Total selected	44		47		48		48
AIC	13129.2		11865.9		11250.0		11234.3

Table 2. Probabilistic widths of length- and weight-based PMRNs. Probabilistic widths are expressed on a log-scale, relative to the average value of the reaction norm midpoint, L_{P50} or W_{P50}. Widths are averaged across all cohorts for each age group. The most right column presents the ratio between the width of weight and length based PMRNs.

Age	PMRN for age and length		PMRN for age and weight		Ratio
	L_{P50} (cm)	$\log L_{P90} / L_{P50} - \log L_{P10} / L_{P50}$	W_{P50} (g)	$\log W_{P90} / W_{P50} - \log W_{P10} / W_{P50}$	(width weight / width length)
2	38.6	0.47	453	n.a.	n.a.
3	34.6	0.45	357	1.34	2.94
4	32.3	0.43	314	1.48	3.43
5	30.7	0.41	269	1.75	4.27
6	29.7	0.43	239	2.26	5.22

Figures

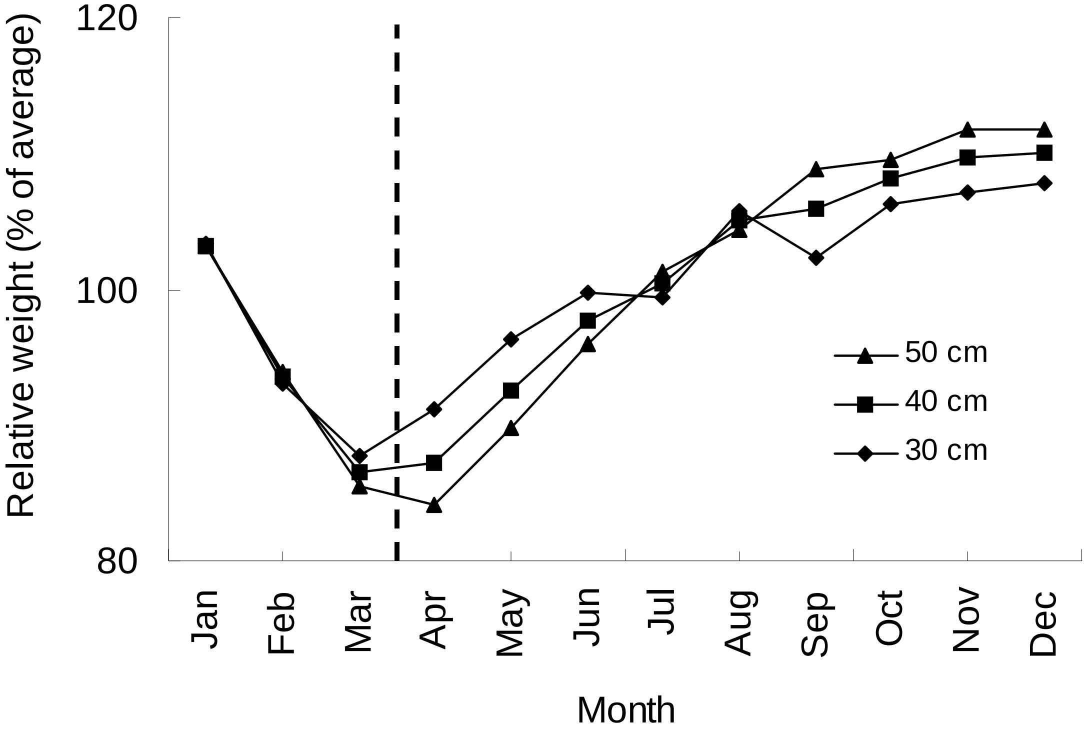

Figure 1. Seasonal changes in the weight of mature female plaice, illustrated for three lengths. For each month and length, weights relative to the yearly average weights were averaged across ages 4 to 10 and years 1990 to 1999. The hatched vertical line indicates the end of the first quarter, to which data used in this study were restricted.

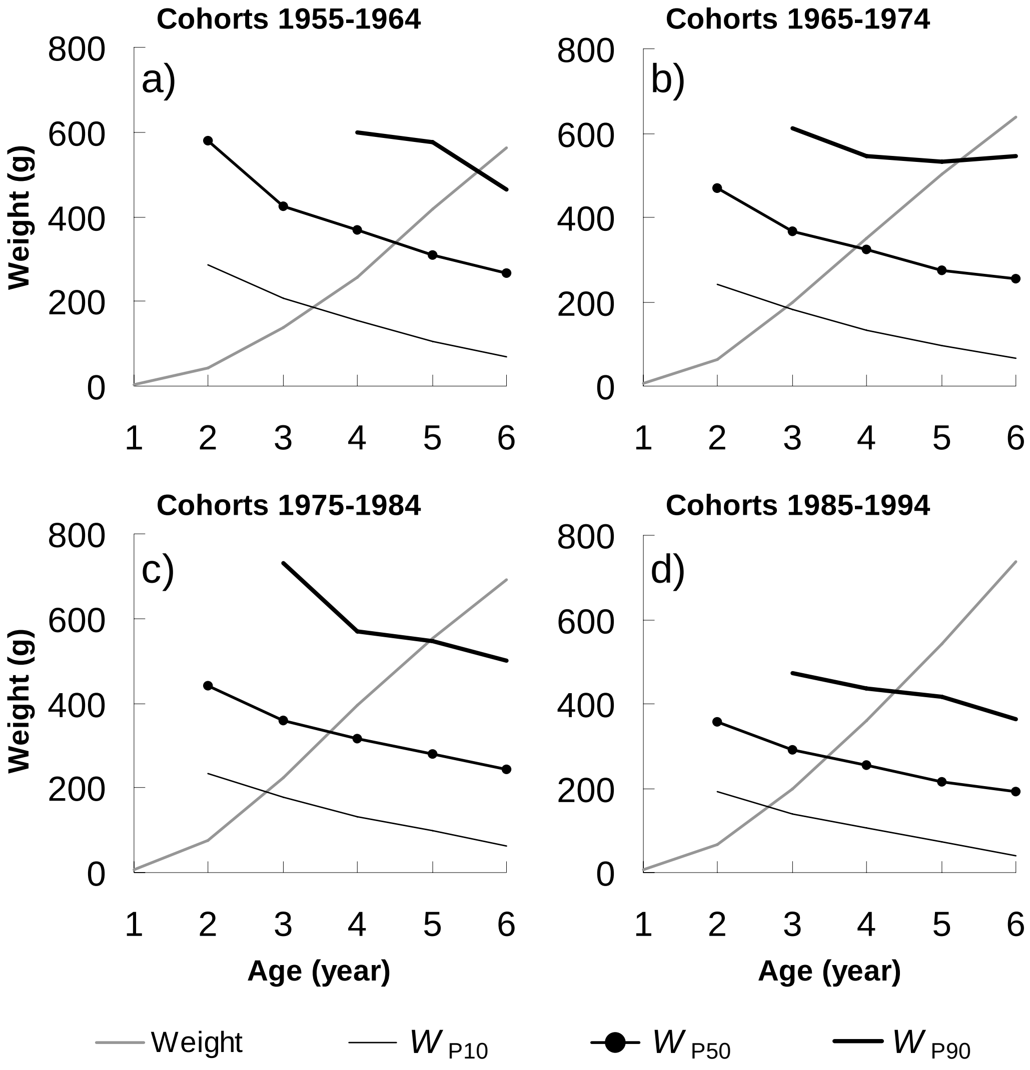

Figure 2. Probabilistic maturation reaction norms (PMRNs) and growth trajectories for weight and age. Weights at which the probability of maturation reaches 10%, 50%, and 90% (W_{P10}, W_{P50}, and W_{P90}) are shown as black curves of increasing thickness. Growth trajectories, depicted as grey curves, are based on averaging age-specific weights across the cohorts indicated for each panel. Values of W_{P90} for age 2 in all periods and for age 3 in the first period could not be estimated.

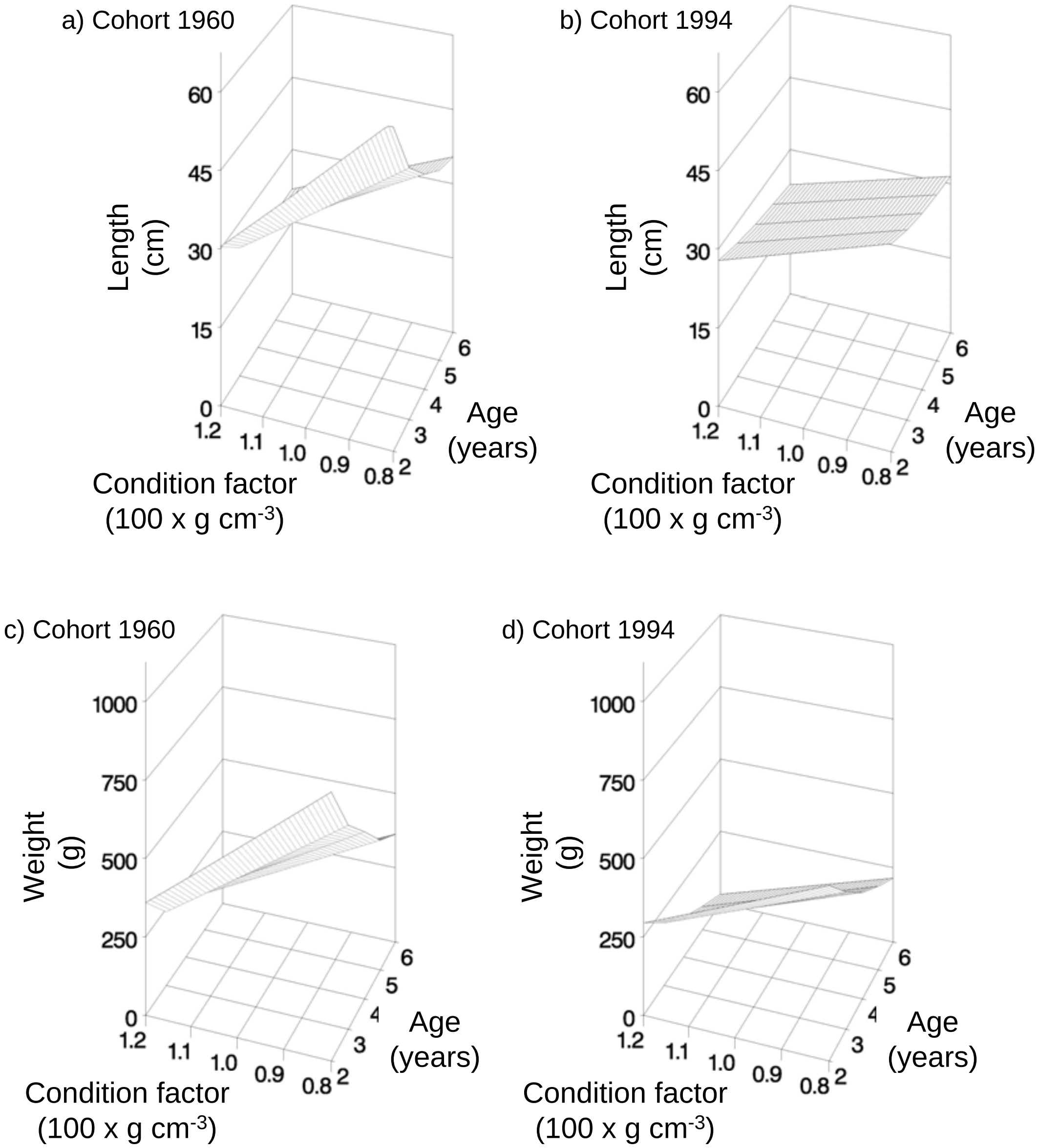

Figure 3. Three-dimensional probabilistic maturation reaction norms (PMRNs) for age, size, and condition, with size being measured as either length (top row) or weight (bottom row). The shown midpoint surfaces indicate the combinations of age, size, and condition for which maturation probabilities equal 50% for the cohorts of 1960 (left column) and 1994 (right column). While these figures transparently highlight the three-dimensional nature of the estimated PMRNs, they are difficult to read. The resultant contour plots in Figures 4 and 5, based on the same information, prove to be more informative.

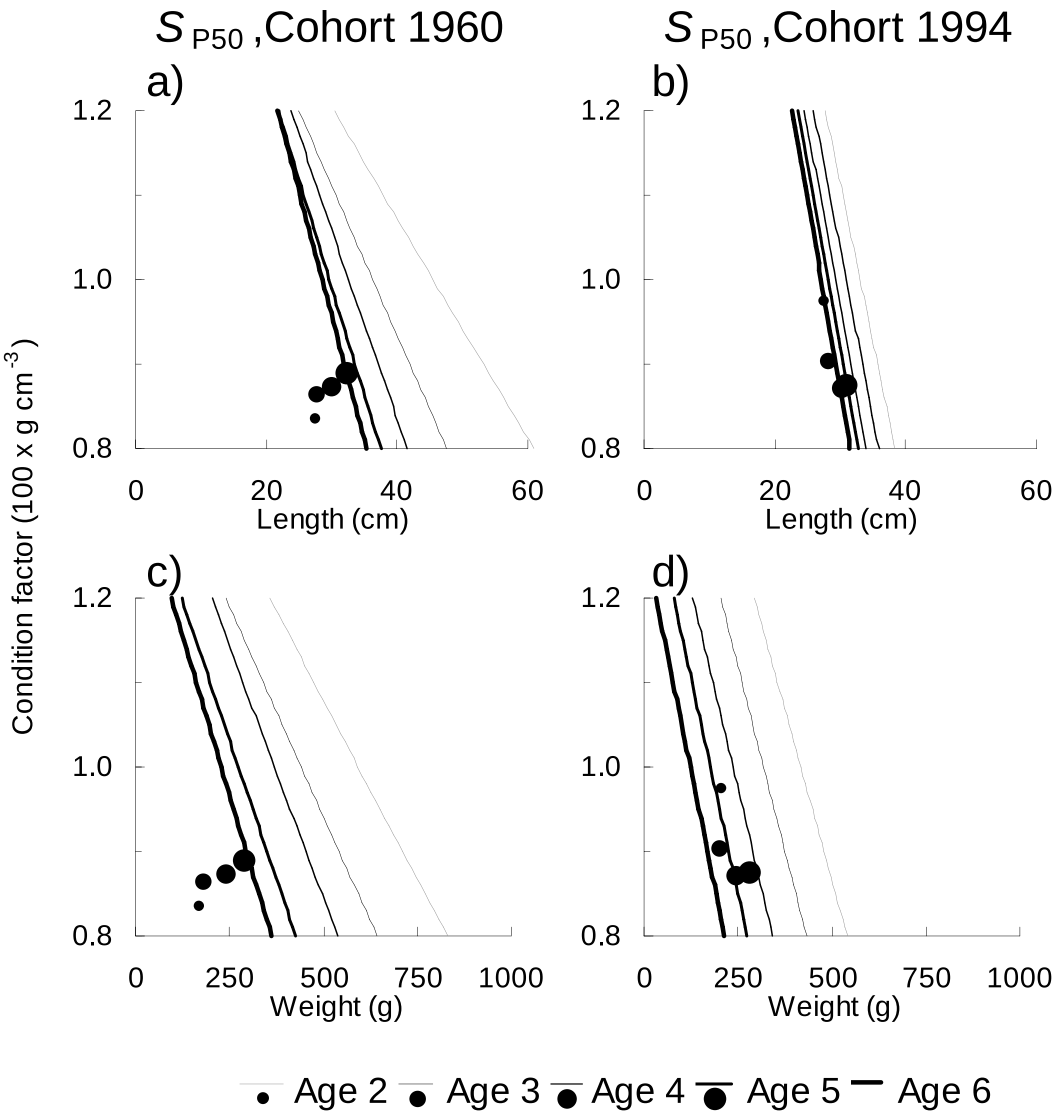

Figure 4. Projections of three-dimensional probabilistic maturation reaction norms (PMRNs) for age, size, and condition onto the size-condition plane, with size being measured as either length (top row) or weight (bottom row). Reaction norm midpoint lines are shown for ages 2 to 6 for the cohorts of 1960 (left column) and 1994 (right column). These lines thus indicate, separately for each age, the combinations of size and condition for which maturation probability equals 50%. Filled circles indicate the median size and condition factor for each age group in the cohort (observations for age-6 females did not occur in the dataset for either cohort).

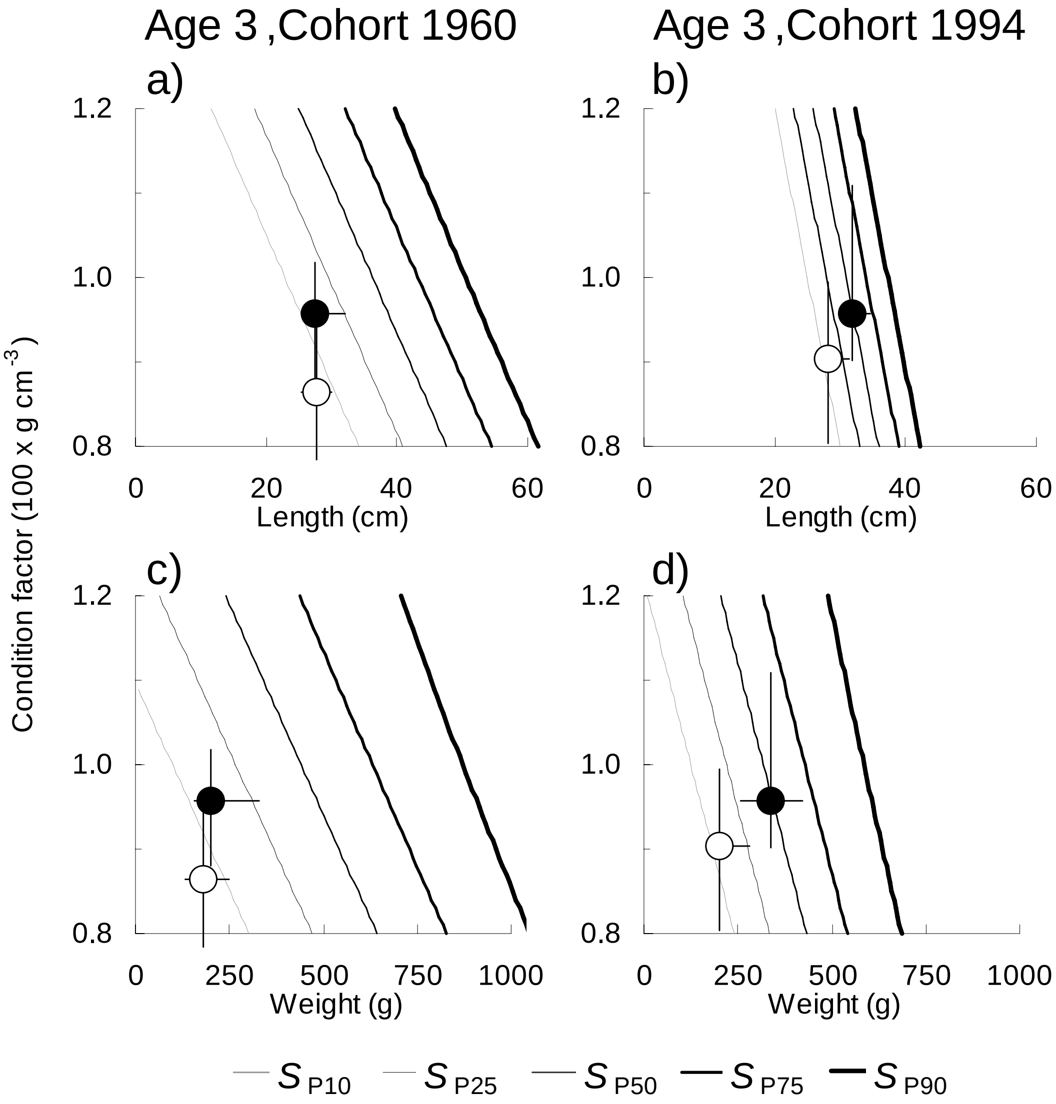

Figure 5. Projections of three-dimensional probabilistic maturation reaction norms (PMRNs) for age, size, and condition on the size-condition plane, with size being measured as either length (top row) or weight (bottom row). Contours for 10%, 25%, 50%, 75%, and 90% maturation probability are shown for age-3 of cohorts 1960 (left column) and 1994 (right column). Open circles indicate the median size and condition factor for immature age-3 females in the cohort, while the attached whiskers indicate the corresponding 10% and 90% percentiles. Filled circles and attached whiskers show the same information restricted to mature females.

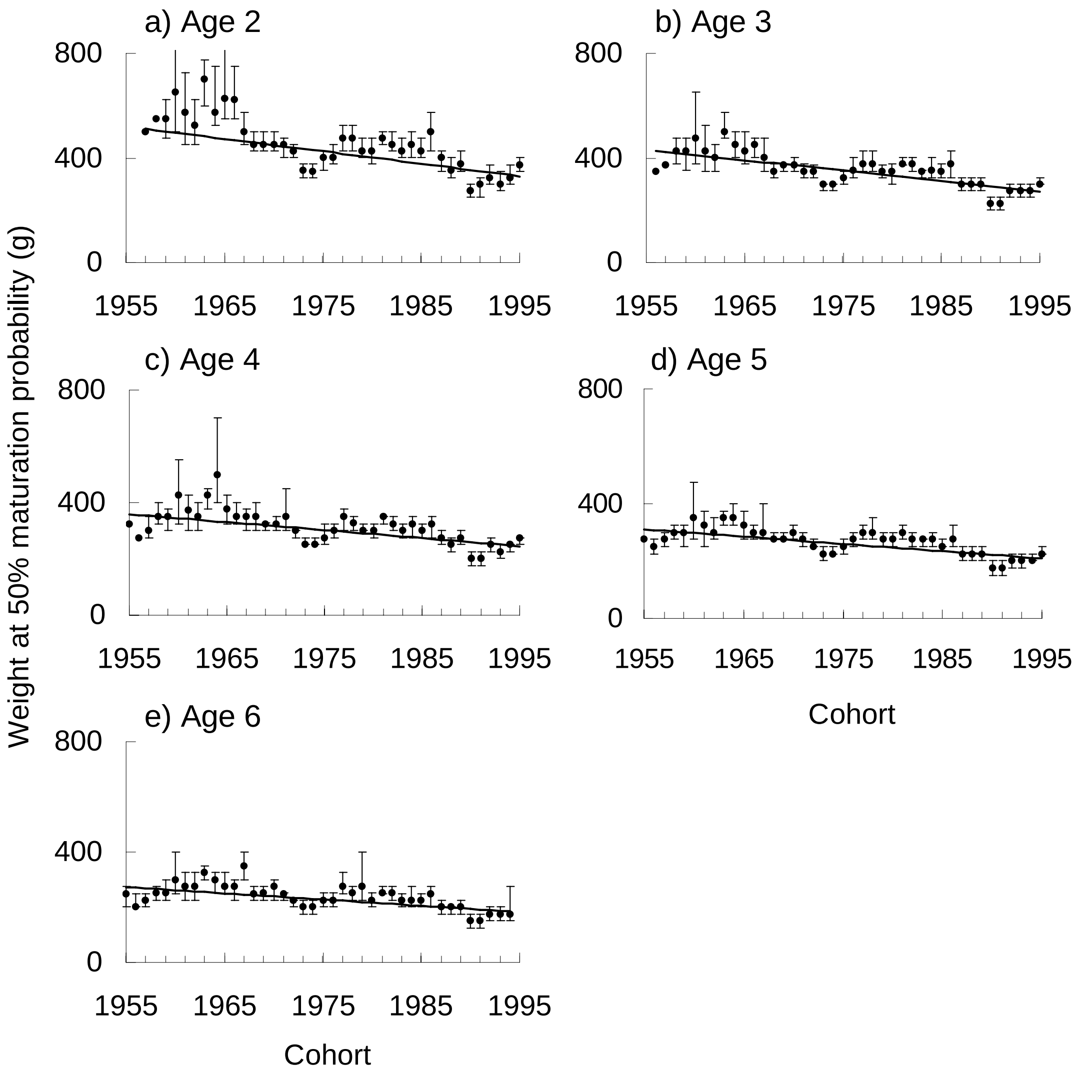

Figure 6. Trends in reaction norm midpoints W_{P50} (filled circles) and 95% confidence limits (error bars) for ages 2 to 6 of all cohorts. Trends in midpoints, based on a linear model, are highlighted by continuous lines.

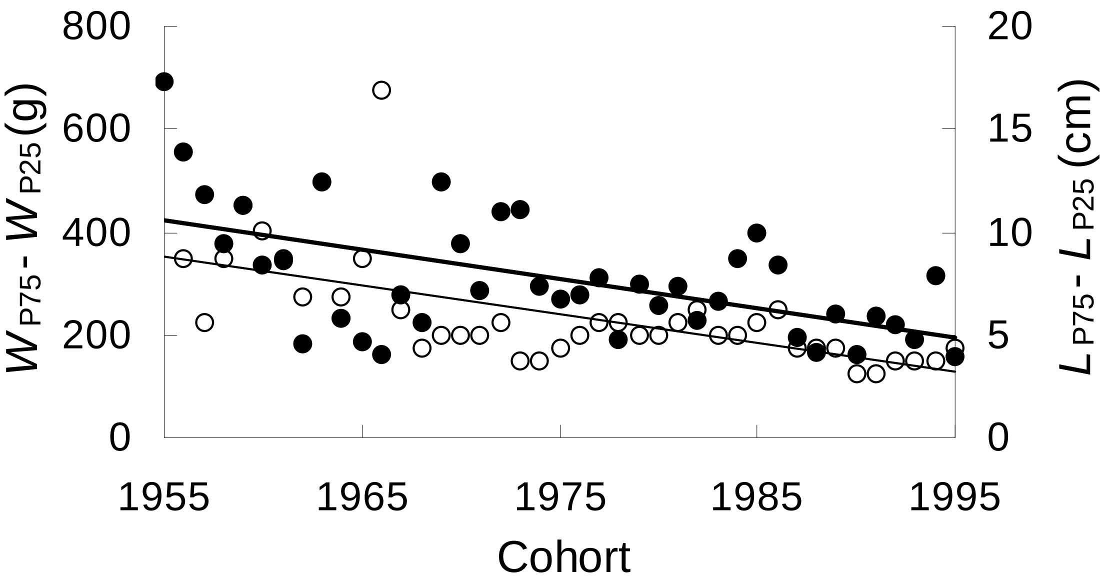

Figure 7. Trends in the width of probabilistic maturation reaction norms (PMRNs) for age and weight (open circles and thin line) and for age and length (filled circles and thick line). These trends are illustrated for age 3 (R^2= 0.15 for W_{P75}–W_{P25}; R^2=0.31 for L_{P75}–L_{P25}).