International Institute for
Applied Systems Analysis
Schlossplatz 1
A-2361 Laxenburg, Austria

Tel: +43 2236 807 342
Fax: +43 2236 71313
E-mail: publications@iiasa.ac.at
Web: www.iiasa.ac.at

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The importance of social dimension and maturation stage for the probabilistic maturation reaction norm in *Poecilia reticulata*

Beatriz Diaz Pauli
Mikko Heino (heino@iiasa.ac.at)

Approved by

Ulf Dieckmann
Director, Evolution and Ecology Program

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The importance of social dimension and maturation stage for the probabilistic maturation reaction norm in *Poecilia reticulata*

Beatriz Diaz Pauli[1]† and Mikko Heino[1, 2, 3]

[1] Department of Biology, University of Bergen, Norway

[2] Institute of Marine Research, Bergen, Norway

[3] International Institute for Applied Systems Analysis, Laxenburg, Austria

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† Author for correspondence.

beatriz.diaz-pauli@bio.uib.no

Department of Biology, University of Bergen, Box 7803, N-5020 Bergen, Norway

Phone: (+47) 55 58 81 37

Fax: (+47) 55 58 44 50

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Abstract

Maturation is an important event in an organism's life history, with important implications on dynamics of both wild and captive populations. The probabilistic maturation reaction norm (PMRN) has emerged as an important method to describe variation in maturation in wild fish. Because most PMRNs are based on age and size only, it is important to understand limitations of these variables in explaining maturation. We experimentally assessed 1) the sensitivity of age- and size-based PMRNs to unaccounted sources of plasticity, 2) the role of social environment on maturation, and 3) the significance of estimating PMRNs early and late in the maturation process (initiation and completion of maturation, respectively). We reared male guppies (*Poecilia reticulata*) under laboratory conditions, subject to two food levels and three different social cues. We found that growth and social environment affected the maturation in ways that could not be accounted for by their effect on age and size. PMRNs estimated for the initiation stage were less plastic (growth differences and social cues influenced the PMRN shape only little) than those for completion. The initiation of maturation is probably closer to the maturation "decision" and allows determining factors influencing maturation decision most accurately.

Keywords: Maturation, age, size, PMRN, social cues, maturation stages

Introduction

Maturation is a key life-history event that determines the beginning of the reproductive part of an individual's life cycle. In addition, maturation is costly. Semelparous organisms pay the highest possible cost: they are physiologically programmed to die after reproduction. Iteroparous organisms that have capacity for multiple reproductive events face less dramatic but still important costs in terms of survival and energetics, typically showing reduced or nil growth after maturation. It is therefore unsurprising that maturation is one of the most studied traits in life-history theory, with the focus on trying to understand the ultimate, eco-evolutionary drivers of variability in maturation (Roff, 1992; Stearns, 1992, 2000). Age and size at maturation depend on 1) resource acquisition and 2) the resource allocation between growth and other competing functions (e.g., build-up of reproductive organs during maturation process; Berner & Blanckenhorn, 2007). Environmental factors, such as food availability, temperature, predator presence, social environment, etc., and genetic factors play a role in both processes (Berner & Blanckenhorn, 2007). The reaction norm for age and size at maturation by Stearns & Koella (1986) allows capturing some of these effects. This reaction norm is represented by a curve in a diagram with age and size as coordinate axes; maturation occurs when an individual's growth trajectory intersects the reaction norm. Age and size are relevant determinants of maturation because the combination of size and age carries information on average growth, summarizing some key environmental influences on maturation.

Another body of literature approaches maturation from a different, more proximate angle (Marshall & Browman, 2007): in the ontogenetic perspective, maturation is seen as a developmental process that starts long before the first reproduction (Thorpe, 2007; Wright, 2007). The development may be halted or triggered to proceed further in response to the

physiological state of an individual, possibly in combination with external signals (e.g., photoperiod). Age and size are seen as only poor proxies of the physiological state. They are particularly problematic when observed at the time of reproduction, which can be long after individuals became physiologically committed to complete maturation, i.e., they "decided" to mature (Thorpe, 2007; Wright, 2007).

Research on more ultimate and proximate determinants of maturation has often taken place in isolation, even though these perspectives are complementary (Berner & Blanckenhorn, 2007; Marshall & Browman, 2007). In field studies, it is convenient, and often the only practical option, to describe the maturation tendency with determinants like age and size, rather than with more proximate, physiological determinants. This is sensible in the eco-evolutionary context because size is an important determinant of many aspects of an individual's interaction with its environment and the combination of age and size describes the conditions for growth. Although the proximate causes of maturation in fish remain unclear, recent research suggests the importance of size and energetic status in the onset of maturation or during periods of liability (thresholds) in the juveniles (Thorpe *et al.*, 1998; Day & Rowe, 2002; Tobin & Wright, 2011 and references therein). Thus, it is suggested that maturation decision depends on the energy state before maturation is completed and on the environment that is experienced during that time (Wright, 2007; Tobin & Wright, 2011).

Understanding drivers of maturation is not only of fundamental scientific interest. Age at maturation is an important production trait in animal husbandry and in aquaculture (Patterson *et al.*, 1992; Taranger *et al.*, 2010). Furthermore, changes in maturation in commercially exploited fish stocks have raised considerable attention during the last two decades; typically, a reduction in size and age at maturation has been observed. Such reductions can be due to demographic truncation, phenotypic plasticity and genetic changes (Trippel, 1995). Changes in maturation can have adverse impacts on productivity of fish populations; therefore,

determining which of these drivers are important is key information for fisheries management (Jørgensen *et al.*, 2007).

The probabilistic maturation reaction norm (PMRN; Heino *et al.*, 2002) is a method for studying maturation schedules that arose from the need to better understand the nature of changes in maturation in commercially exploited fish stocks. It is an extension of the deterministic maturation reaction norm concept of Stearns & Koella (1986) that allows for stochasticity in the process of maturation. Thus, it reflects the realization that maturation tendency can only imperfectly be described by age and size.

The probabilistic reaction norm for age and size at maturation is defined as the probability of an immature individual to mature in a certain age interval given that it has survived and grown to that age and size (Heino *et al.*, 2002). It characterizes maturation schedules dependent on certain combination of age and size, and in this way integrates other variables that might influence reaching such age and size (Heino & Dieckmann, 2008). PMRNs enable one to study changes in maturation regimes while accounting for demographic changes and growth-related plasticity. When growth and mortality are the main sources of phenotypic variation on maturation, changes in the PMRN, e.g., in its midpoint (age and size at which maturation probability is 50%, L_{p50}), suggest underlying genetic changes (Heino *et al.*, 2002; Dieckmann & Heino, 2007). Thus, by studying temporal changes in PMRNs, the plausibility that maturation tendency has evolved can be tested. Most estimations of PMRNs have been conducted in the context of analysing maturation trends in exploited stocks, and the largest body of evidence for fisheries-induced evolution is based on PMRNs (reviewed by Heino & Dieckmann, 2008; for more recent examples see Vainikka *et al.*, 2009; van Walraven *et al.*, 2010; Devine & Heino, 2011; Baulier *et al.*, 2012).

Two challenges in using the PMRN approach are worth highlighting. First, the two-dimensional PMRN method, which only considers age and size in the estimation, acknowledges that age and size cannot account alone for changes in maturation, but treats the remaining variability as noise. However, PMRNs could be systematically altered by sources of variation such as environmental trends in temperature, pollutants, social structure, etc. (Kraak, 2007; Dieckmann & Heino, 2007). PMRNs that are more accurate in describing the maturation tendency might be obtained when these other environmental factors are included as new explanatory variables (Grift *et al.*, 2007; Dieckmann & Heino, 2007). Second, estimations of PMRNs using field data usually require making simplifying assumptions about mortality and growth. Assessing the significance of these challenges requires studies where their effects are quantified.

By minimizing observation and model uncertainty, the estimation of the PMRN from laboratory experiments allows one to characterize intrinsic variability of PMRNs. In addition, laboratory experiments allow adding controlled sources of variation to maturation, including factors that cannot easily be determined in the field. So far only four experimental studies on PMRNs have been published. Van Dooren *et al.* (2005) used springtails, *Folsomia candida*, to study alternative ways of defining reaction norms for age and size at maturation using a rate-based approach, and Harney *et al.* (2013), using different clones of *Daphnia magna* and *Daphnia pulex*, compared rate-based and probabilistic approaches to maturation reaction norms. Beckerman *et al.* (2010) estimated PMRNs of the water flea *D. pulex*, taking into account predator treatment as an additional explanatory dimension. Only Uusi-Heikkilä *et al.* (2011), using zebrafish, *Danio rerio*, specifically conducted an experiment to assess the PMRN method. They found that feeding regime strongly influenced PMRNs when only age and size were used as the explanatory variables, but that including condition removed most of this influence.

In the present study we assessed maturation schedules of male Trinidadian guppies, *Poecilia reticulata*, under experimental conditions. We raised guppies individually under two different food regimes and subjected to three different social cues. Our aim was threefold. First, we wanted to apply the PMRN method to experimental data to assess the effect of different growth trajectories on the shape of the PMRN, as well as the effect of unaccounted sources of plasticity. An experimental validation of this method will bring new insights into the study of the evolution of maturation in general and fisheries-induced evolution in particular. The PMRN approach is so far the best method available for inferring possible genetic changes in maturation when only phenotypic field data are available, as is typical for fisheries. The PMRN method allows controlling for certain plastic effects; the better this plastic variability can be controlled, the more likely the residual component represents genetic differences.

The second objective was to add an extra dimension to the PMRN, the social environment. Understanding social influences on maturation is an important task on its own right. The social environment is known to alter maturation in fish; generally, we can expect that maturation can be inhibited by the presence of large individuals of the same sex, but favoured by the presence of individuals of the opposite sex (e.g., Bushmann & Burns, 1994; Dannylchuk & Tonn, 2001; Aday *et al.*, 2003). However, this impact has never been considered in the estimation of PMRNs. Furthermore, when applying the PMRN approach to fisheries data, the social environment is of particular interest because it might be modified by the fishing pressure due to sex-dependent differences in behaviour, habitat use, growth or size (Rowe & Hutchings, 2003); e.g., fishing mortality has been shown to shift sex ratio in cod, *Gadus morhua*, (Jakobsen & Ajiad, 1999).

Our third goal was to understand the consequences of determining maturation at different points of the development for the estimated PMRNs. Male guppies offer an exceptional opportunity for this because their maturation can be scored at two distinct stages, at the

initiation and at the completion of maturation, without harming the individuals (Houde, 1997; Evans *et al.*, 2002). Because of limitations of available data, PMRNs have so far only been estimated at some specific point in the development, usually close to reproduction when determining maturation status is easy. However, a PMRN estimated late in the maturation process represents the influence of the size after gonadal development has taken place, instead of the size at the time of initial maturation decision (Wright, 2007). This might bias our understanding of the role of size on maturation. Male guppies allow quantifying this bias. While initiation (corresponding to the onset of secondary gametogenesis) is not necessarily coinciding with the maturation decision, we believe that initiation of maturation is close to the maturation decision, as assumed in other studies (e.g., lesser sandeels, *Ammodytes marinus*, Boulcott & Wright, 2008; haddock, *Melanogrammus aeglefinus***,** Tobin *et al.*, 2010).

Materials and methods

The experiment was performed with male Trinidadian guppies that were reared in isolation from birth until they reached maturation. The test males were second-generation offspring (F2) of wild-caught individuals (F0) from a low-predation site in the Yarra River in Trinidad, West Indies. The test fish were obtained by pairing twelve F1 virgin females with a randomly chosen, unrelated F1 male, thus making twelve families. The tanks were checked daily for newborn offspring, which were then collected and reared in isolation in individual two-litre aquaria. Sexing of the offspring was possible when they were 4–5 weeks old; at this stage females were discarded from the experiment. We collected in total eighteen male full-sib offspring from each family. On average, 6.2 ± 1.1 (mean ± SD) broods per family were necessary to obtain the required eighteen males (range: 4–8 broods).

The experiment followed a fully factorial set-up with food level (low, high) and social environment (female cue, male cue, no social cue) as the treatments. Within each family, the test males were randomly allocated to one of the six treatment groups, to a total of three full-sibs from each family in each treatment group (N = 216). The treatments started when individuals were four weeks old, when the sex of the individuals could be assessed. All the males were immature at this stage. During the course of the experiment, eighteen males died before reaching maturation, giving the final sample size N = 198. All families were represented by at least two siblings in each treatment group.

In guppies, it is reported that over subsequent litters, the brood size increases, while the individual size of the offspring decreases (1st litter: 1–4 offspring of 0.85–1.00 mg of dry weight each; 6th litter: 15–25 offspring of 0.75–0.90 mg of dry weight each; Reznick *et al.*, 2001). In our experiment, the randomization of test individuals to treatment groups most likely avoided any systematic brood effect. Fluctuations in offspring size seem to be larger after litter number eight (Reznick *et al.*, 2001); we did not include any fish from beyond litter number eight.

Fish were fed quantified amounts of newly hatched brine shrimp, *Artemia salina* (Silver Star Artemia) in the morning and, on weekdays only, liver paste in the afternoon. Feeding procedure followed that of Reznick (1982) and Reznick (1990). Amounts were measured volumetrically with a Hamilton syringe to the nearest 0.5 µl. High and low food levels were chosen to sustain two differentiated maximum growth rates, with the high level being approximately double the low level. The ration was increased every two weeks. During the first four weeks, from birth until the initiation of the treatments, all fish received the same food quantities (0–2 weeks old: 3 µl of *Artemia* and 2 µl liver paste; 2–4 weeks old: 5 µl of *Artemia* and 3 µl of liver paste). In the high food treatment, starting at the age of 4, 6, 8 and 10 weeks, food quantities were increased to respectively 7.5 µl, 10 µl, 10 µl, and 13 µl of

Artemia and 5 µl, 8 µl, 10 µl and 13 µl of liver paste. In the low food treatment, the corresponding amounts were 5 µl of *Artemia* (all ages) and 3 µl, 4 µl, 5 µl and 5 µl of liver paste. All *Artemia* volumes refer to filtered, undiluted *Artemia*.

The individual aquaria (24x14x10 cm) were divided in two sections with a transparent wall; the bigger section (16x14x10 cm) housed the test male, while the smaller one contained the social cue: a mature male, a mature female, or an empty compartment (control). The cue fish were fully mature, virgin F1 individuals, similar to those used as parents but unrelated to the test males. They were visually size-matched, avoiding using old individuals. Test males were in complete isolation (physical, chemical and visual) with each other, and they were in visual contact with the cue fish. The transparent wall was not completely watertight, but the flow was minimal between the two compartments; food could not move between the compartments (avoiding interference with the feeding treatment), and we assume that diffusion of oxygen and chemical cues was minimal too. The placement of aquaria in the climate-room was randomized. All aquaria were kept at constant temperature of 24 ºC and provided a constant air source by air-stone; they were checked for uneaten food daily and water changes took place every second week.

The test fish were measured for size and assessed for maturity every two weeks. The fish were first anaesthetised with MS-222 and then photographed from the side with a digital camera (Canon EOS 500D) and examined under the stereomicroscope to assess the maturation stage according to gonopodial development. Development of the gonopodium (modified anal fin) is correlated with the maturation of the testes and the gonadotropic zone of the pituitary (Kallman & Schreibman, 1973; Schreibman & Kallman, 1977; Greven, 2011). The initiation of maturation is signalled by the increase from nine to ten segments in the third ray of the anal fin, while maturation is completed when the fleshy hood passes beyond the tip of the gonopodium; the final number of segments can be more than 27 (Turner, 1941;

Reznick, 1990). A male can remain immature (nine segments or less) for months, but the step from nine to ten segments is invariably followed by a linear increase with time in the number of segments until it reaches maturation (Reznick, 1990). Individual size was characterized by standard length, measured from the photographs using ImageJ (Rasband, 2011; version 1.45g).

Statistical analysis

To assess whether the different treatments influenced growth trajectories we fitted a modified von Bertalanffy equation (Caillet *et al.*, 2006) to the data:

$$L_t = L_\infty - (L_\infty - L_0)*e^{-K*t}, \text{ (Eq. 1)}$$

where L_t is the length at age t, L_∞ is the asymptotic length, K is the Brody growth coefficient, and L_0 is the length at birth. The parameters L_∞, L_0 and K were estimated with a non-linear mixed model (R function 'nlmer', R Development Core Team 2011; Pinheiro *et al.*, 2011), with family as a random factor for both L_∞ and K (using fish ID as a random factor led to convergence problems). Food and social cue treatments were combined into a single six-level factor, 'treatment', due to the difficulty of including multiple factors in non-linear mixed models. Treatment as a fixed effect on K yielded the lowest AIC (Akaike information criterion) compared to the model without this effect or to the model with treatment effect on L_∞.

Probability of completion of maturation and probability of initiation of maturation were modelled with generalized linear mixed models with binomial error distribution using the "glmer" function in R (Bates *et al.*, 2011). Family and fish ID were considered random factors. The fixed-effect variables of the full model for the two-dimensional PMRN were: 1)

food, 2) length, 3) age, 4) age-squared, and included all the first-order interactions between the variables; here we follow the established nomenclature by referring the main explanatory variables (age, length) as dimensions of the PMRN; food is not treated as a dimension, as we consider it as a nuisance variable that was included in the experiment to create differential growth (e.g., Heino & Dieckmann, 2008; Grift *et al.*, 2007 Uusi-Heikkilä *et al.*, 2011). The final models were selected using AIC. Three-dimensional PMRNs had the social cue as an additional explanatory dimension, as well as first- and second-order interactions between all the fixed effects. The logistic curve for the probability of maturation is given by equation:

$$\text{logit}(p) \sim c_0 + c_1 l + c_2 a + \ldots + c_n, \text{ (Eq. 2)}$$

where $\text{logit}(p) = \log_e[p/(1-p)]$ is the logit link function, c_0 is the intercept, and c_1 to c_n are the regression parameters of the model for the different explanatory variables (length l, age a, age-squared, food, social cue, interactions, etc). To facilitate the interpretation of the model coefficients, length and age were standardized to zero mean and unity standard deviation (SD; see table 1). The PMRN midpoints (i.e., the estimated length at which the probability of maturing is 50%; also referred as L_{p50}) were used to illustrate the estimated reaction norms:

$$L_{p50} = \frac{-(c_0 + c_2 a + c_3 a^2 + \ldots + c_n)}{c_1}, \text{ (Eq. 3)}$$

where parameter estimates are from equation (2).

Results

Differences in growth trajectories

Both food and social cue had an effect on growth (Fig. 1). While neither the asymptotic average length (L_∞, estimate: 19.1 mm) nor the initial length (L_0, 6.5 mm) differed between

treatments, the rate at which that asymptote was approached (the Brody growth coefficient, K) did differ: the model with treatment effect on K was significantly better than the one without it (likelihood ratio test, χ^2 = 124.29, df = 5, $P < 0.001$, ΔAIC = 114.3). Individuals in the treatment with high food and male social cue had the highest K (0.021 d^{-1}), while those in the treatment with high food and female cue had the lowest one (0.016 d^{-1}; see table S1 in the supplementary material for all values and Fig. 1).

Two-dimensional PMRN

The two-dimensional (age- and length-based) PMRN for initiation of maturation was based on a logistic regression model with length, age, food, and food × length interaction as the explanatory variables; age-squared did not have any effect (Table 1). The variable having the strongest effect on odds of maturing was length; the length effect was stronger in the low food treatment compared to the high food one (high food odds ratio = 327, low food odds ratio = 4402 for an increase of one SD of length). Age had a comparatively weak effect on maturation (odds ratio = 1.93); this is manifested as a relatively flat PMRN (Fig. 2a). Low food decreased the probability of initiation (odds ratio = 0.24) relative to high food, but due to the stronger positive effect of length under the low food treatment, large individuals had higher odds of initiating maturation at low relative to high food (odds ratio = 3.25 for length one SD above the mean).

The two-dimensional PMRN for completion of maturation was estimated from the logistic regression model with length, age, age-squared, food, and length × food interaction as the explanatory variables (Table 1). As with initiation, increasing length always increased the odds of maturing, but more so under low food (odds ratio = 46 for 1 SD increase in length) than high food (odds ratio = 7.46); this effect was thus weaker for completion than for initiation. Age had a stronger effect on maturation than length, although the effect of age

declines at later ages because of a significant negative quadratic effect (Table 1). Individuals with low food had lower overall probability of maturing (odds ratio = 0.16) compared to high food, however, because of the stronger effect of length on maturation, large individuals at low food had higher odds of maturing than those at high food (odds ratio = 1.04 for length 1 SD above the mean; Table 1).

For both initiation and completion of maturation, food level has a statistically significant effect on the PMRN (Table 1). However, the actual effects on maturation probabilities are quite different. For initiation, the effect is subtle and of little practical significance (Fig. 2a). For completion, however, maturation probabilities, as illustrated by the PMRN midpoints, are markedly different between the food levels (Fig. 2b). At younger ages, under high food conditions the probability of maturing is lower than under low food conditions, and the opposite happens at older ages. The PMRN for initiation is thus less plastic in the response to variation in food availability than the PMRN for completion of maturation.

Three-dimensional PMRN with a social cue

The probability of initiation of maturation was significantly influenced by length, length × social cue interaction, and food × social cue × age interaction (Table S2 in the supplementary material). The odds of initiating maturation strongly increased with increasing length (odds ratio = 1587 for 1 SD increase in length for high food and no cue). The effects of other variables were weaker and mostly not statistically significant (Fig. 3a, Table S2 in the supplementary material). Food had an effect on the probability of maturing only through its interaction with age and social cue. The social environment affected the probability of initiating maturation through its interaction with length, as well as with the aforementioned interaction with age and food. Because the positive effect of length on maturation was significantly weaker for individuals with female cue (odds ratio = 86.5), maturation was

delayed for this group, manifested as a slightly elevated PMRN midpoint curve (Fig. 4a). Age had no significant main effect, but it did have a positive effect in the presence of male cue at low food, leading to negatively-sloped PMRN midpoints (Fig. 4c).

The social cue-based three-dimensional PMRNs for initiation presents little plasticity with relation to food. However, PMRNs are different for the different social environments (Fig. 4a-c). The differences between food-levels are not reduced with the incorporation of the social environment as the third dimension. In high food conditions the PMRN is superficially similar in all social environments, whereas the differences become more obvious under low food conditions.

The probability of completion of maturation depended on length, age, age-squared, food and food × length interaction, social cue and food × social cue interaction (Fig. 3b, Table S2 in the supplementary material). The odds of maturing differed between social treatments, being lower in fish with female cue than in fish without cue (odds ratio = 0.40), but not in fish with male cue (Fig. 3b); this is also seen as an elevated PMRN midpoint curve for fish with the female cue (Fig. 4d) compared to the male and no cue (Fig. 4e-f). Low food individuals had lower odds of maturing relative to high food ones (odds ratio = 0.08; Fig. 3b), and this effect depended also on the length × food interaction. The odds of maturing increased with length (odds ratio = 6.6 for 1 SD increase in length for high food), and this increase was stronger under low food conditions (odds ratio = 46). In addition, the interaction food × social cue affected maturation (see Fig. 3b).

The midpoint curves of the PMRNs for completion of maturation differ between high and low food for all the social cues. Thus, the incorporation of the social dimension did not reduce the plasticity of the PMRN with respect to the different food levels (Fig. 4d-f). The social cue

influences the PMRN midpoints only very little, with one exception: under high food, a female cue shifts the PMRN upwards, compared to no cue or with a male cue.

Discussion

This study aimed at 1) assessing the effect of different growth trajectories and unaccounted sources of plasticity in the shape of the PMRN, 2) adding a novel dimension to the PMRN, the social environment, in order to test whether it affects the maturation process independently of growth, and 3) investigating whether detecting maturation at different stages of maturation might lead to different conclusions regarding age and size thresholds of maturation or plasticity in maturation.

Plasticity in the PMRN revealed by food

Following the ideas first presented by Stearns and his co-workers (Stearns, 1983; Stearns & Crandall, 1984; Stearns & Koella, 1986), two-dimensional PMRNs have been used to account for growth-related plasticity in maturation schedules (reviewed by Heino & Dieckmann, 2008). Generally, two-dimensional PMRNs predict maturation probabilities independently of growth trajectory during the juvenile stage, as they are only affected by the endpoints of growth trajectories. However, the growth-related plasticity might not be completely accounted for because different growth trajectories can lead to the same age-size combination (Morita & Fukuwaka, 2006; Heino & Dieckmann, 2008). This seems to be the case in our results.

Growth-related plasticity could not always be controlled for with regards to the maturation schedules of male guppies. The shifting position of the PMRN showed that the PMRN did not completely account for food availability; this happened despite the food availability having

only a weak influence on growth rates. Individuals reared under low food conditions had lower probability of maturing, represented by the higher midpoint curve (Fig. 2). However, this influence depended on which stage of maturation was considered (discussed in the following section).

This result is in concordance with Uusi-Heikkilä *et al.* (2011) and Morita *et al.* (2009). Uusi-Heikkilä *et al.*'s (2011) study is similar to the present one, as they estimated PMRNs of zebrafish under different experimentally controlled food availabilities. Their results showed a diet-dependent position of the PMRN (Uusi-Heikkilä *et al.*, 2011). Thus, the position of the PMRN depended on environmental conditions, as in the present study. Morita *et al.* (2009) estimated PMRNs of white-spotted char, *Salvelinus leucomaenis*, introduced to different rivers. The PMRNs were multidimensional as different environmental factors (river width, depth, velocity, substrate, etc.) were included in the estimation. The differences in the probability of maturation were environmental, as the individuals belonged to a single source population and could be assumed to be genetically uniform (though survival between introduction and observation could have been genotype-dependent). The most important factors affecting the position of PMRN for white-spotted char were river width and fork length (Morita *et al.*, 2009).

Uusi-Heikkilä *et al.* (2011) estimated also three-dimensional PMRNs with condition, which reduced the effect of diet on the position of the PMRN: zebrafish reared under high food conditions presented lower PMRN midpoints than individuals reared under low food conditions; this difference was reduced but not absent in the condition-based three-dimensional PMRN (Uusi-Heikkilä *et al.*, 2011). In the present study weight data to calculate the standard morphometric condition index, the Fulton's condition factor, were unavailable. However, we obtained similar results when we included a crude condition index (calculated as the residuals of the fish length-height regression) in the analyses (results not shown). The

condition proxy had a positive effect on maturation, and the effects of all the other variables were reduced, but the position of the PMRN was still food-dependent.

It is generally found that good condition is associated with early maturation, as it was the case for Uusi-Heikkilä *et al.* (2011) and also for the present study. However, when maturation is scored late in the development, it can be difficult to distinguish whether good condition facilitated maturation or whether good condition reflected altered energy allocation patterns associated with maturation. Our study could partially differentiate between these two scenarios: we found a positive effect of the condition proxy on the initiation of maturation, and non-significant effect on the completion of maturation. Thus, studying completion only could bias our understanding of the role of body condition. However, these findings require further corroboration with better estimation of condition index, which was not possible in the present study.

A puzzling aspect of our results is that food availability influenced growth only marginally. Overall low food availability led to a lower Brody growth coefficient, but the difference between high and low food was on average only 0.001 d^{-1}, although this depended on social environment (discussed bellow). The feeding regimes were designed to create differences in growth, but they were based on guppies from other populations. It is possible that guppies from our study population have lower maximum growth rates, and were unable to take advantage of the higher feeding regime. Furthermore, the feeding regimes were differentiated only when the fish were four weeks old (when it was possible to sex them). The criterion to start the feeding treatment, the ability to sex the fish, was the same as used by Reznick (1990) and Auer (2010). However, an important difference between our guppies and those of Reznick (1990) and Auer (2010) is that our fish developed much slower: Reznick (1990) and Auer (2010) could sex their guppies at the age of two weeks. Thus, our low food treatment

may not have been as restrictive as it was meant to be. We can only postulate that there were differences in energy reserves (e.g., lipids).

Yet the feeding treatment influenced maturation. It seems that the low food treatment was at a level where it was possible for the fish to somehow compensate for reduced food intake in terms of growth but not maturation. This is in agreement with the presence of different thresholds that should be passed for maturation to take place. These thresholds are associated with an energy state and the rate to obtain that state; maturation takes place when this physiological threshold is exceeded (Thorpe *et al.*, 1998; Thorpe, 2007). Our low food treatment seemed to allow for growth rate similar to one under high food, but not to reach the maturation threshold at the same speed.

Differences between maturation stages

We considered two maturation stages in male guppies, initiation and completion of maturation. This is the first study to estimate PMRNs for two different stages of maturation from the same individual fish. We hypothesized that studying different maturation stages could bring more insights to the maturation schedule and maturation decisions.

Maturation stages were determined by visually assessing the development of the gonopodium. This is correlated with the development of the gonadotrophic zone in the adenohypophysis and the maturation of the testis (Kallman & Schreibman, 1973; Schreibman & Kallman, 1977; Greven, 2001). In other poeciliids, the initiation of gonopodium development correlates with initial enlargement of the testis, proliferation of spermagonia and possibly spermatocytes (van den Hurk, 1974; Koya *et al.*, 2003) and increase activity in the stroma of the testis of enzymes responsible for the formation of androgens and estrogens (Schreibman *et al.*, 1982). At the last stage of gonopodium development, several layers of spermagonial cysts, sperm cells, and

developed testicular ducts with enzyme activity and spermatozeugmata (sperm bundles) are present (Schreibman *et al.*, 1982; Koya *et al.*, 2003). Kallman & Schreibman (1973) proposed that the differentiation of gonadotropic zone (genetically determined) in the adenohypophysis leads to testis maturation, which in turn results in gonopodial metamorphosis and decrease in growth rate.

It has been suggested that the PMRNs estimated from field data do not represent maturation schedules properly, as they do not reflect the maturation state but the result of a continuous gonadal development (Wright, 2007). The field-based estimation of PMRN may have extra sources of variability, as changes in energy status around the time of maturation decisions cannot be accounted for (Wright, 2007). Probably the initiation of maturation stage is closer to the maturation decision than the completion and thus, the PMRN for initiation is a better representation of the maturation schedule in male guppies. Other studies also refer to the decision time as the moment when animals commit to vitellogenesis or spermatogenesis, as these are the most energy demanding phases of gonad development (lesser sandeels, *Ammodytes marinus*, Boulcott & Wright, 2008; haddock, *Melanogrammus aeglefinus***,** Tobin *et al.*, 2010). In the case of guppies, an individual can remain immature for months, but once the maturation is initiated the development is not stopped until maturation is completed (Reznick, 1990). Thus, we believe that the moment at initiation of maturation is close to the maturation decision in guppies.

The effect of food on PMRN was different when it was estimated for initiation and for completion of maturation. Overall, the probability of initiation was not as much affected by food as the probability of completion of maturation was: the effect of food on the PMRN for initiation, while statistically significant, was subtle and of little practical significance compared to completion of maturation. Furthermore, the PMRN for initiation displayed weaker age dependence compared to the PMRN for completion, i.e., they were close to

horizontal. Interestingly, most PMRNs estimated with field data (corresponding to completion of maturation) have a significant negative slope (Heino & Dieckmann, 2008; but see Baulier *et al.*, 2012). Our results suggest that these negative slopes may be caused by scoring maturation long after decision to initiate maturation, and that the importance of age in maturation decision has been exaggerated. Similar results have been found in recent estimations of PMRN from different maturation stages in *Daphnia*. Harney *et al.* (2013) showed how PMRNs estimated for later maturation stages (primiparity) were more affected by growth plasticity, compared to those estimated for early stages of maturation (oocyte formation).

One reason for why there is a difference between completion and initiation of maturation might be the differential allocation of resources into growth and maturation at the different stages. Reznick (1990) showed that male guppies reached initiation and complete maturation at different age and weight depending on food availability, but food did not affect the time interval between initiation and maturation. Rapid-growing fish initiated and completed maturation at larger sizes and earlier ages (Reznick, 1990). Similar results are found in this study. In addition, the difference in size between fish of different food regimes was bigger after maturation than at initiation (Reznick, 1990).

Once initiation has taken place there is probably more room for flexibility in how to allocate resources than before initiation. Arisaka & Hamai (1975) observed in guppies a greater variation in body and gonopodium length between initiation and completion of maturation, relative to before initiation and after maturation. They suggested that growth in this interval was very much influenced by the variation in maturation velocity or intensity, due to variable physiological conditions in the individuals. This could be why completion of maturation was more affected by food than initiation of maturation in our study. However, we are unable to conclude what is the driver of the differences between initiation and completion. It has been

suggested that in order for maturation to initiate, the organisms require certain amount of resources, and that such threshold may be insensitive to environmental factors (Berner & Blanckenhorn, 2007). Nevertheless, knowledge on the physiological processes triggered by this threshold and how they are actually affected by the environment and growth rates is scarce (Berner & Blanckenhorn, 2007). The present study and the one by Harney *et al.* (2013) suggest that PMRNs based early signs of maturation, rather than later signs, more accurately reflect factors that are involved in the maturation decision.

The social dimension in maturation

We also assessed how the addition of an extra explanatory dimension, the social environment, shapes the PMRN. The inclusion of such explanatory variables is expected to reduce the plastic effects that remain after accounting for growth-related plasticity. The most common additional dimension in field-based PMRNs is condition, in terms of the Fulton condition index (Grift *et al.*, 2007; Mollet *et al.*, 2007; Vainikka *et al.*, 2009), although condition as hepatosomatic index (Baulier *et al.*, 2006; Olsen *et al.*, 2008), growth (Morita & Fukuwaka, 2006), weight (Grift *et al.*, 2007; Mollet *et al.*, 2007), temperature trends (Kraak, 2007), and habitat characteristics (Morita *et al.*, 2009) have also been included.

The inclusion of the social environment was encouraged by earlier studies demonstrating social influences on maturation and because the social environment is also a factor that is influenced by fishing (Rowe & Hutchings, 2003; Kraak, 2007). Fishing might alter the operational sex ratio if one sex is more vulnerable to fishing, due to differential behaviour, habitat use, growth rates or size (Rowe & Hutchings, 2003). This would be the case in guppies, but also in important fishery species. For cod, *Gadus morhua* it has been estimated that an equal sex ratio shifted towards male-biased sex ratio with increasing fishing mortality (Jakobsen & Ajiad, 1999). Male graysby, *Cephalopholis cruentata* (Côte, 2003) and male

plaice, *Pleuronectes platessa* (Solmundsson *et al.*, 2003) are more vulnerable to fishing due to their higher activity compared to females, thus fishing might alter the sex ratio also in these species.

The social environment in which the males were reared affected the probability of maturing in the present study. This effect was similar for initiation and completion of maturation and could not be accounted for by the other explanatory variables of the PMRN. However, the social effect was highly context-dependent, defying simple generalizations, and did not meet our prior expectations. Moreover, the social effect was not very marked: the differences in the PMRN midpoint curves were larger between the feeding regimes than among the social environments, and the effect of feeding regime, or that of age or length, was not qualitatively altered by the social context.

It is generally accepted that maturation can be inhibited by the presence of large individuals of the same sex, but favoured by the presence of individuals of the opposite sex. Dominant and/or large individuals initiate maturation and inhibit the maturation of subordinate/small individuals, who continue growing. There are many examples of this phenomenon in fish, for both females inhibiting other females (Jones & Thompson, 1980) and males inhibiting other males (Danylchuk & Tonn, 2001, Aday *et al.*, 2003). Slower development of individuals in the presence of adults has been observed in guppies (Magellan & Magurran, 2009) and other poeciliids such as platyfish and swordtails (Borowsky, 1978; Bushmann & Burns, 1994). In the present study the presence of female cues resulted in delayed maturation, while the presence of males did not affect maturation of males. This lack of inhibition caused by male cues may be due to lack of direct, physical interaction and transmission of chemical cues between cue and test individuals in our experimental setting. In bluegills, *Lepomis macrochirus*, the presence of chemical cues, rather than mere visual cues, is responsible for the inhibition of maturation between males (Aday *et al.*, 2003).

Female cue might have resulted in delayed maturation because the test males used their energy in courtship displays, rather than growth and maturation. Bisazza *et al.* (1996) and Evans *et al.* (2002) have shown that male guppies and other poeciliids perform courtship behaviour, which is energetically costly (Abrahams, 1993; Jordan & Brooks, 2010), even though they are not completely mature and not yet capable of inseminating the females. Although male guppies might also perform courtship to other males (Field & Waite, 2004), this is unlikely to have happened in our experiment because only adult males are known to court other males (Field & Waite 2004). Even if male–male courting might have happened, it is probably less intense and costly than courting females. Moreover, male guppies reduce their feeding rate when reared in mixed-sex populations compared to single-sex ones (Griffiths, 1996), which is in accordance with the marginally reduced growth in males with female cue, compared to males with male cue (Brody growth coefficient was on average 0.002 d^{-1} lower in males reared with female cue than those reared with male cue). Abrahams (1993) showed that increased food availability resulted in lower food consumption and higher courtship behaviour in male guppies, probably because risk of starvation becomes low in such conditions, thus allowing males to prioritize other activities. This might explain why the test males of the present study showed the lowest growth rate and lower probability of maturing when reared with a female cue and high food availability, compared to the other treatments.

Implications for the study of fisheries-induced evolution

PMRNs are commonly used in the study of evolutionary effects of fishing. This approach is so far the best method available for inferring the possible presence of genetic changes in maturation when only phenotypic field data are available, a situation which will likely persist for a while because of the lack of historic genetic data on fish stocks and difficulty of running suitable experiments. The PMRN method allows controlling certain plastic effects; the

unaccounted variability is due to unaccounted plasticity, genetic differences, or a mixture of both. The better the plastic variability can be controlled, the more likely the residual component represents genetic differences. Thus, it is important to include factors that can account for extra sources of plasticity in maturation, as also shown by Morita & Fukuwaka (2006) and Uusi-Heikkilä *et al.* (2011). The PMRN allows for the inclusion of any additional factors that would account for plastic effects, but the limiting factor in doing so has been the lack of suitable data (Dieckmann & Heino, 2007). In this context, explanatory variables with similar trends to the trends in maturation are more critical than other variables. Therefore, we join Kraak (2007) and encourage searching and identifying these factors in order to improve the assessment evolutionary changes in maturation. Our study and those by Morita *et al.* (2009) and Uusi-Heikkilä *et al.* (2011) are useful in pointing out which factors might be important. Our results show that the effect of social environment explained variability not accounted for by the other factors. However, this effect was context-dependent and not very strong. Thus, shifts in social environment do not appear to be important drivers of variability in maturation in guppies, although future research will have to show whether this result applies more generally.

In our study the PMRN for initiation of maturation was more robust compared to completion. Thus, using traits that signal the initiation of the maturation (e.g., physiological traits involved in the maturation process), rather than its end, might make the assessment of maturation schedules more accurate, as also shown by Harney *et al.* (2013). In addition, our results show that estimating PMRN with completion of maturation led to a greater effect of age in maturation, irrespective of whether the social environment was considered. Therefore, estimating PMRNs at late stages of maturation could lead to an overestimation of the importance of age, and underestimation of importance of size and growth, relative to PMRNs estimated at early stages of maturation; similar conclusions were drawn by Harney *et al.*

(2013). However, using early stages in the maturation process may not be an option available for long-term studies on fisheries-induced evolution that rely on existing data collection programmes. Fisheries surveys are often conducted near the spawning time when identifying an individual's maturity stage is the easiest. Detecting maturation earlier may require more labour-intensive histological techniques, which severely limit available sample sizes (e.g., Saborido-Rey & Junquera 1998). These limitations notwithstanding, we recommend using individuals at early stages of maturation whenever possible.

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Tables

Table 1. Models for the two-dimensional PMRN for initiation and completion of maturation with estimated coefficients, standard errors, *z* and *P* values, and degrees of freedom (d.f.). The model is a logistic regression and the coefficients express effects in log(odds). To facilitate comparisons, age and length have been standardized to zero mean and unity standard deviation. Mean and SD for length and age: 13.26±3.56 mm and 51.2±35.8 days, respectively.

	Variable	Estimate	SE	*z*	d.f.	*P*
Initiation of maturation	Intercept (Food: high)	-3.50	0.45		1	
	Length	5.79	0.69	8.35	1	<0.001
	Age	0.66	0.33	1.99	1	0.04
	Food: low	-1.42	0.65	-2.17	1	0.02
	Food: low × Length	2.60	1.13	2.29	1	0.02
Completion of maturation	Intercept (Food: high)	-5.21	0.52		1	
	Length	2.01	0.46	4.30	1	<0.001
	Age	3.37	0.48	6.95	1	<0.001
	Age^2	-0.66	0.14	-4.43	1	<0.001
	Food: low	-1.78	0.73	-2.44	1	0.01
	Food: low × Length	1.82	0.76	2.39	1	0.01

Figure legends

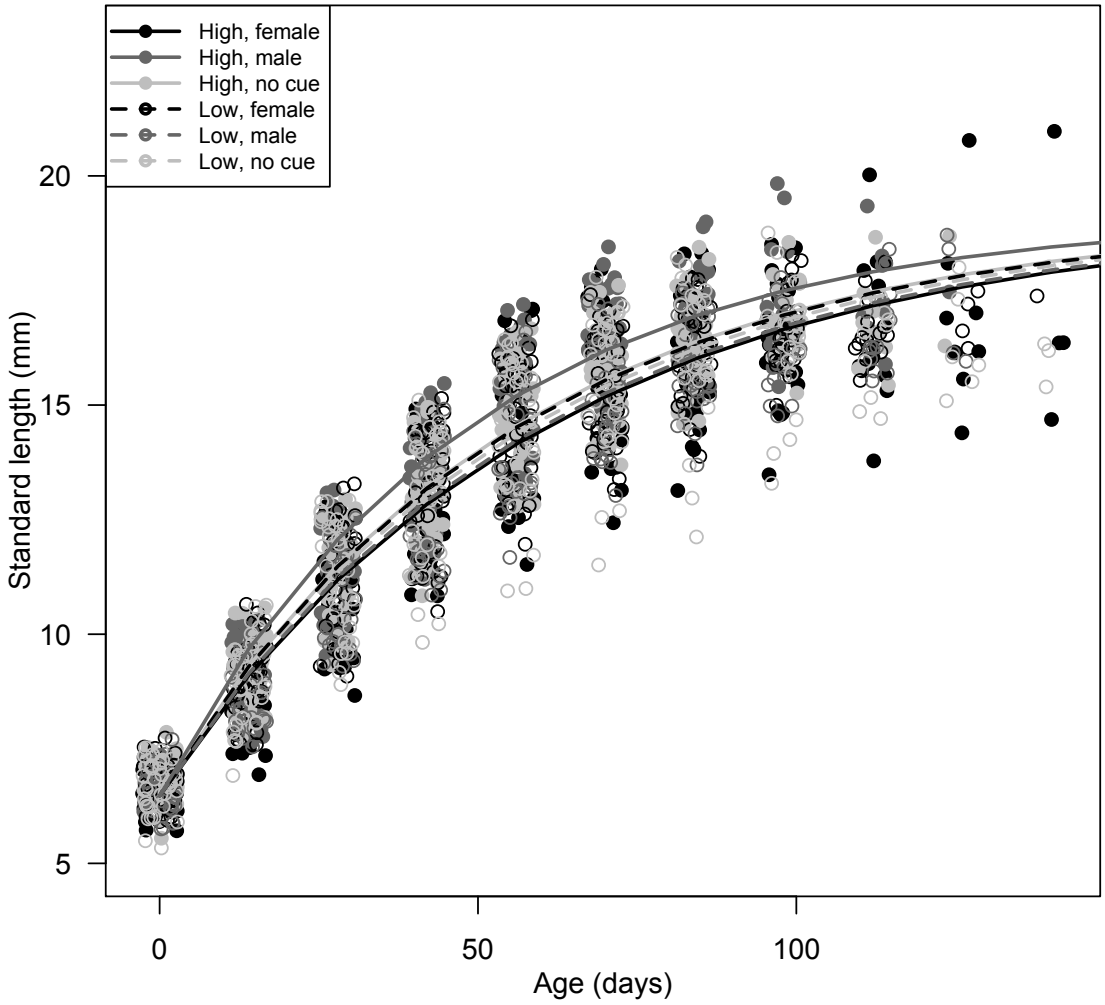

Figure 1: Von Bertalanffy growth trajectories for high (solid line) and low food (dashed line) and female (black), male (dark grey) and no social cue (pale grey) treatments estimated from non-linear mixed effect model. Open and close dots represent the observed growth trajectories for high and low food, respectively, and colours represent social treatment as the lines. The parameter values are given in table S1.

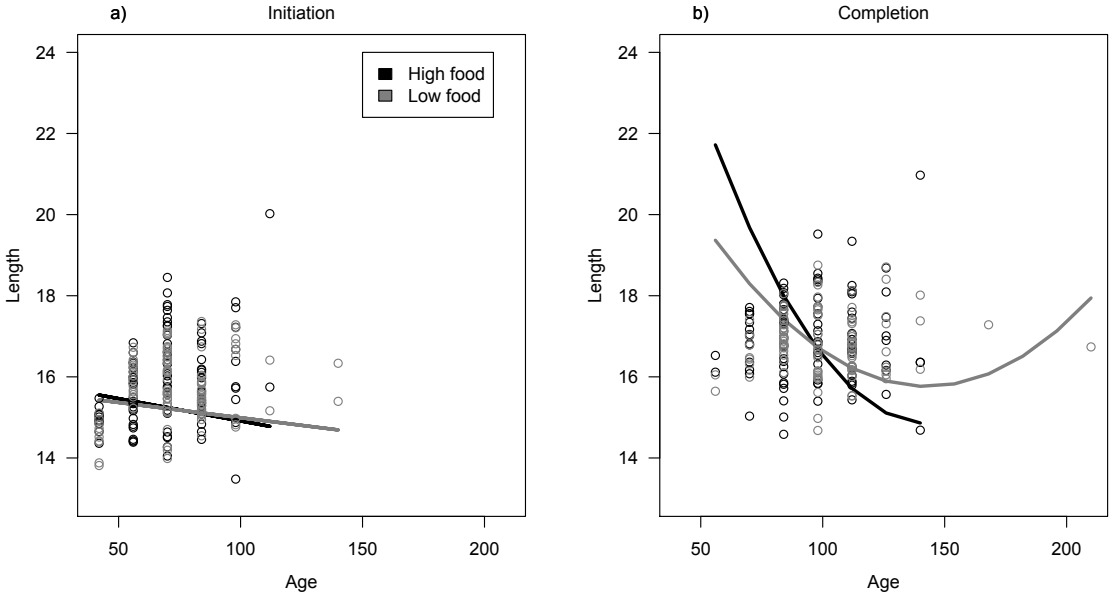

Figure 2: Two-dimensional PMRN for completion a) and initiation b) of maturation. The length and age-based PMRN are represented by the midpoints (length with 50% maturation probability, L_{p50}) at high (black line) and low (grey line) food conditions. Black and grey dots represent the observed lengths and ages at maturation for high and low food, respectively.

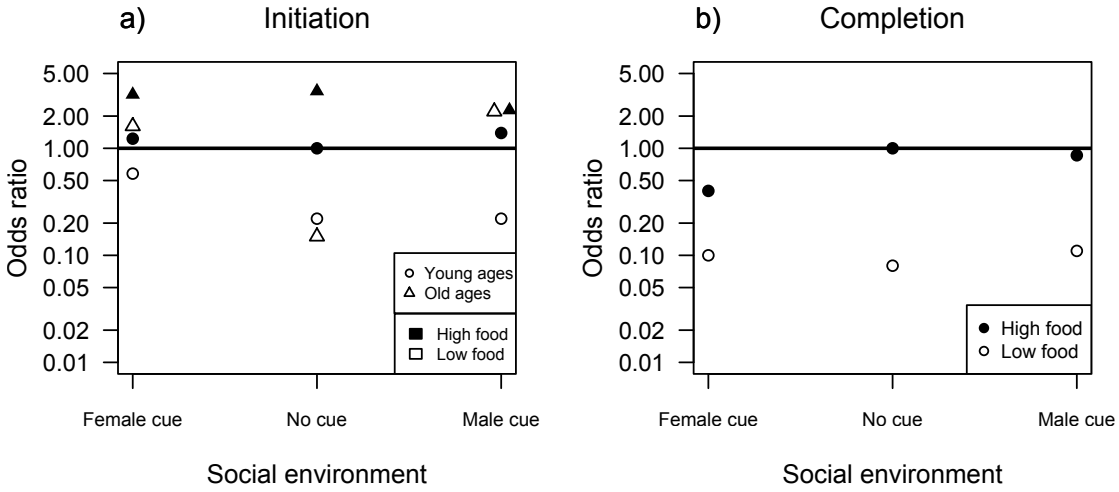

Figure 3. Effect sizes of the social cue and food level illustrated as odds ratios for three-dimensional PMRNs with social cue for initiation and completion of maturation (left and right panels, respectively). The odds ratios are expressed relative to the reference case (odds = 1, thick horizontal line), which is the treatment group with high food and no social cue, and age and length equal to their respective mean values. For the initiation, there was a significant interaction between age and social cue. The effect of cue is therefore shown for 'young' (age equal to the mean age, 51 days) and 'old' (mean age + 1 SD, i.e., 87 days) fish separately. Closed and open symbols represent low food diet and high food, respectively, while dots and triangles (initiation only) are used to separate young and old age, respectively (open and closed triangles for male cue have been jittered for representation). See text for details and the supplementary material for actual estimate values.

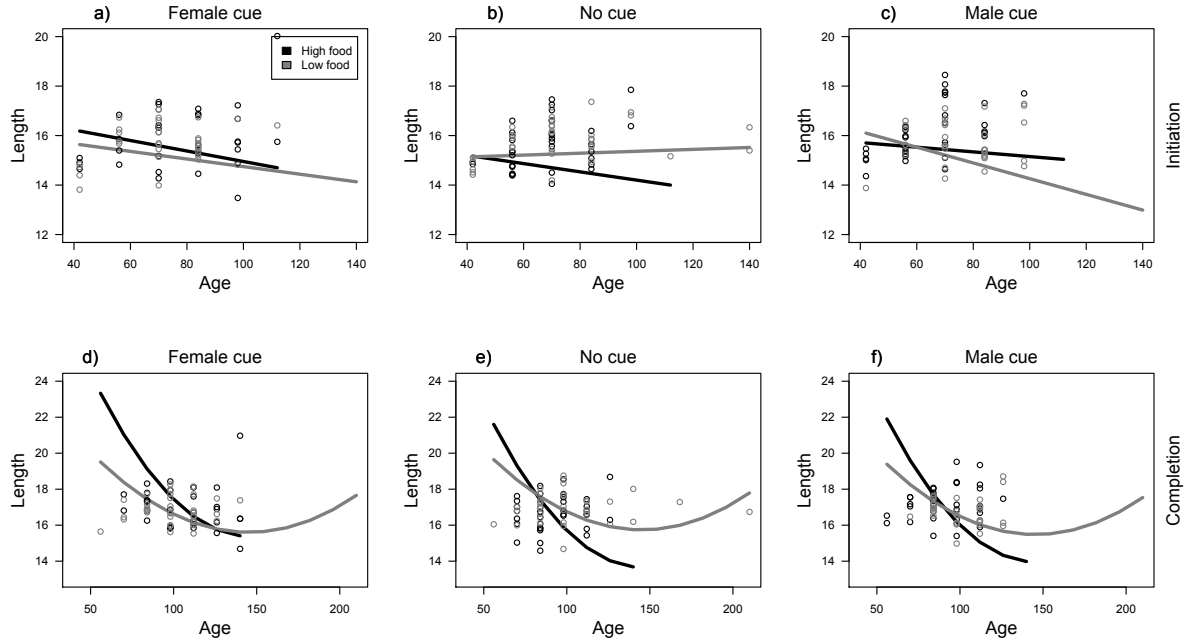

Figure 4: Three-dimensional PMRN with social cue for initiation (a-c) and completion of maturation (d-f) and each social environment, female cue (a and d), no cue (b and e), and male cue (c and f). The length, age and social cue-based PMRN are represented by the midpoints (length with 50% maturation probability, L_{p50}) at high (black line) and low (grey line) food conditions. Black and grey dots represent the observed lengths and ages at maturation for high and low food, respectively.

Supplementary Material

Table S1. Von Bertalanffy growth model values for average asymptotic length, L_∞, Brody growth coefficient, K, and length at birth, L_0, estimated from the non-linear mixed effect model for the different treatments of food and social cue.

Treatment	L_∞ (mm)	K (d^{-1})	L_0 (mm)
High and no cue	19.1	0.018	6.5
Low and no cue	19.1	0.017	6.5
High and female	19.1	0.016	6.5
Low and female	19.1	0.018	6.5
High and male	19.1	0.021	6.5
Low and male	19.1	0.017	6.5

Table S2. Models for the three-dimensional PMRN with social cue for initiation and completion of maturation with estimated coefficients, standard errors, *z* and *P* values, and degrees of freedom (d.f.). Mean and SD for length, 13.26±3.56 mm and age 51.2±35.8 days

	Variable	Estimate	SE	*z*	d.f.	*P*
Initiation of maturation	Intercept (Food: high; Cue: none)	-3.61	0.67		1	
	Length	7.37	1.32	5.55	1	<0.001
	Age	1.23	1.02	1.2	1	0.22
	Cue: female	0.21	0.81	0.26	1	0.79
	Cue: male	0.33	0.82	0.4	1	0.68
	Food: low	-1.5	0.78	-1.91	1	0.055
	Cue: female*food:low	0.75	0.85	0.88	1	0.37
	Cue: male*food:low	-0.32	0.92	-0.34	1	0.72
	Food: low*length	2.18	1.12	1.93	1	0.052
	Cue: female*length	-2.91	1.42	-2.04	1	0.04
	Cue: male*length	-2.38	1.51	-1.57	1	0.11
	Food:low*age	-1.6	1.1	-1.44	1	0.14
	Cue: female*age	-0.28	1.16	-0.24	1	0.8
	Cue: male*age	-0.75	1.33	-0.56	1	0.57
	Cue: female* food:low*age	1.67	1.45	1.15	1	0.24
	Cue: male* food:low*age	3.41	1.7	2	1	0.04

Table S2 continuation

Completion of maturation	Intercept (Food: high; Cue: none)	-4.88	0.53		1	
	Length	1.89	0.49	3.85	1	<0.001
	Age	3.55	0.5	7.05	1	<0.001
	Age^2	-0.67	0.15	-4.43	1	<0.001
	Cue: female	-0.91	0.34	-2.61	1	<0.01
	Cue: male	-0.15	0.35	-0.44	1	0.65
	Food: low	-2.45	0.8	-3.04	1	<0.01
	Cue: female*food:low	1.06	0.50	2.09	1	0.03
	Cue: male*food:low	0.43	0.51	0.84	1	0.39
	Food: low*length	1.95	0.78	2.5	1	0.01