The zebrafish: a new vertebrate model for ageing and systems biology approach*.

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INTRODUCTION

Advances in the understanding of the relationship between genotype and phenotype during the human lifetime depend on a considerable extent on the availability of relevant and appropriate experimental animal models. Each animal model of human ageing and age related diseases has its advantages and limitations, since no single model exactly reflects the age-related processes that occur in humans. The zebrafish (*Danio rerio*) is a small vertebrate that has been long-appreciated as a biological model for developmental biology (Weis, 1968), toxicology (Hisaoka, 1958) and genetic (Kimmel, 1989) studies, but their characteristics make it also an appropriate model for studying ageing (Gerhard et al, 2002; Kishi *et al*, 2003; Keller&Murtha, 2004). Due to the vast developmental biology knowledge already available on this species, the zebrafish is specifically very well suited to study the early developmental events that could have effect later in life (Kishi *et al*, 2009).

Systems biology is a multidisciplinary approach that could be defined as "the study of interactions between the components of a biological system" (Kirkwood, 2008) and it is characterized by the synergistic integration of theory, computational mathematical modelling and experimentation (Kitano, 2002). This approach can provide an efficient analytical framework to better understand all the molecular, genetic and physiological mechanisms influencing ageing, and at the same time it could help to accelerate the sharing, integration and analysis of the data obtained through different research approaches.

As a first step in a systems biology approach on using zebrafish in ageing research, we present a new mathematical model of relevant age-dependant biological processes (growth, reproduction and mortality) in zebrafish, aimed to provide a flexible framework to facilitate the understanding of the age-related mechanisms in this species and its translation to humans.

METHODS

The model has been implemented through a set of nonlinear difference equations which can be solved numerically by iterative methods. The mathematical functions employed to describe the relevant biological processes associated to zebrafish development, growth, reproduction and mortality (metabolic rate, food intake, growth rate, body fat content, reproductive effort and mortality rate) were obtained by analyzing published and/or ZF Biolabs zebrafish selected data from TU, WIK, AB and "wild type" strains, using linear and nonlinear regression analyses.

To develop the model we have followed a "top-down" biological modelling approach starting from the whole system level and then going down into detail as needed. When possible we have selected the mathematical equations based on theoretical biological considerations (Guinea, 1989). Parameter calibration, model validation and performance assessment of the validated model were performed by the use of suitable statistical techniques.

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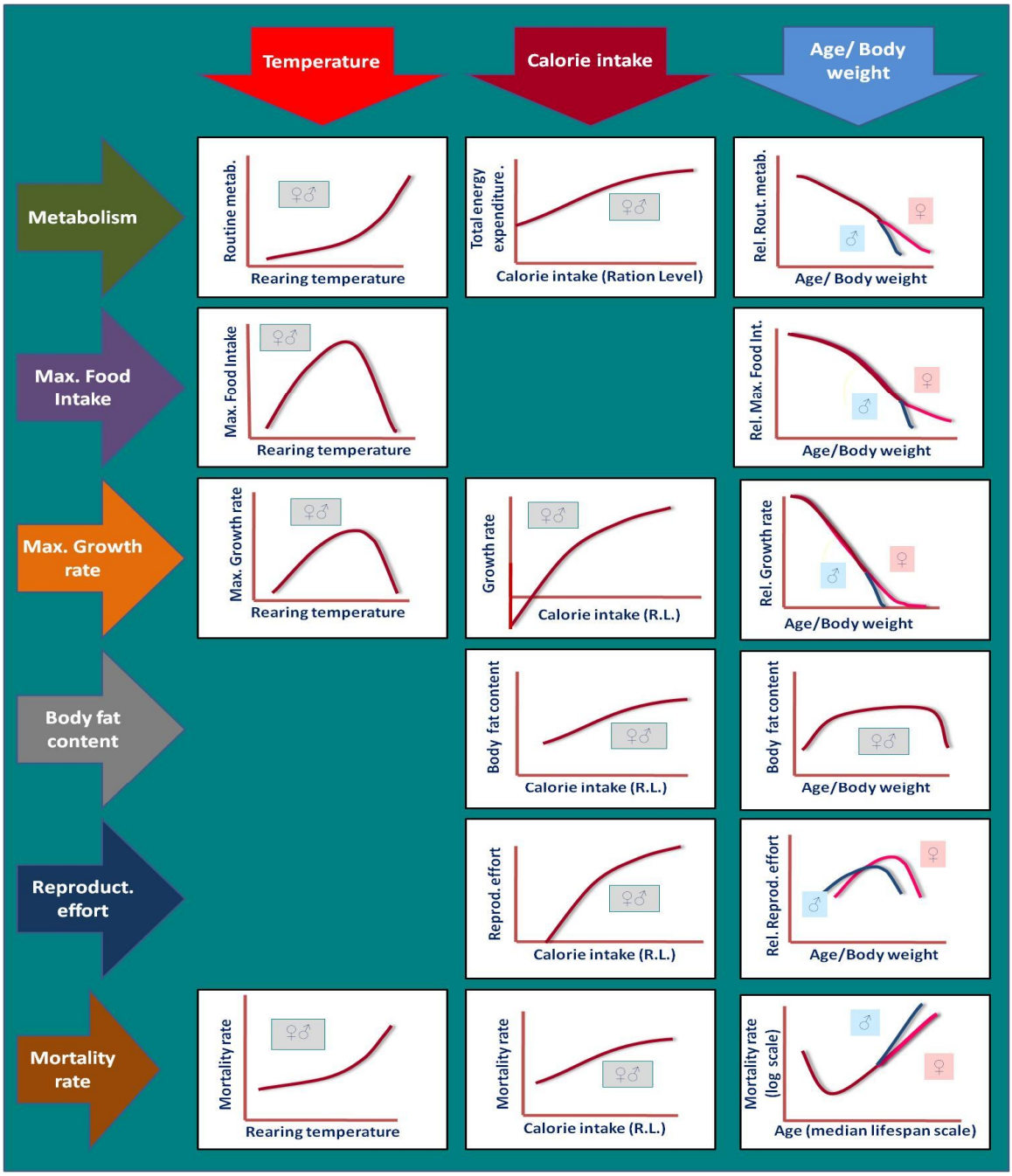
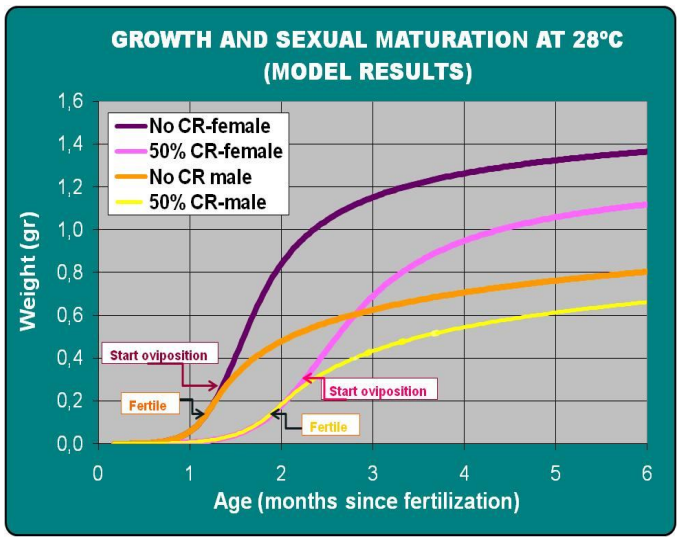
RESULTS

We have developed a discrete-time mathematical model where the zebrafish biological metabolic rate (routine and total), maximum food intake, growth rate, body fat content, reproductive effort and age specific mortality rate are being considered dependant variables of rearing temperature, calorie intake/ration level, age/body weight and sex (independant variables). Early life development (embryonic and eleutheroembryo phases) has also been included as a specific submodel within the main model (details are not given here). The flow-chart of the model is indicated in the following diagram:

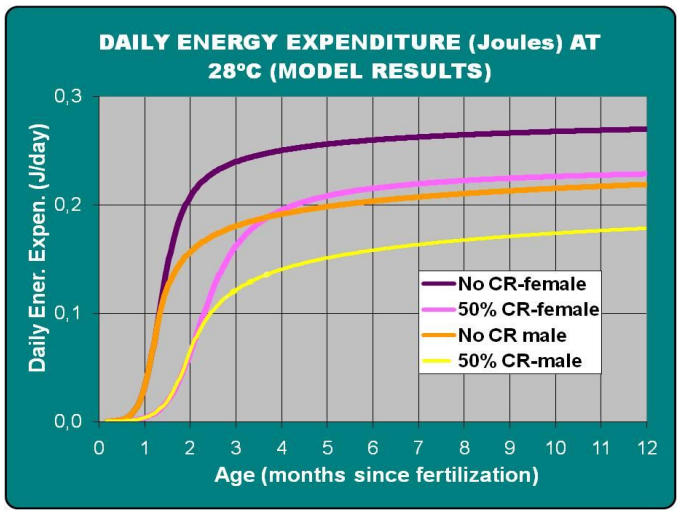


FLOW-CHART OF THE MODEL

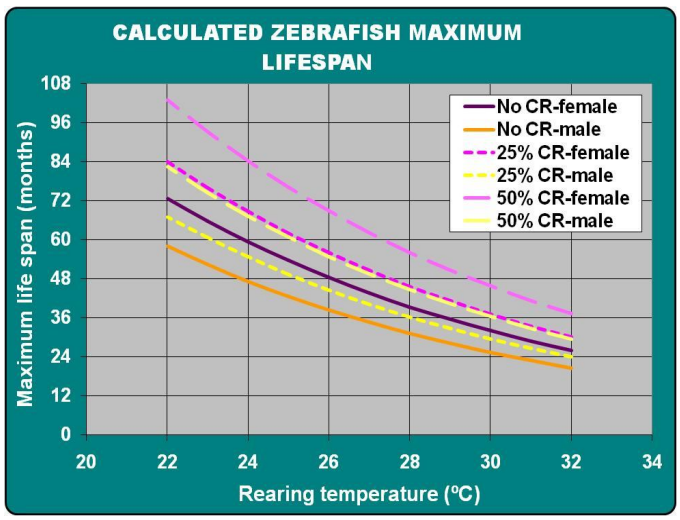
The model could anticipate different growth/reproduction/mortality trajectories for zebrafish by varying the rearing temperature and the calorie intake/ration level. For example in the following chart we show the Calorie Restriction (CR) effect on the growth and sexual maturation of zebrafish reared at 28ºC. The model indicates that zebrafish fed without restriction during the sensitive larval and juvenile periods, grow very fast and start oviposition (females) and produce fertile sperm (males) at very early age (35 and 43 days postfertilization respectively) in comparison with restricted fed zebrafish (50% CR) which starts around a month later. These results are in good agreement with experimental data (Van der Meulen, 2005; Guinea *et al*, unpublished results).



The zebrafish metabolism is also affected by the rearing temperature and the calorie intake/ration level. The results obtained from the model for the first year of age at rearing temperature of 28ºC are shown. Daily energy expenditure increases dramatically during the first weeks of age following the almost exponentially growth in weight during this period. Afterwards 50% of calorie limited adult zebrafish shows a metabolic rate that is 18% depressed in comparison to *ad libitum* feeding. Those results are also in good agreement with experimental data (Lucas&Priede (1992); Guinea *et al*, unpublished results).



Expected maximum lifespan (defined as 10% survival) in laboratory conditions at different rearing temperatures, for *ad libitum* and two calorie restriction levels (25% and 50%) and for both male and female is presented. Although only very few experimental data is available on this issue (Gerhard *et al*, 2002; Kishi *et al*, 2008) we believe that the model data is fairly consistent with those observations after estimating the CR level from the reported growth data.



The model is not intended to be static but, rather, to evolve as it has been designed with the objective to integrate new knowledge coming from genomic, epigenetic studies or other approaches by adding new equations and variables. Furthermore, although the model is deterministic, stochastic modelling could be performed by the introduction of random components, which could add new perspectives to the model.

CONCLUSIONS

The zebrafish has recently attracted attention as a new versatile model for ageing. The conservation of developmental genes across vertebrates, its small size, external fertilization and transparent embryos, rapid development and the ease to carry out analysis of chemicals exposure effects are some of the advantages of this vertebrate that has been mainly used by toxicologists, developmental biologists and geneticists. Those advantages together with the great information already available on zebrafish biology and toxicology make this small fish a very suitable model for studying genetic and early developmental factors that could affect ageing and age related diseases. It has been also verified that the age-dependent degeneration or dysfunction of several organs and age-related pathological changes in zebrafish and other fishes are comparable to those processes described in mammals (Kishi *et al*, 2009).

A further consideration of fish as an ageing model is that their lifespan is modulated and it can be modified by varying environmental factors (i.e. water temperature) and/or by calorie restriction (Reznick *et al*, 2006; Kishi *et al*, 2008). In order to take in account these variables (and their interrelations) when designing ageing experiments and interpreting their results, we have constructed a mathematical model as a first step in a system biology approach on using zebrafish in ageing research. Zebrafish is also being used as a biological model to develop systems biology approaches in other research areas (Ankley *et al*, 2009), which could facilitate the progress in this field.

Mathematical modelling is normally an evolutionary process and therefore successive refinements of the present model are expected. Further model calibration and validation based on new experimental data will help to improve the model. Additional advances could also come by including sound theoretical ageing principles in its framework, as for example those of the disposable soma theory of ageing, which is based on the principle that organisms should optimize the allocation of metabolic resources between somatic maintenance, growth and reproduction (Drenos& Kirkwood, 2005).

The model offers the potentiality to include genomic and epigenetic data and that advantage it is expected to be implemented in future research projects where this type of experimental data is foreseen (i.e. analyzing the impact of the exposure to chemical/drugs early in life on the ageing process).

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