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WHOLE-BODY COMPOSITION OF *XENOPUS LAEVIS* LARVAE: IMPLICATIONS FOR LEAN BODY MASS DURING DEVELOPMENT

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Summary

Body composition in developing animals has been extensively investigated in fish larvae and bird embryos. However, no studies to date have attempted to determine whole-animal body composition or lean body mass (M_{LB}) in developing amphibians. The present study investigates how body composition changes during development in *Xenopus laevis* and the potential implications of M_{LB} for substrate turnover, energy stores, oxygen consumption and other physiological measures. Whole-animal composition was determined during development from eggs (NF stage 1) to 2 weeks post-feeding (NF 50–51), which represents two-thirds of the developmental period. Wet and dry masses were found to be highly correlated, with water content remaining constant at 93 % of wet mass. Whole-animal nucleic acid content was linearly correlated with both wet and dry masses, and declined relative to mass as development progressed. Similarly, total protein content was linearly correlated with wet and dry masses; however, total protein content increased with developmental stage. Amounts of individual neutral lipids were variable although, overall, total neutral lipid content declined progressively with development. The stoichiometric energy balance paralleled the changes seen in mass-specific $\dot{M}_{O_2}$, with the energy primarily from lipids fueling respiration up to NF 44–45. Quantification of total body composition revealed that lipid stores greatly influenced the calculations of M_{LB} and therefore had profound underestimating effects on the mass-specific expression of numerous physiological measures through development.

Key words: *Xenopus laevis*, development, growth, body composition, protein content, lipid content, nucleic acid content, lean body mass, energy balance.

Introduction

A common course of development occurs amongst a wide variety of organisms. However, one of the distinguishing characteristics in vertebrates is the amount and distribution of yolk (Browder *et al*. 1991), which may vary widely both within and among species. Yolk stores ultimately determine the embryo's ability to survive beyond deposition. In most cases, the animal is transformed from a mass of apparently similar cells into a fully functional organism capable of surviving autonomously and most, if not all, of this process is fueled *via* degradation of the yolk. Thus, each embryo must have enough stored fuel to meet its metabolic demands as it undergoes development. Moreover, this fuel must last until the embryo is capable of obtaining extra-embryonic foodstuffs to continue to meet its nutritive requirements.

Body composition and how it changes with progressive development have been extensively investigated in fish (for a review, see Heming and Buddington, 1988) and in chicken embryos (for a review, see Romanoff, 1967). However, all studies to date have utilized centrolecithalic animals, in which the yolk is limited to a yolk sac or specified region of the egg/embryo. This distribution has allowed researchers to excise the yolk and assay for the contents relative to the whole-animal composition. Despite the fact that techniques exist to winnow out true metabolizing tissue mass, no investigators have attempted to express physiological characters such as the rate of oxygen consumption ($\dot{M}_{O_2}$), stroke volume (V_S) and cardiac output ($\dot{Q}$) as a function of the amount of true metabolizing tissue. At best, most developmental studies in fish (Rombough, 1988*b*), amphibians (Burggren and Just, 1992), reptiles (Birchard and Reiber, 1995; Birchard *et al*. 1995; Deeming and Thompson, 1991) and birds (Romanoff, 1960) have ignored the mass component completely (expressing data on a per individual basis) or have chosen to express them on a per egg basis. Unfortunately, discounting yolk stores has led to serious overestimations in metabolizing tissue mass, when expressed on a per gram mass basis, and subsequent underestimations in the variable of interest (e.g. $\dot{M}_{O_2}$, $\dot{Q}$ and V_S). Interestingly, in studies over the past half

century, adult cardiovascular and respiratory physiological quantities have been expressed as mass-specific values.

Although the techniques described for fishes and birds have proved useful, they are not feasible in telolecithalic animals such as amphibians. Moreover, there exists no method to determine 'lean' body mass (M_{LB}) in these developing organisms. The lack of attention to determining yolk-free tissue mass has prompted most developmental physiologists simply to ignore this problem. In this paper, we describe a method of evaluating the yolk-free mass in developing telolecithalic animals.

We hypothesize that yolk lipids provide the primary bulk energy necessary for embryonic development in amphibians, and we suggest that these non-metabolizing energy stores contribute to an overestimation of an animal's true mass. Furthermore, we assert that this overestimation will skew data that are expressed mass-specifically, thereby significantly underestimating the physiological variable of interest.

We tested these hypotheses by measuring total neutral lipids (NL_{tot}), total DNA (DNA_{tot}) and total protein ($Prot_{tot}$) content to determine the whole-body composition of developing *Xenopus laevis*. From this, we estimated lean body mass (M_{LB}), which is a true index of animal mass and which allowed for a quantitative determination of the magnitude of the error encountered when expressing physiological variables in yolk-laden amphibian embryos.

Materials and methods

Experimental animals

Fertilized eggs were obtained, in the late autumn and early winter, from the *in vivo* fertilization of six breeding adult female *Xenopus laevis*, according to the methods of Thompson and Franks (1978). Eggs were transferred into well-aerated holding tanks (25 l), where they were maintained in aged tap water at 24±1 °C. Larvae were fed Nasco frog brittle (Nasco Inc.) *ad libitum*, starting at stage 45 until the end of the development/sampling period. All animals were maintained on a 14 h:10 h light:dark cycle throughout development.

Grouping of developmental animals

Experiments were conducted on *Xenopus laevis* embryos and larvae which ranged in developmental stage from eggs (NF 1) to 2 weeks post-initiation of feeding (NF 51). Stage determination was apportioned according to staging regimes outlined by Nieuwkoop and Faber (1967). Animals were grouped into 13 different ontogenetic categories according to major morphological and physiological characteristics. Developmental groupings and numbers of individual animals (N) are as follows: NF 1–8 (30), 9–12 (30), 13–18 (30), 19–24 (30), 25–38 (30), 39–41 (25), 42–43 (25), 44–45 (25), 45–46 (25), 46–47 (27), 47–48 (25), 48–49 (27) and 50–51 (25). Because the developmental stages, as described by the NF system, are non-uniform with respect to time and are based primarily on morphological changes, we chose to divide some stages into early and late where clearly discernible. This would ensure the high temporal resolution required to characterize small changes in body composition that may occur over very short periods. Readers unfamiliar with the major anatomical and physiological changes associated with the NF stages are referred to the work of Nieuwkoop and Faber (1967) or Burggren and Just (1992).

Experimental procedure

At each sampling period, individual groups of animals were removed from their holding tanks and promptly ($\leqslant$1 min) flash-frozen in liquid nitrogen. The frozen sample was then transferred to a storage vial and kept at −70 °C. All samples were assayed for total neutral lipid content, whole-animal DNA content, total protein content and wet mass within 1 month of storage.

Total neutral lipids (NL_{tot}*)*

Total neutral lipids were prepared by homogenizing 0.5 of the total tissue tissue with a Teflon pestle in a 1.5 ml microcentrifuge tube. Each sample was diluted with exactly 3 volumes of 0.15 mol l^{-1} NaCl (for justification, see nucleic acids section below). Upon completion, the pestle was washed with 200 µl of the diluent. All samples were then lyophilized and the lipids were extracted (Bligh and Dyer, 1959). Extracted lipids were separated according to their polarity (Mason *et al.* 1976). Collected neutral lipids were then dried to completeness under a stream of N_2. Samples were resuspended in 100 µl of chloroform for analysis. Identification of individual neutral lipid species was performed in triplicate using thin layer chromatography (TLC) as described by Mangold (1969). Lipids were detected by conjugation with acidic 2 mol l^{-1} $CuSO_4$, followed by charring for 15–30 min at 190 °C in a convection oven. Images of charred plates were scanned into a personal computer at a resolution of 600 dots per inch using an HP ScanJet 3C (Hewlett Packard Inc). Densitometric analysis was performed with SigmaScan image analysis (Jandel Scientific Inc). Outputs were then read and integrated for concentration using Datacan v5.1 (Sable Systems Inc). In all cases, TLC plates were run with known standard amounts of neutral lipids (Nu-Check-Prep Inc.) ranging from 2 to 240 mg. The mean r^2 for all standards was 0.992±0.007, with a sample size of eight in triplicate. Neutral lipid standards included the following species: mono-olein, diolein, triolein, methyl oleate, oleic acid, cholesterol oleate and cholesterol. Species of lipids without standards were identified on the basis of R_f values according to the method of Mangold and Malins (1960).

Total DNA (DNA_{tot}*)*

Determination of the interaction between diluent strength and yield of nucleic acids revealed that maximum product was achieved by homogenizing tissue in 0.15 mol l^{-1} NaCl; therefore, all tissue samples were ground with this solution to minimize loss. Total nucleic acids were determined fluorimetrically, in triplicate, using binding of Hoechst 33258 (Paul and Myers, 1982) and individual content was determined as the average for the total tissue sampled. Samples were

excited at 350 nm, and emission was read with a Sequoia Turner fluorimeter at 450 nm (10 nm slit width). All samples were compared concurrently with standard curves produced using calf thymus DNA (Sigma Chemical, St Louis, USA), which had a high degree of fit (r^2=0.990, N=8 in duplicate).

*Total protein (*Prot$_{tot}$*)*

Total protein was determined from dried samples (see mass determination) on the basis of a modified Biuret procedure described by Watters (1978). Protein concentrations of samples were determined using standard curves from known quantities of bovine serum albumin (BSA) (r^2=0.999, N=12 in duplicate). Duplicate samples (200 µl) were dispensed into wells of a Corning 96-well microtiter plate and scanned for absorbance at 570 nm on a Dynatech MZ580 micro-ELISA reader. Absorbance for each plate was individually zeroed, and samples were read twice. The results of the four readings were averaged and used to determine concentration.

Mass determination

Wet mass (M_w), used to calculate NL_{tot} and $Prot_{tot}$, was determined by wick-drying animals with a Kimwipe and weighing them (to the nearest milligram) on a Sartorius micro balance, model S120 B. To ensure sufficient quantity of material for all assays, large numbers of animals (see above for actual number per stage) at each stage were pooled and total tissue mass was determined.

A separate population of animals, which ranged in stage from NF 1 to NF 60, was used to determine dry mass (M_d) and water content empirically from wet mass. All samples were dried *via* capillary action, weighed and placed into aluminum planchets. Samples were dried at 90 °C for at least 1 week until a constant mass was achieved. Both wet and dry masses were measured on a Denver Instrument analytical micro balance, model AB-300. Regression analyses were performed, and equations describing the relationship between wet and dry masses were used to calculate dry mass for tissues used in NL_{tot}, $Prot_{tot}$ and DNA_{tot}.

*Lean body mass (*M$_{LB}$*)*

The relationship between wet animal mass and lean body mass (M_{LB}) was described by the following equation:

$$M_{LB} = \left[(b_1 M_w + b_0)\left(1 - \frac{\omega}{100}\right)\right], \quad (1)$$

where, b_1 is the slope of the linear equation between wet and dry masses, b_0 is the intercept for the linear equation between wet and dry masses, ω is the percentage of neutral lipids at a given stage and M_w is wet animal mass in mg.

The relationship between wet and dry masses for *X. laevis* was obtained from Table 1. In order to compare results across studies, the relationship between wet and dry masses was assumed to be constant, and values in the literature were therefore converted to dry mass to ensure equitable comparisons.

*Total gross energy (*E$_{TG}$*) and stoichiometric energy balance (*E$_{SB}$*)*

E_{TG} was calculated as the sum of individual gross energies for lipids and protein. Each individual gross energy was estimated by multiplying the chemical composition by the standard energy equivalents for protein and lipids, 17 J mg^{-1} and 37 J mg^{-1}, respectively (Newsholme and Leech, 1983). E_{SB} consisted of the stoichiometric balance of body composition with routine metabolic rate ($\dot{M}_{O_2}$) and was expressed as a fraction of that rate. This energy balance was based on data from neutral lipids, protein and 'other' bodily constituents. 'Other' consisted of nucleic acids, phospholipids and simple and complex carbohydrates and was determined as the residual energy post-partitioning into lipids and protein.

Statistical analyses

Effects of development on DNA_{tot}, $Prot_{tot}$ and mass were determined by a first-order least-squares linear regression (Statistica v5.0, on a PC). This analysis determines (i) the regression coefficient; (ii) the equation of the line describing the relationship; and (iii) the probability that the slope of the line is not significantly different from zero. A *t*-test was performed to determine whether the slope of the predicted line was significantly different from zero. In all cases, the fiduciary level of significance was taken as $P \leqslant 0.05$.

Results

Mass and water content

Wet mass was linearly correlated with dry mass over a range from 2.1 to 695.4 mg (Fig. 1). These data are described by the equation M_d=0.07M_w+0.03 (r^2=0.99, P<0.001), where M_w and M_d represent wet and dry mass respectively. Results of regression analysis are provided in Table 1.

Total wet tissue mass over the developmental period from NF 1 to NF 51, used for the calculation of NL_{tot} and DNA_{tot}, ranged from 32.55 to 323.40 mg (Table 2). Dry mass over this same developmental range increased 6.5-fold and ranged from 2.31 to 22.72 mg (Table 2). Water content was almost constant at approximately 93 % with development up to NF stage 50–51.

Chemical composition during development

Nucleic acid content showed a strong linear correlation (r^2=0.99, P<0.001) with both wet and dry mass (Table 1). DNA_{tot} ranged from a minimum of 1.35 ng to a maximum of 7.7 ng over a range of individual dry mass from 1.2 to 22.8 mg (17.0–324.7 mg wet mass; Fig. 2) and was described by the allometric equation DNA_{tot}=1.268$M_d^{0.581}$ (Table 1). Analysis of percentage body composition indicated that, relative to dry mass, DNA_{tot} decreased over the range from NF 1 to NF 51. This overall decrease was not simply a linear decline from eggs (NF 1–8) to pre-metamorphic climax (NF 51); instead, DNA_{tot} increased 1.2-fold between NF 1 and NF 18. A similar trend was observed between NF 19 and NF 38, and between NF 42

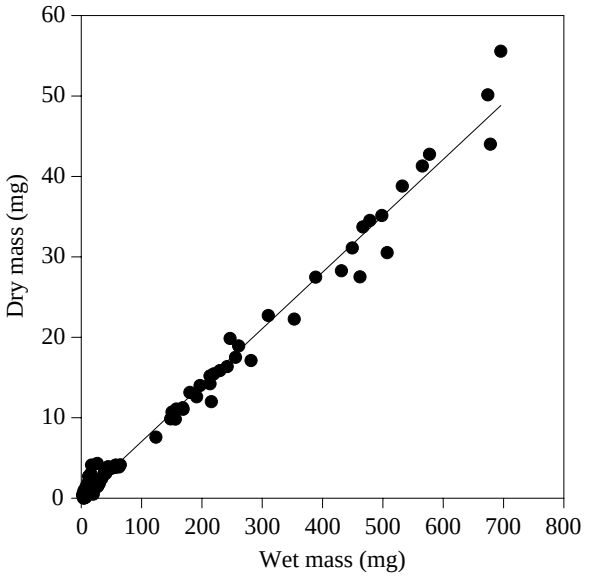

Fig. 1. Plot of wet mass against dry mass for developing *Xenopus laevis* larvae. The linear function of dry mass is plotted against the linear function of wet mass. All data were fitted with a first-order least-squares linear regression and the results are presented in Table 1.

and NF 45, where DNA_{tot} increased by up to twofold over the value for the stages that preceded them (Table 2). Between NF 45 and NF 51, DNA_{tot} showed a consistent decline, achieving a post-absorptive value of 3.63×10^{-5} % of body dry mass.

Protein content, like DNA_{tot}, showed a strong correlation (r^2=0.85, P<0.001) with both wet and dry mass and was described by the equation $Prot_{tot}=0.155M_d^{0.642}$. Total protein content increased by 2.47 mg as dry mass increased from 0.43 to 49.07 mg (2.03–695.4 mg wet mass; Fig. 3). Analysis of overall body composition revealed that $Prot_{tot}$ increased progressively with development between NF 1 and NF 51. However, $Prot_{tot}$ did not change relative to dry mass between

Table 1. *Regression analysis of total protein (*$Prot_{tot}$*) and total nucleic acid (*DNA_{tot}*) contents with dry mass and of wet mass with dry mass (*M_d*) in developing* Xenopus laevis

Stage range	y	N	a	b	r^2	P
1–60	M_d	98	0.028	0.070	0.99	⩽0.001
1–60	$Prot_{tot}$	96	0.155	0.642	0.85	⩽0.001
1–50	DNA_{tot}	13	1.268	0.581	0.99	⩽0.001

Relationships for $Prot_{tot}$ and DNA_{tot} are expressed as $\log y=\log_{10}a+b\log_{10}M_d$, where y is in units of mg for M_d and $Prot_{tot}$, and ng for DNA_{tot}.

The relationship between wet and dry mass is described by the equation $y=a+bx$, such that M_d and M_w are in mg.

N, r^2 and P, are sample size, correlation coefficient, and level of significance, respectively.

NF 1 and NF 18. In contrast, total protein content fell between NF 19 and NF 38. Over the next eight stage ranges (NF 39 to NF 51), $Prot_{tot}$ doubled, reaching a final value of 60.60 % of dry mass (Table 2).

Unlike DNA_{tot} and $Prot_{tot}$, individual neutral lipids did not show a clear linear relationship with either wet or dry mass. Stage-specific analysis indicated that amounts of individual lipid classes were highly episodic with progressive development (Fig. 4A–D).

Levels of triolein, as a proportion of dry mass, declined during development, ranging from a maximum of 20.44±1.2 μg mg^{-1} (NF 9–12) to a minimum of 0.60±0.2 μg mg^{-1} (NF 46–47), and thereafter stabilized up to NF 51 (Fig. 4A). Levels of diolein decreased between NF 1 and NF 13, after which they increased by 1.5-fold to a value of 237.34±1.44 μg mg^{-1} (Fig. 4C). Between stages NF 19 and NF 51, levels of diolein tended to decline with successive stage, reaching a final value of 28.44±1.11 μg mg^{-1}. Levels of mono-olein, by contrast, remained relatively constant at 1.2 μg mg^{-1} between NF stages 1 and 25, after which they increased to a maximal value of 10.89±1.73 at NF 45–46

Table 2. *Body composition and water content during development in* Xenopus laevis

Development NF stage	Wet mass (mg)	Dry mass (mg)	Total body water (%)	Neutral lipids (%)	DNA (%)	Protein (%)	Other (%)
1–8	49.10	3.47	92.93	61.22	1.64×10^{-4}	31.18	7.60
9–12	41.85	2.96	92.92	61.87	1.74×10^{-4}	33.46	4.67
13–18	50.25	3.55	92.93	60.50	1.91×10^{-4}	32.30	7.20
19–24	35.40	2.51	92.90	62.20	1.27×10^{-4}	21.25	16.55
25–38	37.10	2.63	92.91	61.54	1.36×10^{-4}	20.93	17.53
39–41	32.55	2.31	92.90	61.73	0.10×10^{-4}	29.13	9.14
42–43	47.70	3.37	92.93	50.49	0.06×10^{-4}	40.16	9.35
44–45	55.40	3.91	92.93	46.21	1.26×10^{-4}	43.47	10.31
45–46	54.20	3.83	92.93	38.83	0.09×10^{-4}	43.02	18.15
46–47	60.00	4.24	92.94	20.25	0.06×10^{-4}	45.05	34.70
47–48	110.45	7.78	92.96	15.78	0.05×10^{-4}	53.75	30.47
48–49	95.60	6.74	92.95	11.49	0.66×10^{-4}	52.14	30.47
50–51	323.40	22.72	92.98	7.18	0.04×10^{-4}	60.60	32.23

Body composition percentages are expressed with respect to dry mass up to 2 weeks post-initiation of feeding (NF 50–51).

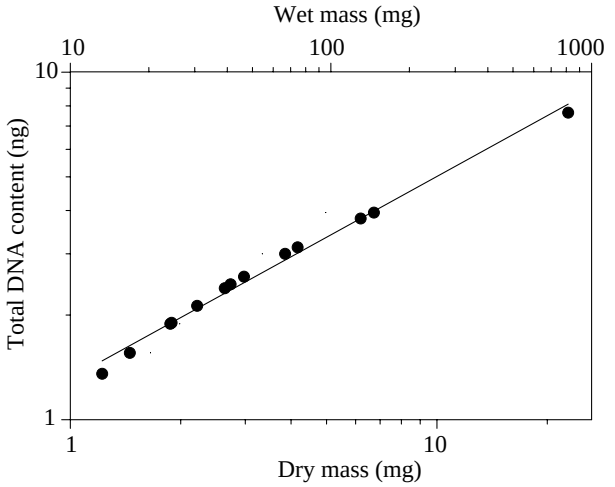

Fig. 2. Plot of total nucleic acid content against wet and dry mass in developing *Xenopus laevis* larvae. Data are plotted as $\log_{10}$[total nucleic acid] against both $\log_{10}$(wet mass) and $\log_{10}$(dry mass). Regression analyses indicating allometric trends are presented in Table 1.

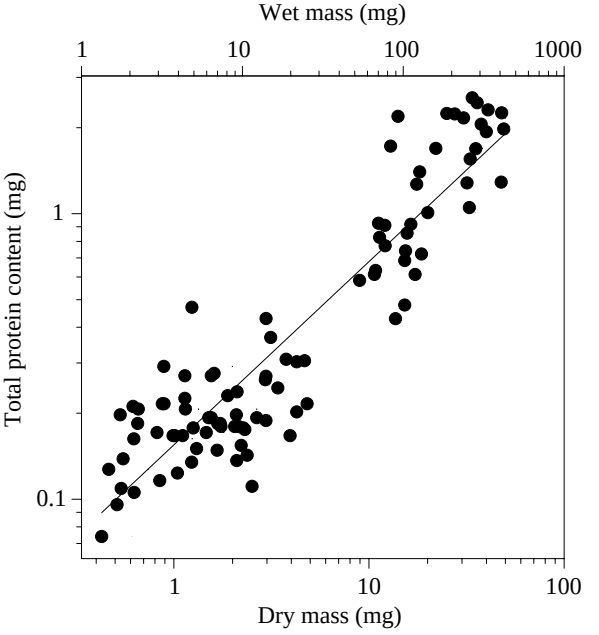

Fig. 3. Plot of total protein content against wet and dry mass in the larvae of *Xenopus laevis*. Data are plotted as $\log_{10}$[total protein] against both $\log_{10}$(wet mass) and $\log_{10}$(dry mass). Results from first-order linear regression analyses indicating allometric trends are presented in Table 1.

(Fig. 4A). Over the next four stage ranges, mono-olein content decreased to a final value of 0.16±0.01 μg mg^{-1}. Of all the individual lipid classes, oleic acid showed the most variability with development. Between NF 19 and NF 38, and NF 42 and NF 45, oleic acid level showed little to no change. In contrast, oleic acid showed a clear reduction between NF stages 1–12, 13–24, 39–43, 45–47 and 48–51 (Fig. 4B).

Methyl oleate content changed very little through most of development (Fig. 4B): the only major deviation from the baseline value of 0.40±0.04 μg mg^{-1} was a sharp increase seen between NF 45 and NF 48, where it reach a maximum of 29.16±0.94 μg mg^{-1}. Like mono-olein, cholesteryl oleate showed an initial increase of 1.3-fold from NF 1 to NF 12, after which it began to decline in concentration, falling from 286.91±30.61 to 19.10±0.1 μg mg^{-1}. Cholesterol displayed a fairly constant level through most of development (NF 1–45). However, between NF stages 44 and 51, cholesterol showed a consistent decline in concentration, at which point it reached a final value of 22.21±2.27 μg mg^{-1}. Diakylmonoacylglycerol (DAG), like methyl oleate, showed little activity and was found only at NF stages 13–18, 25–38 and 50–51. In the stages during which DAG was detected, the concentration never exceeded 6.21±0.14 μg mg^{-1} (Fig. 4D).

Sterols showed a relatively constant level of approximately 15 μg mg^{-1} from NF 1 to NF 38, after which they fell by 60 % to a value of 7.52±1.09 μg mg^{-1}. By stage 44–45, sterol levels had increased to a maximum of 42.42±2.01 μg mg^{-1} and then dropped to a mean value of 0.9 μg mg^{-1} through NF 50–51. Similarly, long-chain alcohols showed a parallel trend to sterols, with concentrations remaining relatively constant at approximately 30 μg mg^{-1} from NF 1 to NF 18. Between NF 13 and NF 43, long-chain alcohol concentration showed a biphasic increase with the first plateau at NF 25–38 (53.23±1.76 μg mg^{-1}) and a second plateau at NF 42–43, at which point it reached a maximum of 84.61±5.94 μg mg^{-1} (Fig. 4D). Over the next six stage ranges, long-chain alcohol levels fell by 83 μg mg^{-1} to a final value of 0.35±0.03 μg mg^{-1}.

Energy content and metabolism

Estimations of stage-specific gross energy from lipids (E_{LG}) and protein (E_{PG}) are presented in Table 3. Gross energy

Table 3. *Lipid, protein and total gross energy during development in* Xenopus laevis

Development NF stage	Lipid gross energy (J mg^{-1})	Protein gross energy (J mg^{-1})	Total gross energy (J mg^{-1})
1–8	22.65	5.30	27.95
9–12	22.89	5.69	29.37
13–18	22.38	5.49	29.10
19–24	23.01	3.61	29.44
25–38	22.77	3.56	29.31
39–41	22.84	4.95	29.35
42–43	22.51	6.83	30.93
44–45	17.10	7.39	26.24
45–46	14.37	7.31	24.77
46–47	7.49	7.66	21.05
47–48	5.84	9.14	20.16
48–49	4.25	8.86	19.30
50–51	2.66	10.30	18.44

All values were expressed per unit dry mass.

Lipid and protein gross energies were calculated on the basis of the energy equivalents provided by Newsholme and Leech (1983).

Total gross energy was calculated as the sum of lipid and protein energies.

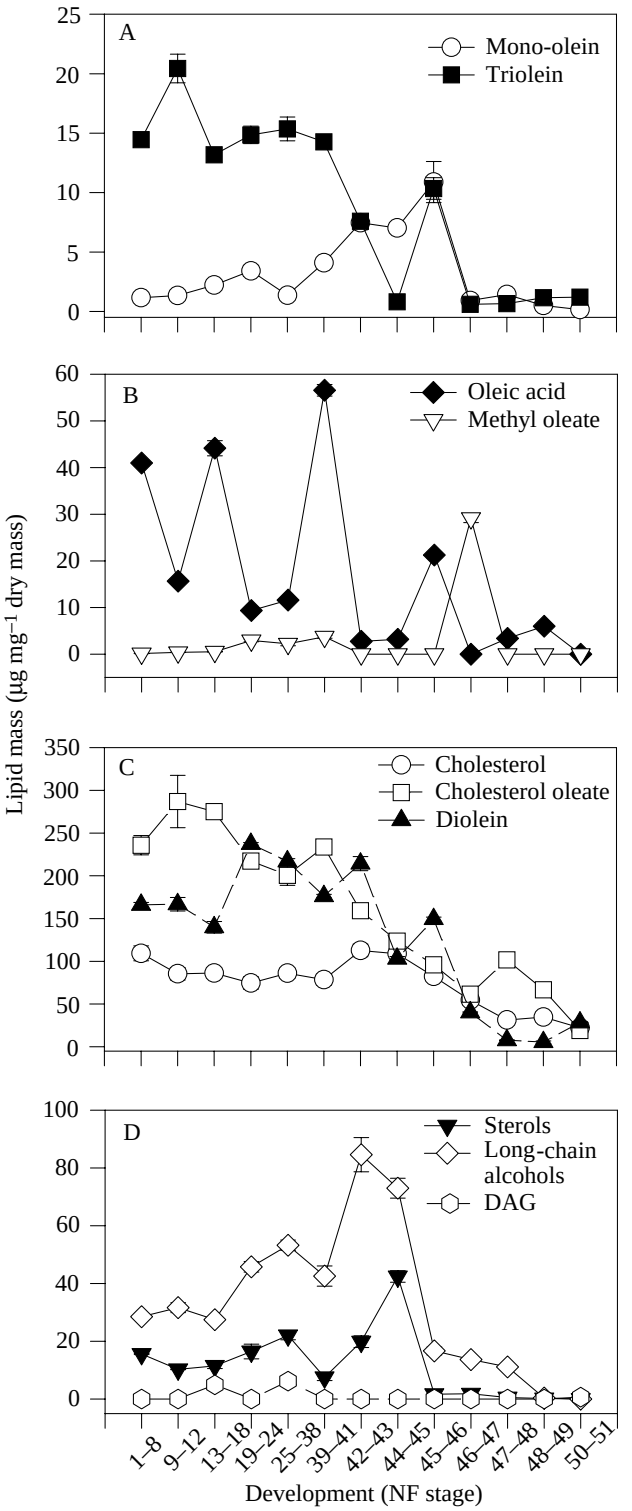

Fig. 4. Lipid composition in developing larvae of *Xenopus laevis*. The linear functions for individual lipid classes are plotted against the linear dimension of development (NF stage ranges). (A) Mono-olein (open circles) and triolein (filled squares); (B) methyl oleate (open triangles) and oleic acid (filled diamonds); (C) cholesterol (open circles), cholesteryl oleate (open squares) and diolein (filled triangles); (D) sterols (filled triangles), long-chain alcohols (open diamonds) and dialkylmonoacylglycerol (DAG; open hexagons).

available from lipids decreased with development and ranged from 23.01 to 2.66 J mg^{-1}. Conversely, the available energy from protein increased approximately twofold with progressive development. This inverse relationship between E_{LG} and E_{PG}, however, did not result in a constant level of E_{TG}. In fact, mass-specific E_{TG} decreased with progressive development (from 30.93 to 18.44 J mg^{-1}), illustrating a clear mismatch between gross energy pools as development progresses.

The relationship between stoichiometric energy balance (E_{SB}) and the rate of aerobic metabolism ($\dot{M}_{O_2}$, from Territo and Burggren, 1998), as illustrated by Fig. 5, shows a clear differential between stored resources and those required for normal maintenance and growth. Clearly, the lipid fraction represents on average 75–80 % of the total energy available for most of development up to NF 45–46, at which point the total lipid energy decreased to less than 15 % of the total energy used for $\dot{M}_{O_2}$, indicating lipid resource depletion. This reduction in lipid energy was concomitant with the onset of feeding and body differentiation, and was largely due to changes in lipid utilization. Over this same stage range, $\dot{M}_{O_2}$ showed a clear increase in energy consumed; it peaked at a maximal rate of 0.69±0.06 μW mg^{-1} (Fig. 5). This peak in $\dot{M}_{O_2}$ was followed by a decline over the next seven stage ranges (NF 42–51). The protein and 'other' (nucleic acids, phospholipids, simple and complex carbohydrates) energy fractions showed a clear increase in amount from NF 19 to NF 51. This increase in protein and 'other' energy fractions indicates consistent growth and shows a controlled synchronization of resource depletion (lipid energy) and production (protein and 'other' energies) with aerobic metabolism.

Discussion

Mass and water content

The relationship between wet and dry mass (Fig. 1) was linear and similar to data reported in the literature for this species (Feder, 1981). The water content of *X. laevis* larvae from NF 1 to NF 51 was constant at approximately 93 %, which is in general agreement with the work of Burggren and Just (1992) and Leist (1970), in which they report similar values up to NF 57. Clearly, these data suggest that embryos and larvae defend their water balance throughout development despite dramatic changes in body composition.

Chemical composition during development

Chemical composition in developing animals has been investigated in a large number of vertebrates, although most work in amphibians has been primarily interested in structural components rather than discrete chemical composition (Deuchar, 1966; Jared *et al.* 1973; Karasaki, 1963; Wallace, 1963, 1965). We chose to evaluate whole-body composition of developing *X. laevis*, thereby allowing the determination of how energy is partitioned and utilized with progressive development in amphibian larvae.

Total DNA content showed a clear allometric relationship with body mass (Table 1). These data are similar to those for

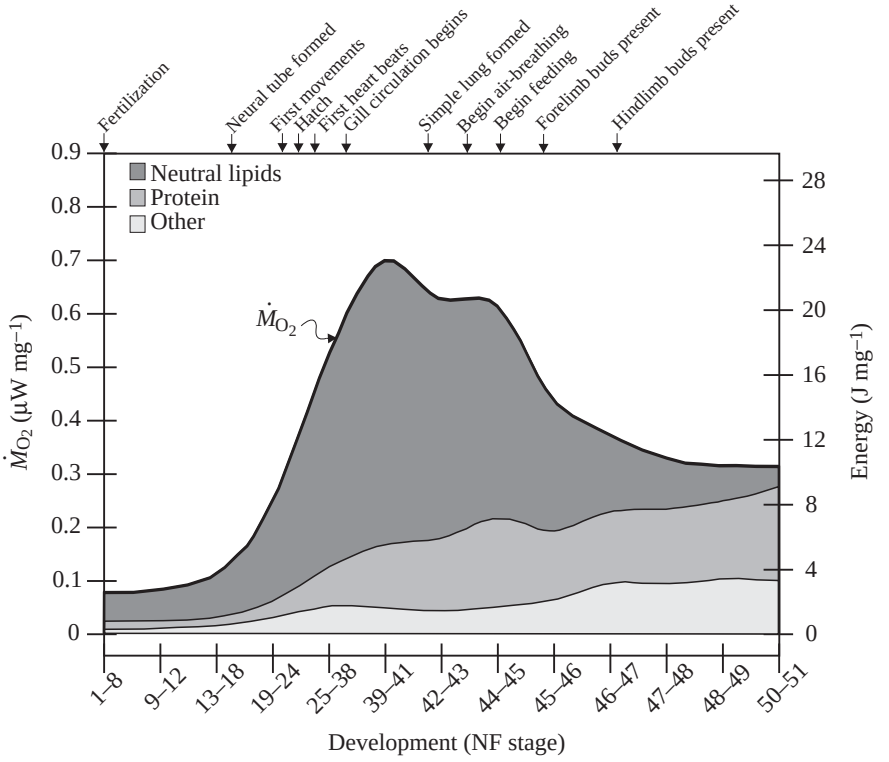

Fig. 5. Catabolic oxidation of endogenous substrates in developing embryos and larvae of *Xenopus laevis* with whole-animal oxygen consumption ($\dot{M}_{O_2}$). All substrates are based on a stoichiometric balance of each substrate with routine metabolic rates (bold solid line) and are expressed as a fraction of that rate. Dark gray hatched area represents neutral lipids; medium and lightly hatched areas represent protein and 'other' bodily constituents, respectively. 'Other' consists of DNA, phospholipids and both complex and simple carbohydrates. The right-hand ordinate represents available energy from each constituent of body composition. Developmental landmarks were based on light microscope observations and detailed descriptions provided by Nieuwkoop and Faber (1967). All $\dot{M}_{O_2}$ data were corrected for lean body mass (M_{LB}) using equation 1 and body compositions presented in Table 2. $\dot{M}_{O_2}$ values in μmol g^{-1} h^{-1} were corrected to energy equivalents (μW mg^{-1}) according to the stoichiometric relationship obtained from Dejours (1981).

larval *Ambystoma tigrinum*, in which DNA content was linearly correlated with wet mass (A. W. Smits, unpublished data). Although few studies exist which have correlated nucleic acid content with wet or dry mass, our data are in the general range reported for developing fish (Zeitoun *et al.* 1977). The percentage of total DNA showed periods of increase (NF 1–18, 19–38 and 42–45) against the general declining trend, suggesting that these punctuated windows of activity occurred concurrently with morphological and structural changes. Inferential support for this premise comes from correlates of morphological changes such as gastrulation, axis formation, gill formation, alimentary tract formation and axial musculature formation (Nieuwkoop and Faber, 1967). Clearly, these data suggest that the process of development is not simply adding new cells and structures associated with them, as standard allometric paradigms would suggest, but is a highly episodic, yet coordinated, process.

Protein content was positively correlated with dry and wet mass during development. Reported levels of embryonic proteins in developing fish larvae ranged from 56 to 67 % of dry mass (Dabrowski *et al.* 1984; Dabrowski and Luczynski, 1984; Smith, 1957; Wang *et al.* 1987; Zeitoun *et al.* 1977). Although these data for fish are similar to our findings for *X. laevis* between NF 47 and 51, it is unclear from reported studies on fish how $Prot_{tot}$ changes in early development, as most reported measurements taken in fish were post-hatch. Additionally, *X. laevis*, unlike meroblastic fish, lack a discrete yolk sac and therefore may also lack the quantity and distribution of yolk proteins associated with stabilizing neutral lipids. However, it is clear from the data for percentage body composition (Table 2) that $Prot_{tot}$ increases with progressive development. Interestingly, there is a slight decline in total protein content between NF 18 and 41, which corresponds well with the dramatic rise in $\dot{M}_{O_2}$ seen over this same range. $Prot_{tot}$ over the last third of development shows a clear rise that is correlated with the start of free swimming and food ingestion, suggesting that both intrinsic and extrinsic foodstuffs are being directed into producing muscle mass.

Whole-animal lipid composition throughout development showed little change up to NF 39–41, at which point it decreased in a linear fashion. By mid yolk absorption (NF 42–43), body composition reached a value of approximately 50 % neutral lipids. This is consistent with the work of Eldridge *et al.* (1981), who showed that bulk yolk lipid constituted 52 % of dry mass in the common carp *Cyprinus carpio*. Although our data are similar to those of Eldridge *et al.* (1981), our values for amounts of lipids in *X. laevis* are significantly higher than those reported for trout (*Salmo gairdneri*), Atlantic salmon (*Salmo salar*) and white sturgeon (*Ancipenser transmontanus*) larvae (Hamor and Garside, 1977; Wang *et al.* 1987; Zeitoun *et al.* 1977). Different reproductive strategies may explain this difference. *X. laevis* routinely lays large numbers of eggs which hatch at 1 day post-fertilization and completely absorb all yolk by day 12 at 25 °C. In contrast, *S. gairdneri* hatches at 20 days post-fertilization (Rombough, 1988*a*) and consumes all yolk by day 45 at 15 °C. If one considers an average Q_{10} of 2 for trout, it is evident that *X. laevis* is clearly consuming more energy in a much shorter time. Although *X. laevis* larvae contained a large quantity of neutral lipids, the lipids that supported metabolism and contributed to the decrease in energy seen overall were fatty acids and triglycerides. These findings are

in good agreement with the work of Finn *et al.* (1996), who demonstrated that more than 90 % of the routine metabolic rates just prior to hatch were supported by fatty acid degradation. The rapid decline in triglyceride and fatty acid levels between NF 13 and 47 (Fig. 4) in *X. laevis* was highly correlated with an increase in metabolic rate over this same stage range, suggesting that overall respiration was fueled *via* the catabolism of these lipids (Fig. 5). Interestingly, long-chain alcohol levels showed a rapid increase up to NF 44–45 (Fig. 4D), consistent with the premise that triglycerides and fatty acids were oxidized to alcohols. Clearly, these data suggest that the bulk oxidation of neutral lipids must be supporting the overall energy requirements of these embryos and indicate that amphibians, unlike teleosts, employ different lipid-utilization strategies at different stages of development.

Energy content and metabolism

Total gross energy content (E_{TG}), which consists of the bulk energy from lipids and proteins, is an important component of total energy reserves in any organism. This measure provides a determinant of total available energy and provides a quantitative measure of its partition. E_{TG} declined in the later stages development, and the majority of this decline was due to a rapid decrease in total neutral lipid content, while $Prot_{tot}$ increased in late development (Table 2). These findings suggest that E_{LG} is the primary contributor to total energy early in development, and by metamorphic climax these reserves are significantly depleted. Conversely, total protein content increased by more than 50 % over these same stage ranges, further suggesting that resource distribution was being put into muscle mass (Table 2). These data are in general agreement with observations in anuran larvae (Burggren and Just, 1992), in which embryos hatch with large quantities of yolk and abstain from feeding for several days post-hatch while lipid reserves are being depleted. Although E_{TG} provides an overall indication of gross energy and its partitioning, it provides little information about what fraction of this energy is utilized in support of respiration.

Stoichiometric energy balance (E_{SB}) affords a quantitative expression of body composition as a function of aerobic metabolism, providing a direct comparison of available energy per unit time, with equivalent energy constituents. Neutral lipid content and $\dot{M}_{O_2}$, both expressed mass-specifically, increased exponentially between NF 1 and 41, with lipid energy making up more than three-quarters of the total body energy for respiration by NF 39–41 (Fig. 5). Similarly, levels of protein and 'other' body constituents increased by more than an order of magnitude over the developmental period. However, by late in development (NF 50–51), neutral lipids only comprised approximately one-sixth of the total energy for respiration, while proteins and 'other' body components made up the rest. This substantial reduction in lipid content and concomitant fractional increase in the content of protein and 'other' body components that occur late in development indicate that lipids are clearly providing the majority of energy necessary for aerobic metabolism and growth. Results from turbot embryos and larvae (Finn *et al.* 1996) are in general agreement with the current study; however, the timing of these events differs and is believed to be due to contrasting morphological and physiological strategies employed through development. Overall, these data indicate that resource depletion and energy stores are in delicate balance that, and unlike most teleost fish larvae, *X. laevis* utilizes lipids as its main energy source to fuel aerobic metabolism and growth.

Table 4. *Lean body mass and its implications for overestimation of physiological variables in developing* Xenopus laevis

Variable	NF stage range	Reported literature value	Reported literature wet mass (mg)	Calculated dry mass (mg)	Calculated value/dry mass	M_{LB} (mg)	Corrected value/ M_{LB}	Percentage error (%)	Reference
$\dot{M}_{O_2}$	37–45	0.25	27	1.9	3.5	0.9	7.1	50.5	Territo and Burggren (1998)
$\dot{M}_{O_2}$	40–44	0.23	26	1.8	3.2	0.7	8.5	61.7	Hastings and Burggren (1995)
$\dot{Q}$	37–41	0.07	3.3	0.3	0.9	0.1	1.8	50.5	Territo (1996)
$\dot{Q}$	45–49	0.15	20	1.4	2.1	1.1	2.6	20.3	Hou and Burggren (1995*b*)
$\dot{Q}$	44–46	2.5	10	0.7	34.3	0.4	63.8	46.2	Orlando and Pinder (1995)
*V*S	45–49	1.5	20	1.4	21.0	1.1	26.3	20.2	Hou and Burggren (1995*b*)
P_{tr}	40–44	1.5	4	0.3	19.5	0.1	50.9	61.7	Fritsche and Burggren (1996)
P_{tr}	46–47	3.5	10	0.7	48.1	0.6	60.3	20.3	Hou and Burggren (1995*a*)

All literature values were expressed per unit wet mass.

Dry mass data were determined on the basis of the regression equations presented in Table 1.

Lean body mass (M_{LB}) was calculated from dry mass using equation 1.

Percentage errors are reported (column 9) relative to M_{LB} and compare calculated values per dry mass (column 6) with corrected values per M_{LB} (column 8).

Rates of oxygen consumption ($\dot{M}_{O_2}$) are reported in $\mu mol\ g^{-1}\ h^{-1}$.

Cardiac output values ($\dot{Q}$) are reported in $(\mu l\ mg^{-1}\ min^{-1}$, and stroke volume (*V*S) is in $nl\ mg^{-1}$.

Pressure measurement from the truncus arteriosus (P_{tr}) are as reported in the literature (mmHg). Note that the net pressure measurements are unaffected by M_{LB}, but mass covaries with pressure and would, therefore, represent a significantly smaller allometric value.

Lean body bass and its implications for expression

The study of development and the physiological processes that underlie it have been actively investigated for more than a century. Over this period, few studies have attempted to deal with the expression of lean body mass (M_{LB}) in amphibians. Extensive studies in fish larvae (Kamler and Kato, 1983; Lapin and Matsuk, 1979; Lasker, 1962; Moroz and Luzhin, 1976; Rombough, 1988*a*) have investigated yolk utilization, from which an index of M_{LB} can be calculated. Although a fairly comprehensive data set exists for fish larvae, we are only aware of one paper (Rombough, 1988*a*) that has directly attempted to compensate for overestimates associated with yolk mass when considering $\dot{M}_{O_2}$. Taken in the context of developing amphibians, there is a clear chasm in our knowledge of how body composition changes with progressive development and, therefore, how this may affect M_{LB}. Consequently, work performed on developing amphibians has largely ignored this problem, yielding to the assumption that wet, or in some cases dry, mass was a true index of metabolizing tissue. Clearly, our work suggests that this is not the case. The observation made by Burggren and Just (1992) that amphibians retain large quantities of yolk is supported by the present study. Applying equation 1 to our data on body composition, we calculated M_{LB} in *X. laevis*. Our data suggest that lipids constitute a major fraction of overall mass and that M_{LB} may be overestimated by more than 50 % over the first half of development (Table 4). The obvious consequence is that overestimating M_{LB} may result in serious underestimation of measured variables such as the rate of oxygen consumption, cardiac output and stroke volume.

Over the past century, measurements of O_2 consumption have revealed that $\dot{M}_{O_2}$ increases sharply during the first third of development, contrary to standard allometric paradigms and, as a result, numerous researchers have commented on the unusual shape of respiratory curves. In some cases, these changes have been attributed to plotting differences (Lovtrup, 1959), whereas others have implicated lipids directly (Atlas, 1938; Wills, 1936). Interestingly, despite the observation that lipid composition may significantly influence M_{LB}, neither of these authors chose to address this problem directly. However, Wills (1936) chose to express $\dot{M}_{O_2}$ per mg of nitrogen, which was believed to be a better index of M_{LB} and, it was hoped, would ameliorate the problems associated with wet or dry mass. Our work has shown that proteins are in dynamic flux through development; hence, expression of $\dot{M}_{O_2}$ per mg of nitrogen would in fact misrepresent M_{LB}. Evaluation of Wills' (1936) plots clearly reveals that this did not afford any greater accuracy and may have exacerbated the shapes of the curves and misrepresented M_{LB}.

In an effort to illustrate this phenomenon more clearly, we have evaluated several contemporary articles which have expressed such physiological variables as rate of oxygen consumption ($\dot{M}_{O_2}$), cardiac output ($\dot{Q}$), stroke volume (V_S) and mean truncus pressure (P_{tr}) mass-specifically. Ignoring lipid composition throughout development can have profound effects on the variable of interest, as indicated in Table 4. In all cases, the extent of the underestimation is related to the stage of development considered. For instance, it is clear that $\dot{M}_{O_2}$ could be 51–62 % lower than would be expected had M_{LB} been considered from NF 37 to NF 45. Similarly, the measures of cardiovascular performance ($\dot{Q}$, V_S, P_{tr}) could also be underestimated by as much as 62 %. As the study of physiology begins to turn to earlier and earlier stages of development in an attempt to clarify the onset and control of fundamental physiological processes, it becomes more imperative that attention be paid to M_{LB}. Moreover, not only does M_{LB} needs to be considered overall, but levels of individual body constituents need to be assessed if an accurate appraisal is to be made of the factors that most influence wet mass.

We have demonstrated that body composition throughout development is a highly dynamic, yet coordinated, process. Furthermore, we have shown that respiration is primarily fueled *via* the degradation of neutral lipids with fatty acids, with triglycerides providing the bulk of this energy. As a result, estimates of lean body mass may be highly skewed during development. In addition, we have demonstrated that, if body mass is not corrected to M_{LB}, measures of physiology could be underestimated by more than 60 % when the variable is expressed mass-specifically.

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