

## Pattern of growth and utilization of abdominal fat bodies during larval development and metamorphosis in five South Indian anurans

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The tadpoles of *Rana tigrina*, *Rana cyanophlyctis*, *Rana curtipes*, *Polypedatus maculatus* and *Bufo melanostictus* grew in size (mass and snout vent length or SVL) progressively until metamorphic climax. The abdominal fat bodies first appeared in stages 25–30; and accumulation/utilization of fat during larval development and metamorphosis varied with the species. In *B. melanostictus*, fat bodies were barely seen. In laboratory-reared *R. curtipes*, body weight and fat body mass were better developed than in the wild caught. The amount of fat deposition was related to the duration of metamorphosis in the various species studied. The findings thus show that the size of fat bodies in the larval anurans is correlated with the body mass, SVL as well as duration of metamorphosis.

THE conspicuous nature of abdominal fat bodies and seasonal and/or annual changes in their mass in adult anurans are well documented<sup>1-3</sup>. Such changes in the fat body mass in amphibians indicate changes in the nutritional status of a given individual<sup>4</sup>. The abdominal

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\*For correspondence.

**Table 1.** Range of SVL, body weight, and fat body mass of tadpoles in relation to the developmental stages in five anuran species

| Species                              | Tadpole stage | SVL (mm) | Body mass (g) | Fat body mass (mg) |
|--------------------------------------|---------------|----------|---------------|--------------------|
| <i>Rana tigrina</i><br>(21)          | 27-44         | 8-26     | 0.09-1.57     | 0.0-8              |
| <i>R. cyanophlyctis</i><br>(28)      | 25-44         | 7-28     | 0.05-3.34     | 0.0-49             |
| <i>R. curtipes</i><br>(33)           | 27-45         | 14-32    | 0.35-4.19     | 0.0-64             |
| <i>Polypedatus maculatus</i><br>(32) | 25-41         | 6-17     | 0.02-1.35     | 0.0-7              |
| <i>Bufo melanostictus</i><br>(30)    | 27-44         | 4-10     | 0.01-0.17     | -                  |

Numbers in parentheses indicate the number of tadpoles.

fat bodies, like the gonads, arise from the mesoderm during embryonic development in amphibians. That the fat bodies have a supportive role in reproduction was recently shown by Saidapur and Prasadmurthy<sup>5</sup>. While the gonadal development and differentiation are studied in a few anurans<sup>6-9</sup>, there are no reports on the pattern of growth and utilization of the fat bodies during their larval development. The present study deals with the changes in fat body mass, and its possible relationship with body weight and snout vent length (SVL) during the various stages of larval development and metamorphosis in five anuran species, *Rana tigrina*, *R. cyanophlyctis*, *R. curtipes*, *Polypedatus maculatus*, and *Bufo melanostictus*. Since *R. curtipes* exhibits a prolonged larval life, the changes in the fat body mass in the laboratory-reared and wild caught tadpoles of *R. curtipes* was also studied.

The tadpoles of different species were collected from ponds around Dharwad and from streams in the western ghats. Each collection included tadpoles in various stages of development. The developmental stages of tadpoles were identified according to Gosner<sup>10</sup>. The tadpoles were autopsied within a few hours of collection. The SVL (mm), body weight (g), and fat body mass (g) were recorded. The fat bodies in *B. melanostictus* were so minute that they could not be weighed.

The data were analysed using Pearson correlation coefficient analysis to determine the relationship between fat body, body weight, and SVL (SPSS for Windows Release 6.13). The changes, if any, in the body weight and fat body mass after metamorphosis were compared with those of the tadpoles at stage 41 (a stage designated as premetamorphic climax), using Mann-Whitney *U* test, in case of *R. cyanophlyctis*, *R. curtipes* and *P. maculatus*. Since data on newly-metamorphosed *R. tigrina* were insufficient, they were not statistically analysed.

For carrying out comparative studies on *R. curtipes* tadpoles (stage 25-30) from nature as well as laboratory-reared individuals, a group of *R. curtipes* tadpoles (stage 25-30) was maintained in cement cisterns (7.8' x 6.8') with 1.5' water. The tadpoles were fed boiled spinach

*ad libitum* on alternate days and reared for 2 months. At weekly intervals they were autopsied. SVL, body weight, and fat body mass of corresponding stages of tadpoles from nature-, and laboratory-reared individuals were compared, using Hotteling's  $T^2$  analysis<sup>11</sup>.

The fat bodies were first observed at stage 25-26 in *R. curtipes*, stage 28-29 in *R. tigrina*, stage 27-28 in *R. cyanophlyctis*, and at stage 29-30 in *P. maculatus* and *B. melanostictus*. The ranges in SVL, body weight and fat body mass of tadpoles of comparable stages in the five species studied are shown in Table 1. All the species showed a high positive correlation between increase in the body weight and SVL (Figure 1). Figure 2 shows that the fat bodies increased progressively in size as the tadpoles grew to their metamorphic climax except in *B. melanostictus*. Further, a positive correlation was observed between SVL and fat body mass (Figure 2) as well as between body weight and fat body mass in the tadpoles of all the species of frogs (Figure 3), however, in *B. melanostictus* fat bodies were barely visible throughout the larval development starting from their appearance at stage 29-30 to the end of metamorphosis.

The observations on the body weight and fat body mass of tadpoles at premetamorphic climax and at metamorphosis (Table 2) showed a significant increase in the fat body mass in *P. maculatus* following metamorphosis, but not in *R. cyanophlyctis* and *R. curtipes*. Also a significant decrease in body weight was observed in *R. cyanophlyctis* and *P. maculatus* following metamorphosis but not in *R. curtipes*. The amount of fat deposition found around premetamorphic climax (stages 39-41 when larval growth is complete) varied within the species (Figure 4). A study comparing the body weight and fat body mass of the laboratory-reared and wild-caught tadpoles of *R. curtipes* (Table 3) showed that in the laboratory-reared tadpoles the body weight and fat body mass were significantly greater ( $F = 3.7485$ ,  $P < 0.05$ ) than in the wild-caught tadpoles at the corresponding stages.

A discussion of our observations is given below. In

adult anurans it is well known that the fat bodies reserve excess energy during the period of food abundance<sup>1,5</sup>. The increase in size of fat bodies with larval growth in *R. curtipes*, *R. cyanophlyctis*, *R. tigrina* and *P. maculatus* suggests that these tadpoles forage well, and store surplus food energy in the form of abdominal fat bodies. However, *B. melanostictus* is an exception. The amount of fat deposition in the anurans during larval development seems to have some bearing on the duration of premetamorphic stage, followed eventually by metamorphosis, in addition to their body size. For instance, in *R. curtipes*, the larval duration is about 6 months, the tadpoles have larger bodies compared to the tadpoles of other four anuran species. Hence, their energy needs are also high. Apparently, these tadpoles feed well and reserve a large amount of energy in fat bodies to overcome unforeseen food draughts, if any, as do the adult amphibians during the periods of food abundance<sup>1,5</sup>. Likewise, a relationship between fat body mass and body weight/duration of larval period in *R. cyanophlyctis*,

*R. tigrina*, and *P. maculatus* is apparent. The larval duration for *R. cyanophlyctis* is about 60–70 days while for *P. maculatus*, and *R. tigrina* it is approximately 45–60 days. The size hierarchy of tadpoles is as follows:

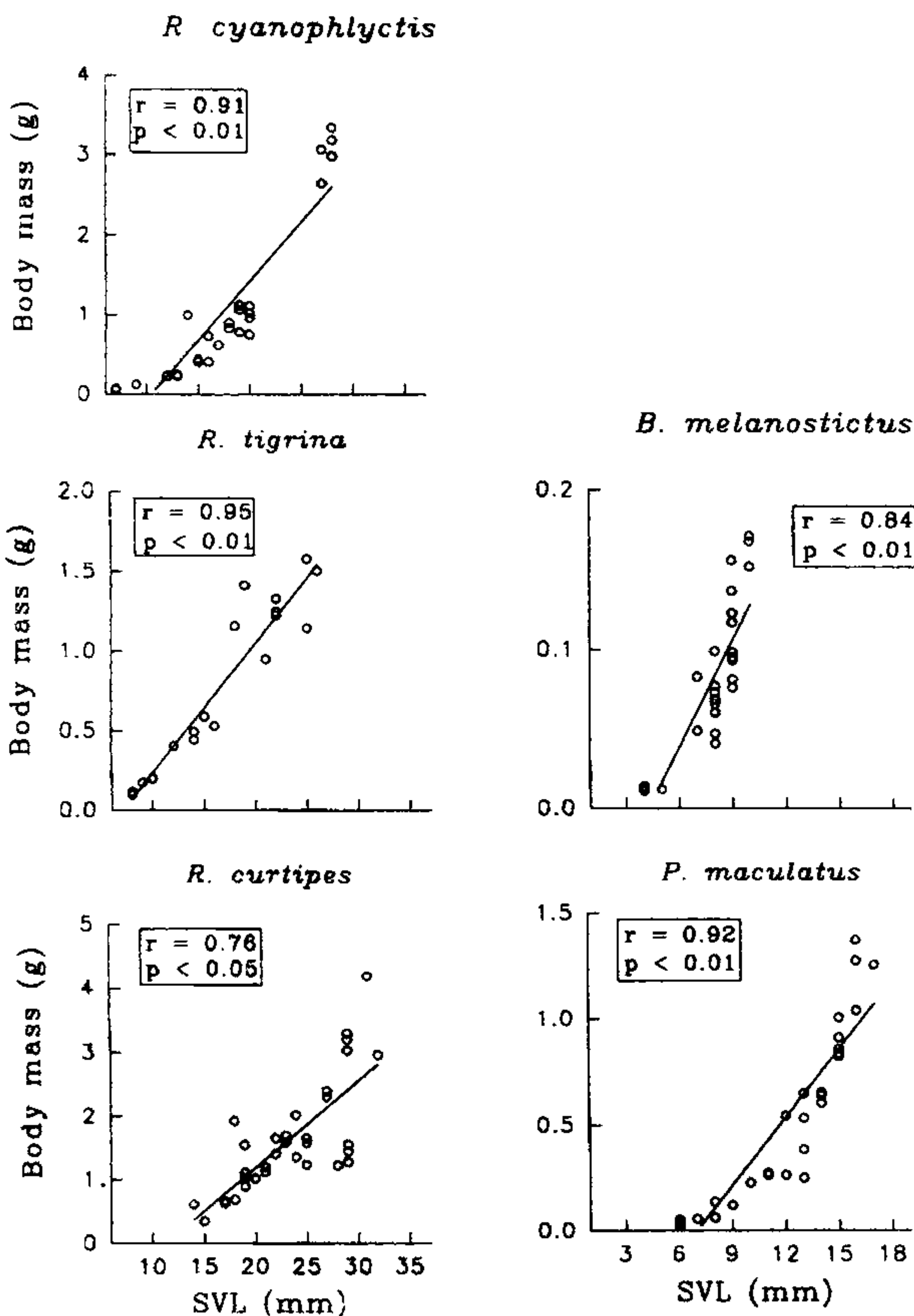


Figure 1. Correlation between SVL and body weight in the tadpoles of *R. cyanophlyctis*, *R. tigrina*, *R. curtipes*, *P. maculatus* and *B. melanostictus*

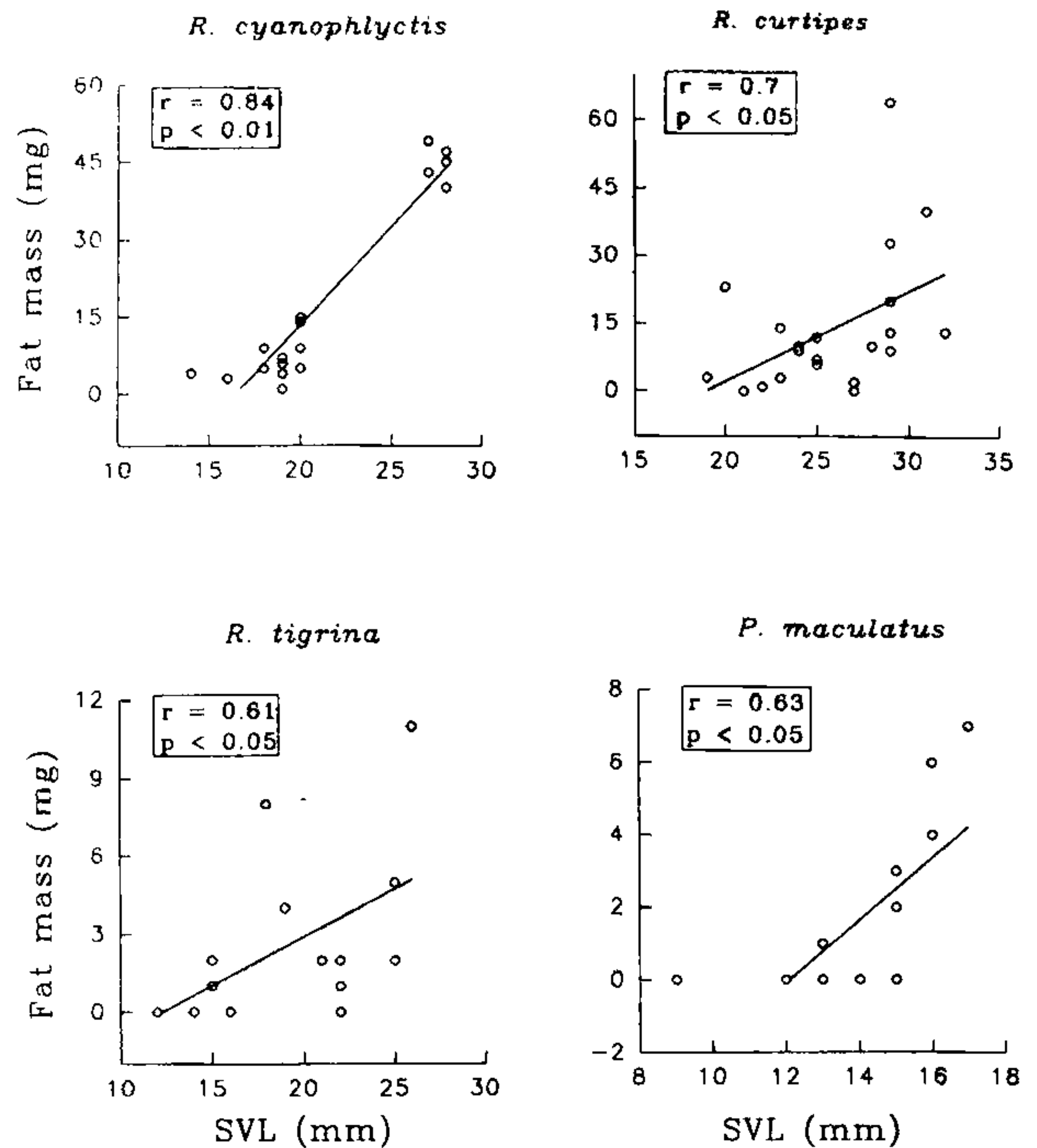


Figure 2. Correlation between SVL and fat body mass in *R. cyanophlyctis*, *R. tigrina*, *R. curtipes* and *P. maculatus*.

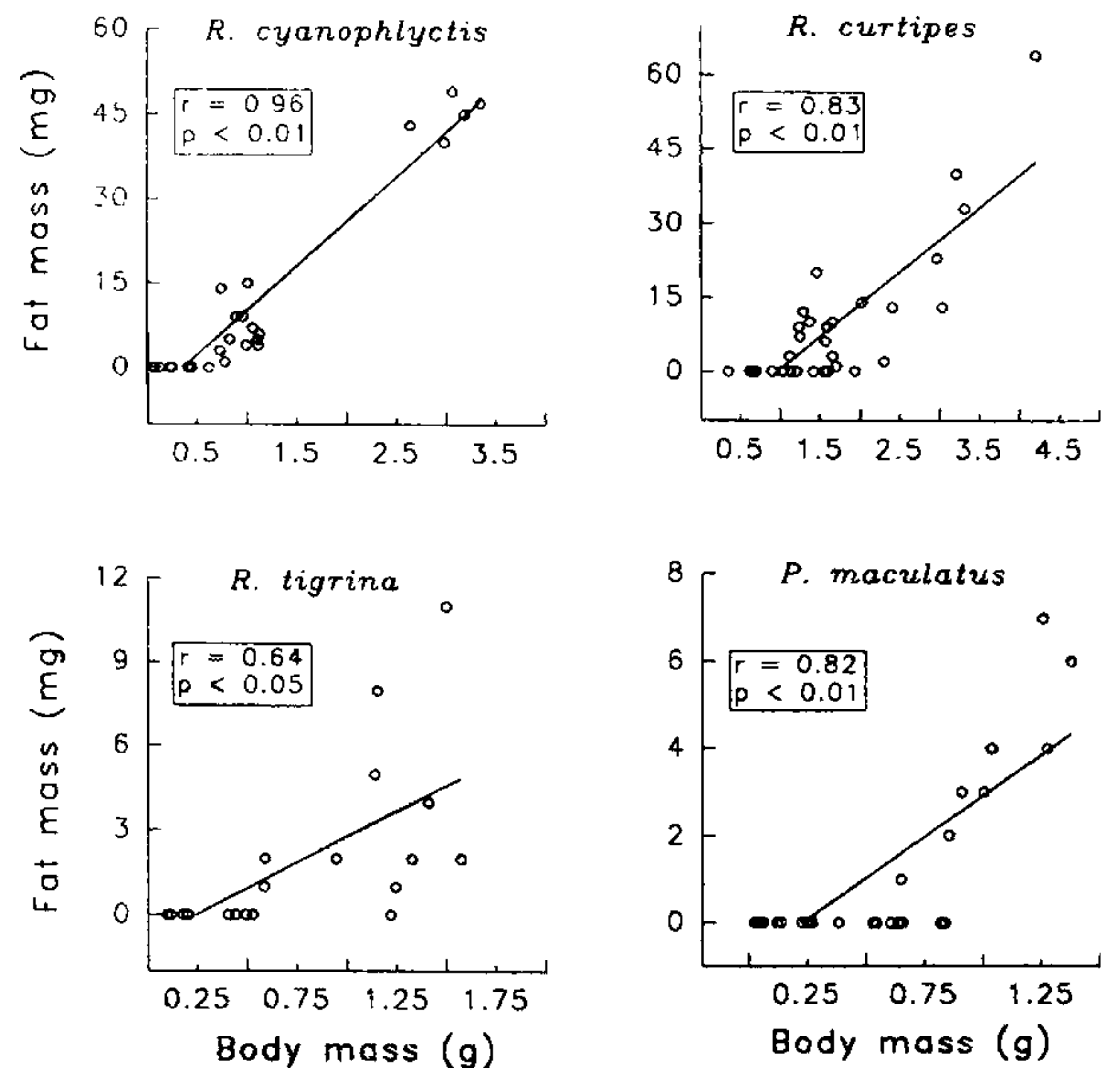
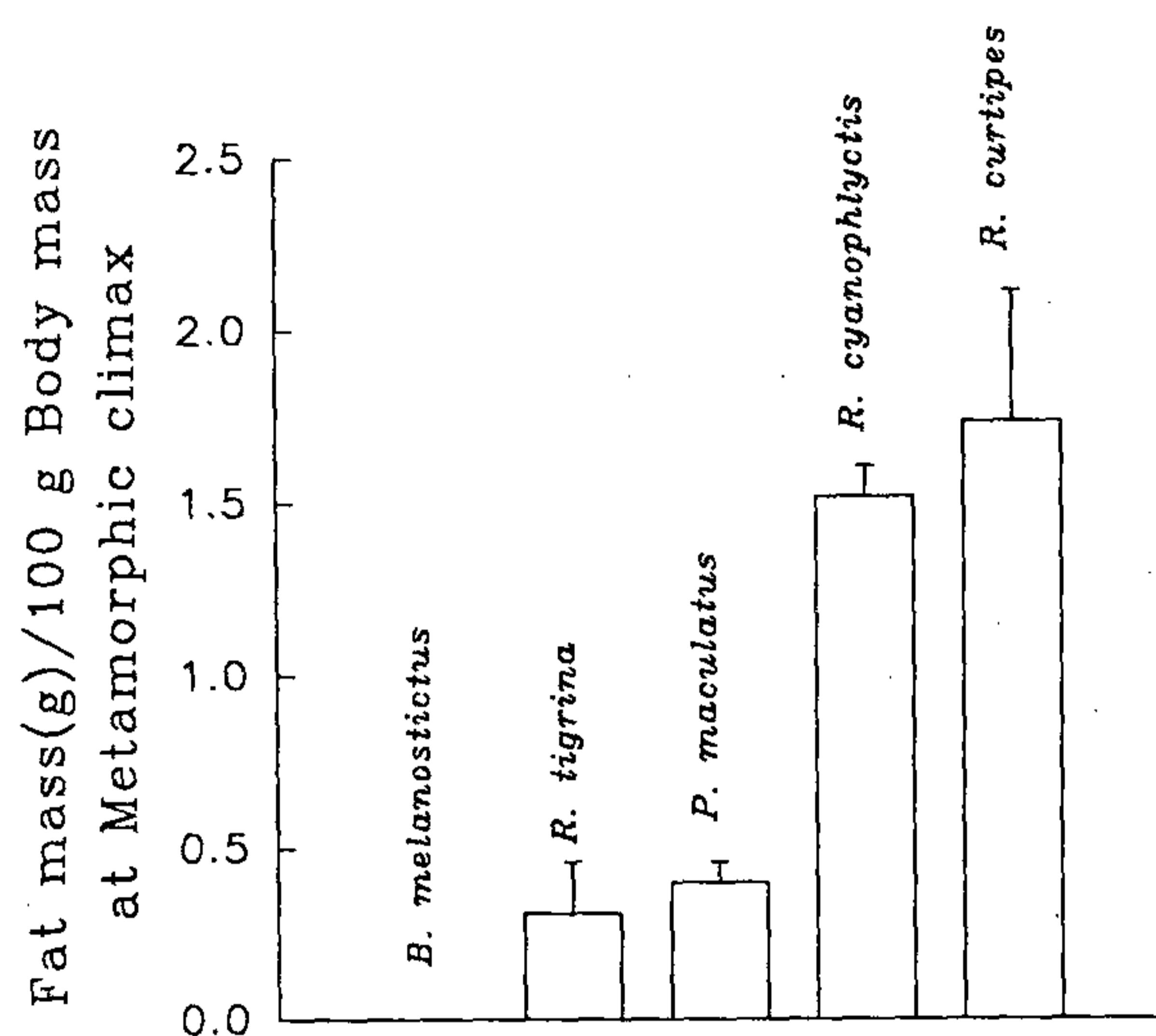


Figure 3. Correlation between body weight and fat body mass in *R. cyanophlyctis*, *R. tigrina*, *R. curtipes* and *P. maculatus*.

**Table 2.** Body weight and fat body mass in tadpoles at premetamorphic climax and at metamorphosis

| Species                 | Stage 41           |                         | Stage 46            |                         |
|-------------------------|--------------------|-------------------------|---------------------|-------------------------|
|                         | Body wt (g)        | Fat wt(g)/100 g body wt | Body wt (g)         | Fat wt(g)/100 g body wt |
| <i>R. cyanophlyctis</i> | 2.97 ± 0.12<br>(4) | 1.50 ± 0.07<br>(4)      | 0.95 ± 0.06*<br>(4) | 0.95 ± 0.26<br>(4)      |
| <i>R. curtipes</i>      | 3.08 ± 0.81<br>(5) | 1.49 ± 0.49<br>(5)      | 2.97 ± 0.31<br>(6)  | 1.21 ± 0.45<br>(6)      |
| <i>P. maculatus</i>     | 1.23 ± 0.08<br>(4) | 0.40 ± 0.06<br>(4)      | 0.72 ± 0.07*<br>(5) | 1.21 ± 0.39**<br>(5)    |

Figures in parentheses indicate the number of tadpoles; Data is given as mean ± SE; \* $P < 0.05$  \*\* $P < 0.001$ ; comparison is made for body weight and fat body mass between stage 41 and 46 of development of the same species.

**Figure 4.** Amount of fat body mass during premetamorphic (39–41) stage in five anuran species.

*R. curtipes* > *R. cyanophlyctis* > *R. tigrina* > *P. maculatus* > *B. melanostictus*. Virtually no fat storage occurs during larval development in *B. melanostictus*, possibly because it has a very short larval duration (3–4 weeks) and small larval body weight.

During the metamorphic climax, the oral apparatus undergoes drastic structural changes and, therefore, the tadpoles consume less<sup>12</sup> or no food<sup>13</sup>. In *R. curtipes* metamorphic changes (stage 42–46) take place between 7–10 days, and in other species studied in 2–3 days. Insignificant change in the fat body mass in recently metamorphosed *R. curtipes* and *R. cyanophlyctis* suggests *albeit indirectly*, that the tadpoles might not utilize energy stored in fat bodies during metamorphosis. Interestingly, the fat bodies in recently metamorphosed *P. maculatus* weighed heavier than those in premetamorphic climax.

**Table 3.** Comparison between the body weight and fat body mass of laboratory-reared and wild-caught tadpoles of *R. curtipes*

| Stage | Wild-caught         |                         | Laboratory-reared    |                         |
|-------|---------------------|-------------------------|----------------------|-------------------------|
|       | Body wt (g)         | Fat wt(g)/100 g body wt | Body wt (g)          | Fat wt(g)/100 g body wt |
| 27–34 | 1.31 ± 0.18<br>(18) | 0.09 ± 0.05<br>(18)     | 1.72 ± 0.11*<br>(33) | 1.68 ± 0.05*<br>(33)    |
| 35–42 | 2.38 ± 0.32<br>(9)  | 0.34 ± 0.11<br>(9)      | 3.38 ± 0.24*<br>(16) | 1.85 ± 0.45*<br>(16)    |
| 43–46 | 2.08 ± 0.23<br>(18) | 0.66 ± 0.18<br>(18)     | 3.41 ± 0.60*<br>(10) | 4.79 ± 2.59*<br>(10)    |

\* $P < 0.05$  (Hotteling's  $T^2$  analysis).

An increase in body size during terminal phase/premetamorphic climax is reported in *R. tigrina*<sup>14</sup>. This rise in body weight prior to metamorphic climax is attributed to the accumulation of energy needed for enhancing metamorphosis in *R. tigrina*<sup>14</sup>. Our present findings on *R. cyanophlyctis*, and *P. maculatus* which show an increase in body weight as well as fat body mass at premetamorphic climax support the above view. Interestingly, there was no significant change in the body weight of tadpoles between premetamorphic and metamorphosed *R. curtipes* in both wild-caught and laboratory-reared individuals. This may be because the duration of metamorphic changes is longer in *R. curtipes* and hence, energy utilization from body source would be at lower pace than in the other two species in which metamorphic duration is very short. The explanation for this observation seems to be that the tadpoles need energy for their growth and metabolism, as well as spend some energy in search of food and predator avoidance. Since the tadpoles reared in the laboratory are exempted from such expenditure, they apparently divert their energy saved in searching food and escaping from predators towards better body growth and fat bodies for storage.

The present study demonstrates a diversity in the deposition of fat and its utilization in anuran tadpoles which, besides the food abundance, may depend on

larval duration, expenditure of energy in search of food, and predator avoidance.

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**Einstein's Miraculous Year – Five Papers That Changed the Face of Physics**, edited and introduced by John Stachel, Princeton University Press, 41 William Street, Princeton, NJ 08540, USA. 1998.

A long time ago the theoretical physicist George Gamow wrote a book with the title *Thirty Years that Shook Physics*. Inspired by his phrase, one may well say that 1905 – *annus mirabilis* – was the one year when Albert Einstein shook the foundations of physics with his five papers, four of them in the then prestigious *Annalen der Physik*, heralding the dawn of a creative career scarcely matched in the annals of science.

A chronological listing of the papers, their respective dates of receipt by and publication details in the *Annalen*, all in 1905, is (in English translation): (i) 'On a heuristic point of view concerning the production and transformation of light' (18 March; vol. 17, p. 132–148); (ii) 'A new determination of molecular dimensions' (University of Zurich dissertation; 30 April); (iii) 'On the motion of small particles suspended in liquids at rest required by the molecular kinetic theory of heat' (11 May; vol. 17, p. 549–560); (iv) 'On the electrodynamics of moving bodies' (30 June; vol. 17, p. 891–921); and (v) 'Does the inertia of a body depend on its energy content?' (27 September, vol. 18, p. 639–641). All these have appeared in 'The Collected Papers of Albert Einstein – Volume 2 – The Swiss Years: Writings, 1900–1909', English Translation, published by Princeton University Press in 1989. What John Stachel's *Einstein's Miraculous Year – Five Papers that Changed the Face of Physics* offers are modern English translations of these classics, an extended introductory essay along with individual ones for each paper, and historical and editorial notes for each. There is a brief preface by Roger Penrose where he points out that we in this century have been privileged to witness two major revolutions in physics, and Einstein had so much to do with both.

Stachel's general introduction compares Newton's and Einstein's personalities, states of preparation and mathematical abilities in their respective miracle years 1664–1666 and 1905. Comparisons are always difficult, especially when the

individuals are separated by two and a half centuries, but Stachel's account is most illuminating. While Newton was twenty two and just beginning his creative efforts in 1664–1666, Einstein in 1905 was twenty six and already a mature physicist with five publications behind him. Newton's innate mathematical strengths were of a supremely high order, while in this respect Einstein often depended on others for help. Stachel groups the five papers of 1905 into three major areas in increasing order of 'distance' from classical Newtonian physics: papers (ii) and (iii) in the above chronological listing (Nos 1, 2 in Stachel's sequence) are in the tradition of classical mechanics and show Einstein's mastery in using statistical ideas and fluctuation methods; papers (iv) and (v) (Nos 3, 4 in Stachel's arrangement) are in the tradition of the classical field theory of electromagnetism, and show the way to the reformulation of mechanics to be in harmony with the former. These four papers bring to a culmination the classical legacy of Galileo–Newton–Faraday–Maxwell–Boltzmann, and display Einstein's extraordinary understanding of this legacy. Paper (i) (No. 5 in Stachel's list) stands by itself, constitutes area three, and in Einstein's own judgement is the only one of the five that is truly revolutionary!

Paper 1 was Einstein's dissertation submitted to the University of Zurich for his Ph.D. Here he suggests using arguments based on phenomena in liquids rather than in gases to arrive at reliable estimates of molecular sizes. He uses detailed knowledge of classical hydrodynamics to calculate the effect of a solute on solvent viscosity, and on the diffusion rate of solute molecules. Comparing these two results with experimental data, he was able to estimate both Avogadro's number and the size of solute molecules. What is rather surprising about this paper, apart from his unerring intuition and choice of physical approximations, are the innumerable errors – in symbols, numerical factors and even in inferences from his own formulae – that are present! His own student Ludwig Hopf was later to recheck and correct many results, in the midst of extensive correspondence with the experimentalist Jean Baptiste Perrin.

Paper 2 on the Brownian motion is a jewel of the kinetic theory of heat. Its

influences on statistical physics, theory of stochastic processes and probability theory have been simply enormous. It was written at a time when there was considerable scepticism concerning the reality of atoms – 'atomism may have a heuristic didactic utility' alone. (In passing one recalls Gell-Mann's initial attitude to quarks in the early 1960s.) Einstein invented and recognized fluctuation as the key concept; and that the most appropriate measurable quantity is the mean square displacement of a Brownian particle, not its speed. The attentive reader will see on page 96 of Stachel's book the seed of the idea of the Dirac delta function – this in 1905! For a long time the experimental studies on Brownian motion were quite poor in resolution, and it took a while before Perrin beginning in 1908 could improve their accuracy and verify Einstein's predictions.

Papers 3 and 4 are landmarks in the evolution of physics. They established the special theory of relativity and one of its most stunning and fateful consequences – the equivalence of mass and energy. Paper 3 is so carefully crafted that it already reads like a review rather than a research paper! Stachel's introduction is an excellent historical overview of the principle of relativity, its origins in mechanics, the conflict with electromagnetism, and the final resolution. It transpires that Einstein had been possessed of these questions for seven long years, until finally a discussion with his friend Michele Besso, during which he aired his difficulties, suddenly showed him the way. This is a splendid instance of the psychological fact that clear enunciation of a problem to a willing listener can itself lead to the way out of the darkness. In a bare six weeks was the paper then composed. Einstein's analysis of space and time measurements (philosophically influenced by his reading of Hume, Mach and Poincaré), his enunciation of the two postulates underlying special relativity, the physical derivation and interpretation of the already known Lorentz transformation equations, are all part of legend. His deep unravelling of the meaning of simultaneity shows a courage in thinking and conception that was to inspire many others. The paper itself – the longest of the five – is made up of Part A setting up the new kinematics of space time, and Part B applying it to electromagnetism. Einstein realized early