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Developmental energetics of zebrafish, *Danio rerio*

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ABSTRACT

Using zebrafish (*Danio rerio*) as a case study, we show that the maturity concept of Dynamic Energy Budget (DEB) theory is a useful metric for developmental state. Maturity does not depend on food or temperature contrary to age and to some extent length. We compile the maturity levels for each developmental milestone recorded in staging atlases. The analysis of feeding, growth, reproduction and aging patterns throughout the embryo, juvenile and adult life stages are well-captured by a simple extension of the standard DEB model and reveals that embryo development is slow relative to adults. A threefold acceleration of development occurs during the larval period. Moreover we demonstrate that growth and reproduction depend on food in predictable ways and their simultaneous observation is necessary to estimate parameters. We used data on diverse aspects of the energy budget simultaneously for parameter estimation using the covariation method. The lowest mean food intake level to initiate reproduction was found to be as high as 0.6 times the maximum level. The digestion efficiency for Tetramin™ was around 0.5, growth efficiency was just 0.7 and the value for the allocation fraction to soma (0.44) was close to the one that maximizes ultimate reproduction.

1. Introduction

Experimental evidence points to developmental processes being heterochronic and they play an important role in evolutionary theory (McKinney and McNamara, 1991). Spicer and Burggren (2003) propose the concept of heterokairy to study how changes in the sequence of developmental events relate to changes in the timing of physiological regulatory systems and/or its components. Theoretical progress in the analysis of these processes lags behind experimental work for lack of a quantifier for internal time which does not necessarily correlate with morphological (e.g. length) or chronological (e.g. age) criteria (Reiss, 1989). The objective of the present study is to provide such a quantifier for the rate of development of the individual. We focus on zebrafish *Danio rerio* since it is widely used to study development (Kimmel et al., 1995; Parichy et al., 2009). A growing number of disciplines use zebrafish as a model with the consequence that numerous observations on life history traits, under laboratory controlled conditions, are available (Laale, 1977; Gerhard and Cheng, 2002; Lawrence, 2007; Spence et al., 2008).

We undertake a theoretical analysis of zebrafish development over its entire life cycle using Dynamic Energy Budget (DEB) theory (Kooijman, 2001, 2010), see Sousa et al. (2010) an introduction to the theory. The theory quantifies the uptake and use of substrates (food) by organisms, see Fig. 1. Stage transitions, such as from embryo to juvenile (defined as the initiation of feeding) and from juvenile to adult (defined as the ceasing of further maturation and the initiation of allocation to reproduction) are linked to the level of maturity. Maturity is quantified as the cumulated energy invested in development. Although the standard DEB model has been applied to a wide variety of animals (Zonneveld and Kooijman, 1993; van der Veer et al., 2010; Bodiguel et al., 2009; Flye-Sainte-Marie et al., 2009; Pecquerie et al., 2009; Rico-Villa et al., 2010), tests that the parameters for the embryo, juvenile and adult are all the same are relatively rare (Kooijman et al., 2011; Lika et al., 2011). We collected a number of detailed studies on the growth, reproduction and aging of zebrafish, and we also did a growth-and-reproduction experiment at three feeding levels, to estimate the parameters of the standard DEB model and judge if the full life cycle could be captured with a single set of parameter values. We discuss the coupling of developmental milestone to age and size at stage transitions (e.g. birth and puberty) and give a mechanistic underpinning of effects of food and temperature.

2. DEB model

The conceptual organization of metabolism, described in the DEB model, is presented in Fig. 1. The mobilization of reserve $\dot{p}_C$ is such that weak homeostasis is respected, i.e. the ratio of the amounts of reserve E (J) and structure V (cm^{-3}), called the reserve density $[E]$, is constant at constant food densities in juveniles and adults. The

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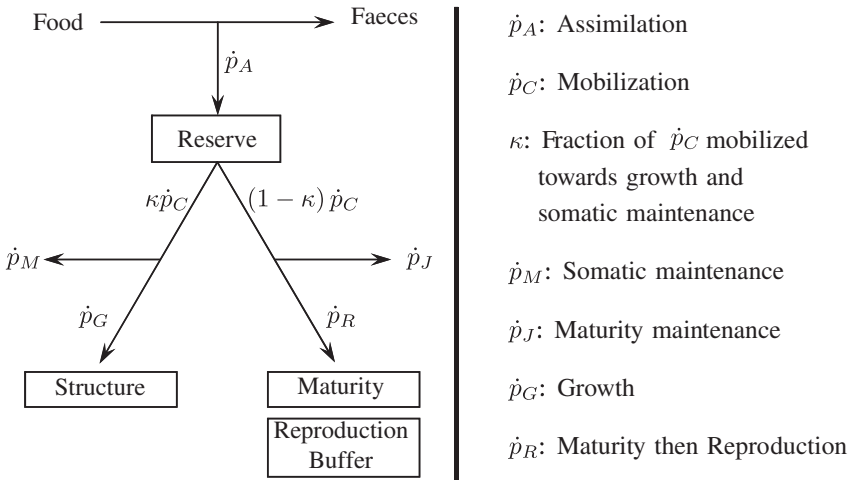

Fig. 1. Conceptual organization of animal metabolism as defined by DEB theory (Kooijman, 2010). Arrows: energy fluxes (J d^{-1}); boxes: state variables of the system. Embryo: $\dot{p}_A = 0$; birth: assimilation is switched on; puberty: allocation to maturity E_H stops and allocation to reproduction E_R starts. Energy allocated to reproduction accumulates in the reproduction buffer and is emptied at spawning.

increase in the maturity level E_H (J), called maturation, is by allocating a fixed fraction of mobilized reserve $(1-\kappa)\,\dot{p}_C$ after subtraction of maturity maintenance costs $\dot{p}_J$ (e.g. immune system, hormonal regulation, anti-oxidative stress system). $\dot{p}_J = \dot{k}_J E_H$, with $\dot{k}_J$ (d^{-1}) the maturity maintenance rate coefficient. Positive maturity maintenance implies that reproduction is absent at low food levels. There is no assimilation during the embryonic period, i.e. when $E_H < E_H^b$, with E_H^b the cumulated amount of energy invested in maturity at birth.

From birth onwards reserve is replenished by assimilation, $\dot{p}_A$ (J d^{-1}). We now explain how assimilation is linked to actual ingestion. Ingestion rate, $\dot{p}_X$ (J d^{-1}), is a function of food density and is taken proportional to surface area L^2, with $L = V^{1/3}$ the (volumetric) structural length of an organism. $\dot{p}_X$ is quantified by the scaled functional response (ingestion level) f, defined as the ratio of actual ingestion rate and the maximum possible one for an individual of that size. This makes $\dot{p}_X = f\{\dot{p}_{Xm}\}L^2$, where $\{\dot{p}_{Xm}\}$ (J d^{-1} cm^{-2}) is the maximum surface area specific ingestion rate. The conversion efficiency of food to reserve (digestion efficiency), κ_X, is specific to each type of food. Finally, $\dot{p}_A = \kappa_X \dot{p}_X$. Digestion efficiency is calculated using the following relationship:

$$\kappa_X = \{\dot{p}_{Am}\} / \{\dot{p}_{Xm}\} \quad (1)$$

where $\{\dot{p}_{Am}\}$(J d^{-1} cm^{-2}) is the maximum surface-area specific assimilation rate and a model parameter (see Table 1).

Growth is defined as the increase of structure. Energy allocated to growth $\dot{p}_G$ is a fixed fraction of mobilized reserve $\kappa\dot{p}_C$ after subtraction of somatic maintenance costs $\dot{p}_M = [\dot{p}_M]V$ (e.g. maintaining intra-cellular concentration, protein turnover, movement), with $[\dot{p}_M]$ (J d^{-1} cm^{-3}) the volume-specific maintenance costs. The cost of the synthesis of a unit of structure is called $[E_G]$ (J cm^{-2}) which indirectly defines growth efficiency κ_G being the ratio of energy fixed and invested in new structure (see Table A.3, Online Appendix A).

Allocation to maturation in juveniles is redirected to reproduction in adults ($\dot{p}_R$) at puberty which occurs at $E_H = E_H^p$. Reserve allocated to reproduction is first accumulated in a buffer which is emptied at spawning. The conversion of reserve to egg (embryo reserve) occurs with efficiency κ_R. The value of 0.95 in Table 1 has been chosen in view of the absence of substantial chemical work. Allocation to growth and somatic maintenance occurs in parallel to allocation to maturation and reproduction (see Fig. 1). The embryo starts its development with zero structure and maturity, and an amount of reserve such that the reserve density at birth equals that of the mother at egg formation. The latter condition is a maternal effect Kooijman (2009).

The standard DEB model assumes that the individual is isomorphic, i.e. it does not change in shape during growth, which makes that surface area is proportional to volume to the power 2/3. Motivated by studies on Anchovy, *Engraulis encrasicolus*, and Bluefin Tuna, *Thunnus orientalis*, (Pecquerie, 2007; Jusup et al., in preparation), we implemented the possibility that development accelerates after birth ($E_H = E_H^b$) by including a V1-morphic stage (surface area grows proportional to volume) for the larva (early juvenile) till maturity reaches a threshold level for metamorphosis ($E_H = E_H^j$), after which growth resumes in an isomorphic fashion (Kooijman et al., 2011). If $E_H^j = E_H^b$ there is no acceleration, but if $E_H^j > E_H^b$ both $\{\dot{p}_{Am}\}$ and energy

Table 1
State variables and primary parameters (affecting changes of state variables at 20 °C) of the zebrafish DEB model, and other parameters (see text).

Symbol	Value	Unit	Name
State variables			
E	–	J	Reserve
V	–	cm^3	Structure
L	–	cm	Structural length $V^{1/3}$
E_H	–	J	Cumulated energy invested in maturity up till puberty and reproduction after puberty
Primary energy parameters			
$\{\dot{p}_{Am}\}$	246.3	Jd^{-1}cm^{-2}	Embryo maximum surface area specific assimilation rate
$\dot{v}$	0.0278	cmd^{-1}	Embryo energy conductance
κ	0.437	–	A specific fraction of energy mobilized from reserve allocated to growth and somatic maintenance
κ_X	0.5	–	Digestion efficiency for Tetramin™
κ_R	0.95	–	Reproduction efficiency
$[\dot{p}_M]$	500.9	Jd^{-1}cm^{-3}	Volume specific somatic maintenance costs
$\dot{k}_J$	0.0166	d^{-1}	Maturity maintenance rate
$[E_G]$	4652	Jcm^{-3}	Cost of synthesis of a unit of structure
E_H^b	0.54	J	Cumulated energy invested in maturity at birth
E_H^j	19.66	J	Cumulated energy invested in maturity at metamorphosis
E_H^p	2062	J	Cumulated energy invested in maturity at puberty
Other parameters			
T_A	3000	K	Arrhenius temperature
$\dot{h}a$	1.96 10^{-9}	d^{-2}	Weibull aging acceleration
s_G	0.0405	–	Gombertz stress coefficient
δ_Y	0.1325	–	Shape coefficient for embryos
δ_M	0.1054	–	Shape coefficient juveniles and adults for total length

conductance $\dot{v}$ (cm d^{-1}) increase with length. $\dot{v}$ controls reserve mobilization.

Auxiliary theory assumes that the shape coefficient δ, i.e. the ratio of structural length (L) and physical observed length L_{obs}, is constant for isomorphs. Shape changes during the early juvenile (V1-morphic) period are described by the empirical function $\delta(L) = \delta_M + (\delta_Y - \delta_M)\frac{L_j - L}{L_j - L_b}$ for $L \in [L_b, L_j]$ with δ_Y and δ_M shape coefficients for embryos and adults respectively, L_b structural length at birth and L_j structural length at metamorphosis.

The effect of temperature on all biological rates is well captured by the Arrhenius relationship (Kooijman, 2010), quantified by the Arrhenius temperature T_A. This relationship only holds within a particular temperature tolerance range.

DEB theory considers development and senescence to be parallel processes. The aging module of DEB theory (Kooijman, 2010; van Leeuwen et al., 2010) specifies that the induction of damage inducing compounds (e.g. modified mitochondrial DNA) is proportional to the mobilization rate of reserve, which is (about) proportional to the use of dioxygen (linking to free radicals) that is not associated with assimilation. Damage inducing compounds can also induce themselves at a rate that is proportional to their concentration and, again, to the mobilization rate of reserve as quantifier for metabolic activity. Damage inducing compounds induce damage compounds (e.g. modified proteins) which accumulate in the body. The hazard rate due to aging is taken proportional to the density of damage compounds. This specifies the aging module, and how aging depends on energetics, and so on the nutritional status of the organism.

3. Materials and methods

3.1. Caloric restriction experiment

We designed a caloric restriction experiment to obtain quantitative data on growth in combination with reproduction at 3 ingestion levels for individual fish.

2 cm (SL) fish were acquired from a commercial fish breeder (Elevage de la grande rivière, Lyon, France) and were 116 days post-fertilization (dpf) upon arrival. Based on this size and age we estimated the mean intake level f_{cult}. Animals were kept in soft water: T = 26 ± 0.6 °C, pH = 6.5 ± 0.4, electrical conductivity 200 μS cm^{-1}. Food given was Tetramin™ (proteins, 46%, lipids 7.0%, ash 10.0%, cellulose 2.0% and moisture 8.0%). We calculated the energetic content of Tetramin as 19.1 kJ g^{-1} with 17.2 and 38.9 kJ g^{-1} for the lipids and proteins respectively (Kooijman, 2010, Table 4.2). This result is used to obtain κ_X (see Eq. (1)) for individuals during acclimatization (116 and 132 dpf), where they were fed ad libitum $f_{pretreat}$. Caloric restriction took place between 132 and 214 dpf.

We started with 6, 4.5 and 3 Tetramin per day per individual fish for the first (f_1), second (f_2) and third (f_3) ingestion level respectively. f_1 and f_2 were gradually raised to 10 Tetramin d^{-1} while f_3 remained constant during the caloric restriction phase, in accordance with the expectation that feeding rate is proportional to squared length. The daily ration was hand weighed in aluminum micro weighing dishes (VWR) with an SE2 ultra-microbalance (Sartorius AG, Göttingen, Germany) and dispensed 2 to 3 times throughout the day. Individuals fasted one day per week. The experimental system is fully characterized in the Online Appendix D. We kept 20 individuals per condition. Reproduction was assessed by forming couples (1:1 male to female ratio) in the evening and counting the number of eggs spawned the following morning. We followed the daily egg output of individual females over two successive breeding trials which lasted 22 and 15 days respectively.

To check the condition of the gonads three males and three females in each condition were sacrificed at the end of the experiment. Gonads were removed and immersed in 2.5% glutaraldehyde sodium cacodylate buffer (0.1 M, pH 7.4) for 24 h at 4 °C then post fixed with 1% osmium tetroxyde for 1 h. The samples were dehydrated through a graded ethanol series and finally embedded in monomeric resin Epon 812. Semi-thin sections for light microscopy analysis (500 nm) were obtained with an ultramicrotome UCT (Leica Microsystems GmbH, Wetzlar, Germany). Plastic sections were stained with aqueous blue toluidine and gonad structure was examined under a light microscope (Leica, DM750) equipped with a Leica camera ICC50 and LAS EZ Software. For each replicate, at least 20 micrographs of local detailed structures were taken, analyzed and compared.

3.2. Data and parameter estimation

We incorporated real and pseudo data into the parameter estimation routine. Real data, compiled from the literature and the caloric restriction experiment, include observed lengths, weights, reproduction, and survival at single (0-variate) or multiple (1-variate) time and/or temperatures points. Pseudo data represent the more conserved aspects of animal metabolism; large deviations from these data are considered to be less likely. The pseudo data concern: $\dot{v} = 0.02$ cm d^{-1}, $[\dot{p}_M] = 18$ J d^{-1} cm^{-3}, $\kappa = 0.8$, $\kappa_G = 0.8$ and $\dot{k}_J = 0.002$ d^{-1} (see Kooijman, 2010, pp.300). The chemical indices (c-mol per c-mol), molar weights (g mol^{-1}), and chemical potentials (J mol^{-1}) and are as given in Lika et al. (2011) (Table A.1, Online Appendix A). The densities d_{Vd} and d_{Ed} (g cm^{-3}) for structure and reserve were derived from Craig and Fletcher (1984) (Table A.1, Online Appendix A).

We allowed expectations to deviate from these values by giving them less weight relative to real data. We converted all rates and ages to a reference temperature of 2 °C. We measured the ratio of standard length SL (tip of snout till base of caudal fin) and total length TL (tip of snout to end of caudal fin) of 70 adult zebrafish (Adam-Guillermin unpublished 2009) and found an average ratio of 0.8. DEB model predictions are given in TL so when necessary the predictions are corrected to standard length SL. We treated forked length FL (tip of snout till fork in the caudal fin) equal to TL.

We applied the method of covariation for parameter estimation (Lika et al., 2011) using the freely downloadable software DEBtool (Kooijman et al., 2008). This software uses the simplex (Nelder-Mead) method to simultaneously minimize the weighted sum of squared deviations between model predictions and observations for a considerable number of data sets. Computations were performed with Matlab© (version 7.9.0.529). DEBtool routines and formula for calculating predictions for data in Table 2 can be found in Table A.3

Table 2
Model predictions for 0-variate data are compared with observations.

Data	Observations	Predictions	Unit	Reference
Size at birth	0.39	0.40	cm	(Schilling, 2002)
Maximum length (TL)	5.00	5.10	cm	(Spence et al., 2008; Schilling, 2002)
Maximum reproduction rate	60–240	113.6	# eggs d^{-1}	(Eaton and Farley, 1974a) and Geffroy (Unpublished 2009)
Egg dry mass	30–106	73		Augustine (Unpublished 2010)
Egg diameter	0.08–0.09	0.10	cm	(Uusi-Heikkila et al., 2010)
Maximum wet weight	1	0.99	g	Pers. Obs

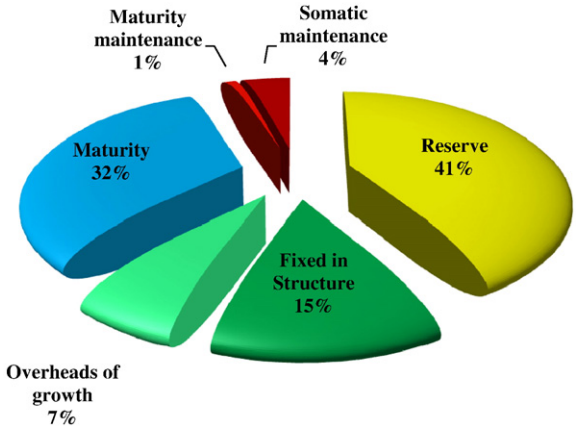

Fig. 2. Energy budget at birth when the mother was at abundant food. Initial reserve in an egg (E_0) is 1.670 J while structure and maturity are zero. Slices represent the cumulated energy investments at birth relative to E_0. Maturity (cumulated energy invested in maturity) as well as maturity and somatic maintenance are dissipated in the environment as minerals (e.g. CO_2). A fraction $1-\kappa_G$ of energy invested in structure is lost as overheads of growth. The reserve and structure contribute to the actual mass of the organism at birth.

(Online Appendix A). Without information on mass and caloric content of food ingested per day we could not calculate the digestion efficiency of each food type described in the data from the literature. We make the assumption that while diets differ between experiments they are similar enough for us to apply a scaled functional response $0 \le f \le 1$ for all data. f is a free parameter and is estimated for each data set. Although we could suppose constant food density and temperature for most data (Section A.1, Online Appendix A), a dynamic formulation of the model was needed to predict growth and reproduction in the caloric restriction experiment (Section A.2, Online Appendix A).

4. Results

The conversion efficiency of Tetramin™ to reserve was found to be $\kappa_X = 0.5$. Parameter estimates (Table 1) show a factor 40 difference between maturity level at metamorphosis and at birth (E_H^j and E_H^b) which translates into a factor 3 difference in structural lengths (L_j and L_b) at abundant food. So specific assimilation and energy conductance also increase by a factor 3 during the early juvenile period. Drain to maturity maintenance amounts to 34.27 in adults and only a fraction $1-\kappa = 0.56$ of mobilized reserve is allocated to maturity maintenance plus reproduction. This implies that puberty is not reached for ingestion levels lower than $f = 0.6$. We were surprised that the lowest intake level for reproduction is that high. The value for κ is very close to the one which maximizes the ultimate reproduction rate (0.48) given the values of the other parameters. The Add_My_Pet collection typically shows values around 0.8 for which reproduction is far below the maximum possible one (Lika et al., 2011). Some 44% of the initial energy in an egg is lost by the end of the embryonic period (birth), see Fig. 2, by mineralization of reserve. Although maintenance is relatively high (see the Add_My_Pet collection) the cumulative amount spent at birth is small due to high costs for growth and maturation. Energy conductance is typical for embryos and relatively high for adults due to the acceleration during the early juvenile period.

Compiled growth curves (Fig. 3) are confronted with DEB predictions and we conclude that general growth patterns are accurately predicted by DEB theory for ingestion levels ranging from

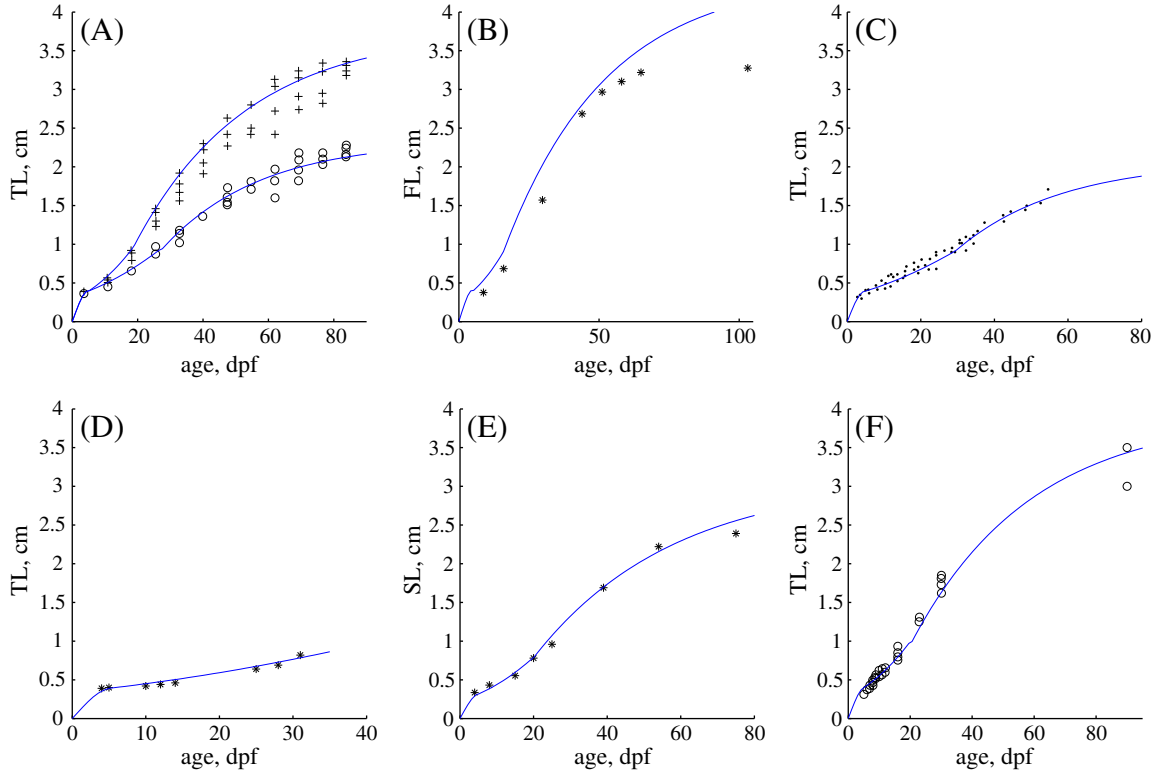

Fig. 3. Synthesis of model predictions against observed lengths of fish from different published studies. Crosses or circles: observations; full lines: model predictions; TL: Total Length (snout till end of caudal fin); FL: Forked Length (snout till fork in caudal fin); and SL: Standard Length (snout till base of caudal fin). For each data set we specify temperature and estimated ingestion level f ($0 \le f \le 1$). (A) Data from (Lawrence et al., 2008) and pers. comm.. $T = 28.5$ °C; (+) individuals fed approximately twice as much as (o). $f_1 = 0.75$ for high ingestion level and $f_2 = 0.65 f_1$ for the lower ingestion level. (B) Data from (Gómez-Requeni et al., 2010). $T = 28$ °C; $f = 0.88$. (C) Data from (Schilling, 2002); $T = 28.5$ °C; $f = 0.44$. (D) Data from (Bagatto et al., 2001). $T = 25$ °C; $f = 0.4$. The type of length measurement is unknown but model predictions are in TL. (E) Data from (Eaton and Farley, 1974a). $T = 25.5$ °C; $f = 0.77$. (F) Data from (Best et al., 2010) but we present the full growth curve provided through pers. comm. with C. Lawrence. $T = 25$ °C; $f = 0.78$.

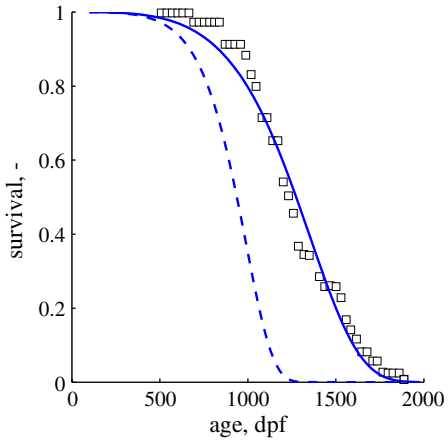

Fig. 4. Survival probability in relation to nutritional status as predicted by the DEB aging module. (□) data from (Gerhard et al., 2002) outbred tank 2; solid line: model prediction for $f=0.8$. Dashed line: predicted survival for $f=1$. T=26 °C.

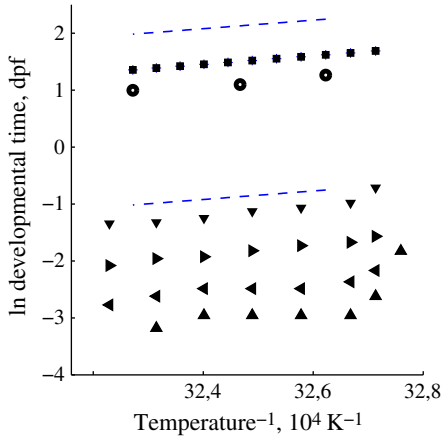

Fig. 5. Ln developmental age as a function of inverse temperature. Model predictions for birth (■) and data: protruding mouth stage (O, from (Kimmel et al., 1995)), 8 cell stage (▲), late cleavage (◄), very late blastula (►), and blastoderme 3/4 yolk sphere (▼), all from (Shirone and Gross, 1968). The slope of the dashed lines gives an Arrhenius temperature of 3000 K.

0.4 in Bagatto et al. (2001) to 0.88 in Gómez-Requeni et al. (2010). Predicted egg dry mass ranges from 58 to 73 ($f=0.5$ to $f=1$). Maximum predicted wet weight for a fish is 0.99 g.

Age dependent survival was correctly predicted (see Fig. 4 and Section A.3, Online Appendix A for equations). Lifespan is expected to decrease for increasing food. The ultimate size of the fish of around 3.5 cm SL (Gerhard et al., 2002, pers. comm.) suggests that $f\approx 0.8$. Fig. 4 also presents model predictions for abundant food.

Predicted length at birth (4 mm at abundant food Table 2) is not very sensitive to the nutritional status of the mother which is in line with both Parichy et al. (2009) (developmental milestone pSB+: inflation of posterior swim bladder, head showing anterior mouth position) and Schilling (2002) (length at first feeding). DEB theory predicts that age at birth also depends on ingestion level of the mother (maternal effect). However the effect is minimal. According to Kimmel et al. (1995) 'birth' (active feeding behavior) is around 5 dpf at 28.5 °C. DEB theory predicts birth in the range of 4.3 to 4.7 dpf ($f=1$ to $f=0.5$) at 28.5 °C and in the range of 4.8 to 5.3 dpf ($f=1$ to $f=0.5$) at 25 °C.

The Arrhenius relationship for the log of developmental age against inverse temperature should be linear. Fig. 5 confirms this for different developmental milestones and also presents model predictions for age at birth. We see (a) that the estimated Arrhenius temperature of 3000 K (which corresponds graphically to the regression slope) is coherent with observations and (b) that birth occurs just after the protruding mouth stage described by Kimmel et al. (1995). Typical Arrhenius temperatures revolve around 8000 K (Add_My_Pet) making zebrafish less sensitive to temperature variations.

For $f=0.77$ (Fig. 3, E) first spawns occurred at 71–75 dpf for females between 2.4 and 2.6 cm SL at 25.5 °C (Eaton and Farley, 1974b). Predicted age and length at puberty for this data set is 88 dpf and 2.7 cm SL. Not only these data but also the complete growth curve is in remarkable agreement.

This agreement also applies to the growth and reproduction in our caloric restriction experiment (Fig. 6) ($f_{cult}=0.55$, $f_{pretreat}=0.9$, $f_1=0.74$ and $f_2=0.69$). Predicted ages at puberty are 160 dpf and 165.5 dpf and predicted reproduction rates are 19.84 and 13.01 eggs per day for f_1 and f_2 respectively at 26 °C. Maximum predicted reproduction rate (Table 2) at 26 °C at abundant food is 113.6 eggs per day. This value represents the maximum daily energy invested in reproduction by the largest possible individual ($L=L_m$) at highest ingestion level ($f=1$).

Length at puberty ranges from 2.9 ($f=0.6$) to 3.8 ($f=1$) cm TL (2.3 − 3 cm SL) and age at puberty ranges from 178.8 to 59.18 dpf at 28 °C. Contrary to age and length maturity levels at various developmental milestones are independent of temperature and nutritional status, see Tables 3 and 4. Maturity levels are computed using parameters values in Table 1. Standardized Standard Lengths (SSL) as

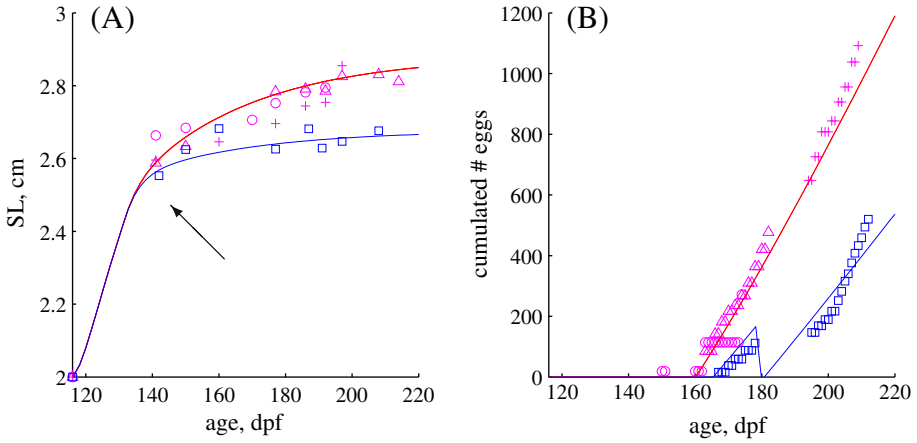

Fig. 6. Observations, the symbols refer to the different individuals, and model predictions, solid lines, for growth (A) and reproduction (B) during 82 days of caloric restriction at two feeding levels which are estimated at $f_1=0.74$ and $f_2=0.69$. Animals are 116 days post-fertilization (dpf) and 2 cm standard length SL (tip of snout till base of caudal fin) at arrival. They are acclimated to laboratory conditions for two weeks. Caloric restriction is initiated at 132 dpf (arrow).

Table 3
Cumulated energy invested in maturation, E_H (mJ) for the embryonic developmental milestones. Developmental stages and ages (28.5 °C) are as defined by (Kimmel et al., 1995). Ages are presented in hours post-fertilization, hpf.

Stage	Age, hpf	E_H, (mJ)
2-cell	0.75	0.01
4-cell	1	0.02
8-cell	1.25	0.02
16-cell	1.5	0.02
32-cell	1.75	0.02
64-cell	2	0.03
128-cell	2.25	0.03
256-cell	2.5	0.04
512-cell	2.75	0.05
1 k-cell	3	0.07
High	3.33	0.088
Oblong	3.66	0.11
Sphere	4	0.14
Dome	4.33	0.171
30%-epiboly	4.66	0.20
50%-epiboly	5.25	0.27
Germ-ring	5.66	0.33
Shield	6	0.38
75%-epiboly	8	0.71
90%-epiboly	9	0.96
Bud	10	1.3
3-somite	11	1.7
6-somite	12	2.1
14-somite	16	4.6
21-somite	19.5	8.0
26-somite	22	11.2
Prim-6	25	16.0
Prim-16	31	29.5
Prim-22	35	41.4
High-pec	42	68.9
Long-pec	48	99.6
Pec-fin	60	180
Protruding-mouth	72	280

given by (Parichy et al., 2009) are converted to total lengths using their photographs for each milestone. The contribution of the caudal fin changes during development from 0.06 at birth to 0.2 at puberty. The estimated ingestion level is based on length at puberty in their staging atlas. We assume abundant food (of mother) for embryonic development in Table 3.

Microscopic analysis of gonads revealed a remarkable effect of ingestion level on male gonads (Fig. 7): f_3 gonads were small empty shriveled versions of healthy male testes such as those observed in f_1 and f_2. The analysis of female gonads was not conclusive.

Table 4
Maturity levels E_H for post embryonic developmental milestones. Standard length SL (tip of snout till base of caudal fin), developmental stages and names as given in (Parichy et al., 2009). Ages, in days post-fertilization (dpf), at $f=0.63$ and $f=1$ as well as SL at $f=1$ are model predictions at 28.5 °C.

Stage	Age, dpf	SL,[a] cm	Age, dpf	SL, cm	E_H(J)
	$f=0.63$		$f=1$		
pSB + swim bladder inflation[b]	4.5	3.8	4.0	3.7	0.5
Fle early flexion	7.2	4.5	6.3	4.6	1.1
CR caudal fin ray	8.9	4.9	7.4	5.1	1.6
AC anal fin condensation	10.5	5.4	8.5	5.9	2.3
DC dorsal fin condensation	12.3	5.7	9.6	5.8	3.3
MMA metamorphic melanophore appearance	12.9	5.9	10.0	6.0	3.8
AR anal fin ray appearance	14.2	6.2	10.9	6.3	5
DR dorsal fin ray appearance	15.0	6.4	11.4	6.5	5.9
PB + following pelvic fin bud appearance	18.7	7.6	13.8	7.7	12.7
PR pelvic fin ray appearance[c]	21.3	8.5	15.5	8.5	21.9
PR + following pelvic fin ray appearance	22.5	9.2	16.3	9.3	27.9
SP onset of posterior squamation	23.8	9.8	17.2	9.8	34.9
SA onset of anterior squamation	25.0	10.4	17.9	10.5	42.3
J juvenile	26.2	11.0	18.7	11.0	50.9
J + following juvenile	30.8	13.0	21.6	13.2	91.7
J++ following juvenile	40.7	16.0	27.5	16.4	221.6
A adults[d]	218	26.0	59.9	30.6	2061

[a] Standardized Standard Lengths as given in (Parichy et al., 2009).
[b] Birth are defined by the DEB model.
[c] Maturity level just above metamorphosis as defined by the DEB model.
[d] Puberty as defined in the DEB model.

5. Discussion

Reports of deviating growth patterns can be found in the literature which we briefly discuss here. Gómez-Requeni et al. (2010) observed growth arrest after 65 dpf, an age at puberty of around 50 dpf and suggest that growth ceases during reproduction. This interpretation is at odds with other data (Barrionuevo and Burggren, 1999; Lawrence et al., 2008; Barrionuevo et al., 2010; Best et al., 2010) and our own experimental results; growth continues during reproduction at rates increasing with food level. Based on their growth data we estimate $f=0.88$ with an ultimate length of 4 cm TL and an age at puberty of 67 dpf. Zebrafish growth was found to be indeterminate Gerhard et al. (2002). Accordingly, we found that length still increased by a factor 1.3 after puberty at abundant food.

Eaton and Farley (1974a); Schilling (2002); Best et al. (2010); Gómez-Requeni et al. (2010) observe juvenile growth that is consistent with our model predictions. Barrionuevo and Burggren (1999); Barrionuevo et al. (2010) however, observed growth arrest for about a month after birth (≈4 mm). They only started to feed the larvae when the yolk sac is completely absorbed, when zebrafish can begin actively feeding as soon the esophagus opens and some of the yolk sac still remains (Lawrence, 2007; Best et al., 2010). Delaying initial feeding beyond this point impacts subsequent growth and survival (Guillaume et al., 1999). Except for a slow start, lengths and wet weight curves of Barrionuevo and Burggren (1999); Barrionuevo et al. (2010) are in agreement with our model predictions. During parameter estimation we assumed that all food types were equivalent. Practice teaches that food quality is an important parameter (Gergs and Rothhaupt, 2008) and it might contribute to deviation from general patterns. Moreover the model does not take digestion into account (i.e. gut residence time). The model can be extended to

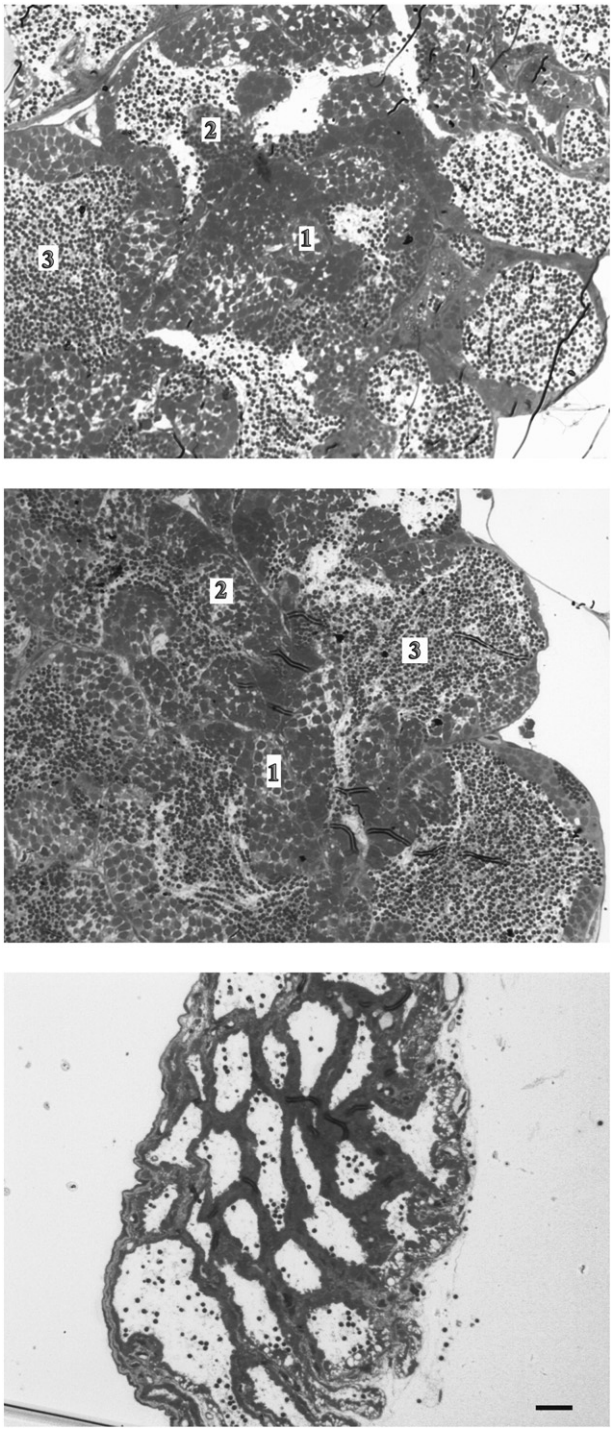

Fig. 7. Microphotographs of male gonads after 82 days of caloric restriction for the ingestions levels f_1 (top), f_2 (middle) and f_3 (bottom). Scale bar: 20; 1: cysts with secondary spermatogonia; 2: cysts with spermatocytes; and 3: cysts with spermatids or spermatozoa. At f_3 structures are shriveled.

capture this aspect of physiology for certain applications (e.g. Evers and Kooijman, 1989).

If well fed individuals of sufficient size are exposed to $f=0.44$ computer simulations suggest that they should reproduce while growth is slightly negative. Pecquerie et al. (2009) consider that starved female Anchovy pay for somatic maintenance ($[\dot{p}_M]$) by reabsorbing energy from the reproduction buffer. This is confirmed by our observations of male gonads. We implemented this behavior in our simulation studies and growth will not become negative in the case of mild starvation.

Reproductive patterns are found highly variable compared to growth. Eaton and Farley (1974b) report a maximum of 60.4 eggs per day (25.5 °C, 497 dpf) over a period of 105 days, Hisaoka and Firlit (1962) observe 37.9 eggs per day over a period of 146 days (26 °C) and Geffroy (unpublished data 2009) found 240 eggs per day over a 10 day period trial for zebrafish fed 5% their body weight (25 °C). Our study shows that the simultaneous observation of food intake, body size and reproductive output provides insight into the degree/nature of this variability. We present such information for individual zebrafish taken from the caloric restriction experiment (Fig. 6) and find results consistent with growth and first spawnings in Eaton and Farley (1974a). The detailed rules for spawning are part of the behavioral repertoire that can be rather stochastic and differ between individuals (Eaton and Farley, 1974b; Gerlach, 2006; Spence and Smith, 2006) calling for advanced statistical methods to detect metabolic perturbations (Paull et al., 2008). The reproduction buffer can contribute up to 15% of adult weight (Forbes et al., 2010). Reproduction increases with size, consistent with the finding of Uusi-Heikkila et al. (2010), and food availability. Our results show that a large variability of the reproduction buffer handling rules can combine with a low variability of the reproduction rate.

Our assumption of a maternal effect is consistent with Brown (1958); Guillaume et al. (1999). It is not sure that this applies equally well to zebrafish. Forbes et al. (2010) suggest that egg size is decided during oocyte maturation and observe that higher food intake comes with the production of smaller eggs of poorer quality.

We measured egg dry mass of mature females (>364 dpf, 26 °C, ad libitum feeding) for each spawning event during a 2 month reproduction trial and found values ranging from 30 to 105 (Augustine unpublished 2010). This large variation makes it difficult to judge the model assumption on the maternal effect. Future extensions of the model might include this variation.

The data did not allow us to study the effect of temperature outside the tolerance range as has been done for marine fish species (Freitas et al., 2010). We suggest that for ecological purposes such a study should be considered. Although our model correctly predicts survival patterns at a single ingestion level, predictions at other levels need confirmation. Reproduction might be impacted by age (Tsai et al., 2007), but more quantitative data is needed for modeling. Gold fish, *Carassius aureatus*, for example have maximum fertility at 3 years, become sterile by 7 and can live as long as 40 (Brown, 1958, pp.370). Effects of toxicants can be captured by linking parameter values to internal concentrations. This can also be done for density of damage compounds to model post reproductive periods (Jager and Klok, 2010; Kooijman, 2010).

We found that puberty, defined as the onset of allocation to reproduction, occurs above $f=0.6$. Lawrence et al. (2008) reported the appearance of secondary sexual characteristics after 4 months while we estimate an ingestion level of $f=0.49$ for some of the fish. Additionally, Schilling (2002) reported such characteristics at TL = 2–3 cm which is 20% lower than predicted. Secondary sexual characteristics might occur at a lower maturity level than for puberty; first spawning events (Eaton and Farley, 1974b) must occur later. The various events might be difficult to recognize accurately which can contribute to the discrepancies.

Even after correcting for effects of temperature, we demonstrated that developmental state at a particular age depends on feeding history. To a lesser extent developmental state at a particular size also depends on feeding history: better fed individuals reach puberty faster and at a larger size. This is in agreement with the observations of Parichy et al. (2009). The impact of food history accumulates over time (see Table 4) and can become irreversible (Kooijman et al., 2011).

Variations in development play an important role in evolutionary theory (McKinney and McNamara, 1991). To study heterochrony it is necessary to distinguish between the "time" spent in a particular stage of development and the rate of growth within it. For instance, embryonic growth between 0.6 and 1.3 dpf (13% of the embryonic

period) is in the order of 3 mm d^{-1} (Kimmel et al., 1995) whereas growth rate between 18 and 40 dpf in a high and low food regime is roughly 0.6 and 0.35 mm d^{-1} (Lawrence et al., 2008) and in all cases growth rate continues to decrease with age (under constant ingestion level). The lower area-specific maximum assimilation and reserve mobilization rates in an embryo can combine with the higher growth rate because volume linked somatic maintenance, which always takes precedence over growth in DEB theory, are very small for embryos relative to juveniles and adults (Kooijman et al., 2011). DEB theory offers the framework for changes in maturity throughout the life cycle that is consistent with observations. The use of reserve, being a good quantifier for metabolic activity, not directly translates in changes in maturity and/or size.

Reiss (1989) proposed 7 criteria which should be respected by a metric for developmental time: (1) independent of morphology, (2) independent of body size, (3) depend on one a priori homologous event, (4) unaffected by changes in temperature, (5) similar between closely related species, (6) increase with clock time, and (7) physically quantifiable.

The maturity concept of DEB theory complies to all criteria. Parameter values are individual specific. Intra-species variations are small relative to inter-species variations and parameter values of similar species are similar (Kooijman, 2010). In the particular case that maturity maintenance $\dot{k}_J E_H$ is no longer be paid (starvation) rejuvenation can occur as has been observed in krill *Euphausia superba* (Thomas and Ikeda, 1987).

We show in this study that maturity is indirectly physically quantifiable see Tables 3 and 4.

"Without a metric for developmental time the extent and meaning of evolutionary change in developmental timing simply cannot be assessed" (Reiss, 1989). We cannot agree more and suggest that maturity is a good candidate for such a metric.

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