A bioenergetic model for zebrafish
Danio rerio (Hamilton)

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A bioenergetics model was developed from observed consumption, respiration and growth rates for zebrafish *Danio rerio* across a range (18–32° C) of water temperatures, and evaluated with a 50 day laboratory trial at 28° C. No significant bias in variable estimates was found during the validation trial; namely, predicted zebrafish mass generally agreed with observed mass. © 2008 The Authors

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INTRODUCTION

Zebrafish *Danio rerio* (Hamilton) is a prominent model vertebrate for studies of development and toxicology. Extensive research has been conducted on zebrafish embryonic and larval development, physiological functions, behaviour and taxonomy (Laale, 1977; Hill *et al.*, 2005). This vast knowledge of zebrafish biology has also made this organism an ideal model in toxicological studies (Spitsbergen & Kent, 2003), including studies of the effects of environmental contaminants on development and reproduction. For example, zebrafish displayed altered morphologies, gonadal differentiation or reproductive performance when exposed to toxicants such as hexahydro-1,3,5-trinitro-1,3,5-triazine (Mukhi *et al.*, 2005a), malathion (Cook *et al.*, 2005) and perchlorate (Mukhi *et al.*, 2005b, 2007; Mukhi & Patiño, 2007) in high concentrations or for extended periods.

Modelling has been previously used to explore the effects of toxicants on fish energetics (Widdows & Donkin, 1991; Beyers *et al.*, 1999; Nisbet *et al.*, 2000; Smolders *et al.*, 2002). Two different approaches have been used for modelling the effects of toxicants: physiological energetics and dynamic energy budgets. Physiological energetics utilize biochemical analyses of proximate composition

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to infer the energy budget of an organism at the cellular level (Smolders *et al.*, 2002, 2003), and have been previously used to investigate changes in the cellular energy budget of fishes exposed to effluents. Changes in lipid budgets were the most sensitive endpoints for evaluating responses of zebrafish to effluents (Smolders *et al.*, 2003). Alternatively, dynamic energy budgets model the acquisition and utilization of energy for survival, growth and reproduction at the individual level (Nisbet *et al.*, 2000). The Wisconsin bioenergetic model, a particular dynamic energy budget predicated on a generalized mass balance equation in which the sum of energy uses equals energy acquired, predicts acquisition and utilization of energy by an individual fish as a function of body mass and water temperature (Hewett & Johnson, 1992). Some studies have used the Wisconsin bioenergetic model to evaluate the potential effect of toxicants on growth of fishes (Rice *et al.*, 1983), and numerous authors have recognized the potential for dynamic energy models as a tool for predicting null responses (metabolism and growth) of fishes in toxicological studies (Beyers *et al.*, 1999; Nisbet *et al.*, 2000; Smolders *et al.*, 2002, 2003). The primary application of the Wisconsin bioenergetic model to toxicology, however, has been the estimation of contaminant uptake in exposed wild fishes (Norstrom *et al.*, 1976; Barber *et al.*, 1991).

Quantifying the energetic cost to the fish of disturbances to its environment provides insight for predicting ecological outcomes of anthropogenic activity (Fry, 1947; Beamish *et al.*, 1975; Rice, 1990; Nisbet *et al.*, 2000; Smolders *et al.*, 2003). An energetics approach has much appeal because it relies on the first principles of thermodynamics (matter and energy are conserved) and provides a common basis for linkages among different levels of biological organization (Nisbet *et al.*, 2000). The metabolic cost hypothesis [changes in energy metabolism will ultimately affect future life characteristics, such as growth and reproduction, of the organism (Koehn & Bayne, 1989; Calow, 1991)] provides a framework for incorporating dynamic energy budget modelling into toxicological assessments. For example, stress (physiological reaction of an organism in which energy is expended to return the organism to its normal state) causes an alternative allocation of energy in an organism that probably will be manifested in an increase in consumption, a reduction in growth or both. Bioenergetics modelling is well suited for investigating these patterns of alternative energy allocation. No dynamic energy budget model, however, is currently available for zebrafish. Given the current interest in how exposure to toxicants can be modelled in fishes and the considerable use of zebrafish in basic and applied research, this study was designed to develop a dynamic energy budget (Wisconsin-like) model for zebrafish, providing a complement to the physiological energetics model for zebrafish (Smolders *et al.*, 2003), that could be applied in future developmental and toxicological studies.

MATERIALS AND METHODS

FISH HUSBANDRY

Wild-type zebrafish (0·18–0·73 g) of both sexes and similar age (90–120 days) were obtained from a local vendor. Fish were acclimatized in 37·9 l glass aquaria with 30 l

of zebrafish water (180 mg of sea salt per 1 l of deionized water). Aquaria were contained in 1135 l water-baths fitted with heater and chiller units. Water quality variables (pH, temperature, dissolved oxygen, specific conductivity and salinity) were measured daily and ammonia-N was measured at least twice a week. Environmental variables were maintained at recommended levels for zebrafish (pH 6·8, 28° C, 12 L:12 D; Mukhi *et al.*, 2005*a*) until consumption and respiration trials began. Fishes were fed until satiation twice daily with adult frozen *Artemia* sp. and Tetramin flakes (Tetra GmbH, Melle, Germany). Uneaten food and faecal material were removed daily. Feeding continued in this manner until consumption and respiration trials began. After the laboratory acclimatization period (14 days), temperature within each water bath was changed at a rate of 1° C day^{-1} until reaching test temperatures of 18, 23, 28, 30 and 32° C. Fish were allowed an additional 2 weeks acclimation after water baths had been adjusted to target temperatures. Zebrafish were transferred to respiration and consumption chambers for experiments.

BIOENERGETIC MODEL

A zebrafish bioenergetic model was developed using the generalized mass balance equation: $G = C - (R + S + F + U)$, where energy available for somatic and reproductive growth G is equal to energy acquired through food consumption C less energy utilized in respiration R, specific dynamic action S, egestion F and excretion U.

Food consumption was measured in 3 l feeding chambers. Two or three zebrafish (total fish mass similar between feeding chambers) of similar size were added to each consumption chamber and allowed to acclimate to test temperatures (18, 23, 28, 30 and 32° C) for 2 weeks. The test chambers were of sufficient size to allow typical movement and feeding activity of the zebrafish. After the acclimation period, initial mass of zebrafish in each chamber was measured (0·17–0·82 g). Pre-measured (mass, g) amounts of thawed *Artemia* sp. were fed twice daily to ensure *ad libtum* feeding in each consumption chamber. Uneaten *Artemia* sp. were removed, excess water was removed from the uneaten *Artemia* sp. using filter paper in a filter pump and weighed daily. Trials lasted from 8 to 17 days depending on the initial size and temperature such that a measurable amount of growth was observed in each consumption chamber. At the end of the consumption trials, fish were removed and weighed to the nearest 0·01 g. Total consumption was determined as the difference of the total mass of *Artemia* sp. added less that recovered, divided by the number of the fish (two or three) in the tank.

Respiration was measured in 0·5 l static respirometers. Two zebrafish of similar size were added to each respirometer and allowed to acclimate for 2 h prior to the onset of measurements. A fish-free respirometer was utilized as a control for each temperature to determine the biological oxygen demand of bacteria in the water. The respirometers were sufficiently small to minimize the movement of the zebrafish. After the acclimation period, the initial O_2 concentration was measured in each respirometer using a YSI 95 metre (YSI Hydrodata Ltd, Letchworth, UK), and then the respirometer was sealed for the trial. After 1 h, each respirometer was unsealed and the final O_2 concentration was measured. The O_2 uptake of fish was calculated as the difference between the concentration change measured in the respirometer with fish and the concentration change in the fish-free (control) respirometer; this difference was divided by number of fish within the respirometer to obtain an estimate of the average O_2 consumption per fish.

Consumption (g *Artemia* sp. day^{-1}) and respiration (mg O_2 day^{-1}) were modelled as a function of mean body mass (M, g) and water temperature (T, ° C): $C = a_c M^{b_c} e^{\theta_c T}$ and $R = a_r M^{b_r} e^{\theta_r T}$. These models were chosen in accordance to the models specified in Munch & Conover (2002). Fitted coefficients are represented by a_c, b_c, θ_c, a_r, b_r, and θ_r. Specific dynamic action (S; mg O_2 g wet *Artemia* sp.$^{-1}$) and energy lost through excretion and egestion (u) were modelled as constant proportions of consumption.

The equation for daily incremental growth was modelled as: $dW(T) = [J_a(1 - u)C - J_o(AR + S)]J_f^{-1}$, where $dW(T)$ is the change in fish mass (g day^{-1}), J_a is energy density of *Artemia* sp. (J g wet mass^{-1}), J_o is oxycaloric conversion, A is

the activity multiplier and J_f is the energy density of fish ($J\ g^{-1}$). Energy density of the zebrafish (J, 4194 $J\ g^{-1}$ wet mass) was estimated from the equation developed by Hartman & Brandt (1995) as: $J = -1.265 + 262.2\%\ M$, where $\%\ M$ is the per cent dry mass of the zebrafish. Zebrafish $\%\ M$ was determined from 100 zebrafish that were dried at 60° C for 4 days. Energy density of the frozen *Artemia* sp. (2047 $J\ g^{-1}$ wet mass) was estimated from energy equivalents, assumed to be 39.56 for fat, 23.65 for protein and 17.16 carbohydrates (Winberg, 1971), from proximate composition obtained from the manufacturer. The standard value for the oxycaloric conversion (0.0136 J mg O_2^{-1}; Elliott & Davidson, 1975) was used.

Growth in this manner provided information that was necessary to fit coefficients μ and A through a penalized likelihood approach; procedures for this approach are described by Munch & Conover (2002). The likelihood modelled varied from that specified by Munch & Conover (2002) because S was modelled as a constant proportion of C. The coefficients for the bioenergetics model were estimated by maximizing the log likelihood function: $L_T = L_C\ L_R\ L_G\ L_{NEG}$, where L_T is the total log likelihood, L_C is the likelihood of the consumption coefficients, L_R is the likelihood of the respiration coefficients, L_G is the likelihood of the growth coefficients and L_{NEG} is the constraints on negative coefficients. Exact specification of likelihood functions is provided by Munch & Conover (2002). To assess the fit of coefficient values, a linear hypothesis test, *i.e.* intercept = 0 and slope = 1 (Fox, 1997), of predicted and observed values was used.

MODEL EVALUATION

Two replicate 50 day growth trials were conducted under typical laboratory conditions for holding zebrafish. For each trial, groups of 75 similarly sized zebrafish (mean ± s.e.; 0.30 ± 0.01 g) were placed in 75 l aquaria (two aquaria for each trial); each group was treated as an experimental unit ($n = 4$). Fish were allowed 2 weeks of acclimation before the start of the experiment. Fish were fed a pre-measured amount of frozen adult *Artemia* sp.two to three times each day. Temperature in each aquarium was recorded daily. Fifty fish (66%) in random aggregates of five were weighed in a small volume of water to the nearest mg every 10 days and returned to their respective tank; subsamples of fish were weighed to minimize number of handlings (and associated stress) for each fish and thus, minimize any bias of the validation experiment. Daily growth of fish will naturally vary day-to-day; hence, comparisons of modelled growth to observed growth require an accounting for daily variation in physiological variables (Munch & Conover, 2002). Monte-Carlo confidence intervals were estimated by sampling from the error distributions generated in fitting the model using a stochastic model (Munch & Conover, 2002): $dW(T) = \{J_a(1 - u)Ce^{E_c 0.5\sigma_c} - J_o[A(R + E_R) + S]\}J_f^{-1}$, where E_C and E_R are normally distributed random variables that were independently sampled at each time step. E_C was back transformed from log–log fits; half of the s.d. was subtracted to minimize bias in simulated data (Hilborn & Mangel, 1997). A 95% CI was calculated from the model using 5000 replicate integrations. This approach assumes that error variances in the measurements were primarily measurement error and daily variation in physiologically variables occurring independently of each other. To assess the fit of predicted to observed growth, a linear hypothesis test *i.e.* intercept = 0 and slope = 1 (Fox, 1997), was used.

RESULTS

MODEL COEFFICIENTS

The best fit allometric and temperature-dependent consumption (C) function was $C = 0.0001\ M^{1.14}\ e^{0.15T}$ (Table I). The consumption model provided a sufficient fit ($r^2 = 0.78$, $n = 34$) to observed consumption data [Fig. 1(a)]. The null

TABLE I. Bioenergetics model coefficients for zebrafish

Model function	Coefficient	Value
Consumption (C; g $Artemia$ sp. day^{-1})	a_c	0·0001
$C = a_c M^{b_c} e^{\theta_c T}$	b_c	1·14
	θ_c	0·15
Sample size (n)	n	34
Error variance (σ_c^2)		0·0127
Coefficient of determination (R^2)		0·99
Respiration (R; mg O^2 day^{-1})	a_r	9·5
$R = a_r M^{b_r} e^{\theta_r T}$	b_r	0·97
	θ_r	0·04
Sample size (n)		50
Error variance (σ_r^2)		9·95
Coefficient of determination (R^2)		0·93
Growth [$dW(T)$ g wet mass]		
$dW(T) = \{J_a(1-u)C - J_o[AR + S(C)]\}J_f^{-1}$	S	0·2
	A	2·2
	u	0·02
	n	34
	R^2	0·93
Energy density of $Artemia$ sp. (J g wet mass^{-1})	J_a	2047
Oxycaloric conversion (J mg O_2^{-1})	J_o	0·0136
Energy density of zebrafish (J g wet mass^{-1})	J_f	4194

hypothesis of the linear hypothesis test was not rejected ($F_{34,32}$, $P > 0·05$); thus, no significant bias was evident between predicted and observed consumption values.

The best fit allometric and temperature-dependent respiration (R) function was $R = 9·5\,M^{0·97}\,e^{0·04T}$ (Table I). The respiration model provided a sufficient fit ($r^2 = 0·69$, $n = 50$) to observed respiration data [Fig. 1(b)]. The null hypothesis of the linear hypothesis test was not rejected ($F_{50,48}$, $P > 0·05$); thus, no significant bias was evident between predicted and observed respiration values.

The other coefficients fit with the whole-model approach were u and A (Table I). The value used for S was 0·2, which is greater than the constant proportion (0·17) used in prior bioenergetics models (Hewett & Johnson, 1992). The best fit activity multiplier was 2·2, which was slightly greater than other published values of activity (Munch & Conover, 2002; Chipps & Wahl, 2004). The fitted value for egestion and excretion, 0·02, was similar to the value used for Atlantic silverside *Menidia menidia* (L.) (Munch & Conover, 2002), but considerably less than the typical value (0·2) used in other bioenergetics models (Hewett & Johnson, 1992). These coefficients combined with the above consumption and respiration coefficients provided a model that explained most of the variation observed in the 8–17 day consumption and growth study ($r^2 = 0·76$, $n = 34$).
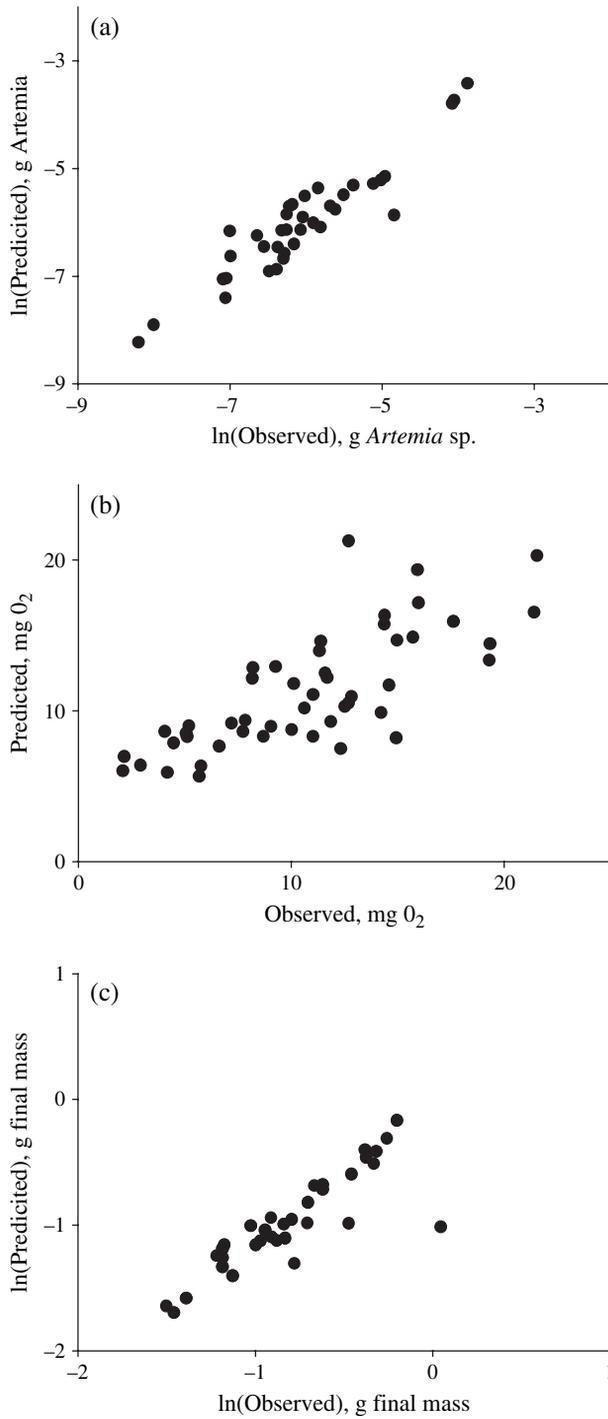
FIG. 1. Predicted and observed values of (a) consumption (*C*), (b) respiration (*R*) and (c) growth (final mass, M_F) for zebrafish.

MODEL EVALUATION

The mean mass (0·30 g) of all fish was used as the initial mass for the model's predictions. Mean ± s.e. daily temperatures in aquaria were 28·07 ± 0·01° C. The observed final mean mass of zebrafish was 0·34 g, resulting in a growth rate of *c.* 0·0008 g wet mass day^{-1}. Model predictions assumed that the fish in the evaluation study were feeding at maximum consumption (*i.e.* $P_{C_{max}} = 1·0$). Model predictions agreed moderately well with the observed growth of the zebrafish in the evaluation study ($r^2 = 0·76$) and the null hypothesis of the linear hypothesis test was not rejected ($F_{6,4}$, $P > 0·05$), indicating that no bias was evident in the developed bioenergetics model for zebrafish. All of the observed points, except the second point (day 10), fit within the 95% CI of the model's projections (Fig. 2).

DISCUSSION

The importance of zebrafish as a model for research of development and toxicology justifies the development of a bioenergetics model for zebrafish for application in this research. The novel approach to modelling energy use, specified in Munch & Conover (2002), was applied to fit coefficients in the present growth model. This approach (complete-model maximum likelihood), which led to the specification of model coefficients that are typically borrowed and assumed to be constant among species, was beneficial because many model coefficients (*F*, *U* and *A*) require expensive and time-consuming assessments to obtain species-specific estimates. The simultaneous whole-model approach of estimating bioenergetics coefficients provides an alternative to borrowing multiple bioenergetics coefficients from other species, a common practice in the development of bioenergetics models that has the potential to bias model predictions (Bajer *et al.*, 2003, 2004*a*, *b*).

Prolonged or severe stress (*e.g.* chronic exposure to a pollutant) will cause zebrafish to alter their energy allocation patterns and hence change
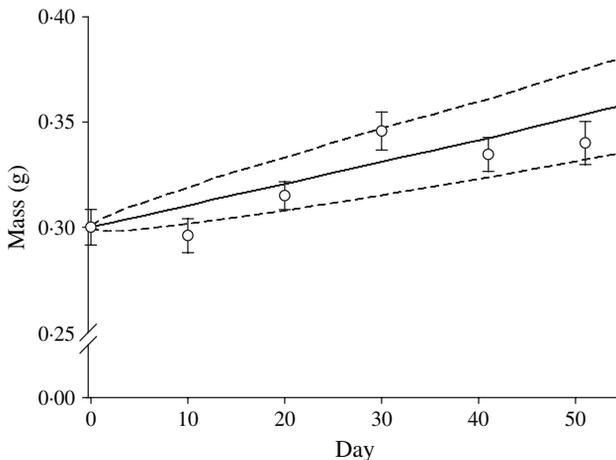

FIG. 2. Predicted (——) and observed (mean ± s.e.; ○) mass of individual zebrafish over a 55 day period with fish reared under typical laboratory conditions. Predicted values (----, the 95% Monte-Carlo C.I. for the model prediction) were obtained from the bioenergetics model.

metabolism, growth or reproduction patterns. The objective of many labora-
tory studies using zebrafish is to understand complex patterns that account
for development and growth. Given the complexity of issues addressed, numer-
ous authors have called for the use of energy budgets to better understand ob-
servations made in the laboratory (Beyers *et al.*, 1999; Nisbet *et al.*, 2000; Smolders
et al., 2003). The zebrafish bioenergetic model developed herein reliably predicts
energy consumption and utilization based on size- and temperature-specific func-
tions and provides a tool for establishing null models (predicted outcomes in the
absence of ecological or environmental mechanisms; Gotelli & Graves, 1996) of
consumption and growth for zebrafish in laboratory settings.

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