Experimental assessment of the probabilistic maturation reaction norm: condition matters

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The probabilistic maturation reaction norm (PMRN) describes an individual's probability of maturing at a given age as a function of size and other relevant phenotypic traits. Population-level shifts in the PMRN are often interpreted to indicate genetic as opposed to phenotypic changes in maturation in fish. Inferences derived from trends in the PMRN have been challenged, warranting an experimental assessment of the method. This was accomplished in a laboratory experiment using zebrafish (*Danio rerio*). Fish were reared under different food levels to induce variation in growth and maturation. Plasticity in maturation was not entirely captured by the demographic age- and length-based PMRN. Adding condition to the PMRN captured a greater amount of environmental variation in maturation probability. Nevertheless, significant differences in the PMRNs among the food levels remained after accounting for the influences of age, size and condition on maturation probability indicating plasticity of the PMRN. This was particularly pronounced between fish held on low food levels as compared with fish experiencing abundant resources, with the latter experiencing higher size-specific maturation probabilities. Our analysis emphasizes the need for incorporating salient physiological traits influencing maturation, such as condition, to make accurate inferences about documented shifts observed in the position of PMRNs on maturation trends in wild fish stocks.

Keywords: growth; size-at-age; body condition; environmental variation; fisheries-induced evolution; phenotypic plasticity

1. INTRODUCTION

Intensive fishing has been proposed to be one of the main reasons for declines in size and age at maturation in exploited fish stocks [1–4]. Fishing, especially when size-selective, shifts the population's age and size distributions towards younger ages and smaller sizes through demographic truncation effects [5,6]. Moreover, when stock biomass declines owing to fishing, individual growth usually increases in response to the greater *per capita* food availability [7]. Enhanced growth rate can result in earlier maturation [8–10], reflecting an individual's response to changing environmental conditions (i.e. phenotypic plasticity). Thus, observed reductions in the average age and size at maturation in exploited fish stocks over time can be caused by demographic and/or environmental factors [5].

In addition to being phenotypically plastic traits [8,11,12], age and size at maturation are also known to

be in part genetically determined [13–15]. From an evolutionary perspective, when a fish population is exposed to elevated or positive size-selective mortality, postponing reproduction for too long may be costly [2,16,17]. Thus, under conditions of high fishing mortality, individuals genetically predisposed to mature early and/or at a small size will have a higher probability of reproducing and passing on genes to the next generation than late-maturing individuals and/or individuals maturing at large size. Therefore, changes in the maturation schedule in response to increased fishing mortality can constitute either a phenotypically plastic response, an evolutionary response or a combination of both [1,2]. Because fisheries-induced evolution may have a number of undesirable consequences, such as reduced yields and speed of stock recovery [18–20], disentangling environmentally induced and genetic changes in traits affected by fishing is not only of academic interest but also of importance for fisheries management [3,4].

The probabilistic maturation reaction norm (PMRN) describes an individual's probability to mature as a function of age, size and other relevant phenotypic variables [21]. PMRN is, in principle, an individual-level property

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but in field studies a population-level PMRN is constructed [22]. This population-level approach constitutes a statistical tool which has been used to make inferences about fisheries-induced evolution of maturation schedules in wild fish stocks [22,23]. In the PMRN estimation process two assumptions are traditionally applied [21,23]: (i) most environmental effects on maturation are linked to plasticity in growth (although other traits and processes can obviously be added in the statistical model), and (ii) most environmental variation in maturation age and size is reflected by variation in size-at-age. Following these two assumptions, a PMRN is assumed to describe phenotypic plasticity in maturation probability as a function of salient traits and underlying physiological processes, such as age, size or condition [21,22]. If a population level PMRN model indeed captures all the phenotypic plasticity in maturation, the position of the PMRN itself should only change when genetic changes in age and size at maturation have occurred in response to natural and/or anthropogenic selection pressures, such as those induced by fishing (figure 1; [21,22]). Displacement of the position of the PMRN is referred to as a shift in the PMRN (figure 1).

While growth rate of individual fish certainly contributes to timing of and size at maturation [8,12], the process of maturation might be influenced by more variables than represented by age and size alone [24–26]. If the maturation process is influenced by variables not included in the statistical estimation of the PMRN, shifts in the position of the PMRN over time may not necessarily indicate genetic changes [23,27]. Indeed, shifts in the position of a PMRN in response to selection pressures in the wild might always be owing to some unaccounted for factor (e.g. condition, temperature) in the statistical model [22,23], which is why the general validity of insights about fisheries-induced evolution using the PMRN method has been challenged [3,25,28,29]. Naturally, if other processes apart from size-at-age influence maturation, the traditionally used age- and length-based (i.e. two-dimensional) PMRN method can be extended to three- or multi-dimensional PMRNs, which incorporate additional variables to control for other salient environmental covariates of maturation [30–33]. However, fisheries databases seldom contain information, which would allow resolving in detail the morphological, physiological or behavioural traits affecting maturation (e.g. weight, condition or hormonal changes that precede maturation; [34,35]). This presumably explains why many applications of the PMRN have focused on the two-dimensional, age- and length-based approach.

The ongoing debate concerning the suitability of the PMRN approach for evolutionary inference warrants for an experimental assessment of a method in a controlled laboratory setting, but so far no such study has been published. To fill this gap, we conducted an experiment under controlled environmental conditions using zebrafish (*Danio rerio*) as a model species. Genetically similar fish from one population were exposed to highly diverse ecological environments (simulated by varying food levels), which were expected to induce phenotypic variation in growth and maturation. Under these conditions and assuming that the PMRN model captures all or most phenotypic plasticity in maturation, no genetic change in age and size at maturation and thus no displacement of the

Figure 1. Schematic of the principal idea of the probabilistic maturation reaction norm (PMRN) approach. Lines indicate the midpoint of a hypothetical PMRN illustrating the combination of age and size at which the maturation probability is 0.5. Variability in growth (growth curves in thin solid lines) determines the part of the PMRN that can be observed (indicated by the thick lines). Over time, a PMRN is not expected to shift owing to differences in growth rates but a genetic change in age and size at maturation is assumed to result in its displacement [22]. By comparing two PMRNs from different time periods (the thick solid and the dashed lines), one can assess whether the data support the hypothesis of genetic changes in response to selection pressures. In this example, the PMRN shifts downwards, indicating earlier maturation at smaller size.

PMRN midpoints (figure 1) estimated for fish exposed to different ecological environments is to be expected. Based on phenotypic information about maturity status, age, length and body condition we constructed two-dimensional (based on age and length) and three-dimensional (based on age, length, and condition) PMRNs using the demographic estimation method [36] to determine whether in zebrafish the PMRN approach fully captured the phenotypic plasticity in maturation stemming from experimentally induced variation in growth and condition.

2. MATERIAL AND METHODS

(a) Experimental design

The fish used in the experiment were third-generation offspring from a wild zebrafish population captured from a river system west of Coochibhar (West Bengal, India, 22.56° N, 87.67° E) reared under laboratory conditions. No obvious phenotypic traits were selected for in the experimental population and fish were assumed to have the same genetic background.

At age 85 days post fertilization (dpf) when zebrafish were still immature, a feeding experiment was initiated. This age was chosen when initiating the experiment to assure that juvenile fish were large enough to resist unfavourable environmental conditions, i.e. starvation resulting from low food amounts in some feeding treatments. Individual fish were then randomly assigned to five different feeding groups and fed with 0.5, 1, 2, 4 or 8 per cent dry food of fish biomass per aquarium per day. Food amount was adjusted throughout the experiment (see the electronic supplementary material). Each diet was applied in five replicated aquaria with 50 fish per aquarium (density of 1.1

individuals l^{-1}). Every 10–15 days, 25–50 randomly selected fish from each diet were culled and their standard length was estimated to the nearest mm and wet mass to the nearest 0.1 mg. The sample size was not fixed for each sampling period but it was adjusted to the expected maturation rate of the fish. Fish on the high food levels (2%, 4% and 8%) were detected to mature earlier and thus collected more intensively at the beginning of the experiment to obtain a representative proportion of immature and mature individuals than fish on the low food levels (0.5% and 1%). After culling, the fish were opened and the sex determined visually as described in the electronic supplementary material. Since maturity status of males could not be accurately estimated macroscopically, maturity data were collected from females only. Batch-spawning zebrafish have several oocytes simultaneously at different developmental stages when they are about to spawn [37], so females were classified as immature or mature, rather than maturing. Fish on high food levels (2%, 4% and 8%) were sampled a total of seven times from day 103 to 197 after initializing the experiment. Fish fed on 0.5 and 1 per cent diets were very small and generally had undeveloped gonads. Therefore, fish in these diet treatments were not lethally sampled as often as fish in the other diet treatments to avoid sacrificing the fish prior to them achieving the critical stage of potential maturation. As a result, female maturity data were only obtained five times from 1 per cent diet fish and three times from 0.5 per cent diet fish. Each sampling event took an average of five days. More details about fish rearing, feeding and determination of maturity status can be found in the electronic supplementary material.

(b) Estimation of probabilistic maturation reaction norms

The PMRN is defined in the majority of the studies by age- and size-specific probabilities that an immature individual matures during a given discrete time interval [21]. To estimate PMRNs, we used the demographic estimation method [36], which involves three steps: (i) estimation of the probability of being mature at a particular combination of variables such as age and size (i.e. estimation of maturity ogives), (ii) modelling growth rates, and (iii) estimation of the PMRNs based on the maturity ogives and the growth model. The demographic estimation method has been used in previous PMRN studies based on a simplifying assumption that the growth rate and survival is the same for immature and mature individuals. Although this assumption is not expected to apply accurately in fish [38], Barot *et al.* [36] showed that the method is fairly robust. In the current application, we estimated the maturity ogive by including the variables age and length in the two-dimensional models, and age, length and condition in the three-dimensional model. The condition factor was included because previous research has found that condition serves as a surrogate for the nutritional status of fish and may represent a valid determinant of maturation probability [30,31,39]. In our study, the condition factor was represented by the relative condition factor [40,41]. The relative condition factor was calculated for each female as

$$\frac{W}{aL^b}, \quad (2.1)$$

where W is the wet mass (g), L the standard length (mm), a the intercept and b the slope of a linear

regression of $\ln(W)$ on $\ln(L)$ for females originating from the same population used in the present experiment but reared outside our experimental approach in typical feeding conditions in our laboratory ($F_{1,156} = 1352$, $p < 0.01$). The standard length–weight regression parameters of our zebrafish population were estimated as $a = 0.00003$ and $b = 2.907$.

Maturity ogives required for the calculation of the PMRNs were estimated using logistic regression. The ogive models $o(a, l)$ and $o(a, l, c)$ were estimated for the traditional, two-dimensional PMRN relating the maturation probability to age (a) and length (l) exclusively (equation (2.2)) and for the three-dimensional PMRN to age-, length- and relative condition (c) (equation (2.3)):

$$o(a, l, d) \sim a + l + d \quad (2.2)$$

and

$$o(a, l, c, d) \sim a + l + c + d, \quad (2.3)$$

where age (a) in days, standard length (l) in mm and relative condition factor (c) were continuous variables, and diet treatment (d) was a categorical variable. Additionally, the quadratic terms of age (a^2), length (l^2) and relative condition factor (c^2) were added to the models to test for non-linearity in the predicted relationships. On relatively low sample sizes the estimation of a full model may no longer be robust [36] and therefore interactions were omitted from the models. Maturity ogives were not estimated for each diet separately owing to low numbers of observations of mature fish in low food diets (i.e. 0.5% and 1% diets). This problem was surmounted by combining data from all the diet treatments as suggested in Barot *et al.* [36] and as would be typical when constructing PMRNs from phenotypic data collected in the wild. The potential variance in the probability of being mature associated with the replicate aquaria in the diet treatments were accounted for by considering replicates as a random effect in the models and estimating its variance component.

As the second step in the PMRN estimation, growth in length was modelled testing both nonlinear and linear multiple regressions with age, diet and their interaction as predictor variables. Changes in relative condition were modelled similarly by using age, length, diet and their interactions as predictor variables. As in the maturity ogive calculations, growth and relative condition models were based on data collected from females only. There were no mortalities during the experiment.

In the ogive model, age was used as a continuous variable instead of estimating ogives for each age class separately (as in Barot *et al.* [16,36]) owing to the relatively low number of females per age class. Using age as a continuous variable also helped to avoid biases in maturity ogive estimations arising from sampling design. This is because for logistical reasons sampling did not take place for each diet exactly the same day but the low food diets were always sampled a few days later than high food diets. Therefore, using age as a discrete variable would have resulted in a few extra days to mature for fish on low food diets in each sampling period. By contrast, following the commonly applied demographic estimation method [36] PMRNs were estimated for discrete age classes. The age classes i were here represented by the midpoint of the sampling period lasting on average 5 days. The PMRNs were constructed for the two-dimensional

Figure 2. Growth curves for (a) 0.5%, (b) 1.0%, (c) 2.0%, (d) 4.0% and (e) 8.0% diet treatments in zebrafish (*Danio rerio*). Open circles indicate the observations (offset to improve the visualization) and filled circles the estimated mean values for standard length used in the probabilistic maturation reaction norm estimations. The solid lines represent growth curves predicted by the growth model (see electronic supplementary material).

length-based (equation (2.4)) and the three-dimensional (equation (2.5)) approach for each diet treatment separately using the approach by Barot *et al.* [36]:

$$m(a, l) = o(a, l, d) - o(a[i] - a[i - 1], l - \Delta l) / 1 - o(a[i] - a[i - 1], l - \Delta l) \quad (2.4)$$

and

$$m(a, l, c) = o(a, l, c, d) - o(a[i] - a[i - 1], l - \Delta l, c - \Delta c) / 1 - o(a[i] - a[i - 1], l - \Delta l, c - \Delta c), \quad (2.5)$$

where m refers to the probability of maturing. The probability of being mature at a given age and size was calculated from the maturity ogives, $o(a, l, d)$, $o(a, l, c, d)$, and the mean age-specific growth increments in length (Δl) and the mean age-specific changes in relative condition (Δc) were included from the final growth and condition models. To visualize the PMRN and its shape and position as a function of size, age and relative condition, the lengths at which the probability of maturing would be 25, 50 and 75 per cent were estimated using equations (2.4) and (2.5) for a range of standard lengths (10–26 mm). Following the approach by Barot *et al.* [36], a logistic regression was fitted to the estimated probabilities for the length range to derive the desired quantiles. The logistic regression model was described as

$$\text{logit}(p) = \alpha + \beta \times l, \quad (2.6)$$

where the logit link function is given by $\text{logit}(p) = \ln[p / (1 - p)]$, α and β are the parameters to be estimated and l notates for standard length (mm). To test whether fish that were fed with different diets had different PMRNs, a randomization test as described by Barot *et al.* [16], was used (see the electronic supplementary material for details). All statistical analyses were performed using R 2.10.0 with packages MASS and lme4 [42].

3. RESULTS

By the end of the experiment 37.9 per cent (122 individuals) had matured and 62.1 per cent of the females (200 individuals) remained immature. While fish on the 0.5 and 1 per cent diets exhibited minimal growth in length during the experiment, zebrafish in the high food treatments (2%, 4%, 8% diets) showed positive growth in terms of body length (figure 2). This resulted in significant differences in mean standard length of zebrafish among the various diet treatments. When pooled across the experimental period (103–197 days) fish from the 0.5 per cent (16.5 ± 1.81 mm, mean $\pm$ s.d.) and 1 per cent diet treatments (16.6 ± 1.80 mm) were on average smaller, in terms of standard length, than fish from the 2 per cent (16.7 ± 2.91 mm), 4 per cent (18.2 ± 2.88 mm) and 8 per cent diets (19.9 ± 2.21 mm). When modelling growth in length, linear and nonlinear

Table 1. Two- and three-dimensional maturity ogive models. Nagelkerke R^2 -values [57] for the two-dimensional model: 0.39 and for the three-dimensional model: 0.42. AIC-values for the two-dimensional model: 282 and for the three-dimensional model: 260.

approach	variable	^a deviance (d.f.)	^b p -value	null deviance	residual deviance
two-dimensional	age ²	4.37(1,315)	0.04	427.3	268.5
	length	50.1(1,315)	<0.01		
	diet	32.0(4,318)	<0.01		
three-dimensional	age	18.8(1,319)	<0.01	427.3	252.2
	length	91.0(1,319)	<0.01		
	condition	47.9(1,319)	<0.01		

^aIncrease in residual deviance upon deletion from the full model.

^b p -values from χ^2 -test.

The values for diet refer to all diet treatments.

models were virtually overlapping, therefore a linear growth model was used (figure 2). Length increased in the course of the experiment, but this was dependent on the diet treatment as indicated by a significant age $\times$ diet interaction (see the electronic supplementary material, table S1). Similar to length, pooled across the experimental period the relative condition factor was higher among fish held on 2 per cent (0.86 ± 0.15 , mean $\pm$ s.d.), 4 per cent (1.03 ± 0.20) and 8 per cent (1.18 ± 0.24) diets compared with fish held on 0.5 per cent (0.76 ± 0.19) and 1 per cent (0.69 ± 0.14) diets. As in growth model, the interaction of age $\times$ diet significantly correlated with the relative condition (see the electronic supplementary material, table S1), implying that the effect of the diet on zebrafish condition differed at varying ages or stages of the experiment. Relative condition was independent of body length and not significantly related to it (see the electronic supplementary material, table S1).

The maturity ogive models used in the estimation of the two-dimensional, age- and length-based PMRN included the main effects of age, length and diet treatment. In the age- and length-based ogive model, length and the quadratic term of age were both important determinants of maturity (table 1; see the electronic supplementary material, table S2), but these did not entirely encompass the variation in maturity status among the diets. This was reflected by the significant effect of the diet treatment in the ogive model after controlling for variation in age and length (table 1). Age (ANOVA, D (deviance)_{1,315} = 2.747, p = 0.097) and quadratic length (D _{1,314} = 0.381, p = 0.537) did not significantly affect the probability of being mature. In the three-dimensional maturity ogive model the main effects of age, length, relative condition factor, and diet were included. Age, length and relative condition were found to be significant covariates of maturity probability (table 1; see the electronic supplementary material, table S2), but the effect of diet was not significant (D _{4,318} = 8.780, p = 0.066) and was therefore omitted from the three-dimensional ogive model. The quadratic effects of age (D _{1,314} = 0.639, p = 0.424), length (D _{1,312} = 0.012, p = 0.912), and relative condition (D _{1,313} = 0.820, p = 0.365) were not significant. Systematic variation among the replicates could not be detected, i.e. there was no indication of a substantial tank effect influencing the results, because the variance component of replicates was virtually zero (less than 0.0001%).

The two-dimensional, age- and length-based PMRNs for the fish exposed to medium and abundant food levels (2%, 4% and 8% diets) exhibited lower 50 per cent quantiles (i.e. PMRN midpoints) compared with the PMRNs constructed for fish held at restricted food levels (0.5% and 1% diets, figure 3a; 25% and 75% quantiles are presented in the electronic supplementary material, figure S3a,b). This reflected the significant effect of diet on maturity after the removal of variation in maturation probability resulting from age and length. The significant diet-dependency of the position of the PMRN indicated that the two-dimensional PMRN did not fully capture the phenotypic plasticity in maturation of zebrafish. In other words: the two-dimensional PMRN was found to be plastic to some degree. The distance among midpoints of the PMRN constructed for high and low food levels observed in the two-dimensional length-based PMRN (randomization test, p < 0.01) notably decreased in the three-dimensional PMRN (figure 3b; 25% and 75% quantiles are presented in the electronic supplementary material, figure S3c,d), which accounted not only for age and length, but also relative condition. Although the difference in the PMRN quantiles at rich and poor feeding environments was still statistically significant (randomization test, p < 0.01), the smaller distance among the diet-specific quantiles of the three-dimensional age, length and condition-based PMRN (figure 3) indicated that the covariates in the three-dimensional model explained a larger amount of variation in maturation probability in zebrafish. As a result the three-dimensional PMRN exhibited less plasticity in response to environmental variation induced by food availability than the two-dimensional PMRN.

4. DISCUSSION

The PMRN estimation approach has been repeatedly used to study and interpret changes in age and size at maturation in exploited fish stocks [2,16,17] despite the fact that the method has not been tested experimentally. Our assessment of a population-level PMRN approach was accomplished by exposing laboratory-held zebrafish experimentally to environments that strongly differed in food availability to induce plasticity in growth and maturation. We found that the two-dimensional, age- and length-based PMRN did not account for all the phenotypic plasticity in maturation probability in zebrafish. In particular, the diet-dependency of the position of the two-dimensional PMRN reflected environmentally

Figure 3. Probabilistic maturation reaction norms with 50% quantiles (i.e. midpoints) estimated for (a) two-dimensional, age- and length-based and (b) three-dimensional, age-, length- and relative condition-based PMRN models. Standard length on the y-axis represents the length at 50% maturation probability. PMRNs were estimated for the time periods data were available. In the three-dimensional PMRN the midpoints for different diets were closer to each other than in the two-dimensional PMRN in the considered scale of the y-axis, but, nonetheless, differences were significant (see text). The PMRNs are nonlinear owing to the age $\times$ diet interactions in the underlying growth and condition models (thin dashed line, 0.5%; thin solid line, 1% thick solid line, 2%; thick dashed line, 4%; bold dots, 8%).

induced and hence plastic, rather than genetic, variation in maturation, which was not accounted for by differential growth. However, we found that incorporating relative condition into the PMRN estimation reduced the diet-dependent differences in the vertical position of the midpoints between high and low food level treatments. Hence, the three-dimensional PMRN better explained environmental variation in maturation probability compared with the two-dimensional, age- and length-based maturation model. Adding relative condition, however, did not entirely remove the diet-dependency of the position of the PMRN, indicating that neither of the PMRN models we tested succeeded in encompassing all the phenotypic plasticity in maturation.

The assumption that variation in age and size capture most, if not all, of the environmentally induced variation in maturation probability in fish is challenged by our findings, which revealed a diet-dependency in the vertical position of the age- and length-based PMRNs constructed for zebrafish exposed to different food levels. The differences in the vertical position of the midpoints between high and low food levels were evident despite the fact that all the experimental fish were from a common gene pool, thus no genetic response in maturation schedule was possible in our within-generation experiment. Our findings agree with a study by Morita *et al.* [27], who transplanted genetically similar white-spotted charr (*Salvelinus leucomaenis*) males to five different sites in a natural river to subsequently study maturation schedules. They showed that plasticity in the position of a multi-dimensional PMRN estimated for fish exposed to different environments was caused by a habitat characteristic (river width), not by genetic changes in the maturation schedule. In our study, the midpoints of both the two- and the three-dimensional PMRNs constructed for fish exposed to rich food conditions were found to be consistently lower than the ones for the slow-growing zebrafish indicating higher size-specific maturation probabilities of fast-growing fish compared with fish growing slower owing to low food levels. These differences were evident despite statistically controlling

for variation in growth and condition, but were less pronounced in the three-dimensional PMRN that included relative condition to explain maturation.

In time series from heavily exploited wild fish populations a downward shift of the PMRN has been interpreted as fisheries-induced evolutionary change towards earlier maturation at smaller size [2,16,17]. The removal of biomass through fishing usually reduces intraspecific competition for food and elevates growth rate [7], which, according to our results, may also contribute to downward shifts in the PMRN. However, in our study adding relative condition in the estimation model captured a greater fraction of the phenotypic plasticity in maturation probability than the two-dimensional PMRN based on age and length alone. This resulted in a smaller distance among the PMRN midpoints estimated for zebrafish exposed to poor and abundant food levels (figure 3). Previous field studies have similarly shown that adding condition as a third dimension in the statistical model of the maturation process improved the model fit and the accuracy of predicting maturation probabilities in fish [30–32]. Therefore, as long as researchers add all salient traits affecting maturation in fish, PMRN will capture a large degree of phenotypic plasticity in maturation. In the absence of alternative methods to disentangle phenotypic plasticity and evolutionary change in age and size at maturation, the PMRN thus remains a useful tool to study the evolutionary consequences of fishing as long as researchers carefully consider the possibility for shifts in the position of the PMRN being caused by plasticity rather than genetic change.

In the three-dimensional PMRN model, the decrease of the differences among the PMRNs estimated for the different food levels indicated that the effect of condition on maturation was more important in our experiment than has been found in previous studies, where condition only explained marginal variance in maturation probability [30–32]. This might be owing to the extreme feeding regimes in our experiment, which could have translated into larger differences in relative condition among fish than is typically seen in natural populations.

However, the occurrence of severe food limitations in nature is not uncommon and may result in stunted fish or very poor growth [43,44]. Therefore, feeding conditions comparable to those in our study could potentially also occur in nature. However, owing to the experimental set up, the importance of relative condition in explaining maturation probability was probably stronger than should be expected in the wild. For example, at the time our feeding trials were initiated, all the experimental fish were immature and of equal size. Fish cannot shrink in length even when starving, thus the different feeding treatments translated mainly into differences in an individual's relative condition. Starting the experiment earlier could have led to larger differences in body length among treatments. This could have decreased the importance of condition relative to the length in predicting maturation, but this assumption needs to be tested in the future.

One can argue that change in relative condition over time is a consequence rather than a cause of maturation (e.g. gonad weight can increase relative condition), such that there is no causal relationship between condition and maturation probability in zebrafish. Indeed, a correlative estimation method, such as the PMRN, cannot uncover the causality between the variables used for its estimation. Our study nevertheless shows that relative condition helped to explain variation in maturation probability, which was not captured by age and length alone. Condition, independent of growth rate, can represent the nutritional status of an individual [35,45] and may correlate with tissue fat level [46,47]. It has been shown that nutritional state strongly affects the maturation processes in various fish species [48–50]. It has also been suggested that an energy storage threshold must be surpassed for sexual maturation to occur in fish [46,51]. Therefore, we interpret the significant effect of relative condition on maturation probability in zebrafish in the light of its importance for affecting energy allocation towards gonad growth rather than maintenance or somatic growth. Fish continuously exposed to food limitation, as in our 0.5 and 1 per cent diet treatments, might therefore allocate energy mainly to maintenance of body functions. This might explain the higher midpoints of PMRNs estimated for zebrafish held at low food levels compared with zebrafish at high food levels, indicating lower size-specific maturation probabilities by fish experiencing restricted food conditions. To fully understand the underlying determinants of the maturation process in various fish species, a deeper understanding of the physiological processes governing maturation and how these processes respond to environmental factors and interact with growth rate is needed.

Limitations of our work are attributed to the characteristics of the model species. These limitations may restrict the implications of our findings to exploited fish stocks. Firstly, unlike many species of fisheries importance, laboratory-reared zebrafish spawn year-around on a daily basis, and thus its reproductive cycle lacks seasonality that is typical for many commercial stocks. Secondly, in many seasonally reproducing species reproductive decisions can take place early in life or during a time period before the next breeding season [35]. In zebrafish, however, maturation seems not to be determined early in ontogeny [52]. This might explain why in our study

experimentally induced differences in maturation schedule were expressed even though the feeding experiment was initiated at the end of the juvenile period at 85 dpf. However, the underlying physiological mechanisms of maturation should still be similar in zebrafish compared with other fish species with a seasonal breeding cycle traditionally used in PMRN applications. A third difference among our study species and many commercially important species concerns the fact that zebrafish establish dominance hierarchies with dominant individuals controlling the feeding opportunities [53]. This may result in dominant individuals maturing larger, earlier and in better condition than subdominant fish, which might have influenced the PMRN estimation processes in the present study. However, dominance hierarchies are also common in other species, for example in cod (*Gadus morhua*) and plaice (*Pleuronectes platessa*) [54,55]. Despite the differences between our model species and other fish species, the purpose of our study was to experimentally assess the PMRN method by using a short-lived species suitable for a laboratory study. Due to the generality of the method, we do not expect substantial differences in the most important factors influencing maturation between our study system and natural fish populations.

The final limitation of the study is related to the relatively small amount of maturation data from individuals on low food levels, which might have contributed to the diet-dependency of the vertical position of the PMRN midpoints. This issue could not be avoided because fish on low food levels were consistently smaller with a lower condition factor than fish on the high food levels and had lower maturation probabilities. The low sample size of mature fish held on low food levels restricted the use of flexible ogive models with interactions between the variables so that the estimation models became biased and were no longer robust when including all two-way interactions (see [36]). Low sample size also restricted the estimation of ogive models for each diet separately. Therefore, the data collected from all diets were combined as it would be the case in phenotypic data collected from the wild allowing more robust estimation of PMRNs (e.g. [2,16]). The problem of low sample sizes in our study calls for careful evaluation of the results and upon replicating the experiment with larger numbers of observations.

PMRN estimations for wild fish stocks have often been based on length-at-age information (but see [30,31]), because sufficiently long time series of other phenotypic traits that may be important for modelling maturation are often not available [56]. However, as our study showed, age and length may not be sufficient for constructing a PMRN that is assumed to fully capture phenotypic plasticity in maturation, and a more integrated view of maturation involving indices of condition and potentially other traits may be required. This could be relevant especially among fish stocks experiencing high temporal variation in food availability, resulting in environmentally induced variation in condition among individuals. A practical implication of our study would therefore be to measure individual weight in addition to length in surveys and to estimate a condition index for maturation analyses to help providing robust inferences from PMRN analyses in wild fish stocks. There is a need to perform further assessment studies of the PMRN

method with sufficiently large amount of data to better understand the degree of plasticity that might be expected in PMRN analyses in wild fish stocks. These assessments should ideally be species-specific and conducted for commercially and recreationally exploited species, which are anticipated to be affected by fisheries-induced evolutionary changes in age and size at maturation [4].

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REFERENCES

- Law, R. 2000 Fishing, selection, and phenotypic evolution. *ICES J. Mar. Sci.* **57**, 659–668. (doi:10.1006/jmsc.2000.0731)
- Olsen, E. M., Heino, M., Lilly, G. R., Morgan, M. J., Brattey, J., Ernande, B. & Dieckmann, U. 2004 Maturation trends indicative of rapid evolution preceded the collapse of northern cod. *Nature* **428**, 932–935. (doi:10.1038/nature02430)
- Kuparinen, A. & Merilä, J. 2007 Detecting and managing fisheries-induced evolution. *Trends Ecol. Evol.* **22**, 652–659. (doi:10.1016/j.tree.2007.08.011)
- Jørgensen, C. *et al.* 2007 Managing evolving fish stocks. *Science* **318**, 1247–1248. (doi:10.1126/science.1148089)
- Beacham, T. D. 1983 Variability in size and age at sexual maturity of argentine, *Argentina silus*, on the Scotian Shelf in the Northwest Atlantic Ocean. *Env. Biol. Fish.* **8**, 67–72. (doi:10.1007/BF00004948)
- Trippel, E. A., Kjesbu, O. S. & Solemdal, P. 1997 Effects of adult age and size structure on reproductive output in marine fishes. In *Early life history and recruitment in fish populations* (eds R. C. Chambers & E. A. Trippel), pp. 29–62. London, UK: Chapman and Hall.
- Trippel, E. A. 1995 Age at maturity as a stress indicator in fisheries. *Bioscience* **45**, 759–770. (doi:10.2307/1312628)
- Alm, G. 1959 Connection between maturity, size and age in fishes. *Rep. Inst. Freshwat. Res. Drottningholm* **40**, 5–154.
- Healey, M. C. 1980 Growth and recruitment in experimentally exploited lake whitefish (*Coregonus clupeaformis*) populations. *Can. J. Fish. Aquat. Sci.* **37**, 255–267. (doi:10.1139/f80-033)
- Haug, T. & Tjemsland, J. 1986 Changes in size- and age-distributions and age at sexual maturity in Atlantic halibut, *Hippoglossus hippoglossus*, caught in North Norwegian waters. *Fish. Res.* **4**, 145–155. (doi:10.1016/0165-7836(86)90039-1)
- Reznick, D. N. 1983 The structure of guppy life histories: the trade-off between growth and reproduction. *Ecology* **64**, 862–873. (doi:10.2307/1937209)
- Reznick, D. N. 1990 Plasticity in age and size at maturity in male guppies (*Poecilia reticulata*): an experimental evaluation of alternative models of development. *J. Evol. Biol.* **3**, 185–203. (doi:10.1046/j.1420-9101.1990.3030185.x)
- Aubin-Horth, N., Landry, C. A., Letcher, B. H. & Hofmann, H. A. 2005 Alternative life histories shape brain gene expression profiles in males of the same population. *Proc. R. Soc. B* **272**, 1655–1662. (doi:10.1098/rspb.2005.3125)
- Consuegra, S., García de Leániz, C., Serdio, A. & Verspoor, E. 2005 Selective exploitation of early running fish may induce genetic and phenotypic changes in Atlantic salmon. *J. Fish Biol.* **67**(Suppl. A), 129–145. (doi:10.1111/j.1095-8649.2005.00844.x)
- Basolo, A. L. 2008 Evolution of pleiotropic alleles for maturation and size as a consequence of predation. *Biol. Lett.* **4**, 200–203. (doi:10.1098/rsbl.2007.0638)
- Barot, S., Heino, M., O'Brien, L. & Dieckmann, U. 2004 Long-term trend in the maturation reaction norm of two cod stocks. *Ecol. Appl.* **14**, 1257–1271. (doi:10.1890/03-5066)
- Olsen, E. M., Lilly, G. R., Heino, M., Morgan, J., Brattey, J. & Dieckmann, U. 2005 Assessing changes in age and size at maturation in collapsing populations of Atlantic cod (*Gadus morhua*). *Can. J. Fish. Aquat. Sci.* **62**, 811–823. (doi:10.1139/F05-065)
- Hutchings, J. A. 2001 The influence of population decline, fishing, and spawner variability on the recovery of marine fishes. *J. Fish Biol.* **59**(Suppl. A), 306–322. (doi:10.1006/jfbi.2001.1756)
- Conover, D. O., Munch, S. B. & Arnott, S. A. 2009 Reversal of evolutionary downsizing caused by selective harvest of large fish. *Proc. R. Soc. B* **276**, 2015–2020. (doi:10.1098/rspb.2009.0003)
- Enberg, K., Jørgensen, C., Dunlop, E. S., Heino, M. & Dieckmann, U. 2009 Implications of fisheries-induced evolution for stock rebuilding and recovery. *Evol. Appl.* **2**, 394–414. (doi:10.1111/j.1752-4571.2009.00077.x)
- Heino, M., Dieckmann, U. & Godø, O. R. 2002 Measuring probabilistic reaction norms for age and size at maturation. *Evolution* **56**, 669–678.
- Heino, M. & Dieckmann, U. 2008 Detecting fisheries-induced life-history evolution: an overview of the reaction-norm approach. *Bull. Mar. Sci.* **83**, 69–93.
- Dieckmann, U. & Heino, M. 2007 Probabilistic reaction norms: their history, strengths, and limitations. *Mar. Ecol. Prog. Ser.* **335**, 253–269. (doi:10.3354/meps335253)
- Morita, K. & Fukuwaka, M. 2006 Does size matter most? The effect of growth history on probabilistic reaction norm for salmon maturation. *Evolution* **60**, 1516–1521.
- Kraak, S. B. M. 2007 Does the probabilistic maturation reaction norm approach disentangle phenotypic plasticity from genetic change? *Mar. Ecol. Prog. Ser.* **335**, 295–300. (doi:10.3354/meps335295)
- Marshall, T. C. & McAdam, B. J. 2007 Integrated perspectives on genetic and environmental effects on maturation can reduce potential for errors in inference. *Mar. Ecol. Prog. Ser.* **335**, 301–310. (doi:10.3354/meps335301)
- Morita, K., Tsuboi, J. & Nagasawa, T. 2009 Plasticity in probabilistic reaction norms for maturation in a salmonid fish. *Biol. Lett.* **5**, 628–631. (doi:10.1098/rsbl.2009.0290)
- Marshall, C. T. & Browman, H. I. 2007 Disentangling the causes of maturation trends in exploited fish populations. *Mar. Ecol. Prog. Ser.* **335**, 249–251.
- Kuparinen, A. & Merilä, J. 2008 The role of fisheries-induced evolution. *Science* **320**, 47–48.
- Grift, R. E., Heino, M., Rijnsdorp, A. D., Kraak, S. B. M. & Dieckmann, U. 2007 Three-dimensional maturation reaction norms for North Sea plaice. *Mar. Ecol. Prog. Ser.* **334**, 213–224. (doi:10.3354/meps334213)
- Mollet, F., Kraak, S. B. M. & Rijnsdorp, A. D. 2007 Fisheries-induced evolutionary changes in maturation reaction norms in North Sea sole *Solea solea*. *Mar. Ecol. Prog. Ser.* **351**, 189–199. (doi:10.3354/meps07138)
- Vainikka, A., Gårdmark, A., Bland, B. & Hjempl, J. 2008 Two- and three-dimensional maturation reaction norms

- for the eastern Baltic cod, *Gadus morhua*. *ICES J. Mar. Sci.* **66**, 248–257. (doi:10.1093/icesjms/fsn199)
- 33 Pardoe, H., Vainikka, A., Thórdarson, G., Marteinsdóttir, G. & Heino, M. 2009 Temporal trends in probabilistic maturation reaction norms and growth of Atlantic cod (*Gadus morhua*) on the Icelandic shelf. *Can. J. Fish. Aquat. Sci.* **66**, 1719–1733. (doi:10.1139/F09-132)
 - 34 Thorpe, J. E. 2007 Maturation responses of salmonids to changing developmental opportunities. *Mar. Ecol. Prog. Ser.* **335**, 285–288. (doi:10.3354/meps335285)
 - 35 Wright, P. J. 2007 Understanding the maturation process for field investigations of fisheries-induced evolution. *Mar. Ecol. Prog. Ser.* **335**, 279–283. (doi:10.3354/meps335279)
 - 36 Barot, S., Heino, M., O'Brien, L. & Dieckmann, U. 2004 Estimating reaction norms for age and size at maturation when age at first reproduction is unknown. *Evol. Ecol. Res.* **6**, 659–678.
 - 37 Selman, K., Wallace, R. A., Sarka, A. & Qi, X. 1993 Stages of oocyte development in zebrafish *Brachydanio rerio*. *J. Morphol.* **218**, 203–224. (doi:10.1002/jmor.1052180209)
 - 38 Lester, N. P., Shuter, B. J. & Abrams, P. A. 2004 Interpreting the von Bertalanffy model of somatic growth in fishes: the cost of reproduction. *Proc. R. Soc. Lond. B* **271**, 1625–1631. (doi:10.1098/rspb.2004.2778)
 - 39 Baulier, L., Heino, M., Lilly, G. R. & Dieckmann, U. 2006 Body condition and evolution of maturation of Atlantic cod in Newfoundland. *ICES CM 2006/H:19*.
 - 40 Le Cren, E. D. 1951 The length–weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). *J. Anim. Ecol.* **20**, 201–219.
 - 41 Froese, R. 1998 Length–weight relationship for 18 less-studied fish species. *J. Appl. Ichthyol.* **14**, 117–118. (doi:10.1111/j.1439-0426.1998.tb00626.x)
 - 42 R Development Core Team. 2009 R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. See <http://www.R-project.org>.
 - 43 Ylikarjula, J., Heino, M. & Dieckmann, U. 1999 Ecology and adaptation of stunted growth in fish. *Evol. Ecol.* **13**, 433–453. (doi:10.1023/A:1006755702230)
 - 44 Amundsen, P.-A., Knudsen, R. & Klemetsen, A. 2007 Intraspecific competition and density dependence of food consumption and growth in Arctic charr. *J. Anim. Ecol.* **76**, 149–158. (doi:10.1111/j.1365-2656.2006.01179.x)
 - 45 Lambert, Y. & Dutil, J.-D. 1997 Condition and energy reserves of Atlantic cod (*Gadus morhua*) during the collapse of the northern Gulf of St. Lawrence stock. *Can. J. Fish. Aquat. Sci.* **54**(Suppl. 1), 104–112. (doi:10.1139/cjfas-54-10-2388)
 - 46 Silverstein, J. T., Shimma, H. & Ogata, H. 1997 Early maturity in amago salmon (*Oncorhynchus masu ishikawai*): an association with energy storage. *Can. J. Fish. Aquat. Sci.* **54**, 444–451. (doi:10.1139/cjfas-54-2-444)
 - 47 Silverstein, J. T., Shearer, K. D., Dickhoff, W. W. & Plisetskaya, E. M. 1998 Effects of growth and fatness on sexual development of chinook salmon (*Oncorhynchus tshawytscha*) parr. *Can. J. Fish. Aquat. Sci.* **55**, 2376–2382. (doi:10.1139/cjfas-55-11-2376)
 - 48 Bernardo, J. 1993 Determinants of maturation in animals. *Trends Ecol. Evol.* **8**, 166–173. (doi:10.1016/0169-5347(93)90117-8)
 - 49 Metcalfe, N. B. 1998 The interaction between behavior and physiology in determining life history patterns in Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* **55**(Suppl. 1), 93–103. (doi:10.1139/cjfas-55-S1-93)
 - 50 Marteinsdóttir, G. & Begg, G. A. 2002 Essential relationships incorporating the influence of age, size and condition on variables required for estimation of reproductive potential in Atlantic cod *Gadus morhua*. *Mar. Ecol. Prog. Ser.* **235**, 235–256. (doi:10.3354/meps235235)
 - 51 Rowe, D. K., Thorpe, J. E. & Shanks, A. M. 1991 Role of fat stores in the maturation of male Atlantic salmon (*Salmo salar*) parr. *Can. J. Fish. Aquat. Sci.* **48**, 405–413. (doi:10.1139/f9052)
 - 52 Wang, X. G., Bartfai, R., Sleptsova-Freidrich, I. & Orban, L. 2007 The timing and extent of 'juvenile ovary' phase are highly variable during zebrafish testis differentiation. *J. Fish Biol.* **70**(Suppl. A), 33–44. (doi:10.1111/j.1095-8649.2007.01363.x)
 - 53 Pritchard, V. L. 2001 Behaviour and morphology of the zebrafish (*Danio rerio*). PhD dissertation, University of Leeds.
 - 54 Holdway, D. A. & Beamish, F. W. H. 1985 The effect of growth rate, size and season in oocyte development and maturity in Atlantic cod (*Gadus morhua* L.). *J. Exp. Mar. Biol. Ecol.* **85**, 3–19. (doi:10.1016/0022-0981(85)90010-3)
 - 55 Rijnsdorp, A. D. 1991 The mechanism of energy allocation over reproduction and somatic growth in female North Sea plaice, *Pleuronectes platessa* L. *Neth. J. Sea Res.* **25**, 279–290. (doi:10.1016/0077-7579(91)90045-3)
 - 56 Hilborn, R. & Walters, C. J. 1992 *Quantitative fisheries stock assessment: choice, dynamics and uncertainty*. New York, NY: Chapman and Hall.
 - 57 Nagelkerke, N. J. D. 1991 A note on a general definition of the coefficient of determination. *Biometrika* **78**, 691–692. (doi:10.1093/biomet/78.3.691)