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Relationship Between Body Size, Growth Rate, and Maximal Enzyme Activities in the Brine Shrimp, *Artemia franciscana*

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Abstract. Activity-body size relationships for eight enzymes (*citrate synthase*, CS; *lactate dehydrogenase*, LDH; *pyruvate kinase*, PK; *alanine aminotransferase*, ala AT; *aspartate aminotransferase*, asp AT; *glutamate dehydrogenase*, GDH; *glucose-6-phosphate dehydrogenase*, G6Pdh; and *nucleoside diphosphate kinase*, NDPK) were examined in the brine shrimp, *Artemia franciscana*. The animals were fed on the alga *Dunaliella salina*, which was provided in three concentrations representing a 25-fold range. Enzyme activities per animal (Y) were regressed against body size (M, expressed as dry mass or protein) in the form of the allometric equation, $\log Y = \log a + b \log M$, where a and b are fitted constants. For all enzymes considered, the value of the scaling exponent (b) was significantly higher when dry mass was used, as a body size index, than when protein mass was used. Therefore, the index of body size chosen can influence the exponent obtained in allometric studies. Although specific growth rates of different cultures varied greatly, no significant differences in scaling relationships were found between cultures for any enzyme. For many enzymes, growth rate may not be a source of variation in scaling relationships. Unlike the other enzymes examined, the log-transformed NDPK activity *versus* log-transformed mass was not linear; NDPK activity reached a plateau. Variation in NDPK scaling relationships with growth may provide a means to predict growth rate in *Artemia*.

Introduction

Relationships between metabolic rate processes and body size are usually described by an allometric equation of the form:

$$Y = aM^b \quad (1)$$

where Y is the rate process, M is body mass and a and b are empirically derived constants (*e.g.*, Brody, 1945; Kleiber, 1961; Peters, 1983). A much-disputed feature of this equation is the precise value of the exponent b (here referred to as the "scaling exponent"). For interspecific studies of respiration in mammals, for example, the exponent 0.75 is often cited (Peters, 1983), yet arguments have been made that the true exponent is 0.67, based on dimensional analyses (*e.g.*, Mahon and Bonner, 1983) or statistical considerations (*e.g.*, Heusner, 1982).

Disagreements such as these are difficult to resolve for a number of reasons. The measurement of a rate variable, such as respiration, has several potential sources of variation including the organism's nutritional status and activity level (Peters, 1983). The result is that data in the literature relating metabolic rate and body size may not be directly comparable. Careful analyses of the sources of variation in scaling relationships are needed to overcome these problems.

At least two sources of variation have not been accounted for in previous scaling studies: the index of body size used and the growth rate. The index of body size used may be an important source of variation, particularly in intraspecific studies where different developmental stages are compared. In mammals, for example, body composition varies widely as they mature; bone and other structural tissues comprise a larger portion of the mass of young mammals, while lipid stores increase disproportionately with size and age (Taylor, 1980). In invertebrates, body composition is much more variable, even within adult organisms, and it is also affected by food concentration (Mayzaud, 1986). The variable traditionally selected for use in scaling studies is fresh mass. Because a large portion of fresh mass is water, substantial error can be introduced due to dehydration or, in the case of aquatic organisms, water associated with the outer surface. As an alternative, dry mass is more reproducible, particularly for small or-

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ganisms (Downing and Rigler, 1984), but comparisons of dry mass imply, often unjustifiably, a constancy of composition. Ideally, what is required is a mass variable highly correlated with the metabolically active mass of tissue (because this is most relevant to a metabolic rate variable). Protein constitutes a relatively constant proportion of mass across developmental stages in mammals (Brody, 1945; Kleiber, 1961; Schmidt-Nielsen, 1984), and may also meet the requirements for a mass variable in invertebrates.

Growth has been defined by Bertalanffy (1957) as the difference between catabolic and anabolic processes. Bertalanffy suggested that these processes vary with body size in different ways. If this is true, then relationships between integrative measures of metabolism (*i.e.*, respiration) and body size may change with growth rate of the organisms. For example, comparing the respiration-body size relationships of a fast-growing group of animals in which anabolic processes dominate, with a more slowly growing group in which catabolism and anabolism are more nearly balanced, may yield conflicting results.

In this study, we address the issue of the sources of variation in scaling relationships. We ask, in particular, whether the index of body size used or the growth rate of the organism can affect the scaling exponent. Because of the wide range of parameters that can influence an index of metabolic rate, such as respiration, we examined the maximal activities of selected enzymes as alternative rate variables. Enzyme assays can be conducted under defined conditions, and afford high precision. For an experimental organism we selected the brine shrimp, *Artemia franciscana*, a crustacean whose growth rate can be manipulated easily in the laboratory. Growth in *Artemia* is exponential for the early part of its life history (Berges, Roff, and Ballantyne, unpub.), which means that, over a particular range of body size, growth rate is not correlated with body mass. This independence is a necessary precondition for distinguishing the effect of growth rate from the effects of body mass. By comparing enzyme activity-body size relationships among groups of animals growing at different rates, we could determine whether growth rate can be considered an important source of variation in scaling relationships. In addition, by measuring both protein and dry mass we could examine the effects of different mass variables on scaling relationships.

Enzymes selected for assay were as follows: *citrate synthase* (CS), a rate-limiting enzyme in the Krebs cycle that provides an index of aerobic metabolic rate (Hochachka *et al.*, 1970); *lactate dehydrogenase* (LDH), which allows an assessment of anaerobic metabolic capacity (Somero and Childress, 1980); *pyruvate kinase* (PK), which provides an indication of maximal potential flux through glycolysis; *aspartate* and *alanine aminotransferase* (ala AT and asp AT), which function in amino acid metabolism in both synthetic and degradative pathways; *glutamate dehydrogenase* (GDH), which may provide an index of nitrogen excretion and, therefore, of the potential flux toward amino acid degradation; and *glucose-6-phosphate dehydrogenase* (G6Pdh) and *nucleoside diphosphate kinase* (NDPK), two primarily anabolic enzymes whose activities might be expected to respond to growth differences. G6Pdh is the rate-limiting enzyme in the hexose monophosphate shunt (Hochachka and Somero, 1984), providing both NADPH, the primary reducing power for anabolic processes, and ribulose sub-units for nucleic acid synthesis. NDPK acts to transfer energy from ATP into the other triphosphate nucleotides (*e.g.*, CTP, UTP, ITP) that are used preferentially in synthesis pathways; it may therefore control the allocation of energy to growth processes.

Materials and Methods

Cysts of *Artemia franciscana* SFB were obtained from San Francisco Bay Brand Inc. (Newark, California); all animals used were hatched from a single lot. Brine shrimp were hatched in 18 ‰ seawater (Instant Ocean Brand, Aquarium Systems, Mentor, Ohio) at 25°C in a 60-l common aquarium. Approximately 1000 animals were transferred to each of three 20-l aquaria for the feeding experiments. The green alga, *Dunaliella salina*, which is a suitable food item for *Artemia* (Mason, 1963; Reeve, 1963), was grown in 35 ‰ seawater in f/2 medium (Guillard and Ryther, 1962).

On two separate occasions (trials 1 and 2), three food levels were established to provide a range of growth conditions based on growth results obtained by Reeve (1963). These were: 2000 cells · ml^{-1} (low food, L), 10,000 cells · ml^{-1} (medium food, M) and 50,000 cells · ml^{-1} (high food, H). Shrimp were placed in aquaria with food soon after hatch. The batch culture was sieved with a 350 μm net to ensure that only hatched nauplii of uniform size were introduced to each culture. The concentration of algal cells was monitored with a light microscope and a Levy Ultraplane haemocytometer (Guillard, 1978), and the cells were replenished once daily. Each day of the experiment, 6 random samples of 25 shrimp each were taken from each culture. Three samples from each culture were analyzed for protein content and, on alternate days, for enzyme activities. The remaining three groups from each culture were preserved in 2% formalin for later dry mass measurement.

Brine shrimp were homogenized in 50–1000 μl of ice-cold 50 m*M* imidazole buffer, pH 7.4, using a Megason PA-300 sonicator at maximum setting for three bursts of 10 s each. The homogenates were of sufficiently low optical density that centrifugation was not required.

The protein level of the homogenates was found by microassay, the procedure based on the Coomassie bril-

liant blue assay of Bradford (1976) [Bio-Rad Laboratories (Canada) Inc., Mississauga, Ontario] with bovine serum albumin as the standard. Preliminary experiments showed that imidazole buffer did not affect the linearity of the assay.

Maximum enzyme activities were determined using a Varian DMS 100 UV-Visible spectrophotometer equipped with a thermostatted cell changer maintained at 25° (±0.1°) C with a Haake D8 circulating temperature bath. Reaction rates were determined by increase or decrease in absorbance of NADH or NADPH at 340 nm. *Citrate synthase* was monitored at 412 nm using 5,5′ dithiobis 2-nitrobenzoic acid (DTNB). Enzyme activity was expressed as units per animal, where one unit equals 1 μmol substrate converted to product per minute. Conditions for assay procedures were adapted from Hochachka *et al.* (1970) and Emmett and Hochachka (1981), unless otherwise indicated, with substrate and cofactor concentrations optimized to give maximum activity. All assays were conducted in 50 m*M* imidazole pH 7.2, except CS, which was assayed at pH 8.2. Other conditions were as follows:

Citrate synthase (CS), EC 4.1.3.7: 0.1 m*M* DTNB, 7.0 m*M* $MgCl_2$, 0.3 m*M* acetyl coenzyme A, 0.5 m*M* oxaloacetate (omitted from control).

Lactate dehydrogenase (LDH), EC 1.1.1.27: 0.2 m*M* NADH, 100 m*M* KCl (Somero and Childress, 1980), 2.0 m*M* sodium pyruvate (omitted from control).

Pyruvate kinase (PK), EC 2.7.1.40: 0.2 m*M* NADH, 5.0 m*M* ADP, 50 m*M* KCl, 10 m*M* $MgCl_2$, 5.0 m*M* phosphoenolpyruvate (omitted from control) and excess lactate dehydrogenase.

Alanine aminotransferase (ala AT), EC 2.6.1.2: 200 m*M* L-alanine, 0.2 m*M* NADH, 0.025 m*M* pyridoxal phosphate, excess LDH and 10.5 m*M* alpha-ketoglutarate (omitted from control).

Aspartate aminotransferase (asp AT), EC 2.6.1.1: 0.2 m*M* NADH, 7.0 m*M* alpha-ketoglutarate, 0.025 m*M* pyridoxal phosphate, excess malate dehydrogenase, and 30 m*M* L-aspartate (omitted from control).

Glutamate dehydrogenase (GDH), EC 1.4.1.2: 0.2 m*M* NADH, 250 m*M* ammonium acetate, 0.1 m*M* Na_2EDTA, 1.0 m*M* ADP, and 14.0 m*M* alpha-ketoglutarate (omitted from control).

Nucleoside diphosphate kinase (NDPK), EC 2.7.4.6: 0.2 m*M* NADH, 20 m*M* $MgCl_2$, 2.0 m*M* ATP, 70 m*M* KCl, 1.1 m*M* phosphoenolpyruvate, excess LDH, excess PK, and 0.7 m*M* thymidine diphosphate. Two controls were run; homogenate was omitted from one, and thymidine diphosphate from the other (Agarwal *et al.*, 1978).

Glucose - 6 - phosphate dehydrogenase (G6Pdh), EC 1.1.1.49: 0.4 m*M* NADP, 7.0 m*M* $MgCl_2$, 1.0 m*M* glucose-6-phosphate (omitted from control).

Because the cultures differed markedly in their concentrations of *Dunaliella salina,* we determined whether enzymes in the algae could bias determinations of enzymes in *Artemia.* Known volumes of culture were filtered and homogenized, as for *Artemia,* and were centrifuged at 18,000 × *g* for 10 min in a Sorvall RCB-5 refrigerated centrifuge. Enzyme assays were performed on the supernatants under conditions identical to those used for *Artemia.*

In the smallest post-yolk sac nauplii, *Dunaliella* could contribute, at most, 1.2% of asp AT activity measured in *Artemia;* this is well within the error for replicate determinations. Similar analyses for other enzymes show even less potential for interference.

The mass of preserved samples of brine shrimp was determined about 3 weeks after fixation. Animals were rinsed well in deionized water, placed in pre-massed pans, and dried for 12 h at 50°C. Immediately upon removal from the drying oven, samples were brought to room temperature in a desiccator, then allowed to equilibrate with room humidity for 30 min before final determination of mass. Room temperature was 24°C and relative humidity 50%. This procedure is preferable to the time-dependent bias in mass that results from sequential removal of samples from a common desiccator. The mass added by room humidity is about 6% and is highly reproducible between samples (Chisholm and Roff, 1990). To estimate errors due to preservation, mass was determined for freshly dried, and preserved-then-dried animals from a separate culture growing under high food conditions.

Statistical analyses and model fitting were carried out according to the GLM (general linear model) and NLIN (non-linear fitting) procedures (SAS Institute, Cary, North Carolina). A linear regression of dry mass on protein mass was performed using a functional regression; such a regression is appropriate in cases where both variables have similar associated error (Ricker, 1973). Similarly, functional regression was used to compare freshly dried animals with preserved-then-dried animals.

Changes in dry mass and protein mass over time were modeled for each culture treatment using the simple exponential growth model:

$$M_t = M_o e^{gt} \qquad (2)$$

where M_t is mass at time t, t is expressed in days, and M_o and g are fitted constants. The Gauss-Newton method (SAS Institute) was used for model fitting. For each enzyme, activity per whole animal was regressed against dry or protein mass for individual culture treatments and for pooled data. Tests of homogeneity of regression slopes and intercepts for culture treatments were performed in an analysis of covariance. Where found, differences were investigated using Fisher's least significant difference

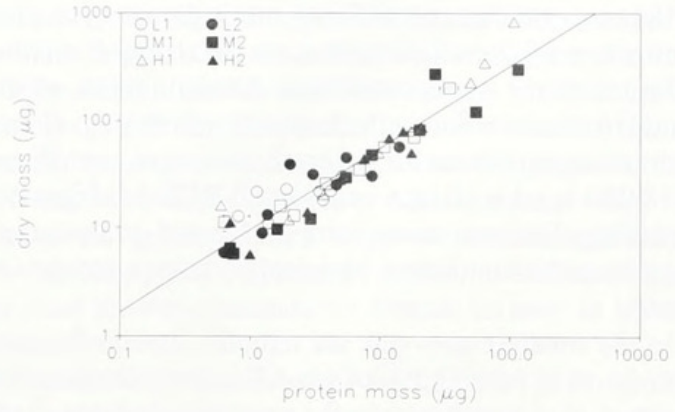

Figure 1. Dry mass *versus* protein mass for *Artemia franciscana* SFB under different culture treatments (L = 2000, M = 10,000 and H = 50,000 cells · ml^{-1} of *Dunaliella salina*). Error bars give standard errors of mean mass (n = 3). Equation of the functional regression line is: log Y = 1.663 + 0.763 log X, with r^2 = 0.893.

(LSD) test (Steel and Torrie, 1980). For all comparisons, the probability of a type I error was set at 0.05.

Results

Dry- and protein mass relationships and preservation effects

Regression analysis revealed non-normality and increasing variance with mean mass. Data (Fig. 1) fit the linear functional regression model,

$$\log(\text{dry mass in } \mu g) = 1.663 + 0.763 \log(\text{protein mass in } \mu g)$$

with r^2 of 0.893 and 22.4 percent standard error of the estimate. This relationship was used to convert protein mass to equivalent dry mass without introducing additional variation.

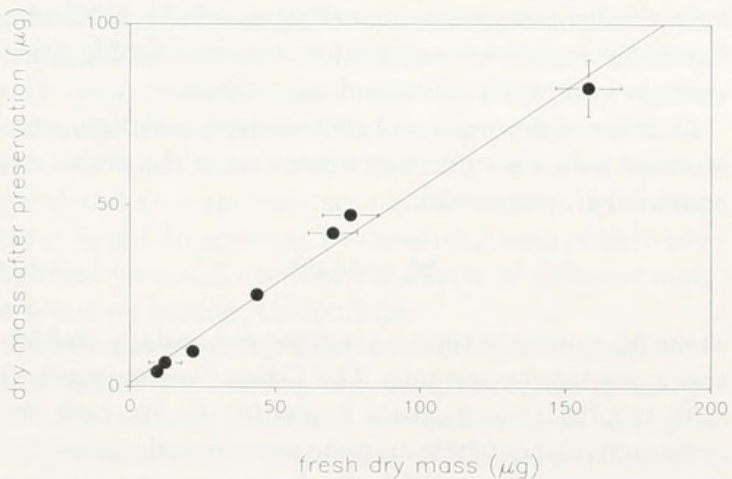

Figure 2. Dry mass after 3 weeks preservation in 2% formalin *versus* dry mass in freshly killed *Artemia franciscana* SFB in a culture fed 50,000 cells · ml^{-1} of *Dunaliella salina*. Error bars give standard errors of mean mass (n = 3). Functional regression equation is Y = 0.567 X, with r^2 = 0.985.

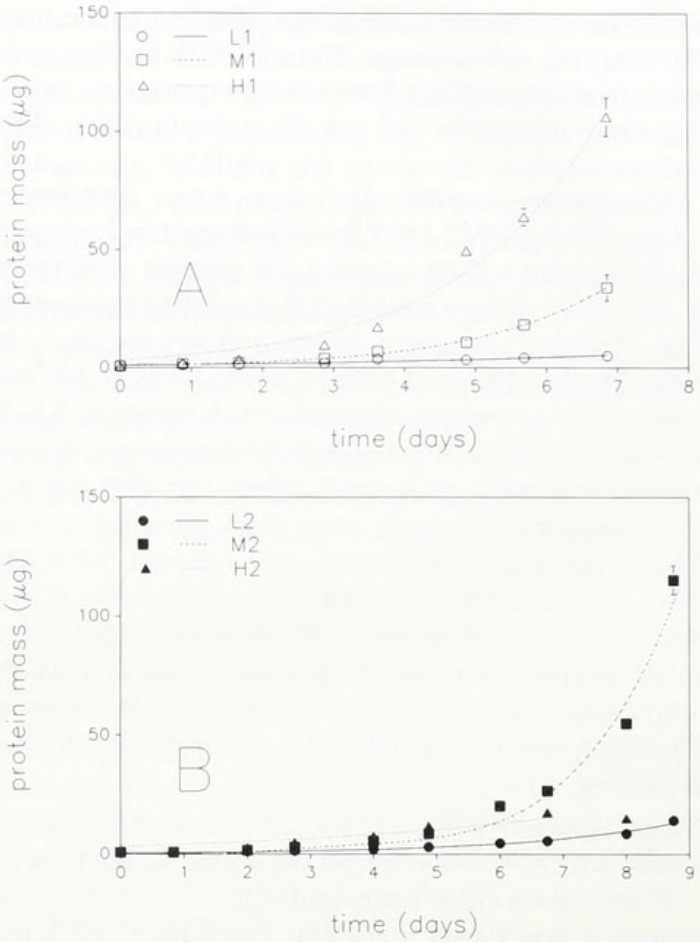

Figure 3. Protein mass growth of *Artemia franciscana* SFB under different culture treatments (L = 2000, M = 10,000 and H = 50,000 cells · ml^{-1} of *Dunaliella salina*) for trial 1 (A) and trial 2 (B). Error bars give standard errors of mean mass (n = 3 groups of 25 animals). Curves represent best fits of an exponential growth model. Model parameters are given in Table I.

For the loss-of-mass experiment, functional regression gave the relationship:

$$\text{preserved dry mass} = 0.567 \text{ fresh dry mass}$$

with r^2 of 0.985 (Fig. 2). Thus, preservation introduced a mass loss of 43.3%, consistent across all sizes of animals. This correction factor was therefore applied to all masses of preserved animals.

Culture growth rates

Identical food concentrations did not produce identical growth rates in separate trials (Fig. 3 for protein mass; Fig. 4 for dry mass). Water conditions in the high food culture in trial 2 became poor, and subdued animal swimming activity was noted on day 7. Therefore, the data obtained after day 6 were excluded from the exponential growth model for culture H2 (although points are plotted in Figs. 3 and 4). An examination of exponential

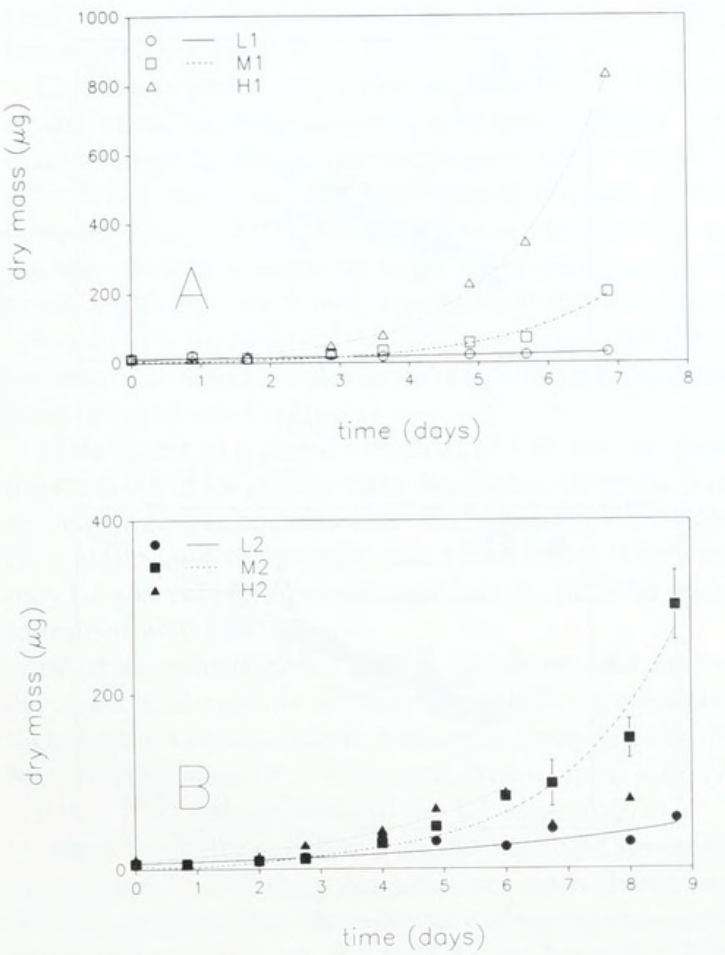

Figure 4. Dry mass growth of *Artemia franciscana* SFB under different culture treatments (L = 2000, M = 10,000 and H = 50,000 cells · ml^{-1} of *Dunaliella salina*) for trial 1 (A) and trial 2 (B). Error bars give standard errors of mean mass (n = 3 groups of 25 animals). Curves represent best fits of an exponential growth model. Model parameters are given in Table I.

growth models (Table I) demonstrates a broad range of growth rates. The parameters for the models were consistent with a more rapid increase in protein mass than in dry mass; in all cases except H2, the value of the exponent g for protein is higher than that for dry mass.

Despite differences in growth rate, a qualitative examination of preserved individuals indicated that the cultures did not have clearly different development. Compound eye and limb bud development was more advanced in individuals in the higher food cultures, but these differences could not be defined based on the stages of development in *Artemia* indicated by Heath (1924) and Anderson (1967).

Enzyme activities

For the majority of enzymes, there were no visually discernible differences between activities in animals from different cultures. This generalization held true for CS (Fig. 5A), LDH (Fig. 5B), PK (Fig. 5C), asp AT (Fig. 5D), ala AT (Fig. 5E), GDH (Fig. 5F), and G6Pdh (Fig. 5G) and even for the abnormal culture conditions for H2.

Only in the case of NDPK were differences in enzyme activity apparent between cultures. For clarity, only data from trial 1 (which exhibited the greatest range of growth rates) are presented in Figure 6A, B. At low mass, enzyme activities for different treatments overlapped, but, as animal mass increased, activities in different culture treatments became distinct. For NDPK activity, non-linearity in both log-transformed and in untransformed data was apparent, and activities reached a plateau at higher mass. This is most clearly seen in non-transformed data (Fig. 6B).

Although protein and dry mass yielded similar enzyme activity-body size relationships, slopes were greater for dry mass. Statistical analyses of regression data (Table II) confirm this. In general, while the slopes of regression of log-transformed data (scaling exponents) were greater than or equal to 1.0 for the dry mass data, for the protein mass data the exponents tended to be equal to or less than 1.0. On average, dry mass scaling exponents were 0.29 higher than those calculated for protein. Statistically significant differences were found for scaling exponents, but, intercepts (a) were not different in any case ($P > 0.07$ in all cases). In the following discussion, the term "global" refers to the scaling exponent for all data combined, while "spe-

Table I

Parameters (with asymptotic 95% confidence intervals) describing growth of Artemia franciscana *SFB under different culture treatments, using the exponential growth model* $M_t = M_0 e^{gt}$*

A) Protein mass

Culture	M_0 (asymptotic 95% CI)	g (asymptotic 95% CI)
L1	0.962 (0.195–1.72)	0.279 (0.077–0.482)
L2	0.560 (0.431–0.689)	0.342 (0.303–0.380)
M1	1.06 (0.52–1.60)	0.479 (0.363–0.595)
M2	0.626 (0.256–0.995)	0.560 (0.466–0.652)
H1	0.881 (0.782–0.979)	0.826 (0.802–0.850)
H2	1.050 (0.54–1.56)	0.495 (0.387–0.605)

B) Dry mass

Culture	M_0 (asymptotic 95% CI)	g (asymptotic 95% CI)
L1	12.5 (7.94–14.3)	0.110 (0.036–0.256)
L2	5.81 (3.15–9.09)	0.305 (0.180–0.429)
M1	7.84 (5.92–9.82)	0.399 (0.298–0.501)
M2	3.38 (2.74–4.04)	0.524 (0.469–0.587)
H1	5.83 (3.40–9.84)	0.783 (0.708–0.859)
H2	5.80 (3.66–7.94)	0.509 (0.363–0.656)

* M_0 is initial mass (μg), M_t is mass at time t, and t is time in days. Culture treatments are concentrations of the alga *Dunaliella salina* (L = 2000 cells · ml^{-1}, M = 10, 000 cells · ml^{-1} and H = 50,000 cells · ml^{-1}).

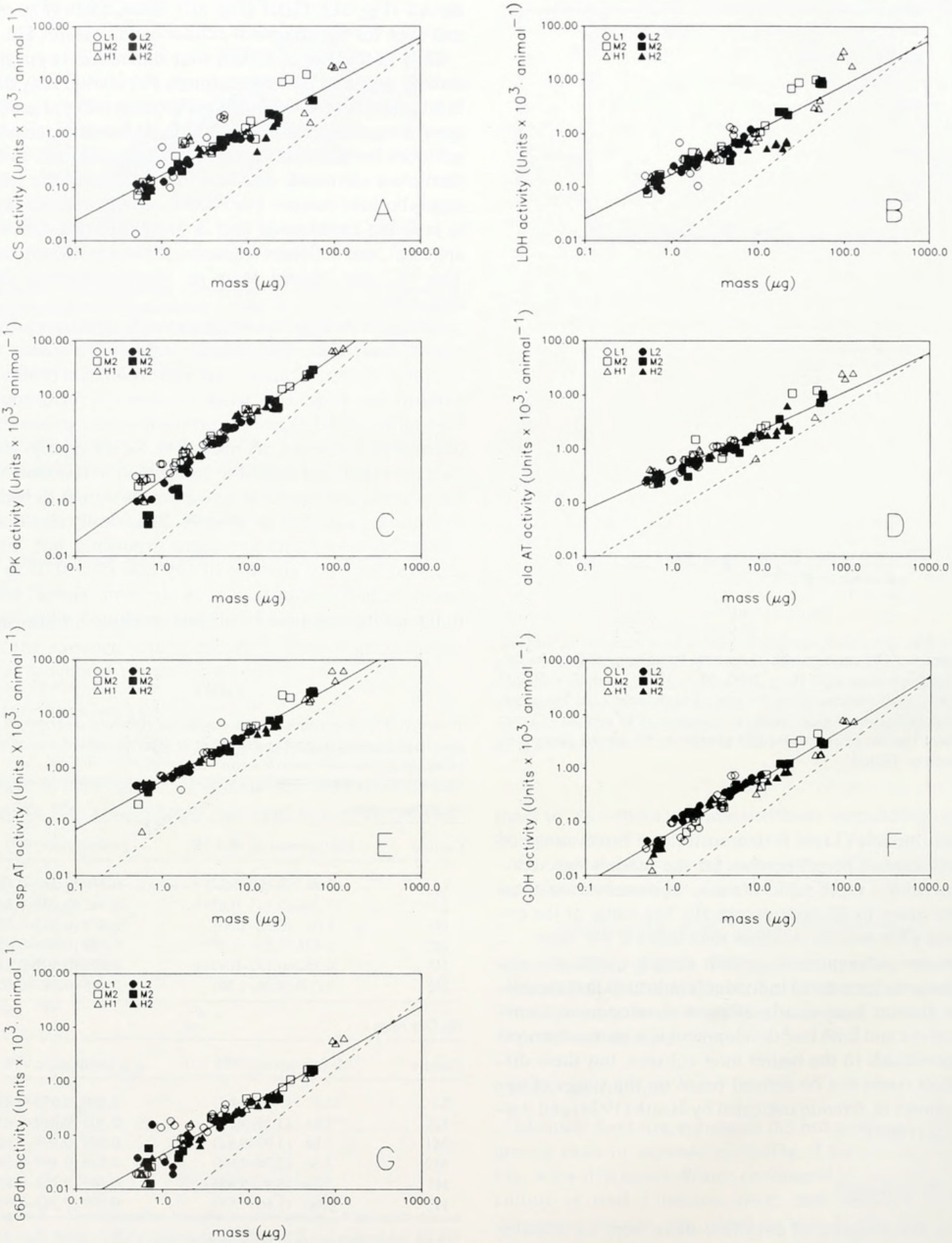

Figure 5. Log-transformed enzyme activity *versus* log-transformed dry mass and protein mass for A, citrate synthase (CS); B, lactate dehydrogenase (LDH); C, pyruvate kinase (PK); D, alanine aminotransferase (ala AT); E, aspartate aminotransferase (asp AT); F, glutamate dehydrogenase (GDH); and G, glucose-6-

cific" refers to scaling relationships in individual culture treatments (see Table II).

CS activity scaled to a global exponent of 1.13 based on dry mass. Specific exponents were generally near 1.0, except culture L1, which was significantly higher than 1.0 ($P < 0.02$). Tests revealed significantly different slopes between cultures ($P < 0.03$). LSD tests identified L1 as the sole distinct culture. In terms of protein mass, the global exponent was 0.864, significantly different from 1.0 and 0.75. As observed for dry mass data, scaling exponents of L1 and H2 cultures were significantly different from those of other cultures.

LDH scaled to a global exponent of 1.07 for dry mass data and 0.812 for protein data. Significant differences in specific scaling exponents were found for dry mass data ($P < 0.01$), and for protein data ($P < 0.01$). However, only L1 and H2 cultures were significantly different when examined with LSD tests.

PK data yielded global scaling exponents of 1.56 for dry mass, and 1.18 for protein mass, both significantly higher than 1.0. Significant differences were detected in both the dry mass ($P < 0.01$) and protein mass data ($P < 0.01$). LSD tests showed culture L1 to be distinct.

Asp AT activity scaled to a global exponent generally higher than 1.0 (1.21) for dry mass, yet lower than unity (0.94) for protein mass. Heterogeneity of scaling exponents was found in both cases: $P < 0.01$ for dry mass, $P < 0.01$ for protein, with LSD tests identifying H2 and L2 cultures as significantly different from others.

For ala AT activity, the global scaling exponent was 0.984 for dry mass (not significantly different from 1.0, $P < 0.67$), but 0.747 (significantly lower than 1.0, but not different from 0.75 ($P < 0.01$, $P < 0.91$, respectively) for protein data. In both cases, specific scaling exponents were not significantly different from one another ($P < 0.39$ for dry mass, $P < 0.39$ for protein).

GDH activity scaled to a global exponent of 1.28 for dry mass, significantly higher than 1.0 ($P < 0.01$); and 0.978 for protein data, not significantly different from 1.0 ($P < 0.45$). As was the case for CS activity, specific slope differences were detected for dry mass ($P < 0.02$) and protein mass ($P < 0.03$). LSD tests showed that only culture H2 was significantly different.

NDPK data were more variable with respect to specific scaling exponents for both protein and dry mass than for any other enzyme. The global exponent for dry mass was 1.24, significantly greater than 1.0 ($P < 0.01$). Specific exponents varied from 0.327 to 2.06 and significant differences were found ($P < 0.01$). A similar picture emerged from protein mass analysis; the global exponent of 0.942 (not significantly different from 1.0, $P < 0.35$) had a specific range of 0.189 to 1.57 (significant differences found, $P < 0.01$). LSD tests identified four groupings for both protein and dry mass data: L1 and L2 were distinct, while H1 and M1 fell together, and H2 and M2 formed another grouping.

For G6Pdh, activity scaled to a global exponent of 1.26 for dry mass (significantly greater than 1.0, $P < 0.01$) and 0.961 for protein mass (not significantly different from 1.0, $P > 0.21$). The specific scaling exponents were not

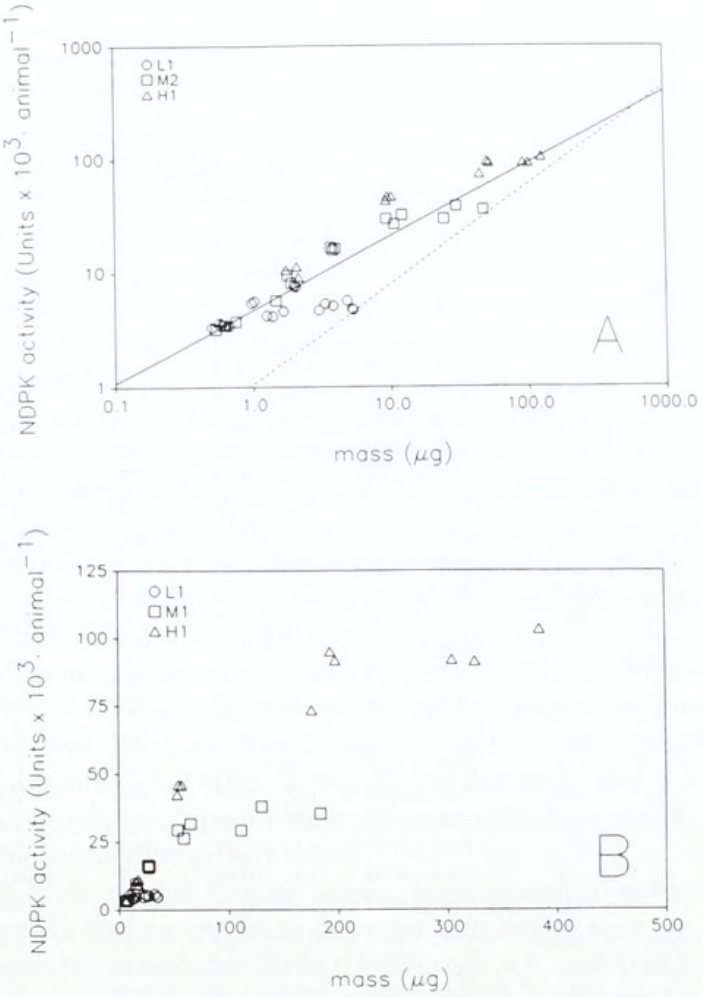

Figure 6. A, Log-transformed nucleoside diphosphate kinase (NDPK) activity versus log-transformed dry mass and protein mass in *Artemia franciscana* SFB under different culture treatments (L = 2000, M = 10,000 and H = 50,000 cells · ml^{-1} of *Dunaliella salina*) for trial 1 data. Solid line represents protein mass regression, dashed line represents dry mass regression. Data points are presented only for protein mass data. Linear regression parameters are given in Table II. B, Linear plot of the same data, dry mass only.

phosphate dehydrogenase (G6Pdh) in *Artemia franciscana* SFB under different culture treatments (L = 2000, M = 10,000 and H = 50,000 cells · ml^{-1} of *Dunaliella salina*). Solid line represents protein mass regression, dashed line represents dry mass regression. Data points are presented only for protein mass data. Linear regression parameters are given in Table II.

Table II

Parameters (±S.E.M.) for regression of enzyme activity (Units · animal^{-1}) versus *mass (μg)* *for* Artemia franciscana *SFB under different culture treatments*

		Protein mass				Dry mass					
				H_0 test				H_0 test			
Enzyme	Culture	log a	b	b = 0.75	b = 1.00	log a	b	b = 0.75	b = 1.00	r^2	%SEE
CS	ALL	−0.78 ± 0.04	0.86 ± 0.05	0.02	0.01	−1.61 ± 0.08	1.13 ± 0.06	0.01	0.02	0.83	75.0
	H1	−0.71 ± 0.13	0.85 ± 0.10	0.34	0.16	−1.53 ± 0.21	1.12 ± 0.13	0.01	0.39	0.84	101.6
	M1	−0.65 ± 0.13	0.86 ± 0.17	0.54	0.43	−1.48 ± 0.28	1.13 ± 0.23	0.13	0.59	0.73	78.4
	L1	−0.96 ± 0.11	1.71 ± 0.27	0.01	0.03	−2.62 ± 0.34	2.24 ± 0.36	0.01	0.01	0.80	94.3
	H2	−0.67 ± 0.07	0.61 ± 0.08	0.09	0.01	−1.26 ± 0.14	0.80 ± 0.10	0.64	0.07	0.84	39.7
	M2	−0.84 ± 0.04	0.88 ± 0.05	0.02	0.04	−1.70 ± 0.08	1.16 ± 0.06	0.01	0.02	0.97	27.2
	L2	−0.81 ± 0.02	0.82 ± 0.05	0.19	0.01	−1.60 ± 0.07	1.07 ± 0.06	0.01	0.27	0.97	16.1
LDH	ALL	−0.76 ± 0.03	0.81 ± 0.04	0.16	0.01	−1.56 ± 0.08	1.07 ± 0.06	0.01	0.21	0.83	71.5
	H1	−0.74 ± 0.11	0.91 ± 0.09	0.09	0.30	−1.61 ± 0.18	1.20 ± 0.11	0.01	0.11	0.90	81.6
	M1	−0.73 ± 0.09	0.87 ± 0.12	0.32	0.31	−1.58 ± 0.19	1.16 ± 0.16	0.03	0.34	0.86	48.9
	L1	−0.67 ± 0.10	0.66 ± 0.25	0.73	0.21	−1.21 ± 0.31	0.73 ± 0.39	0.97	0.51	0.28	90.3
	H2	−0.68 ± 0.06	0.43 ± 0.06	0.01	0.01	−1.12 ± 0.12	0.58 ± 0.09	0.07	0.01	0.79	32.5
	M2	−0.78 ± 0.07	0.91 ± 0.07	0.04	0.21	−1.67 ± 0.12	1.20 ± 0.08	0.01	0.04	0.95	11.9
	L2	−0.77 ± 0.03	0.79 ± 0.07	0.36	0.02	−1.56 ± 0.09	1.06 ± 0.09	0.01	0.56	0.92	24.3
PK	ALL	−0.59 ± 0.03	1.18 ± 0.04	0.01	0.01	−1.74 ± 0.06	1.56 ± 0.05	0.01	0.01	0.93	58.0
	H1	−0.33 ± 0.03	1.04 ± 0.02	0.01	0.10	−1.34 ± 0.05	1.36 ± 0.03	0.01	0.01	0.99	16.7
	M1	−0.40 ± 0.04	1.05 ± 0.05	0.01	0.33	−1.43 ± 0.08	1.39 ± 0.06	0.01	0.02	0.98	18.3
	L1	−0.40 ± 0.06	0.92 ± 0.12	0.21	0.47	−1.24 ± 0.17	1.13 ± 0.18	0.06	0.47	0.81	34.4
	H2	−0.62 ± 0.07	1.17 ± 0.08	0.01	0.04	−1.77 ± 0.14	1.55 ± 0.09	0.01	0.01	0.95	39.1
	M2	−0.92 ± 0.08	1.41 ± 0.08	0.01	0.01	−2.33 ± 0.15	1.87 ± 0.11	0.01	0.01	0.97	55.1
	L2	−0.80 ± 0.05	1.40 ± 0.09	0.01	0.01	−2.20 ± 0.13	1.87 ± 0.13	0.01	0.01	0.95	34.9
ala AT	ALL	−0.42 ± 0.03	0.75 ± 0.03	0.91	0.01	−1.16 ± 0.05	0.98 ± 0.04	0.01	0.67	0.90	47.4
	H1	−0.33 ± 0.10	0.73 ± 0.08	0.80	0.01	−1.04 ± 0.16	0.96 ± 0.09	0.05	0.70	0.89	68.9
	M1	−0.40 ± 0.10	0.82 ± 0.14	0.59	0.23	−1.21 ± 0.22	1.09 ± 0.18	0.09	0.64	0.80	57.7
	L1	−0.40 ± 0.04	0.78 ± 0.10	0.77	0.04	−1.18 ± 0.14	1.05 ± 0.15	0.08	0.73	0.84	28.2
	H2	−0.40 ± 0.05	0.66 ± 0.06	0.13	0.01	−1.05 ± 0.11	0.87 ± 0.07	0.13	0.11	0.92	28.1
	M2	−0.47 ± 0.06	0.77 ± 0.06	0.89	0.01	−1.22 ± 0.11	1.00 ± 0.08	0.06	0.97	0.94	37.6
	L2	−0.45 ± 0.03	0.70 ± 0.05	0.39	0.01	−1.14 ± 0.07	0.93 ± 0.07	0.02	0.29	0.95	17.0
asp AT	ALL	−0.25 ± 0.02	0.92 ± 0.03	0.01	0.01	−1.15 ± 0.05	1.21 ± 0.04	0.01	0.01	0.94	41.1
	H1	−0.37 ± 0.09	1.03 ± 0.07	0.01	0.72	−1.38 ± 0.15	1.36 ± 0.09	0.01	0.01	0.95	65.3
	M1	−0.31 ± 0.05	1.07 ± 0.07	0.01	0.36	−1.34 ± 0.12	1.40 ± 0.09	0.01	0.01	0.96	26.7
	L1	−0.21 ± 0.07	1.02 ± 0.18	0.16	0.91	−1.23 ± 0.26	1.37 ± 0.28	0.05	0.22	0.72	59.3
	H2	−0.15 ± 0.02	0.71 ± 0.02	0.03	0.01	−0.84 ± 0.03	0.93 ± 0.02	0.01	0.01	0.99	7.1
	M2	−0.26 ± 0.03	0.91 ± 0.03	0.01	0.02	−1.16 ± 0.05	1.20 ± 0.04	0.01	0.01	0.99	16.8
	L2	−0.23 ± 0.03	0.80 ± 0.05	0.38	0.01	−1.00 ± 0.07	1.05 ± 0.07	0.01	0.48	0.95	17.8
GDH	ALL	−1.19 ± 0.03	0.98 ± 0.03	0.01	0.47	−2.14 ± 0.05	1.28 ± 0.04	0.01	0.01	0.93	44.5
	H1	−1.38 ± 0.08	1.07 ± 0.06	0.01	0.28	−2.41 ± 0.13	1.40 ± 0.08	0.01	0.01	0.96	51.5
	M1	−1.29 ± 0.06	1.12 ± 0.08	0.01	0.17	−2.38 ± 0.13	1.47 ± 0.10	0.01	0.01	0.95	30.9
	L1	−1.27 ± 0.07	1.35 ± 0.17	0.01	0.07	−2.57 ± 0.21	1.77 ± 0.22	0.01	0.01	0.85	51.6
	H2	−1.18 ± 0.07	0.90 ± 0.07	0.06	0.22	−2.05 ± 0.14	1.19 ± 0.09	0.01	0.08	0.93	38.0
	M2	−1.13 ± 0.05	0.95 ± 0.05	0.01	0.32	−2.04 ± 0.09	1.24 ± 0.07	0.01	0.01	0.97	30.5
	L2	−1.07 ± 0.03	0.95 ± 0.05	0.01	0.36	−1.99 ± 0.07	1.25 ± 0.07	0.01	0.01	0.97	17.1
NDPK	ALL	1.60 ± 0.05	0.94 ± 0.06	0.01	0.35	−0.52 ± 0.11	1.24 ± 0.08	0.01	0.01	0.76	112.4
	H1	1.16 ± 0.06	0.62 ± 0.04	0.01	0.01	1.76 ± 0.09	0.82 ± 0.06	0.24	0.01	0.95	34.7
	M1	1.24 ± 0.05	0.64 ± 0.07	0.15	0.01	1.87 ± 0.11	0.84 ± 0.09	0.31	0.11	0.91	25.3
	L1	1.35 ± 0.04	0.19 ± 0.10	0.01	0.01	1.59 ± 0.13	0.33 ± 0.14	0.01	0.01	0.38	25.6
	H2	−0.06 ± 0.14	1.37 ± 0.15	0.01	0.03	−1.39 ± 0.27	1.81 ± 0.19	0.01	0.01	0.87	90.0
	M2	1.91 ± 0.16	1.21 ± 0.17	0.02	0.24	−1.08 ± 0.30	1.59 ± 0.22	0.01	0.02	0.84	139.0
	L2	1.93 ± 0.09	1.57 ± 0.18	0.01	0.01	−1.45 ± 0.25	2.06 ± 0.24	0.01	0.01	0.87	74.0
G6Pdh	ALL	−1.40 ± 0.03	0.96 ± 0.03	0.01	0.21	−2.33 ± 0.05	1.25 ± 0.04	0.01	0.01	0.93	46.1
	H1	−1.41 ± 0.06	1.01 ± 0.05	0.01	0.88	−2.38 ± 0.10	1.32 ± 0.06	0.01	0.01	0.97	38.8
	M1	−1.34 ± 0.05	0.98 ± 0.07	0.02	0.66	−2.28 ± 0.11	1.27 ± 0.09	0.01	0.01	0.96	24.8
	L1	−1.29 ± 0.08	1.06 ± 0.21	0.17	0.77	−2.32 ± 0.30	1.39 ± 0.33	0.08	0.27	0.67	71.2
	H2	−1.42 ± 0.04	0.89 ± 0.05	0.01	0.04	−2.29 ± 0.08	1.18 ± 0.06	0.01	0.02	0.97	21.9
	M2	−1.53 ± 0.06	0.99 ± 0.06	0.01	0.84	−2.48 ± 0.10	1.30 ± 0.08	0.01	0.01	0.97	34.5
	L2	−1.45 ± 0.07	0.95 ± 0.14	0.18	0.75	−2.37 ± 0.19	1.25 ± 0.18	0.02	0.20	0.80	53.5

Regression model was log Y = log a + b log X. %SEE represents the standard error (as a percentage) of an estimate using the regression equation. Results of tests of the null hypotheses (H_0) b = 0.75 and b = 1.00 are given as P-values. Culture treatments are concentrations of the alga *Dunaliella salina* (L = 2000 cells · ml^{-1}, M = 10,000 cells · ml^{-1} and H = 50,000 cells · ml^{-1}).

significantly different in either case ($P < 0.48$ for both dry and protein mass data).

Discussion

Artemia growth and development

Because development occurred synchronously regardless of culture treatment, enzyme activities could be compared directly in terms of size without need to consider developmental stages. The substantial independence of development rate from food concentration suggests a "growth at all cost" strategy. This would be essential for organisms such as *Artemia*, inhabiting temporary environments. Upon hatching, a premium would be placed on developing to a reproductive stage, regardless of food environment, in order to produce cysts which could survive desiccation. In the Copepoda, another crustacean group in which growth has been more extensively studied, developmental rates for certain species are substantially determined by temperature, while growth rate is a variable more sensitive to food concentration (Miller and Johnson, 1977; Vidal 1980).

Effects of different body size indices

This study documents differences between dry mass- and protein mass-based scaling exponents. This is clearly due to a change in body composition over the developmental period examined; protein mass increased more rapidly than dry mass. An increase in percentage protein as animals mature has previously been found for *Artemia* (Persoone *et al.*, 1980).

Differences observed between intra- and interspecific exponents may be largely due to differences in body composition. Where the composition of a multispecific group of animals has been successfully standardized (*e.g.*, mammals; see Taylor, 1980), reproducible and comparable scaling exponents have resulted. The relatively low variation in the composition of mammals contrasts with that of invertebrates, such as copepods, where body composition is highly variable (see Mayzaud, 1986). This may explain the many different scaling exponents derived for invertebrates, in general (see Peters, 1983). The traditional 0.75 exponent is applicable only where body size is expressed as wet mass. Clearly other exponents can be found if protein or dry mass are used. Thus, arguments as to the significance of the 0.75 exponent should be reconsidered, particularly in groups of animals where reliable wet mass cannot be determined, or where body composition varies widely or changes with size. Thus, the selection of a mass variable is worthy of at least as much consideration as the standardization of conditions of measurement of a rate variable (*e.g.*, selecting a "basal" metabolic rate for respiration studies). Protein content may offer a suitable index of body size.

Enzyme response to culture treatments

Animals fed identical diets did not respond with identical growth rates. The reasons for this are unclear, but are not of particular concern, because it is the independently measured growth rates resulting from food levels that are of primary interest. For this reason, the term "culture treatment" will be used in discussion to distinguish the growth rate effects from the food level treatment themselves. Thus, the experiment consisted of six culture treatments.

On the basis of regression analyses, enzymes may be placed into three groups: those in which variation in maximal activity across size is completely independent of treatment, those exhibiting some statistical differences between treatments (but neither predictably nor consistently with growth rate), and those showing consistent differences related to culture treatments.

The first group includes ala AT and G6Pdh. No differences in scaling exponents were found between culture treatments. Because growth rates varied between culture treatments (see Tables Ia and b), we can state that the scaling relationships for these enzymes do not appear to be influenced by growth rate.

For the second enzyme group, some statistical differences in scaling exponent between treatments were detected; these include CS, LDH, PK, asp AT, and GDH. These statistical differences usually identified cultures L1 and H2 as distinct. In terms of growth rates, however, neither L1 nor H2 cultures were distinct (based on 95% confidence intervals of the exponent g). Thus, whatever the differences indicate, they do not appear to be related to growth rate.

NDPK alone (the sole member of the third group) showed variation that could be attributed to differences in growth rates. In trial 1 (Fig. 6 A, B), for a given body mass, culture H1 has a higher activity than M1 which is again higher than L1, for both protein and dry mass data. For trial 2, (data not shown) this pattern was less clear because cultures M2 and H2 had similar exponential growth coefficients (Table I), however their enzyme activities were also similar (52 ± 8 units at 100 μg mass). Clearly NDPK activity does not vary *directly* with growth because, within cultures, it changes with body size, whereas the measured specific growth rate is constant (because growth is exponential). Rather, the differences between cultures are related to the changes in enzyme activities with size. These differences do not appear statistically, as differences between regression parameters, due to a clear lack of fit in allometric models.

NDPK activity reached a plateau in all cultures. Therefore, enzyme activity per unit mass must actually *decrease* from the point at which the plateau is reached. The significance of such a plateau in enzyme activity is unclear.

It may be that there is some physiological mechanism for sensing available food resources and altering development to achieve enzyme levels that are sufficient for possible future growth under prevailing conditions. Enzyme activity may simply anticipate a future decline in animal growth rate. Alternatively, it may mark the beginning of a transition from somatic growth to reproduction. It is clear, however, that NDPK scaling is affected by differences in growth rate. NDPK activity may in fact be useful as a predictor of growth rate.

In summary, among eight enzymes representative of both anabolic and catabolic processes, only one (NDPK) showed a relationship between maximal activity and growth rate. We shall elaborate on this relationship elsewhere. The meaning of particular values obtained for individual enzyme scaling exponents will be considered in a separate study (Berges and Ballantyne, unpub.) for a variety of crustacean species.

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