Size Scaling of Whole-Body Maximal Enzyme Activities in Aquatic Crustaceans

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The relationships between body size and maximal activities of eight enzymes were measured in whole-body homogenates of the crustaceans *Macrobrachium rosenbergii*, *Artemia franciscana*, and *Daphnia magna*. Interspecifically and intraspecifically, enzyme activities per animal (Y) scale with protein weight (W) according to the allometric relationship $Y = aW^b$. Scaling exponents (b) varied with the enzyme examined and were usually different from 0.75. For enzymes such as citrate synthase, intraspecific and interspecific exponents were similar, but for enzymes associated with pathways other than aerobic metabolism, significant differences were found between species. For anaerobic enzymes such as lactate dehydrogenase, these differences may relate to interspecific differences in life history and ecology. For anabolic enzymes such as glucose-6-phosphate dehydrogenase and nucleoside diphosphate kinase, differences may relate to differences in growth rates between species.

Les relations entre la taille et l'activité maximale de huit enzymes ont été établies dans des homogénats d'organismes entiers des crustacés *Macrobrachium rosenbergii*, *Artemia franciscana* et *Daphnia magna*. Interspécifiquement et intraspécifiquement, l'activité des enzyme par animal Y est ramenée à l'échelle de la masse des protéines W selon la relation allométrique $Y = aW^b$. Les exposants de mise à l'échelle b varient selon l'enzyme examinée, différant en général de 0,75. Pour des enzymes telles que la citrate synthase, les exposants intraspécifique et interspécifique sont semblables, mais pour les enzymes associées à des voies autres que le métabolisme aérobie, des différences importantes ont été trouvées entre les espèces. Pour les enzymes anaérobies comme la lactate déshydrogénase, ces différences pourraient tenir à des différences interspécifiques tant génétiques qu'écologiques. Pour les enzymes anaboliques comme la glucose-6-phosphate déshydrogénase et la nucléoside diphosphate kinase, les différences pourraient tenir à des différences de taux de croissance entre les espèces.

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The phenomenon of disproportionate change in metabolic rate processes with body mass has been established in animal groups representing many phyla (Peters 1983). Such "size scaling" may be described using the allometric equation

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

where Y is the metabolic rate, W is some measure of body mass, and a and b are empirically derived constants. The constant b (here referred to as the "scaling exponent") is often found to be near 0.75 for whole-organism measures of aerobic metabolic rate (e.g. oxygen consumption) (Schmidt-Nielsen 1984).

Attention has been given to establishing precise values for the scaling exponent, explaining why the 0.75 exponent is found (see Peters 1983) and comparing inter- and intraspecific size scaling in both vertebrate and invertebrate taxa (Hemmingsen 1960; Heusner 1982; Wheatly 1989). Only in vertebrate groups, however, has research focussed on suborganismal processes.

A particularly interesting line of inquiry in suborganismal work concerns the size scaling of maximal enzyme activities. While processes such as oxygen consumption are confined to aerobic metabolism, enzymes in different pathways may be used to examine specific components of metabolism (i.e. anaerobic, anabolic, catabolic, etc.). Enzyme scaling analyses in fish and mammals have shown that the 0.75 paradigm does not always hold; lactate dehydrogenase activity scales to exponents greater than 1.0 (Somero and Childress 1980; Emmett and Hochachka 1981).

In the present study we extend examination of enzyme size scaling to one invertebrate group, the Crustacea. Crustaceans represent a useful experimental group in which respiratory scaling has been carefully examined (Weymouth et al. 1944; Hemmingsen 1960; Banse 1982; Wheatly 1989). Rapid growth rates within species allow specimens to be obtained over a large size range in short periods of time, while within the group, large variations in size may be found. From a practical viewpoint, crustaceans are economically important both as food items for human populations as well as intermediates in food webs in aquatic ecosystems (Rigler and Downing 1984). In programs of aquaculture or environmental monitoring, metabolic rates and correlates such as enzyme activity may be extremely useful. Therefore, detailed knowledge of how different types of metabolism and enzyme activity vary with body size is essential.

Invertebrates such as crustaceans present two practical problems for enzyme scaling studies. First, there is the need for an

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accurate means of assessing size which will apply across species of varying life history and morphology. Crustaceans are much more highly variable in body composition than are mammals (Corner and O'Hara 1986) and this can lead to greater variation in size scaling relationships. One common index, live or fresh weight, cannot be applied to smaller aquatic organisms, since it is often difficult to uniformly remove associated water (Rigler and Downing 1984). Dry weight cannot be directly determined if enzyme activity is to be measured in the same animal, and may be a biased estimate of the mass of metabolically active tissue, particularly in crustaceans where the exoskeleton is heavily calcified or large stores of lipid are found. Body protein content was selected for use in the present study, since it is readily measured in homogenates of whole animals and is generally a more constant fraction of organism composition (Schmidt-Nielsen 1984). The second problem concerns the use of tissue-specific enzyme activities. For smaller crustaceans, dissection of individual tissues is impractical. Furthermore, because the metabolism of individual tissues is not predictably related to that of other tissues nor to that of the whole animal in terms of respiration rate (Schmidt-Nielsen 1984), or enzyme activity (Somero and Childress 1980; Emmett and Hochachka 1981; Ewart et al. 1988), the results obtained in tissue analyses are not clearly applicable to whole-animal size. One alternative is to attempt a whole-animal enzyme measurement.

To examine the relationship between whole-animal enzyme levels and body size in invertebrates, enzymes were selected on the basis of their metabolic functions. Citrate synthase (CS) is a key regulatory enzyme in the citric acid cycle and gives a quantitative estimate of aerobic capacity (Somero and Childress 1980). Pyruvate kinase (PK) was chosen as an indicator of glycolytic capacity, while lactate dehydrogenase (LDH) served as an index of anaerobic potential. To assess amino acid metabolism the enzymes alanine aminotransferase (ala AT), aspartate aminotransferase (asp AT), and glutamate dehydrogenase (GDH) were selected. GDH may also be related to excretory processes (Bidigare and King 1981). As indices of general biosynthesis, two enzymes were chosen: nucleoside diphosphate kinase (NDPK), which provides nucleoside triphosphates for use in a variety of synthetic pathways, and glucose-6-phosphate dehydrogenase (G6Pdh), a hexose monophosphate shunt enzyme which provides NADPH for fatty acid, amino acid, and nucleic acid synthesis.

Materials and Methods

Experimental Animals

The crustaceans selected were freshwater forms with the exception of *Artemia franciscana* which was grown in half-strength seawater. Prawns (*Macrobrachium rosenbergii*) were obtained from Ontario Hydro, Toronto, Ontario, and maintained for 2 wk at 25°C prior to use. Animals were fed a commercial salmonid diet (Diet 84G, Martin Feed Mills Ltd., Elmira, Ontario) ad libitum. Brine shrimp (*Artemia franciscana* SFB, San Francisco Bay Brand, Newark, CA), hatched from cysts, were maintained in 15‰ seawater (Instant Ocean Brand, Aquarium Systems, Mentor, IL) at 25°C and fed an infusorium (Lawrence 1981). *Daphnia magna* (Northwest Biological Supply, Guelph, Ontario) were kept at 25°C following the culture techniques of Lawrence (1981) for 3 mo before sampling. At this point, individuals were reproducing parthenogenically. Freshwater copepods (*Cyclops* spp. and *Eurytemora* spp.) and ostracods (unidentified) were isolated from local ponds and maintained at 25°C.

Enzyme Extraction and Assay Procedures

Prawns were killed by decapitation, blotted dry, dissected as necessary, and weighed. Whole animals or body parts were homogenized in ice-cold 50 mM imidazole buffer (5 mL of buffer to 2 g wet weight), pH 7.2, using a Polytron PT10 unit (Kinematica GMBH, Luzern, Switzerland) for three bursts of 10 s each. Homogenates were centrifuged at 18 000 *g* for 20 min and the supernatant used directly in enzyme assays. Preliminary experiments showed that if the pellets were rehomogenized, extracted, and assayed for citrate synthase, the activity was less than 5% of that recovered in the supernatant. To verify that insoluble protein lost in the pellet did not bias body size estimates for larger animals, body wet weight was regressed against protein weight. Microcrustaceans (*Artemia, Daphnia*, copepods, and ostracods) were homogenized whole in 50 mM imidazole buffer (50–1000 $\mu L \cdot individual^{-1}$) using a Megason PA-300 sonicator (Ultrasonic Instruments International, Farmingdale, NY) at maximum setting for three bursts of 10 s each. For copepods and ostracods, it was necessary to pool between 10 and 25 similar-sized individuals, which were separated into size classes using 175-, 250-, and 350-μm sieves. Because preliminary centrifugation of microcrustacean homogenates produced no pellet or visible precipitate, centrifugation was not performed for subsequent measurements.

Maximum enzyme activities were determined using a Varian DMS 100 UV-Visible spectrophotometer (Varian Canada, Inc., Georgetown, Ontario) equipped with a thermostatted cell changer maintained at 25 (±0.1)°C. Reaction rate was 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 of substrate converted to product per minute. Conditions for assay procedures were adapted from Hochachka et al. (1970) and Emmett and Hochachka (1981) with substrate and cofactor concentrations optimized to give maximum activity. All assays for cytoplasmic enzymes were conducted in 50 mM imidazole, pH 7.2, while the mitochondrial enzyme citrate synthase was assayed at pH 8.2. Such pH values approximate in situ pH for the respective enzymes (Roos and Boron 1981). Other conditions were as follows:

Citrate synthase (CS), EC 4.1.3.7: 0.1 mM DTNB, 7.0 mM $MgCl_2$, 0.3 mM acetyl coenzyme A, 0.5 mM oxaloacetate (omitted for control).

Lactate dehydrogenase (LDH), EC 1.1.1.27: 0.2 mM NADH, 100 mM KCl, 2.0 mM sodium pyruvate (omitted for control).

Pyruvate kinase (PK), EC 2.7.1.40: 0.2 mM NADH, 5.0 mM ADP, 50 mM KCl, 10 mM $MgCl_2$, 5.0 mM phosphoenolpyruvate (omitted for control), and excess LDH.

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

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

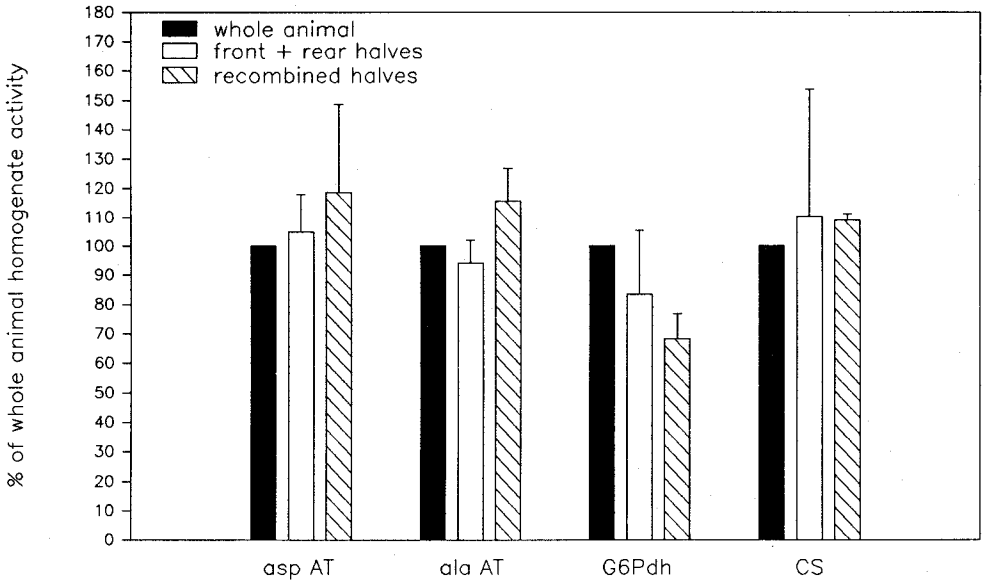

FIG. 1. Relative activities of aspartate aminotransferase (asp AT), alanine aminotransferase (ala AT), glucose-6-phosphate dehydrogenase (G6Pdh), and citrate synthase (CS) in *Macrobrachium rosenbergii* using different homogenization procedures. Activities are expressed as percentages of the activity of the whole-animal homogenates. Values are means ± SE for four determinations.

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

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

Glucose-6-phosphate dehydrogenase (G6Pdh), EC 1.1.1.49: 0.4 mM NADP, 7.0 mM $MgCl_2$, 1.0 mM glucose-6-phosphate (omitted for control).

Protein assays were performed on homogenates with a microassay procedure using coomassie brilliant blue dye (Bio-Rad Laboratories (Canada) Inc., Mississauga, Ontario) with bovine serum albumin as the standard. Preliminary experiments showed that imidazole buffer did not affect linearity of the assay.

Validation of Whole-Body Enzyme Measurements

The use of whole-animal versus single-tissue homogenates was conveniently validated in *Macrobrachium rosenbergii*. The posterior body segment of *Macrobrachium* is composed predominantly of the abdominal flexor muscle (virtually a single tissue), while most of the intestine and the hepatopancreas are localized in the anterior segments. Pairs of animals, closely matched in size, were selected over as large a range of body size as possible. One animal in each pair was homogenized whole, while the second was separated into two parts at the posterior margin of the carapace and each part homogenized separately. For a representative subset of enzymes, asp AT, ala AT, G6Pdh, and CS, four different assays were performed in duplicate. Whole-animal homogenate was used in the first assay. The second and third assays were performed on anterior body homogenate and posterior body homogenate individually. To differentiate homogenization artifacts (for example, decreased whole-animal activity due to increased time to homogenize) from unfavorable interactions of homogenate components, anterior and posterior body homogenates were mixed in proportions relative to anterior/posterior wet weights and assayed. Activities were statistically compared within each enzyme in a one-way ANOVA, with $P < 0.05$ considered to represent a significant difference.

Scaling Experiments

Duplicate or triplicate assays for CS, LDH, PK, ala AT, asp AT, GDH, NDPK, and G6Pdh were performed on animals over as wide a size range as possible within *Macrobrachium* ($n = 18$ or $n = 22$), *Artemia* ($n = 46$), and *Daphnia* ($n = 20$). Copepods ($n = 6$) and ostracods ($n = 2$) were included for comparison only and were not used in subsequent regression analyses due to the small sample size.

Protein weights and enzyme activities per whole animal were log-transformed (common logarithm) to linearize the allometric equation (Peters 1983). Activity of each enzyme was regressed against protein weight for each species and for all species combined using SAS General Linear Models procedure (SAS Institute Inc., Cary, NC). Tests of the null hypotheses $b = 1.0$, indicating direct proportionality of enzyme activity with body weight, and $b = 0.75$ (the scaling exponent often found for metabolism–weight relationships) were performed in all cases. Tests of homogeneity of slope ($b_{Daphnia} = b_{Macrobrachium} = b_{Artemia}$) were performed and differences were investigated using paired Student t-test comparisons for unequal variance (Steel and Torrie 1980). For all statistical comparisons, $P < 0.05$ was considered to represent a significant difference.

Results

Validation of Whole-Body Enzyme Measurements

Mean activity of homogenates (Fig. 1) demonstrate the similarity of results for each homogenization procedure. In the

TABLE 1. Summary of regression analyses, fitting the model $\log Y = \log a + b \log W$.[a]

Enzyme	Species	Model parameters[b]				P values for H_0[d]	
		log a	b	r^2	%SEE[c]	$b = 0.75$	$b = 1.00$
CS	*Daphnia*	−3.58 ± 0.34	0.814 ± 0.188	0.69	327.0	0.74	0.36
	Macrobrachium	−4.06 ± 0.98	0.892 ± 0.175	0.69	80.4	0.44	0.55
	Artemia	−3.97 ± 0.11	0.975 ± 0.064	0.88	35.4	0.01	0.70
	All species	−3.86 ± 0.08	0.865 ± 0.025	0.96	120.0	0.01	0.01
LDH	*Daphnia*	−3.55 ± 0.33	0.916 ± 0.179	0.76	76.0	0.39	0.66
	Macrobrachium	−4.12 ± 0.27	1.120 ± 0.050	0.98	48.3	0.01	0.03
	Artemia	−3.34 ± 0.12	0.984 ± 0.071	0.87	40.1	0.01	0.82
	All species	−3.61 ± 0.08	1.050 ± 0.020	0.98	102.1	0.01	0.03
PK	*Daphnia*	−2.66 ± 0.26	0.227 ± 0.141	0.16	55.8	0.01	0.01
	Macrobrachium	−3.39 ± 0.23	1.080 ± 0.040	0.98	40.6	0.01	0.08
	Artemia	−2.86 ± 0.18	0.696 ± 0.106	0.59	64.9	0.62	0.01
	All species	−3.61 ± 0.06	1.120 ± 0.020	0.98	76.4	0.01	0.01
ala AT	*Daphnia*	−2.77 ± 0.10	0.540 ± 0.063	0.85	36.5	0.01	0.01
	Macrobrachium	−3.68 ± 0.16	1.010 ± 0.030	0.99	27.4	0.01	0.80
	Artemia	−3.87 ± 0.13	1.150 ± 0.080	0.88	45.0	0.01	0.07
	All species	−3.52 ± 0.03	0.973 ± 0.011	0.99	51.4	0.01	0.01
asp AT	*Daphnia*	−3.21 ± 0.14	0.835 ± 0.078	0.93	27.7	0.31	0.07
	Macrobrachium	−3.60 ± 0.17	1.010 ± 0.030	0.99	28.7	0.01	0.67
	Artemia	−2.93 ± 0.13	0.710 ± 0.077	0.74	44.0	0.60	0.01
	All species	−3.35 ± 0.03	0.967 ± 0.011	0.99	42.4	0.01	0.01
GDH	*Daphnia*	−3.19 ± 0.20	0.652 ± 0.111	0.81	41.8	0.41	0.02
	Macrobrachium	−4.73 ± 0.34	0.949 ± 0.060	0.96	64.9	0.01	0.42
	Artemia	−4.44 ± 0.17	1.020 ± 0.100	0.79	59.4	0.01	0.84
	All species	−4.15 ± 0.05	0.848 ± 0.015	0.98	61.1	0.01	0.01
NDPK	*Daphnia*	−1.91 ± 0.09	0.194 ± 0.054	0.49	30.8	0.01	0.01
	Macrobrachium	−2.18 ± 0.10	0.888 ± 0.018	0.99	16.1	0.01	0.01
	Artemia	−2.33 ± 0.08	0.857 ± 0.049	0.91	25.2	0.04	0.01
	All species	−2.50 ± 0.04	0.919 ± 0.017	0.97	96.7	0.01	0.01
G6Pdh	*Daphnia*	—	—	—	—	—	—
	Macrobrachium	−4.42 ± 0.21	0.907 ± 0.037	0.98	36.2	0.01	0.03
	Artemia	−4.53 ± 0.18	1.070 ± 0.100	0.78	63.9	0.01	0.54
	All species	−4.25 ± 0.05	0.883 ± 0.017	0.98	69.0	0.01	0.01

[a]Y is enzyme activity (μmol substrate converted·min^{-1}·animal^{-1}); W is protein weight (μg).
[b]Fitted parameters a and b are presented ± SE.
[c]Percent standard error of the estimate.
[d]Results of tests of the null hypotheses (H_0), $b = 0.75$ and $b = 1.00$, are given as P values.

whole-animal homogenates which are set to 100% in Fig. 1, actual mean activities (± SE) were as follows: asp AT, 0.302 (±0.031) units·mg protein^{-1}; ala AT, 0.220 (±0.026) units·mg protein^{-1}, G6Pdh, 0.021 (±0.003) units·mg protein^{-1}; and CS, 0.029 (±0.007) units·mg protein^{-1}. No significant differences were found between homogenization procedures within enzymes (asp AT, $P < 0.73$; ala AT, $P < 0.70$; G6Pdh, $P < 0.56$; CS, $P < 0.49$).

Scaling Experiments

The relationship between wet weight and protein weight in *Macrobrachium* was linear ($r^2 = 0.92$, $n = 24$). Protein comprised an average 17% of wet weight.

For all enzymes examined, regression statistics are provided in Table 1.

Regressions of CS activity versus body weight (Fig. 2) show similar results intra and interspecifically. The scaling exponent for the interspecific relationship is 0.865 which is statistically different from both 0.75 and 1.0. In intraspecific relationships, scaling exponents fall numerically between 0.75 and 1.00; however, no intraspecific exponent is statistically different from 1.0, and only for *Artemia* is the exponent greater than 0.75. No significant differences were detected between intraspecific scaling exponents ($P > 0.53$).

For interspecific regressions of both LDH and PK activity versus body weight (Fig. 3 and 4), scaling to exponents greater than 1.0 is found (1.05 and 1.12, respectively). For intraspecific regressions of LDH activity, only *Macrobrachium* shows an exponent greater than 1.0. *Artemia* and *Daphnia* have scaling exponents not significantly different from 1.0, and lower than *Macrobrachium* ($P < 0.03$, $P < 0.02$), but not significantly different from each other ($P < 0.51$). All intraspecific PK activity scaling exponents are significantly different from each other ($P < 0.01$ in all cases). The exponent for *Daphnia* is lower than 0.75, the exponent for *Artemia* is not different from 0.75, and the exponent for *Macrobrachium* is not different from 1.0.

Ala AT and asp AT activities (Fig. 5 and 6) show patterns of scaling similar to each other. Interspecific scaling exponents are numerically close, 0.973 for ala AT and 0.967 for asp AT, both significantly less than 1.0 and greater than 0.75. For

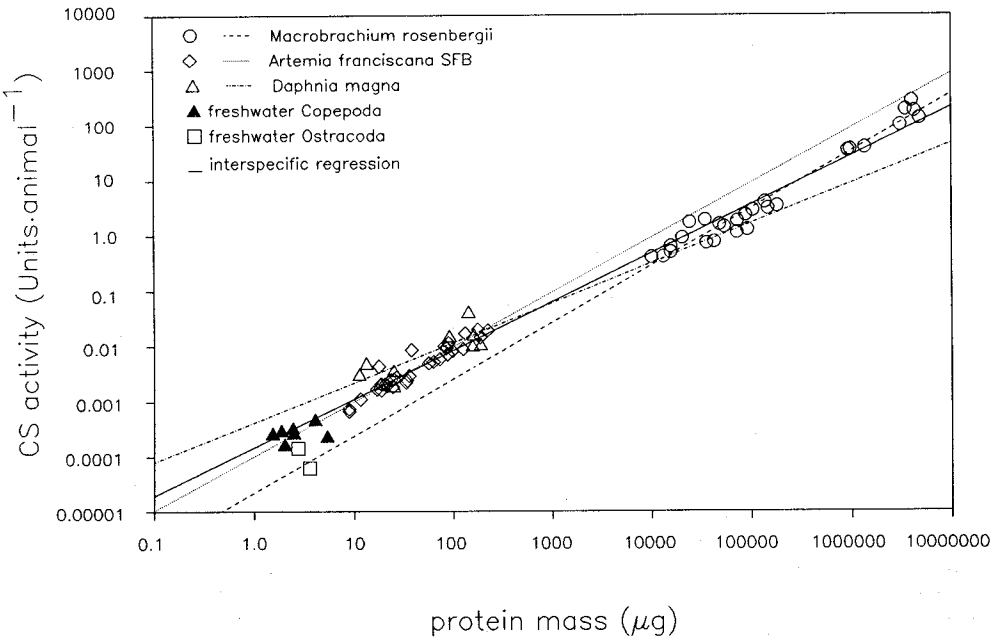

FIG. 2. Whole-animal citrate synthase (CS) activity versus protein weight for five crustacean species. Regression statistics are given in Table 1.

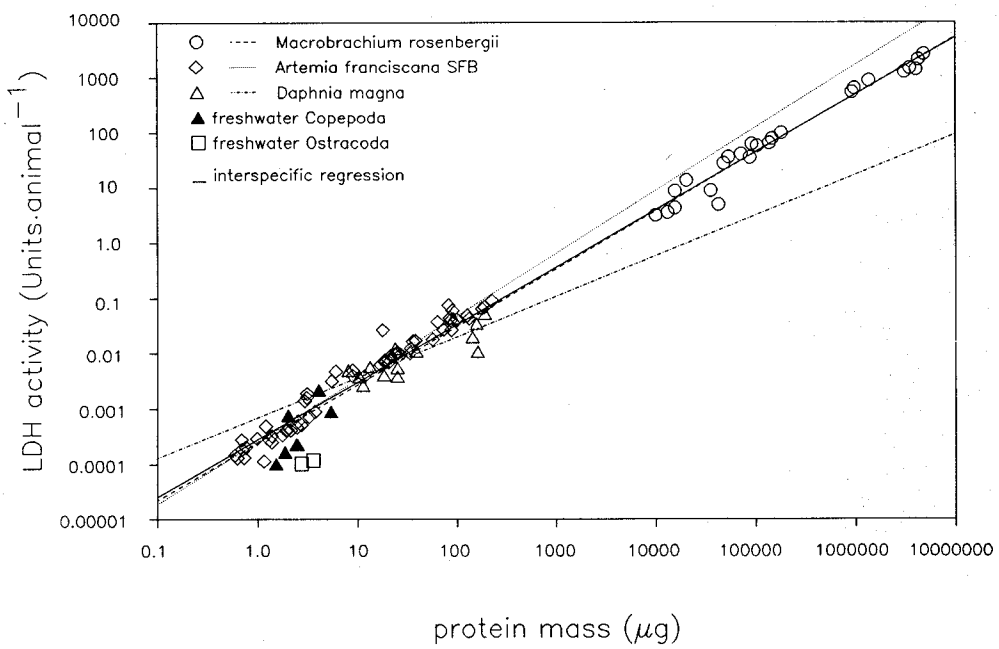

FIG. 3. Whole-animal lactate dehydrogenase (LDH) activity versus protein weight for five crustacean species. Regression statistics are given in Table 1.

ala AT activity the scaling exponent for *Daphnia* lies between 0.75 and 1.0 and is distinct from the other two species ($P < 0.01$ in both cases). *Macrobrachium* and *Artemia* have scaling exponents not different from 1.0 and not significantly different from each other ($P > 0.09$). For asp AT, both *Daphnia* and *Macrobrachium* activities scale to exponents significantly different from 1.0, and not different from each other ($P > 0.12$). The scaling exponent for *Artemia*, which is not significantly different from 0.75, is lower than that of *Macrobrachium* ($P < 0.01$) but not different from *Daphnia* ($P < 0.22$).

GDH activity gives an interspecific scaling exponent of 0.848 (Fig. 7), significantly different from both 0.75 and 1.0. While *Macrobrachium* and *Artemia* showed exponents not significantly different from 1.0, *Daphnia* appeared no different from 0.75. However, no statistical differences are detectable between intraspecific scaling exponents ($P < 0.09$).

The interspecific exponent for NDPK, 0.919, is between 0.75 and 1.0 (Fig. 8). Intranspecific scaling exponents follow the interspecific trend, and are not statistically different from each other ($P < 0.35$). The exponent for *Daphnia* is significantly lower than 0.75 and distinct from *Artemia* and *Macrobrachium* ($P < 0.01$ in both cases).

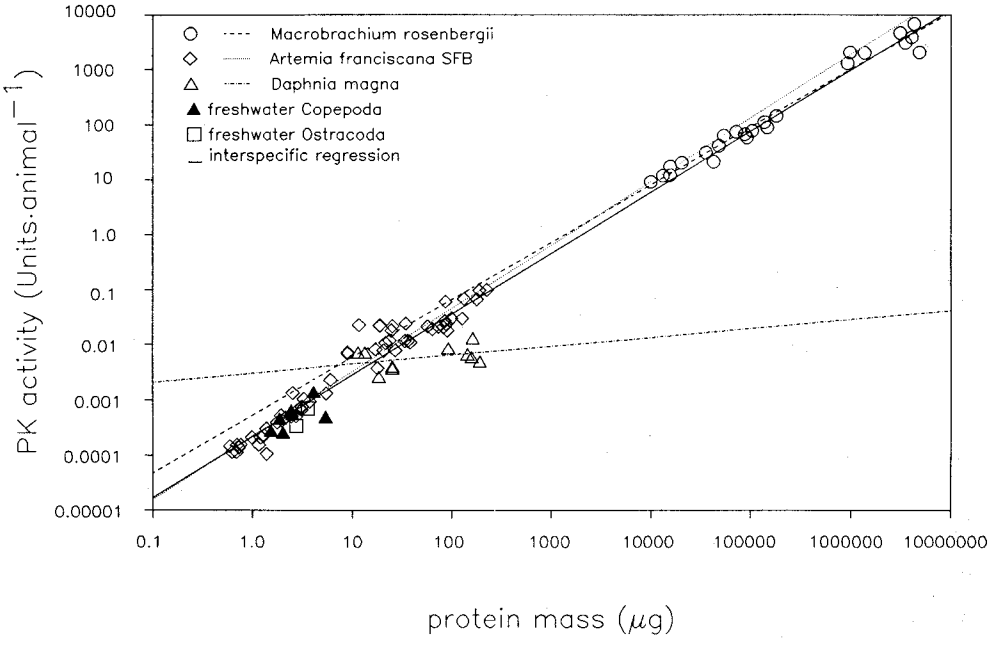

FIG. 4. Whole-animal pyruvate kinase (PK) activity versus protein weight for five crustacean species. Regression statistics are given in Table 1.

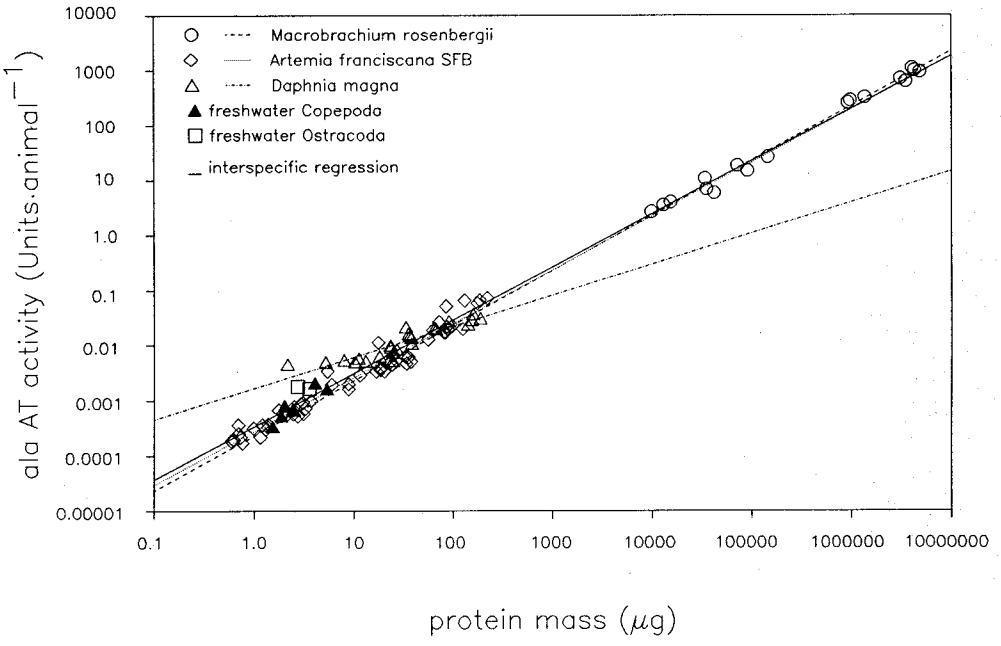

FIG. 5. Whole-animal alanine aminotransferase (ala AT) activity versus protein weight for five crustacean species. Regression statistics are given in Table 1.

G6Pdh (Fig. 9) could not be reliably detected in *Daphnia* due to very low activity and the small amounts of homogenate available for assay. Interspecifically, G6Pdh activity scales to the exponent 0.883, significantly higher than 0.75 and lower than 1.0. Scaling exponents for *Artemia* and *Macrobrachium* were not significantly different from each other ($P < 0.09$), although the exponent for *Macrobrachium* was different from 0.75 and 1.00, and *Artemia* was not different from 1.0.

Based on graphical relationships, enzyme activities of copepod and ostracod species appear close to values which would be predicted on the basis of their size by interspecific scaling relationships (Fig. 2–9).

Discussion

Whole-Animal Homogenization

For the enzymes tested, whole-animal homogenization appears to provide a reasonable index of total enzyme activity. This is true for enzymes with different substrate and cofactor requirements (i.e. coenzyme A for CS, pyridoxal phosphate for ala AT and asp AT, and NADPH for G6Pdh, as well as those in different cellular compartments (mitochondrial for CS versus cytoplasmic for other enzymes).

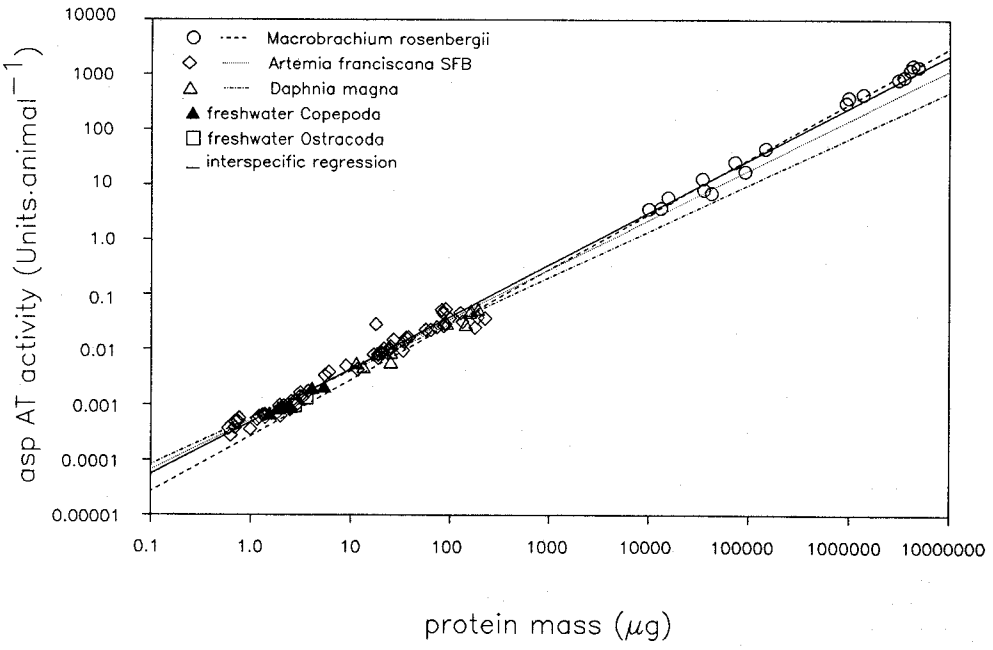

FIG. 6. Whole-animal aspartate aminotransferase (asp AT) activity versus protein weight for five crustacean species. Regression statistics are given in Table 1.

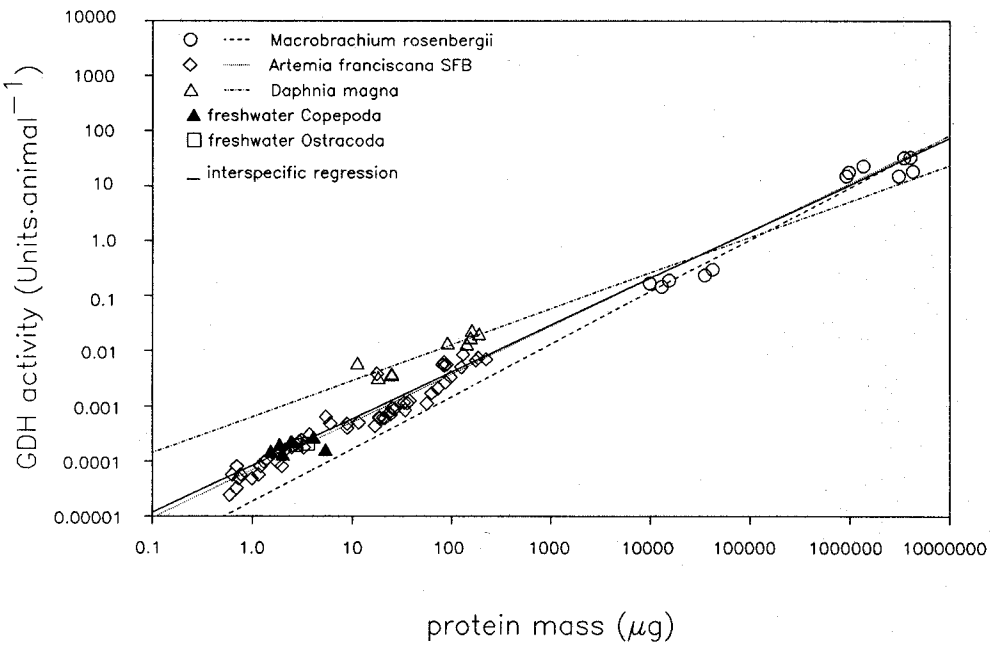

FIG. 7. Whole-animal glutamate dehydrogenase (GDH) activity versus protein weight for five crustacean species. Regression statistics are given in Table 1.

Enzyme–Scaling Relationships

It has been argued that relationships between metabolic rate and body size should not be compared within and between animal groups, since interspecific scaling may involve differences in developmental stages (Hemmingsen 1960; Heusner 1982). Since the case for enzyme activity has not yet been considered in this context, and mindful of this potential problem, we have chosen to examine and compare both inter- and intraspecific scaling relationships in this study. Intraspecific comparisons are most relevant, but general trends may be most clearly demonstrated with interspecific comparisons.

Citrate synthase

CS activity is representative of maximal aerobic capacity and has been found to account for 90% of total respiration (Hochachka et al. 1970). As such, it might be expected that CS activity would scale with body size to the same exponent as that found for oxygen consumption. While mammalian interspecific data clearly indicate an exponent near 0.75 for oxygen consumption, wide variation and generally higher exponents have been reported for crustaceans. Zeuthen (1953) cited scaling exponents of 0.76 for a freshwater crayfish, 0.881 for *Daphnia*, and 1.0 for *Artemia*. Alternatively, Gilchrist (1959) found 0.883 for *Artemia*. Interspecifically, Weymouth et al.

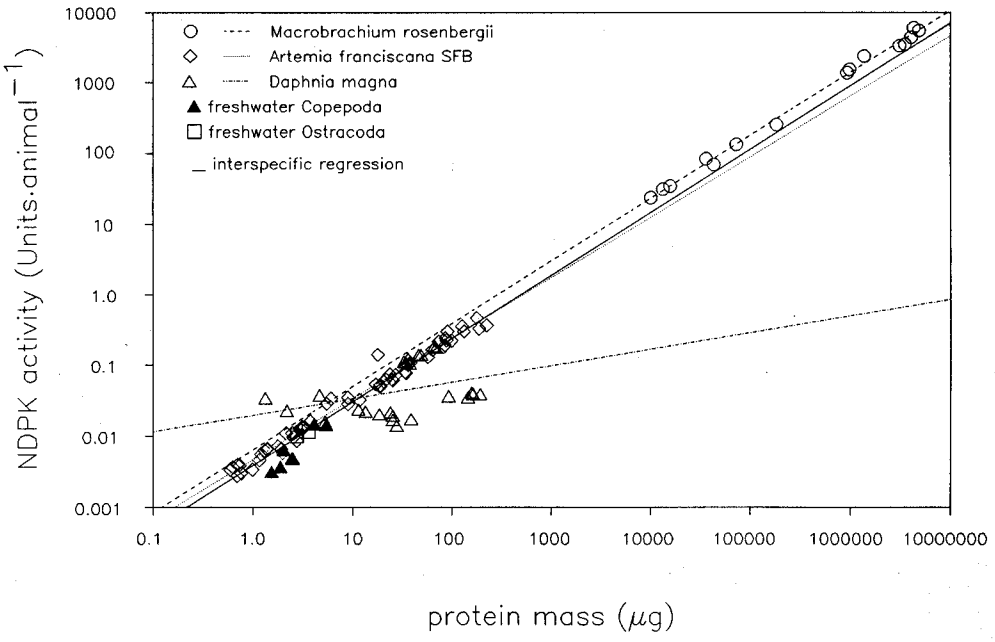

FIG. 8. Whole-animal nucleoside diphosphate kinase (NDPK) activity versus protein weight for five crustacean species. Regression statistics are given in Table 1.

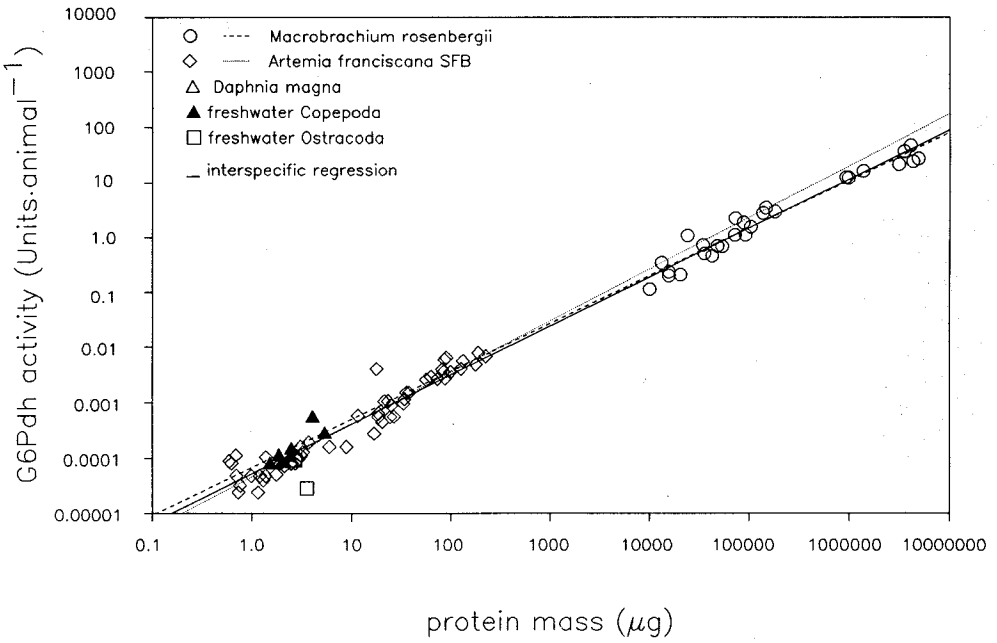

FIG. 9. Whole-animal glucose-6-phosphate dehydrogenase (G6Pdh) activity versus protein weight for five crustacean species. Regression statistics are given in Table 1.

(1944) found an exponent of 0.75 for several species of crab, and Vidal and Whitledge (1982) and Blazka et al. (1982) have shown slopes between 0.88 and 1.15 for marine and freshwater planktonic crustaceans. More recently, Wheatly (1989) calculated an exponent of 0.88 using several decapod crustacean species. These findings are compatible with scaling relationships for CS activity determined in the present study.

Scaling relationships for CS activity in fish and mammal tissues indicate an exponent of 0.75, closely matching the exponent for mammalian oxygen consumption (Somero and Childress 1980; Emmett and Hochachka 1981). Houlihan et al. (1985) reported a wet-weight-specific exponent corresponding to 0.84 in the extensor muscle of the crab *Carcinus*, close to the interspecific value of 0.87 obtained in the present study.

The similarity of intraspecific scaling exponents may indicate a fundamental similarity in the response of aerobic metabolism to changing body size.

Lactate dehydrogenase

Scaling relationships for LDH activity in fish and mammal species have been shown to vary with the tissue examined. For example, Somero and Childress (1980) noted a lack of size scaling for LDH in the brain of certain fish species, while LDH activity in muscle scaled to an exponent greater than 1.0. LDH has different functions in different tissues: it may act primarily in the lactate-to-pyruvate direction in gluconeogenic tissues or in the opposite direction in tissues engaged in anaerobic glycolysis, or in both forward and reverse directions at different times in the same tissue. In a whole-animal measurement, LDH may be more closely related to the relative proportions of different tissues, or the mode of activity of the organism. Thus, interspecific comparisons of LDH activity may be less meaningful. The fact that *Daphnia* and *Artemia* show little size scaling of LDH activity may relate to their small size; energetic requirements of small planktonic organisms relying on diffusion may not require increases in anaerobic capacity over their size ranges. Alternatively, for *Macrobrachium*, a benthic organism which relies on burst swimming responses to escape predators, size variation of LDH and hence anaerobic capacity may be greater. Somero and Childress (1980) argued that anaerobic energetic requirements for burst swimming increase as size increases. This is reflected in the exponent greater than 1.0 observed for *Macrobrachium* in the present study.

Pyruvate kinase

The role of PK in metabolism is not uniquely aerobic because oxidation of glucose via PK may proceed under anaerobic conditions. As in the case of LDH, there is tissue-specific variation; Somero and Childress (1980) reported that PK activity scales with a slope greater than 1.0 in fish muscle, but is virtually constant over size in the brain. Emmett and Hochachka (1981) and Houlihan et al. (1985) also found a positive scaling (slope greater than 1.0) in mammalian and crustacean muscle, although Ewart et al. (1988) demonstrated a slope of 0.81 for sea raven (*Hemitripterus americanus*) heart muscle. Following arguments developed for LDH, if smaller species are predominantly aerobic, PK activity might scale in a similar manner to CS, as is seen for *Artemia*. For larger crustaceans such as *Macrobrachium*, relying increasingly on anaerobic metabolism as body size increases, scaling exponents closer to 1.0 would be expected for PK, since PK does function both in anaerobic as well as aerobic glycolysis. The differences in intraspecific exponents may also be due to variation in molt status, since PK is known to vary in activity across stages of the molt cycle (Lesicki 1977). Interspecific trends are less easily compared in these conditions.

Alanine and aspartate aminotransferases

Intraspecifically, there is little pattern to variations of ala AT or asp AT scaling exponents. For the interspecific comparison, exponents are close to 1.0. Since neither enzyme can be considered to have a regulatory role in metabolism, it is difficult to predict a scaling relationship based on scaling of a particular metabolic process. Thus, an exponent near 1.0 might be what is expected for an enzyme capable of functioning either catabolically or anabolically. Presumably, in high-food environments where all sizes of animals are receiving adequate food, differences in enzyme activity across animal size based on variations in pathways of degradation or synthesis of amino acids would not be expected. Since alanine is involved in the osmoregulatory response in crustaceans (Hochachka and Somero 1984), differences in patterns of activity of ala AT between freshwater and saltwater species might have been anticipated; however, this is not apparent in the present study.

Glutamate dehydrogenase

In crustaceans where the major excretory product is ammonia, GDH activity has been suggested as an indicator of ammonia excretion (Bidigare and King 1981). Nitrogen excretion is generally found to scale to the exponent 0.75 in mammals, as does oxygen consumption (Peters 1983); thus, similar exponents for CS and GDH might be expected. In fact, Banse (1982) has argued that nitrogen excretion must scale with the same exponent as oxygen consumption; otherwise, organisms of different sizes feeding on food of similar composition would differ much more in body oxygen to nitrogen ratios than is observed in the Crustacea. Interspecifically, the present study does indeed show similar scaling exponents for both CS and GDH. Given the wide confidence intervals for intraspecific CS exponents, intraspecific CS and GDH exponents may also be comparable. Interspecific ammonia excretion exponents from Vidal and Whitledge (1982) and Blazka et al. (1982) for marine and freshwater plankton are also in this range.

Nucleoside diphosphate kinase and glucose-6-phosphate dehydrogenase

Both NDPK and G6Pdh are associated with anabolic pathways. The interspecific trend in both cases indicates exponents less than 1.0. Because anabolic processes control the amount of material available for growth, it is useful to consider the growth patterns of individual organisms with respect to differences in scaling relationships of anabolic enzymes. An exponent of less than 1.0 means that weight-specific enzyme activity decreases with increasing body size. This is compatible with the observation that larger species tend to have lower specific growth rates (Banse 1982; Peters 1983). Because growth of organisms in this study was not monitored, it is impossible to attribute differences in scaling relationships to this variable. As well, tissue-specific scaling of protein synthesis in fish indicates exponents less than 1.0, and similar to those of aerobic metabolism (Houlihan et al. 1986). Interestingly, however, in the case of both enzymes, *Daphnia* does not fit the interspecific relationships: G6Pdh could not be detected and the NDPK activity is practically invariant with body protein weight. In terms of growth and development, *Daphnia* is distinct in that it has a direct development instead of a series of larval stages. Scaling studies have paid very little attention to enzymes in anabolic pathways.

More generally, in scaling studies, differences in growth rates between animals are often ignored. Bertalanffy (1957) has suggested that anabolism should follow the surface rule (0.67 exponent), while catabolism will be directly proportional to body weight (1.0 exponent). If this is true, then animal metabolism might be expected to display scaling values near 0.67 during rapid growth when anabolism predominates and nearer 1.0 during senescence when catabolic processes are more important. Evidence from Schmidt-Nielsen (1984) shows that mammalian growth rate itself scales with body mass during postnatal linear growth phases to an exponent between 0.67 and 0.75. In another context, Goolish and Adelman (1987) have suggested that different individual tissue growth rates may confound attempts to establish relationships between tissue enzyme

activity and body size. This may apply to whole-animal studies as well. Thus, future studies of scaling relationships should take growth rate into account.

In summary, from the present study, the following conclusions may be drawn. First, whole-animal homogenization is a valid method for estimating whole-animal enzyme activity, in the crustaceans examined. Such measurements can provide a means of investigating scaling by dividing metabolism into its component anabolic and catabolic processes while overcoming the problems of extrapolating from single-tissue measurements. Second, size scaling of enzyme activity has been demonstrated in crustaceans for a variety of enzymes. This implies that use of enzymes to assess metabolic rates, or responses to environmental change, must take account of body size effects. Third, while intraspecific scaling is similar among species for enzymes such as CS, for others such as PK it is quite different. In cases of high intraspecific variability, the meaning of interspecific exponents must be carefully interpreted.

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