doi:10.1111/j.1558-5646.2012.01758.x

HOW TO MEASURE MATURATION: A COMPARISON OF PROBABILISTIC METHODS USED TO TEST FOR GENOTYPIC VARIATION AND PLASTICITY IN THE DECISION TO MATURE

Ewan Harney,[1] Tom J. M. Van Dooren,[2] Steve Paterson,[1] and Stewart J. Plaistow[1,3]

[1]*Institute of Integrative Biology, University of Liverpool, Biosciences Building, Crown Street, Liverpool, L69 7ZB, United Kingdom*

[2]*UMR7625 Laboratory of Ecology and Evolution, École Normale Supérieure, Rue d'Ulm 46, 75005 Paris, France*

[3]*E-mail: s.plaistow@liv.ac.uk*

Received March 20, 2012
Accepted July 13, 2012
Data Archived: Dryad doi:10.5061/dryad.r13f5

Maturation is a developmental trait that plays a key role in shaping organisms' life-history. However, progress in understanding how maturation phenotypes evolve has been held back by confusion over how best to model maturation decisions and a lack of studies comparing genotypic variation in maturation. Here, we fitted probabilistic maturation reaction norms (PMRNs) to data collected from five clones of *Daphnia magna* and five of *Daphnia pulex* collected from within and between different populations. We directly compared the utility of modeling approaches that assume maturation to be a process with an instantaneous rate with those that do not by fitting maturation rate and logistic regression models, and emphasize similarities and differences between them. Our results demonstrate that in *Daphnia*, PMRNs using a logistic regression approach were simpler to use and provided a better fit to the data. The decision to mature was plastic across a range of growth trajectories and dependent upon both body size and age. However, the age effect was stronger in *D. magna* than *D. pulex* and varied considerably between clones. Our results support the idea that maturation thresholds can evolve but also suggest that the notion of a threshold based on a single fixed state is an oversimplification that underestimates the adaptability of these important traits.

KEY WORDS: **Age and size at maturity, *Daphnia*, life-history evolution, maturation thresholds, phenotypic plasticity, probabilistic maturation reaction norms.**

The age and size at which an organism matures are key life-history traits influencing fitness (Bernardo 1993; Roff 2001; Berner and Blanckenhorn 2007). Age and size upon reaching maturity are remarkably plastic, and maturation reaction norms are commonly used to describe the response of these traits to environmental variation (Stearns and Koella 1986; Perrin and Rubin 1990; Berrigan and Charnov 1994; Olsen et al. 2004; Plaistow et al. 2004; Beckerman et al. 2010). There is also increasing awareness of the importance of underlying ontogenetic processes in shaping these reaction norms (Reznick 1990; Johnson and Porter 2001; Wolf et al. 2001; West-Eberhard 2003; Berner and Blanckenhorn 2007). Maturation is rarely a simple transition from juvenile to adult, but rather a process consisting of a number of co-ordinated and potentially heritable endocrinological and neurophysiological changes, controlling the allocation of resources to growth, maintenance, and reproductive function (Bernardo 1993; Stern and Emlen 1999; Nijhout 2003). The limited studies carried out to date (Boorse and Denver 2004; Davidowitz and Nijhout 2004;

EVOLUTION
INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

Mirth and Riddiford 2007; Nijhout 2008) suggest that understanding the proximate causes of maturation is essential to explaining variation in the age and size at which individuals achieve maturity (Marshall and Browman 2007).

Wilbur and Collins (1973) first suggested that individuals must achieve a minimum size (a size threshold) before they are able to initiate ontogenetic transitions. Since then, evidence that organisms must reach a minimal size or state before maturing or metamorphosing has been found in biennial plants (Werner 1975; Klinkhamer et al. 1987; Wesselingh and Klinkhamer 1996), crustaceans (Ebert 1992, 1994), acarids (Plaistow et al. 2004), insects (Nijhout and Williams 1974; Bradshaw and Johnson 1995; Davidowitz et al. 2003; Etilé and Despland 2008), fish (Policansky 1983; Reznick 1990), and amphibians (Travis 1984; Denver 1997; Morey and Reznick 2000), suggesting that these thresholds are ubiquitous. Studies demonstrating that thresholds can vary between populations (McKenzie et al. 1983; De Moed et al. 1999; Piché et al. 2008; Skilbrei and Heino 2011) and closely related species (Morey and Reznick 2000) further suggest that variation in the position or severity of thresholds is important in shaping the evolution of reaction norms for age and size upon reaching maturity. However, sufficient knowledge of how underlying ontogenetic processes are translated into observed plasticity is not usually available and our understanding of the evolution of age and size upon reaching maturity relies heavily on more phenomenological descriptions of patterns (although for an exception refer to work carried out on the tobacco hornworm moth *Manduca sexta*: Nijhout 2003; Davidowitz and Nijhout 2004; Nijhout et al. 2010) with determinants of the onset of maturation more often assumed than tested for (Ebert 1994; Plaistow et al. 2004; Etilé and Despland 2008; Kuparinen et al. 2008). Quantification of determinants across individuals within a population, rather than at the level of the genotype (e.g. Klinkhamer et al. 1987; De Moed et al. 1999; Morey and Reznick 2000; Engelhard and Heino 2004; Olsen et al. 2004; Mollet et al. 2007; Etilé and Despland 2008) is defensible when data per genotype are difficult to collect (Dieckmann and Heino 2007), but limits our ability to explore their evolutionary potential (Berner and Blanckenhorn 2007; but see Wesselingh and de Jong 1995; Wesselingh and Klinkhamer 1996). Finally, it is still unclear how genetic variation and environmental sensitivity in maturation processes should be quantified and qualified (Van Dooren et al. 2005; Dieckmann and Heino 2007; Kraak 2007; Kuparinen et al. 2008; Uusi-Heikkilä et al. 2011). Adopting the correct methodology is likely to be important, for example, when attempting to disentangle phenotypic plasticity from genetic adaptation to harvesting of fish stocks (Rijnsdorp 1993; Grift et al. 2003; Engelhard and Heino 2004; Ernande et al. 2004; Olsen et al. 2004).

The optimality models first used to study reaction norms for age and size at maturity (Stearns and Koella 1986; Berrigan and Koella 1994; Sibly and Atkinson 1994) assumed that maturation occurs as a deterministic process but in reality the timing of maturation may be influenced by many factors, some of which will invariably be stochastic (Bernardo 1993) and that affect the likelihood of maturing at a given age and size. Therefore, individuals with comparable juvenile growth trajectories may still differ in the size and age at which they mature (Ebert 1991; Morita and Morita 2002). To deal with stochasticity in maturation schedules, the response variable becomes a probability of maturing (Heino et al. 2002; Dieckmann and Heino 2007). The concept of probabilistic maturation reaction norms (PMRNs) based on logistic regression, a class of generalized linear model (GLM), was developed by Heino et al. (2002) to describe the probability of maturing as a function of age and size. PMRNs are extensively used to model data from fish stocks with time as a discrete variable, and the probability of maturing at a given size-at-age is assessed for each age class (Grift et al. 2003; Engelhard and Heino 2004; Olsen et al. 2004). However, there is increasing awareness that factors other than age and size may influence the decision to mature, including recent growth history (Morita and Fukuwaka 2006), condition (Mollet et al. 2007; Uusi-Heikkilä et al. 2011), or other physiological features (Van Dooren et al. 2005). Furthermore, because the exact age or size at which an individual matures during the interval between observations is often not observed (interval censoring), this approach to PMRNs may be problematic if individuals maturing at unobserved ages within a given time interval are pooled and given the same age at maturity. Although complementary methodologies based on demographic data have been devised to bypass this problem (Barot et al. 2004) and produce results comparable to the traditional PMRN concept (Pérez-Rodríguez et al. 2009), they may still suffer from bias when observation intervals vary in length and therefore in the risk of an individual maturing (Van Dooren et al. 2005). One solution to overcoming these biases in interval-censored data is to use time-to-event analysis, focussing on instantaneous rates of maturing (Van Dooren et al. 2005; Kuparinen et al. 2008). Alternatively, logistic regression can be modified to represent such maturation rate models (Lindsey and Ryan 1998; Collett 2003), notably by including an "offset" that corrects for interval length variation, although this approach has yet to be applied to studies of PMRNs.

If maturation events are stochastic processes, then time-to-event analysis appears to be a natural candidate to describe them. This approach is appealing because it focuses on conditional probabilities, that is, the probability of maturing given that it has yet to happen. Specifically, maturation is modeled as a process, determined by an instantaneous rate of maturing, which can depend on the developmental histories of individuals. A maturation status is obtained when the rate of maturation is integrated over an appropriate time scale. This may be the time interval between observations but in some cases it is advantageous to integrate over

a time scale other than time/age if it characterizes the operational history of the maturation process better (Duchesne and Lawless 2000). The instantaneous rate of maturing can also depend on a number of maturation determinants, including age and/or size, as well as other explanatory variables. The fit of different maturation rates and time scales can be compared through hypothesis testing rather than being assumed (Van Dooren et al. 2005), such that a comparison of different types of integration can give evidence of certain maturation mechanisms. Although "rate models" have been successfully used to model the maturation process (Van Dooren et al. 2005; Kuparinen et al. 2008), they can be time-consuming and difficult to fit (Van Dooren et al. 2005) unless numerous assumptions about the underlying maturation rate and time scale are made such that standard approaches of survival analysis can be applied (Kuparinen et al. 2008). Approximations of rate models through the use of standard GLMs with an offset term are considerably easier to fit, and still allow the importance of different rate effects (covariates in a standard GLM) and time scales (the offset) to be determined. However, they necessarily require certain assumptions to be made about maturation rates, including constancy of maturation rates within growth intervals. To our knowledge, a comparison of alternative time scales for the maturation process using GLMs has not yet been carried out.

Another possibility to consider is that probabilistic maturation processes do not operate continually with a certain rate. For example, when the maturation decision is taken within a certain time window of fixed length, a "sensitive period" at a molt for example, it makes no sense to integrate rates over the entire length of time between observations. In this case, and when the intervals between observations are longer than the maturation time window, a GLM without an offset should fit the data better, and the model should not be interpreted as representing a rate. Maturation is then better understood as a probabilistic switch that can be flipped at certain instances. When the dependence of the maturation rate on a determinant rises from zero to a very high value (resembling a threshold), the switch process may even be considered nonprobabilistic.

Models for probabilistic reaction norms can thus either represent a process with a rate or not. The second possibility can be modeled using GLMs without offset, whereas rate models can either be fitted using different methods of survival analysis (Kuparinen et al. 2008), integrated parametric maturation rate models (Van Dooren et al. 2005), or using GLMs with an offset. Comparison of deviances and Akaike information criteria (AIC) values can reveal which alternative explains the data best; yet, no study has compared the applicability of the different approaches to modeling the maturation process, or tested the validity of the assumptions made using a GLM approach. Here, we address this problem by comparing fits of maturation rate models and GLMs with and without offsets for maturation data collected from five clones of *Daphnia magna* Straus and five of *D. pulex* Leydig. We did not apply other methods of survival analysis suggested by Kuparinen et al. (2008), because we wanted to compare alternative time scales.

In *Daphnia*, the importance of size as a maturation determinant or status has been demonstrated previously (Green 1956; Lynch 1989; Ebert 1991), and descriptive models incorporating a size threshold have been used to explain variation in age and size upon reaching maturity in *D. magna* (Ebert 1992, 1994). Due to its parthenogenetic reproduction, *Daphnia* represents a particularly useful organism for the study of maturation reaction norms, as phenotypic effects of environmental variation can be investigated in genotypically identical individuals. The full extent of plasticity can thus be revealed and comparisons between genotypes (clones) can be drawn. However, the effect of threshold variation on maturation reaction norms has only previously been carried out for a maximum of two clones from the same population (Ebert 1994). Moreover, the existence of an exclusively size-dependent threshold was assumed rather than being explicitly tested for, leaving the role of age in the maturation process unclear (Morita and Fukuwaka 2006). As well as comparing the fit of maturation rate models and GLMs with and without offsets for *Daphnia* maturation data, we also investigate the roles that age and size play in shaping their PMRNs and explicitly determine whether these roles vary across a range of individual growth rates, and whether maturation differs between the two species. Finally, we examine clonal variation in PMRNs within each species and discuss the implications this may have for the evolution of age and size upon reaching maturity.

Material and Methods

EXPERIMENTAL ANIMALS

Five laboratory clones of both *D. magna* and *D. pulex* were used in this study. Clones originated from a variety of geographic locations across Europe. *Daphnia magna* clone DKN 1–3 came from Kniphagen, Ostholstein, Germany (54°10′36″N, 10°48′24″E); clone Ness1 from Ness, Cheshire, UK (53°16′16″N, 3°2′47″W); clone H01 from Bogarzo-to, Kikungsagi-nemzeti park, Hungary (46°48′N, 19°08′E); and clones B5 and B7 both originated from Weston Park, Sheffield, UK (53°38′20″N, 1°49′07″W). *Daphnia pulex* clones Cyril, Chardonnay, and Carlos originated from Crabtree pond, Sheffield, UK (53°24′17″N, 1°27′25″W), whereas Boris came from another pond in Sheffield, UK (53°24′18″N, 1°27′27″W). Bierbeek was collected from Bierbeek, Belgium (50°49′60″N, 4°46′0″E). All clones were cultured and experiments were carried out at 21 ± 1°C with a 14:10 light:dark photoperiod. *Daphnia* were maintained individually in 150 mL of hard artificial pond water media (OECD 1984) enriched with a standard organic extract (Baird et al. 1989). *Daphnia* were fed

Chlorella vulgaris Beijerinck (quantified by haemocytometer) on a daily basis and media was totally replaced every other day. Clones were acclimated for a minimum of three generations under ad libitum food rations of 200 cells/μl per day. Experimental animals were obtained from the third clutch.

EXPERIMENTAL DESIGN

For each clone, 64–80 neonates were isolated from three to five mothers (from the same maternal cohort). These were randomly assigned to one of the following eight food rations: for *D. magna* 200, 133, 89, 59, 40, 26, 18, and 12 cells/μl, and for *D. pulex* 89, 59, 40, 26, 18, 12, 8, and 5 cells/μl. Rations differed between species because *D. pulex* is known to have a lower incipient limiting concentration of food (Porter et al. 1982), and a preliminary study suggested that, prior to reproduction, this limit occurred below 89 cells/μl (E. Harney, unpubl. data). This variation in ration generates a wide variety of growth trajectories and resultant ages and sizes at the onset of maturity. All individuals were checked every day and photographed after molting for all instars up to primiparity (deposition of eggs in the brood chamber). Body size was estimated as the distance from the top of the head to the base of the tail spine and measured from photos using the image analysis software ImageJ (Rasband 1997). Experiments were staggered over a 16-week period due to the amount of work involved in conditioning and assaying clones.

MATURATION INDICATORS IN *DAPHNIA*

Daphnia are not constrained by a fixed number of juvenile instars (Green 1956), but once the maturation process is initiated, they commonly achieve maturity within three instars (Bradley et al. 1991). In the first of these instars, nurse cells begin to differentiate into oocytes. The first clearly visible sign of maturation is during the subsequent instar when oocytes are provisioned with yolk, resulting in the enlargement and darkening of the ovaries, and maturity is achieved when eggs are deposited in the brood chamber in the following instar (Bradley et al. 1991; Ebert 1997). These key developmental instars have previously been referred to as IM-1 (oocyte formation), IM-2 (oocyte provisioning), and IM-3 (primiparity) (Bradley et al. 1991; Enserink et al. 1995; Barata and Baird 1998), a system of classification we shall adopt. Any of these maturation "indicators" can be used to model PMRNs. We would expect those based on IM-1 to most accurately describe the role of age and size in initiating maturation (Davidowitz and Nijhout 2004; Wright 2007; Tobin et al. 2010), but those based on IM-3 may be useful in understanding the trade-off between growth and reproduction. PMRNs based on all three maturation indicators were investigated, to describe how effects of age and size changed over the course of the maturation process. In each analysis, developmental histories were censored; individual ages and sizes of the instars following an event for the indicator were not included, as models would then predict the probability of being mature, rather than becoming mature.

STATISTICAL ANALYSES I: MATURATION RATE MODELS AND THEIR GLM APPROXIMATIONS

Maturation rate models (Van Dooren et al. 2005) allow one to investigate the determinants of maturation and choose between different ways of obtaining the maturation status variable from instantaneous rates. Typical of such time-to-event models is that the probability that an individual matures within a certain interval (given that it has not done so before that) is equal to $1 - \exp(-S)$, where S is the total change in maturation status: the maturation rate integrated over the interval duration on the chosen operational time scale. We focused on age or size integration. For an interval between a pair of age–size observations (a_1, x_1) and (a_2, x_2) the change in maturation status across the interval becomes either

$$\int_{a_1}^{a_2} h\,(a, x(a))\,da \tag{1a}$$

$$\int_{x_1}^{x_2} h(a(x), x)dx \tag{1b}$$

for age- and size-dependent integration, respectively, with $h(a, x)$ the rate at which the maturation status changes instantaneously, and where $x(a)$ and $a(x)$ denote that size (age) is seen as a function of age (size). The rate $h(a, x)$ corresponds to the "hazard" in survival analysis. It can depend on age and size, and on other explanatory variables that are not changing during the interval. Within the interval, the growth curve describes how size changes with age. We assume that size increases linearly with age during an interval between observations, to make integration more straightforward and to avoid choosing and fitting growth curve models.

Maturation rate models can be fitted to data using maximum likelihood (ML) methods (Van Dooren et al. 2005) and when the data are interval-censored zero-one observations, they become specific nonlinear regression models fitted to binomial (Bernoulli) distributed data. Van Dooren et al. (2005) focused on a set of hazard functions that can easily be integrated analytically. Time-to-event data can also be modeled by means of more standard binomial GLMs (Lindsey and Ryan 1998; Collett 2003). In the case of the proposed maturation rate models, where rates can be integrated over age or size, and where different time-dependent covariates can exist, two binomial GLMs can be interpreted as approximations of maturation rate models.

To first order of approximation, we can assume that the maturation rate remains constant between observations. The change in maturation status S can then be approximated by the rate at some point during a given interval $h(a', x')$ times the interval length

or duration Δ on the appropriate time scale. This approximation requires that maturation rates change very gradually with age and size and that rate values at the age and size of the interval midpoint are used. This approximation thus implies

$$p = 1 - \exp(-S) = 1 - \exp[-\Delta h(a', x')] \quad (2)$$

and after transforming the maturation probability p, one sees that this approximation produces in fact the model structure of a complementary log-log (cloglog) binomial GLM (equation 3), where the logarithm of the duration Δ is present as an offset term and where the sum of all other model terms in the linear predictor corresponds to the log of the maturation rate

$$ln\,(-ln\,(1 - p)) = ln\Delta + ln\,h(a', x') \quad (3)$$

In this approximation, changing the operational time scale corresponds to changing the offset term, for which no coefficient parameter is estimated in the GLM. When all interval lengths are equal and scaled to unit length, the offset has zero value and can be dropped. In this case, it is impossible to distinguish between a maturation process with a rate and a more switch-like process. If we make an additional assumption, namely that the product of maturation rate and interval length is relatively small, then we find the following first-order approximation

$$ln\left(\frac{p}{1-p}\right) \cong ln\Delta + ln\,h(a', x') \quad (4)$$

Here, we obtain the structure of a binomial GLM with logit link and an offset.

The conclusion is that when we fit these binomial GLMs to maturation data, we can interpret the results as representing maturation rate models. We can plot $ln\ h(a', x')$ estimated by the GLMs to check if the rate function likely satisfies the two assumptions mentioned. We can compare the fit of these GLMs to maturation rate models as described by Van Dooren et al. (2005) where we may still make different assumptions such as a specific parametric shape of the rate, or compare them to other binomial GLMs that are not approximations of rate models.

Even when rates are not small or not constant, it may still be useful to fit a standard GLM with an offset and to inspect $ln\ h(a', x')$. For example, if there is a threshold present, and all individuals mature at ages and/or sizes just above this threshold, the maturation rate is very small below the threshold and is large enough to produce a maturation probability, which is nearly one above it. For these last observations, the linear predictor of both GLMs will also be very large, and the plot of $ln\ h(a', x')$ will reproduce the rise at the threshold reasonably. In other cases, where maturation rates are increasing nonlinearly and not very gradually, GLMs that do not assess rates at interval midpoints but at start- or endpoints might fit data better.

It is also possible for rate models to assume that the maturation rate is determined anew at each observation, which may be appropriate in organisms such as *Daphnia* where observations coincide with discrete instars. Then the change in maturation status per stage is equal to the interval duration times the rate at molt. In the case of a cloglog GLM, there is no further assumption involved, in the case of a logit GLM one still assumes that the product of rates and interval lengths is small. Next to these possibilities, it is of course possible that maturation is not occurring on the basis of a maturation status variable that increases continually, as discussed above. We note that data containing variable interval lengths enables one to reject the possibility of a maturation process with rates.

STATISTICAL ANALYSES II: FITTING THE MODELS

All models discussed so far can be fitted using ML methods. Likelihood ratio tests can thus be used to compare nested models and AIC or likelihood comparisons can be used to compare nonnested models. When fitting maturation rate models, convergence to the ML parameter estimates can be slow and often different initial conditions have to be tried before an ML estimate has been found, which is likely global. In our experience, this is much less of a problem for cloglog GLMs, and the least of all with logit GLMs. When maturation rates have thresholds, predicted maturation probabilities per interval can be zero or one. This leads to a problem called separation, which occurs when explanatory variables predict outcomes perfectly. In this case, there are no ML parameter estimates. Various solutions have been proposed to circumvent separation (Heinze and Schemper 2002). We observed that logit GLMs hardly suffer from separation because the shape of the link function keeps p fractionally away from zero and one, such that boundary probabilities still make very small contributions to the deviance. Rate model fits suffered the most. We made a simple ad hoc adjustment, not to give logit GLMs a systematic advantage. For rate models, observations with predicted values of zero or one did not contribute to the deviance of maturation rate models anymore.

Note that both rate models and GLMs assume specific parametric forms of the maturation rate, which might not be representative of the true functional form and that can affect the significance of contributions of some effects. When maturation data are fitted using GLMs with an offset, the assumed hazard functions are often different from the few specific possibilities considered by Van Dooren et al. (2005). To assess whether the fit of a GLM is dependent on the assumptions required to arrive at a cloglog or logit model, or on differences in assumed rate functions, one can numerically integrate the rate suggested by the GLM and compare it with alternatives. We carried out such numerical integration using adaptive quadrature (Piessens et al. 1983).

STATISTICAL ANALYSES III: PREDICTED REACTION NORMS

From the previous section, it has become clear that many models can be fitted to maturation data. However, the goal is to predict reaction norms from the model that fits the data best, or which we assume to be true. In many cases, ages and sizes at which there is a 50% probability to mature within an age interval of fixed length are plotted as reaction norms (Heino et al. 2002; Beckerman et al. 2010). Unfortunately, these ages and sizes then change when a different age interval is assumed. Alternatively, one can simulate growth curves and track the probability that an individual with that growth curve would have matured already. For a range of simulated growth curves, one can then plot the ages and sizes connecting the points on the different growth curves where individuals have a 50% probability of having matured (Van Dooren et al. 2005).

When maturation rate models are estimated, rates have to be integrated along a growth curve to obtain the 50% percentile. With a fitted GLM, we can obtain probabilities p_i to mature predicted by this model for a series of small successive age-size intervals (a_i, s_i), $i = 1, \ldots, m$, on a growth curve. From these predicted values, we can calculate the probability that an individual with that growth curve would be mature at the end of an interval i as

$$1 - \prod_{\tau=1}^{i} (1 - p_\tau) \tag{5}$$

From these probabilities per growth curve, the 50% percentiles constituting the PMRN can be approximated or interpolated.

All statistical analyses were carried out using R (R Development Core Team 2011). Maturation rate models and GLMs were fitted separately to both the *D. magna* and *D. pulex* datasets for all three maturation indicators (IM-1, IM-2, and IM-3). We initially compared maximal models, containing all explanatory variables and including pairwise interactions between categorical explanatory variables and covariates. Maturation rates were fitted with analytically integrated Weibull, Gompertz, and generalized functions (Sparling et al. 2006); and age and size were fitted as covariates (except with the generalized function, where only one covariate can be integrated). Furthermore, clone identity was included as a categorical variable. Categorical variables can influence the maturation rate through interactions with age and/or size or through effects on shape parameters. Food ration was not included in analyses, as its purpose was to generate variation in growth trajectories. Maturation rates were integrated over size or age. GLMs with either cloglog or logit link functions were also fitted to the data. Age, size, or age and size were included as covariates, using either age/size interval start-, mid- or endpoints, and with values either untransformed or log-transformed, and clone was included as a categorical variable. GLMs were fitted with either an offset, that is, log age or log size difference per interval, or not. Once the best fitting rate model, GLM with offset and GLM without offset had been chosen based on AIC, model simplification was carried out using likelihood ratio tests. Goodness-of-fit tests for models with binary data are the subject of debate and their power depends strongly on the aspect that differs between the true and the fitted model (Hosmer et al. 1997; Hosmer and Hjort 2002). To make an assessment, we calculated Hosmer-Lemeshow's C and H statistic as well as the le Cessie-van Houwelingen-Copas-Hosmer test (Kohl 2012) on the models obtained after model selection. The calculation of the C and H statistics requires arranging the data in a number of groups, which can affect significance of the tests, therefore we repeated the calculations for a range of numbers of groups (8–15) and only conclude a lack of fit when the tail probability was below 0.05 for all values.

Results

COMPARISON OF DIFFERENT MODELING APPROACHES

Comparison of AIC values between rate models and GLMs reveals that maturation was best modeled using GLMs with logit-link functions. This was true for both species and for all three maturation indicators (Table 1). GLMs with offsets yielded lower AIC values when considering IM-1 in *D. pulex*, IM-2 in *D. magna*, and IM-3 in both species (Table 1). In the cases of IM-1 (*D. pulex*) and IM-2 (*D. magna*), size offsets were preferred to age offsets, that is, increases in size were more important than increases in age in determining changes in maturation status; in these models, size is acting as an operational time scale. Conversely, when modeling the data with IM-3 (both species), age offsets were preferred, that is, increases in age were more important in determining maturation status changes. Thus in four of six cases, GLMs with offsets were preferred, suggesting that in *Daphnia*, maturation is likely to be a process with a rate, especially later during development. However, in two cases GLMs without offsets provided a better fit to the data. Inspecting our data revealed that the range of age and size interval variation was smallest for *D. magna* IM-1, which could explain why the model without an offset was preferred there. Also, the number of intervals increased between models when later maturation indicators were used, which is expected to increase the power to discriminate alternative models, whereas the proportion of observations where maturation probabilities between 0.1 and 0.9 were predicted decreased, which tends to decrease discrimination power. Not selecting a model with an offset might therefore be due to a lack of statistical power. However, for both species, the range of sizes for which models with an offset predicted intermediate maturation probabilities seemed narrowest for IM-1, indicating that earlier on in development,

Table 1. A comparison of rate models and GLMs with and without offsets for both species of *Daphnia*, across all three maturation indicators. Lowest AIC values for each species and indicator combination are highlighted in boldface type. GLMs always have lower AIC values than rate models, although the presence of an offset did not always reduce AICs in models using maturation indicators IM-1 and IM-2. In both species, similar models are preferred when considering a given maturation indicator.

Model type	GLM offset	Description	AIC	Number of parameters
Daphnia pulex IM-1				
Rate	–	Generalized function, size integration, size rate effects	438.95	11
GLM	Size	response∼offset(log(size))+(clone)×(log(age ends)+log(size ends))	**427.63**	15
GLM	No	response∼(clone)×(log(age ends)+log(size ends))	434.85	15
D. pulex IM-2				
Rate	–	Weibull function, size integration, age and size rate effects	352.37	15
GLM	Size	response∼offset(log(size))+(clone)×((age ends)+(size ends))	350.23	15
GLM	No	response∼(clone)×((age ends)+(size ends))	**346.34**	15
D. pulex IM-3				
Rate	–	Weibull function, age integration, age and size rate effects	331.04	15
GLM	Age	response∼offset(log(age))+(clone)×(log(age mids)+log(size mids))	**317.90**	15
GLM	No	response∼(clone)×(log(age mids)+log(size mids))	327.82	15
D. magna IM-1				
Rate	–	Generalized function, size integration, size rate effects	311.92	11
GLM	Size	response∼offset(log(size))+(clone)×(log(age ends)+log(size ends))	288.61	15
GLM	No	response∼(clone)×(log(age ends)+log(size ends))	**284.58**	15
D. magna IM-2				
Rate	–	Weibull function, size integration, age and size rate effects	261.72	15
GLM	Size	response∼offset(log(size))+(clone)×(log(age mids)+log(size mids))	**246.77**	15
GLM	No	response∼(clone)×((age ends)+(size ends))	247.90	15
D. magna IM-3				
Rate	–	Weibull function, age integration, age and size rate effects	236.26	15
GLM	Age	response∼offset(log(age))+(clone)×(log(age mids)+log(size mids))	**204.33**	15
GLM	No	response∼(clone)×(log(age mids)+log(size mids))	216.08	15

maturation may be more analogous to a probabilistic switch. Tables S1 and S2 show AIC values of models with different combinations of offsets and covariates (GLMs) and different combinations of time scales and rate effects (maturation rate models).

When numerically integrating the hazard function obtained from the logit GLMs, and using the GLM parameter estimates as initial values, integrations often did not converge, likely due to the threshold-like nature of the function. For *D. magna* and for IM-1, the AIC of the fitted model was 312.59; for *D. pulex*, we obtained an AIC of 461.684 for IM-1 and 518.91 for IM-2. In all cases, these values were larger than of the GLMs approximating rates, such that the overall differences in fit between GLMs and maturation rate models are not due to slightly different hazard specifications. Goodness-of-fit tests on the selected models for each species and for each maturation status variable were all nonsignificant, meaning that our best models fit the data satisfactorily.

CHOICE OF MATURATION DETERMINANTS

In both species and for all maturation indicators, the best fitting GLMs included both age and size as covariates. Model simplification was carried out and in all cases the minimum models retained the clone:age interaction but not the clone:size interaction. For both species, interval endpoints were preferred when using IM-1 as the maturation indicator, while interval midpoints were preferred with IM-3. For IM-2, maturation was modeled using interval endpoints for *D. pulex* and interval midpoints for *D. magna*. Interval start points were never preferred. Models with log-transformed age and size were always preferred to those with untransformed values except in the case of *D. pulex*, IM-2.

Aside from these minor differences, the best fitting models for a given maturation indicator were similar in both species (Table 1). However, plotting PMRNs based on predicted values from these GLMs reveals within- and between-species differences. Clonal variation in age effects (the clone:age interaction) is present in the PMRNs for IM-1 in both *D. magna* (Fig. 1) and *D. pulex* (Fig. 2). Certain clones initiate maturation at smaller sizes at younger versus older ages, resulting in positively sloped PMRNs (e.g., H01, Fig. 1A; Carlos, Fig. 2A), while others do the opposite, resulting in negatively sloped PMRNs (e.g., B7, Fig. 1E;

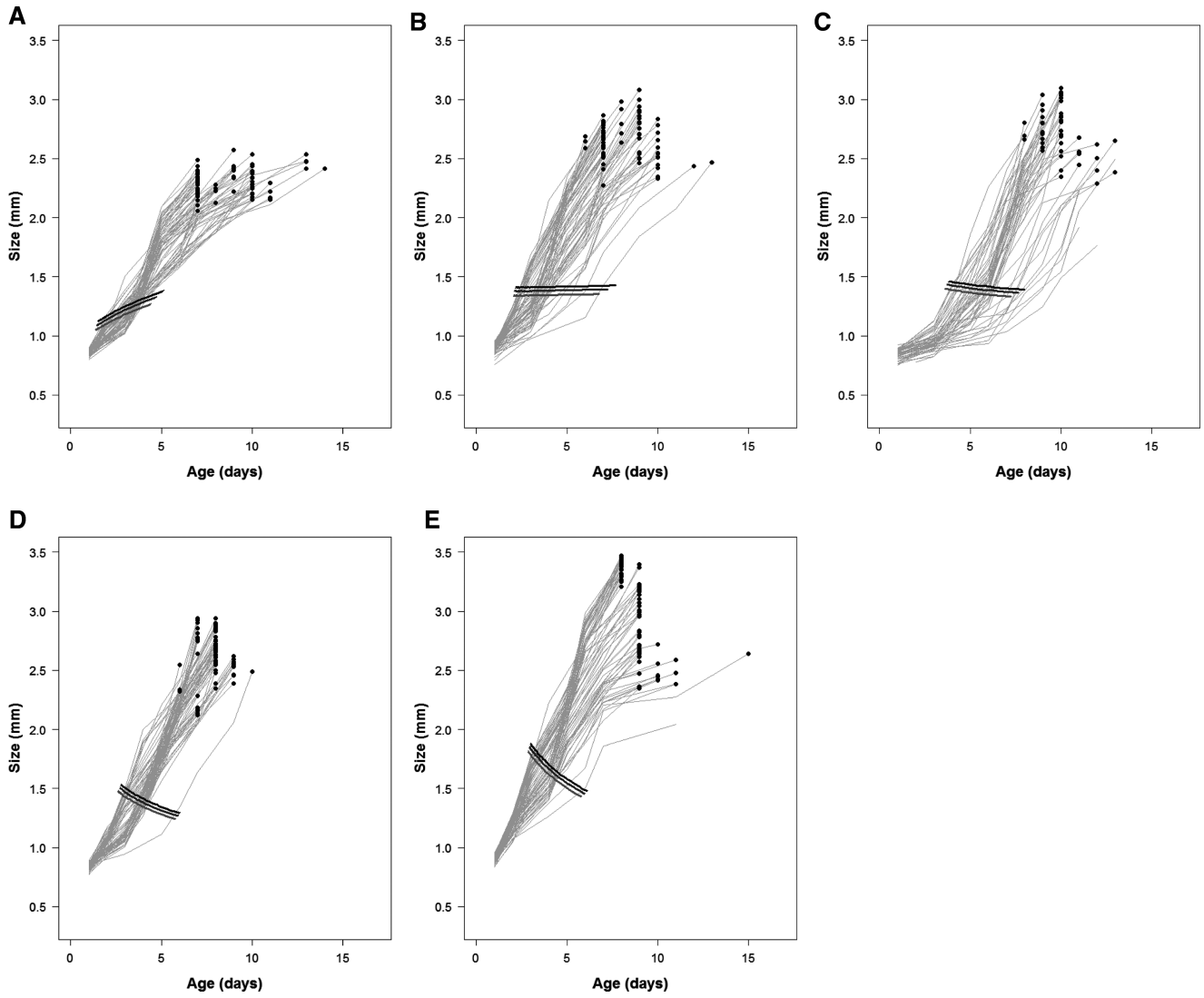

Figure 1. **PMRNs and their consequences for age and size at maturity in five clones of *Daphnia magna*: (A) H01, (B) DKN1–3, (C), B5, (D) Ness1, and (E) B7. Light gray lines are individual growth trajectories, black circles are age and size upon reaching maturity (IM-3), and increasingly dark gray lines represent 25, 50, and 75% probabilistic maturation reaction norms for IM-1 based on the best fitting GLM (no offset, age and size covariates, clone:age interaction). PMRNs vary between clones in terms of both threshold size and the importance of age in determining threshold shape. Variation in PMRNs has consequences for age and size at maturity. PMRNs with negative slopes result in primiparity occurring at a broader range of sizes and/or narrower range of ages, compared with PMRNs with positive slopes.**

Cyril, Fig. 2E). Some clones appear to have maturation thresholds that are at a fixed size (e.g., B5, Fig. 1C; Chardonnay, Fig. 2C). There is greater variation in age effects in *D. magna* (Fig. 1) than *D. pulex* (Fig. 2), and consequently the relationship between age and size upon reaching maturity (IM-3, represented by black points in Figs. 1, 2) appears to be more variable in *D. magna*.

Discussion

STATISTICALLY MODELING MATURATION

Maturation is increasingly recognized as an important heritable developmental trait underpinning the plastic response of a genotype to its environment (Berner and Blanckenhorn 2007; Nijhout et al. 2010). However, our understanding of how maturation phenotypes evolve is still hindered by the debate regarding the best way to quantify and compare maturation reaction norms for age and size upon reaching maturity (Heino et al. 2002; Van Dooren et al. 2005; Dieckmann and Heino 2007; Heino and Dieckmann 2008; Kuparinen et al. 2008; Uusi-Heikkilä et al. 2011). Here, we show that in *Daphnia*, GLM approximations of rate models provide the best fit to maturation data. A comparison of models containing different combinations of age and size suggests that size is the most important maturation determinant, but that age also plays a role in the maturation process. This was true

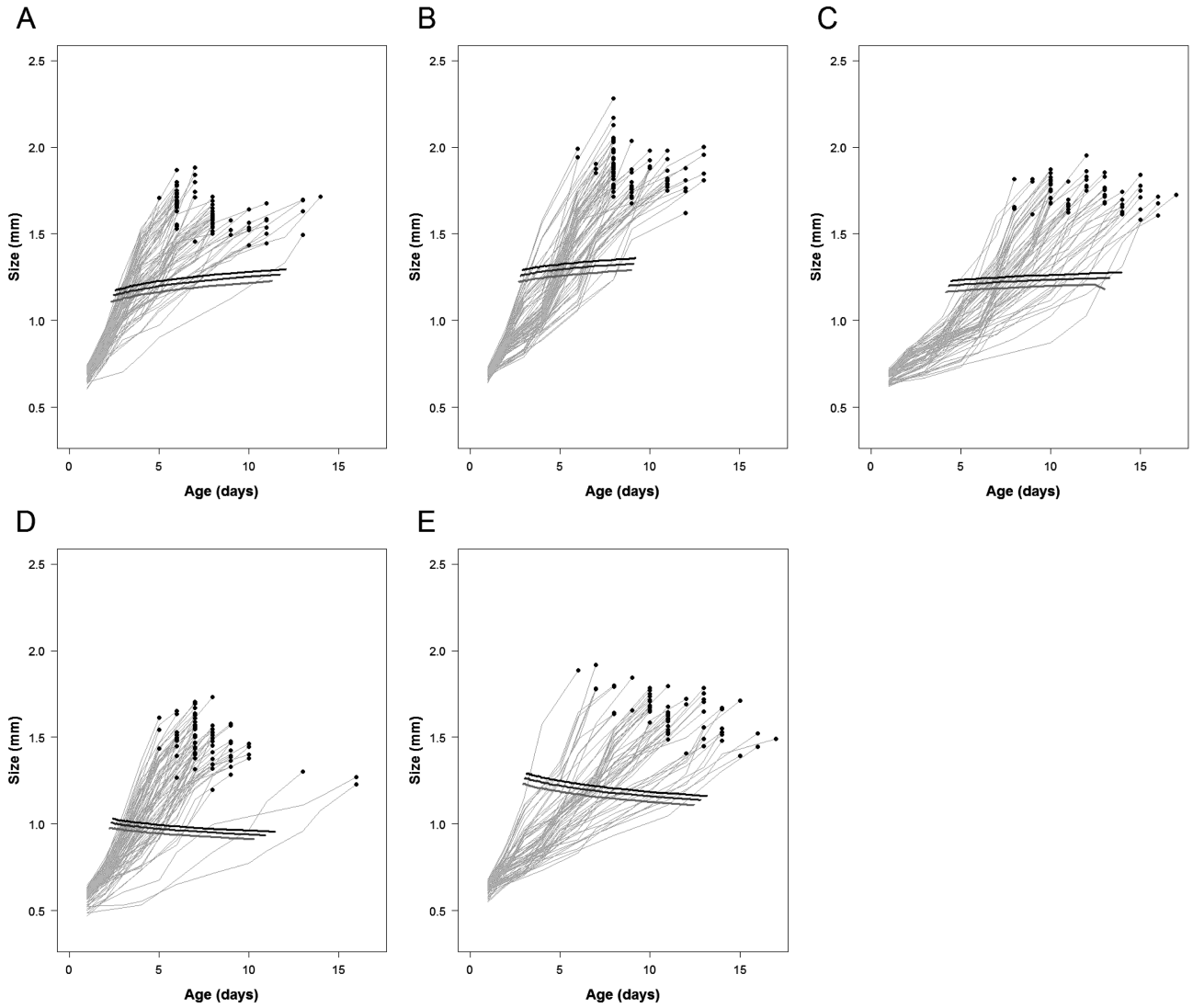

Figure 2. **PMRNs and their consequences for age and size at maturity in five clones of *Daphnia pulex*: (A) Carlos, (B) Boris, (C) Chardonnay, (D) Bierbeek, and (E) Cyril. Light gray lines are individual growth trajectories, black circles are age and size upon reaching maturity (IM-3), and increasingly dark gray lines represent 25, 50, and 75% probabilistic maturation reaction norms for IM-1 based on the best fitting GLM approximation of a rate model (size offset, age and size covariates, clone:age interaction). As with *D. magna*, PMRNs vary between clones in both threshold size and the importance of age. However, these differences and subsequently clonal variation in age and size at primiparity are less pronounced in *D. pulex* than *D. magna*.**

for some clones more than others, demonstrating variation in the position and nature of PMRNs at the level of the genotype.

Logit-link GLMs fitted our data better than rate models, suggesting that the additional assumptions involved in these models versus rate models and cloglog GLMs are generally valid and that the nonlinear functional dependence on age and size implicit in the logit-link fits the data better than the functional forms implied by the cloglog link or rate models. This could be because the maturation rate follows a step-like function indicative of a strong size threshold and relatively deterministic maturation, given the maturation determinants we selected. Because the shape of maturation rate functions has yet to be examined in other systems, we cannot currently comment on the generality of our findings. If maturation rate functions are not step-like in other systems, maturation may be better modeled by the functions contained within rate models, and model comparison will remain an important step in quantifying and comparing maturation phenotypes. In general, most of our GLM models were improved by the inclusion of an offset term, indicating that maturation is generally more analogous to a rate than a switch. However, some of our models based on earlier maturation indicators were not improved by the inclusion of an offset. This may be due to a lack of statistical power, or because the time window for maturation is restricted to a fixed interval length, but could also be indicative of stage-specific switches rather than a

continually changing maturation status. However, the importance of correcting for interval bias is highlighted by the fact that analyses of maturation using the latest possible indicator of maturation (IM-3), that most closely resembles the sorts of indicators used in other studies, were improved by including age interval offsets. Even when the offset does not improve the fit of the model, the corresponding models with an offset should be inspected and presented to assess the strength of evidence for a switch-like process. Furthermore, lacking a discussion of offset effects, the majority of studies that utilize GLMs to predict PMRNs (Grift et al. 2003; Engelhard and Heino 2004; Olsen et al. 2004; Mollet et al. 2007; Beckerman et al. 2010), cannot investigate potentially insightful alternative time scales.

CLONAL VARIATION IN MATURATION DETERMINANTS

Although evidence of between-population (Piché et al. 2008) and within-population (Skilbrei and Heino 2011) variation in PMRNs is emerging, few studies are able to compare maturation thresholds of different genotypes. Using parthenogenetic organisms such as *Daphnia* allows us to demonstrate within- and between-population genotypic variation in the position and nature of maturation thresholds and may improve our understanding of how maturation decisions evolve and influence the evolution of age and size at the onset of maturity. Clonal variation in the position of the threshold has previously been shown to differ for two clones from the same population (Ebert 1994). However, unlike previous studies (Ebert 1992, 1994), we found that maturation thresholds in *Daphnia* varied across a range of growth trajectories and that in some clones the decision to mature depended on both size and age. The strength of this effect was itself variable between different clones and was less apparent in *D. pulex* than *D. magna* (Figs. 1, 2), although it is unclear whether differences between the two species are due to the narrow geographic origin of our *D. pulex* clones, or reflective of constrained threshold feeding and incipient feeding concentrations in smaller species (Porter et al. 1982; Gliwicz 1990; Dudycha and Lynch 2005; Hart and Bychek 2010).

Our finding that age can be an important maturation determinant in *Daphnia* demonstrates that the fixed size thresholds previously assumed in studies of maturation thresholds in *D. magna* (Ebert 1992, 1994, 1997) are an oversimplification. Under a fixed size threshold model, maturation thresholds (and subsequent sizes and ages upon reaching maturity) can only evolve through upward or downward shifts in threshold size. The extreme L-shaped reaction norm predicted by Day and Rowe (2002), and previously observed in some studies (Plaistow et al. 2004), is assumed to be the result of growth plasticity, such that fast growing individuals overshoot the threshold more than slow growing individuals. In this study, we use a PMRN approach that explicitly corrects for such growth bias, yet we still see curvature in some PMRNs at the earliest stages of maturation (see Figs. 1, 2). This suggests that negatively sloped reaction norms in age and size at the completion of maturation may be generated by the shape of the maturation threshold itself. Organisms that are able to include age (or a correlate of age) as a maturation determinant may be able to reduce the size at which they mature to maintain their development rate, or alternatively to maintain or even increase size at maturation at the expense of increasing their development time (Morita and Fukuwaka 2006). Variation in the extremes of these two strategies can be seen in our data by comparing the clone B7 (Fig. 1E; black points), which varies more in body size than development time upon reaching maturity, with clone H01 (Fig. 1A), which varies more in development time than body size. Such patterns have previously been predicted by life-history theory (Wilbur and Collins 1973; Stearns and Koella 1986) but the proximate mechanisms underpinning these responses are generally not understood.

If age can have an effect on the decision to mature, it raises the question: "what other factors can influence this decision?" It is well understood that maturation itself involves the co-ordination of a number of endocrinological and neurophysiological processes that control changing patterns of resource allocation to growth, maintenance, and reproductive function (Bernardo 1993; Stern and Emlen 1999; Nijhout 2003). Indeed, the development of the PMRN approach was a response to the realization that just measuring size and age may not be sufficient for predicting maturation decisions (Morita and Fukuwaka 2006). We have therefore tried to create a general modeling framework that utilizes PMRNs and encourages exploration of various and numerous maturation determinants. Having said that, it appears that in *Daphnia*, provided there is good data on the age and size of individuals throughout their life, age and size alone can be used to accurately predict PMRNs. This is reflected in the fact that the 25, 50, and 75% probability contours are always very closely associated with each other and suggests that *Daphnia* may be a useful and relatively simple model in which to investigate the evolutionary ecology of maturation thresholds. This is especially true because the transparent cuticle of daphniids allows us to observe the progress of the maturation process (IM-1–IM-3) in a manner often not possible in other systems.

PMRNs based on early maturation indicators should provide the best description of which factors are involved in the maturation decision, yet in many studies maturation is only scored at the end of the maturation process, marked by the appearance of secondary sexual characters or offspring. This is a problem when there is a lag between the initiation of the maturation process and its conclusion (Wright 2007), because the allocation of resources to reproduction can alter the growth curve (Day and Taylor 1997), and because further maturation might be a simple matter of time (Davidowitz and Nijhout 2004), blurring the effects of maturation determinants

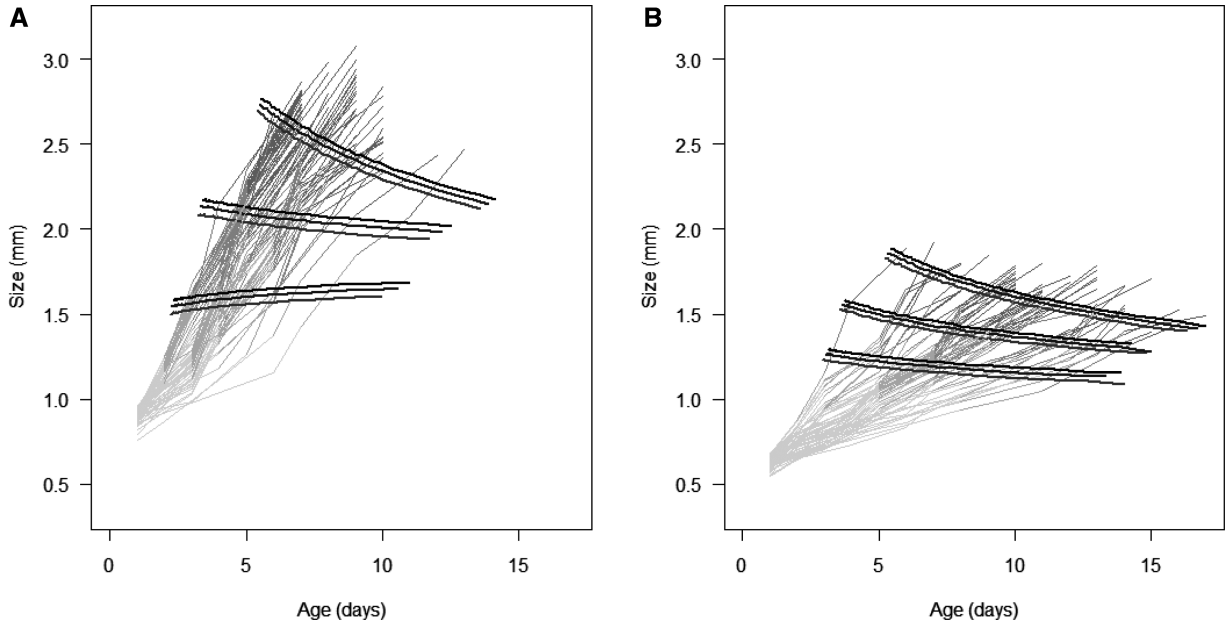

Figure 3. PMRNs for three different maturation indicators IM-1, IM-2 and IM-3 in two species of *Daphnia*. (A) *Daphnia magna* clone DKN1–3 and (B) *D. pulex* clone Cyril. PMRNs for all three maturation indicators have been generated using the model: response ~ offset(log(size)) + (clone) × (log(age ends) + log(size ends)). Differences between PMRNs highlight the effects of growth during maturation. In both species, later maturation indicators have PMRNs with more pronounced L-shapes. Prior to the maturation threshold at IM-1 growth differences do not influence the shape of the PMRN. After reaching IM-1, however, individuals with high growth rates achieve IM-2 and IM-3 at larger sizes, and over a wider range of sizes but narrower range of ages than individuals with low growth rates. This pattern is true for all clones.

that led to the decision to mature. Thus, if individuals are scored as immature after they have initiated maturation but before they display any evidence of maturation, or if the maturation process itself lasts through a number of intervals (e.g., three instars, as in this study), but only states at later ages and sizes are included as maturing, one could expect stronger age effects because later size increases are less relevant for maturation. Such an effect can be observed in this study, where PMRNs estimated using IM-3 are more L-shaped than those estimated using IM-1 (Figs. 3, S1, S2) and feature age integration (Table 1). This finding highlights the importance of using traits at the beginning of maturation rather than the end (Tobin et al. 2010). Approaches such as measuring changes in hormone titers or patterns of gene expression may allow more accurate estimation of when the maturation process begins.

DEVELOPMENTAL PLASTICITY IN MATURATION

Ultimately, even subtle differences in how maturation decisions are made draws attention to the fact that the proximate mechanisms leading to developmental plasticity in maturation schedules are often poorly understood (Berner and Blanckenhorn 2007). While factors underpinning the decision to mature will inevitably vary across taxa (Nijhout 2008), in-depth investigation in a few key species, such as the spadefoot toad, *Spea hammondii* (Denver 1997; Denver et al. 1998; Boorse and Denver 2004) and the tobacco hornworm moth *M. sexta* (Nijhout 2003; Davidowitz and Nijhout 2004; Nijhout et al. 2010) is helping us to understand how maturation phenotypes are assembled during the course of development, from initial maturation decisions through to the completion of the maturation process, and how different environmental variables influence this process. Deconstructing the maturation phenotype in this way will be critical to understanding which parts of the process can evolve and which parts are simply the product of environmental fluctuation and constraints.

The proximate mechanisms operating in *Daphnia* must be able to explain the strong effect of size on the decision to mature (Ebert 1994), our finding that in some clones older individuals mature at smaller sizes and the fact that individuals can sometimes exceed the typical "threshold body size" without maturing when resources are particularly scarce (Enserink et al. 1995). We suggest that one likely mechanism is a minimum "state" or "condition" below which maturation is unviable. Assuming the level of stored energy reserves individuals can possess is constrained by their body size, this would explain the strong influence of body size on maturation decisions in *Daphnia* (Ebert 1992, 1994, 1997) and is in accordance with the more switch-like nature of the maturation process we suggest for IM-1. The apparent age-dependence observed in the PMRNs of some clones could arise if individuals growing in resource-poor environments increase the proportion of resources allocated to storage at the expense of

growth. In this way, slower growing individuals could potentially exceed a threshold state at a smaller body size. This mechanism may also explain why individuals that are big enough to mature but experience extremely resource poor environments could be constrained from maturing, as they have insufficient resources.

We conclude that to understand how the important life-history traits of age and size at the onset of maturity evolve, one must further investigate the underlying ontogenetic processes that produce these phenotypes. Statistically, modeling the maturation process using PMRNs can help elucidate the importance of age, size, or other maturation determinants in maturation decisions. We compared the utility of three different approaches to PMRNs: maturation rate models, GLM approximations of maturation rate models, and GLMs. In *Daphnia*, GLM approximations of maturation rate models often provided a better fit to the data and suggest that maturation, particularly later on during development, is best modeled as a process with a rate. Because *Daphnia* are clonal, PMRNs also reveal how maturation decisions differ between genotypes. Our results suggest that in *Daphnia*, maturation thresholds are variable across growth environments and between genotypes, and therefore may play an important role in the evolution of age and size at the onset of maturity.

ACKNOWLEDGMENTS

SJP was funded by a NERC postdoctoral fellowship grant (NE/ C518214/1). We would like to thank H. Collin, M. Heino, T. Lenormand, and an anonymous reviewer for providing helpful comments that enabled us to improve earlier versions of this article.

LITERATURE CITED

Baird, D. J., A. M. V. M. Soares, A. Girling, and I. Barber. 1989. The long-term maintenance of *Daphnia magna* Straus for use in ecotoxicity tests: problems and prospects. Pp. 144–148 *in* Proceedings of the First European Conference on ecotoxicology. Lyngby, Denmark.

Barata, C., and D. J. Baird. 1998. Phenotypic plasticity and constancy of life-history traits in laboratory clones of *Daphnia magna* Straus: effects of neonatal length. Funct. Ecol. 12:442–452.

Barot, S., M. Heino, L. O'Brien, and U. Dieckmann. 2004. Estimating reaction norms for age and size at maturation when age at first reproduction is unknown. Evol. Ecol. Res. 6:659–678.

Beckerman, A. P., G. M. Rodgers, and S. R. Dennis. 2010. The reaction norm of size and age at maturity under multiple predator risk. J. Anim. Ecol. 79:1069–1076.

Bernardo, J. 1993. Determinants of maturation in animals. Trends Ecol. Evol. 8:166–173.

Berner, D., and W. U. Blanckenhorn. 2007. An ontogenetic perspective on the relationship between age and size at maturity. Funct. Ecol. 21:505–512.

Berrigan, D., and E. Charnov 1994. Reaction norms for age and size at maturity in response to temperature: a puzzle for life historians. Oikos 70:474–478.

Berrigan, D., and J. C. Koella. 1994. The evolution of reaction norms: simple models for age and size at maturity. J. Evol. Biol. 7:549–566.

Boorse, G. C., and R. J. Denver. 2004. Endocrine mechanisms underlying plasticity in metamorphic timing in spadefoot toads. Integr. Comp. Biol. 43:646–657.

Bradley, M., D. J. Baird, and P. Calow. 1991. Mechanisms of energy allocation to reproduction in the cladoceran *Daphnia magna* Straus. Biol. J. Linn. Soc. 44:325–333.

Bradshaw, W. E., and K. Johnson. 1995. Initiation of metamorphosis in the pitcher-plant mosquito: effects of larval growth history. Ecology 76:2055–2065.

Collett, D. 2003. Modelling survival data in medical research. Chapman and Hall/CRC, Boca Raton.

Davidowitz, G., and H. F. Nijhout. 2004. The physiological basis of reaction norms: the interaction among growth rate, the duration of growth and body size. Integr. Comp. Biol. 44:443–449.

Davidowitz, G., L. J. D'Amico, and H. F. Nijhout. 2003. Critical weight in the development of insect body size. Evol. Dev. 5:188–197.

Day, T., and L. Rowe. 2002. Developmental thresholds and the evolution of reaction norms for age and size at life-history transitions. Am. Nat. 159:338–350.

Day, T., and P. D. Taylor. 1997. Von Bertalanffy's growth equation should not be used to model age and size at maturity. Am. Nat. 149:381–393.

De Moed, G. H., C. L. J. J. Kruitwagen, G. De Jong, and W. Scharloo. 1999. Critical weight for the induction of pupariation in *Drosophila melanogaster*: genetic and environmental variation. J. Evol. Biol. 12:852–858.

Denver, R. J. 1997. Proximate mechanisms of phenotypic plasticity in amphibian metamorphosis. Am. Zool. 37:172–184.

Denver, R. J., N. Mirhadi, and M. Phillips. 1998. Adaptive plasticity in amphibian metamorphosis: response of *Scaphiopus hammondii* tadpoles to habitat desiccation. Ecology 79:1859–1872.

Dieckmann, U., and M. Heino. 2007. Probabilistic maturation reaction norms: their history, strengths, and limitations. Mar. Ecol. Prog. Ser. 335:253–269.

Duchesne, T., and J. Lawless. 2000. Alternative time scales and failure time models. Lifetime Data Anal. 6:157–179.

Dudycha, J. L., and M. Lynch. 2005. Conserved ontogeny and allometric scaling of resource acquisition and allocation in the Daphniidae. Evolution 59:565–576.

Ebert, D. 1991. The effect of size at birth, maturation threshold and genetic differences on the life-history of *Daphnia magna*. Oecologia 86:243–250.

———. 1992. A food-independent maturation threshold and size at maturity in *Daphnia magna*. Limnol. Oceanogr. 37:878–881.

———. 1994. A maturation size threshold and phenotypic plasticity of age and size at maturity in *Daphnia magna*. Oikos 69:309–317.

———. 1997. The evolution and genetics of maturation in *Daphnia*. Pp. 151–178 *in* B. Streit, T. Städler, and C. M. Lively, eds. Evolutionary ecology of freshwater animals: concepts and case studies. Birkhäuser Verlag, Basel.

Engelhard, G. H., and M. Heino. 2004. Maturity changes in Norwegian spring-spawning herring *Clupea harengus*: compensatory or evolutionary responses? Mar. Ecol. Prog. Ser. 272:245–256.

Enserink, L., M. J. J. Kerkhofs, C. A. M. Baltus, and J. H. Koeman. 1995. Influence of food quantity and lead exposure on maturation in *Daphnia magna*; evidence for a trade-off mechanism. Funct. Ecol. 9:175–185.

Ernande, B., U. Dieckmann, and M. Heino. 2004. Adaptive changes in harvested populations: plasticity and evolution of age and size at maturation. Proc. R. Soc. Lond. B Biol. Sci. 271:415–423.

Etilé, E., and E. Despland. 2008. Developmental variation in the forest tent caterpillar: life history consequences of a threshold size for pupation. Oikos 117:135–143.

Gliwicz, Z. M. 1990. Food thresholds and body size in cladocerans. Nature 343:638–640.

Green, J. 1956. Growth, size and reproduction in *Daphnia* (Crustacea: cladocera). Proc. Zool. Soc. Lond. 126:173–204.

Grift, R., A. D. Rijnsdorp, S. Barot, M. Heino, and U. Dieckmann. 2003. Fisheries-induced trends in reaction norms for maturation in North Sea plaice. Mar. Ecol. Prog. Ser. 257:247–257.

Hart, R. C., and E. A. Bychek. 2010. Body size in freshwater planktonic crustaceans: an overview of extrinsic determinants and modifying influences of biotic interactions. Hydrobiologia 668: 61–108.

Heino, M., and U. Dieckmann. 2008. Detecting fisheries-induced life-history evolution: an overview of the reaction-norm approach. Bull. Mar. Sci. 83:69–93.

Heino, M., U. Dieckmann, and O.R. Godo. 2002. Measuring probabilistic reaction norms for age and size at maturation. Evolution 56:669–678.

Heinze, G., and M. Schemper. 2002. A solution to the problem of separation in logistic regression. Stat. Med. 21:2409–2419.

Hosmer, D. W., and N. L. Hjort. 2002. Goodness-of-fit processes for logistic regression: simulation results. Stat. Med. 21:2723–2738.

Hosmer, D. W., T. Hosmer, S. Le Cessie, and S. Lemeshow. 1997. A comparison of goodness-of-fit tests for the logistic regression model. Stat. Med. 16:965–980.

Johnson, N. A., and A. H. Porter. 2001. Toward a new synthesis: population genetics and evolutionary developmental biology. Genetica 112–113:45–58.

Klinkhamer, P. G. L., T. J. de Jong, and E. Meelis. 1987. Delay of flowering in the "biennial" *Cirsium vulgare*: size effects and devernalization. Oikos 49:303–308.

Kohl, M. 2012. MKmisc: Miscellaneous functions from M. Kohl. R package version 0.91. Available via http://www.stamats.de.

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.

Kuparinen, A., R. B. O Hara, and J. Merilä. 2008. Probabilistic models for continuous ontogenetic transition processes. PLoS One 3:3677.

Lindsey, J. C., and L. M. Ryan. 1998. Tutorial in biostatistics methods for interval-censored data. Stat. Med. 17:219–238.

Lynch, M. 1989. The life history consequences of resource depression in *Daphnia pulex*. Ecology 70:246–256.

Marshall, C. T., and H. I. Browman. 2007. Disentangling the causes of maturation trends in exploited fish populations. Mar. Ecol. Prog. Ser. 335:249–251.

McKenzie, W. D., D. Crews, K. D. Kallman, D. Policansky, and J. J. Sohn. 1983. Age, weight and the genetics of sexual maturation in the platyfish, *Xiphophorus maculatus*. Copeia 1983:770–774.

Mirth, C. K., and L. M. Riddiford. 2007. Size assessment and growth control: how adult size is determined in insects. BioEssays 29:344–355.

Mollet, F. M., S. B. M. Kraak, and A. D. Rijnsdorp. 2007. Fisheries-induced evolutionary changes in maturation reaction norms in North Sea sole *Solea solea*. Mar. Ecol. Prog. Ser. 351:189–199.

Morey, S. R., and D. N. Reznick. 2000. A comparative analysis of plasticity in larval development in three species of spadefoot toads. Ecology 81:1736–1749.

Morita, K., and M. -A. Fukuwaka 2006. Does size matter most? The effect of growth history on probabilistic reaction norm for salmon maturation. Evolution 60:1516–1521.

Morita, K., and S. Morita. 2002. Rule of age and size at maturity: individual variation in the maturation history of resident white-spotted charr. J. Fish Biol. 61:1230–1238.

Nijhout, H. F. 2003. The control of body size in insects. Dev. Biol. 261:1–9.

———. 2008. Size matters (but so does time), and it's OK to be different. Dev. Cell 15:491–492.

Nijhout, H. F., and C. M. Williams. 1974. Control of moulting and metamorphosis in the tobacco hornworm, *Manduca sexta* (L.): growth of the last-instar larva and the decision to pupate. J. Exp. Biol. 61:481–491.

Nijhout, H. F., D. A. Roff, and G. Davidowitz. 2010. Conflicting processes in the evolution of body size and development time. Philos. Trans. R. Soc. Lond. B 365:567–575.

OECD. 1984. Daphnia sp., acute immobilisation test and reproduction test. Pp. 1–16 *in* Annex I: OECD test guidelines for studies included in the SIDS. OECD, Paris.

Olsen, E. M., M. Heino, G. R. Lilly, M. J. Morgan, J. Brattey, B. Ernande, and U. Dieckmann. 2004. Maturation trends indicative of rapid evolution preceded the collapse of northern cod. *Nature* 428:932–935.

Perrin, N., and J. Rubin. 1990. On dome-shaped norms of reaction for size-to-age at maturity in fishes. Funct. Ecol. 4:53–57.

Piché, J., J. A. Hutchings, and W. Blanchard. 2008. Genetic variation in threshold reaction norms for alternative reproductive tactics in male Atlantic salmon, *Salmo salar*. Proc. R. Soc. Lond. B. Biol. Sci. 275: 1571–1575.

Piessens, R., E. DeDoncker-Kapenge, C. Uberhuber, and D. Kahaner. 1983. Quadpack: a subroutine package for automatic integration. Springer V., Berlin.

Plaistow, S.J., C. T. Lapsley, A. P. Beckerman, and T. G. Benton. 2004. Age and size at maturity: sex, environmental variability and developmental thresholds. Proc. R. Soc. Lond. B. Biol. Sci. 271:919–924.

Policansky, D. 1983. Size, age and demography of metamorphosis and sexual maturation in fishes. Am. Zool. 23:57–63.

Porter, K. G., J. Gerritsen, and J. D. Orcutt Jr. 1982. The effect of food concentration on swimming patterns, feeding behavior, ingestion, assimilation, and respiration by *Daphnia*. Limnol. Oceanogr. 27:935–949.

Pérez-Rodríguez, A., M. J. Morgan, and F. Saborido-Rey. 2009. Comparison of demographic and direct methods to calculate probabilistic maturation reaction norms for Flemish Cap cod (*Gadus morhua*). Evol. Appl. 2: 291–298.

R Development Core Team. 2011. R: a language and environment for statistical computing. R Foundation for Statistical Computing Vienna, Austria.

Rasband, W. S. 1997. ImageJ, U.S. National Institute of Health, Bethesda, Maryland, USA. Available via http://rsb.info.nih.gov/ij/.

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.

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

Roff, D. A. 2001. Age and size at maturity. Pp. 99–112 *in* C. W. Fox, D. A. Roff, and D. J. Fairbairn, eds. Evolutionary ecology: concepts and case studies. Oxford Univ. Press, Oxford, U.K.

Sibly, R. M., and D. Atkinson. 1994. How rearing temperature affects optimal adult size in ectotherms. Funct. Ecol. 8:486–493.

Skilbrei, O.T., and M. Heino. 2011. Reduced daylength stimulates size-dependent precocious maturity in 0+ male Atlantic salmon parr. Aquaculture 311:168–174.

Sparling, Y. H., N. Younes, J. M. Lachin, and O. M. Bautista. 2006. Parametric survival models for interval-censored data with time-dependent covariates. Biostatistics 7:599–614.

Stearns, S. C., and J. C. Koella. 1986. The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at maturity. Evolution 40:893–913.

Stern, D. L., and D. J. Emlen. 1999. The developmental basis for allometry in insects. Development 126:1091–1101.

Tobin, D., P. J. Wright, and M. O'Sullivan. 2010. Timing of the maturation transition in haddock *Melanogrammus aeglefinus*. J. Fish Biol. 77:1252–1267.

Travis, J. 1984. Anuran size at metamorphosis: experimental test of a model based on intraspecific competition. Ecology 65:1155–1160.

Uusi-Heikkilä, S., A. Kuparinen, C. Wolter, T. Meinelt, A. C. O'Toole, and R. Arlinghaus. 2011. Experimental assessment of the probabilistic maturation reaction norm: condition matters. Proc. R. Soc. Lond. B. Biol. Sci. 278:709–717.

Van Dooren, T. J. M., T. Tully, and R. Ferrière. 2005. The analysis of reaction norms for age and size at maturity using maturation rate models. Evolution 59:500–506.

Werner, P. A. 1975. Predictions of fate from rosette size in teasel (*Dipsacus fullonum* L.). Oecologia 20:197–201.

Wesselingh, R., and T. J. de Jong. 1995. Bidirectional selection on threshold size for flowering in *Cynoglossum officianale* (hound's-tongue). Heredity 74:415–424.

Wesselingh, R., and P. G. L. Klinkhamer. 1996. Threshold size for vernalization in *Senecio jacobaea*: genetic variation and response to artificial selection. Funct. Ecol. 10:281–288.

West-Eberhard, M. J. 2003. Developmental plasticity and evolution. Oxford Univ. Press, New York.

Wilbur, H. M., and J. P. Collins. 1973. Ecological aspects of amphibian metamorphosis: nonnormal distributions of competitive ability reflect selection for facultative metamorphosis. Science 182:1305–1314.

Wolf, J.B., W. A. Frankino, A. F. Agrawal, E. D. Brodie, and A. J. Moore 2001. Developmental interactions and the constituents of quantitative variation. Evolution 55:232–245.

Wright, P. 2007. Understanding the maturation process for field investigations of fisheries-induced evolution. Mar. Ecol. Prog. Ser. 335: 279–283.

Associate Editor: N. Perrin

Supporting Information

The following supporting information is available for this article:

Figure S1. PMRNs for three different maturation indicators IM-1, IM-2 and IM-3 in five clones of *Daphnia magna*: (A) H01, (B) DKN1-3, (C), B5, (D) Ness1, and (E) B7.

Figure S2. PMRNs for three different maturation indicators IM-1, IM-2 and IM-3 in five clones of *Daphnia pulex*: (A) Carlos, (B) Boris, (C) Chardonnay, (D) Bierbeek, and (E) Cyril.

Table S1. A comparison of GLMs with and without offsets and maturation rate models for all three maturation indicators (IM-1, IM-2 and IM-3) for *Daphnia magna*.

Table S2. A comparison of GLMs with and without offsets and maturation rate models for all three maturation indicators (IM-1, IM-2 and IM-3) for *Daphnia pulex*.

Supporting Information may be found in the online version of this article.