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## Measurement of the carbon balance in *Daphnia*<sup>1</sup>

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### Abstract

Short term measures of assimilation and respiration rates of four species of *Daphnia* consistently lead to the prediction that the relation between body mass and net carbon intake,  $F(B)$ , should be close to linear. Yet a more direct estimation of  $F(B)$  based on observed investments in growth and reproduction indicates that the true relationship is nonlinear with a roughly constant value of  $F$  maintained beyond the size at maturity. Several experiments demonstrate that our direct measures of  $F(B)$  are not greatly influenced by the experimental protocol. Hence, we conclude that short term measures of physiological parameters cannot be extrapolated to estimate growth and reproductive rates of *Daphnia*. Despite the existence of significant physiological differences between species, our results further reveal a conservative relation between age and the pattern of energy allocation to growth and reproduction in the genus and suggest that the evolution of *Daphnia* life histories is strongly regulated by one key character—the size at maturity.

The intermediate position of cladocerans in the food chain and their amenability to experimental manipulation have stimulated a long tradition of research on their energetics. A general assumption of this work is that the difference between short term measures of assimilation and respiration can be extrapolated to provide more long term estimates of secondary production (growth plus reproduction). Unfortunately, research in this area has progressed in a rather disorganized fashion. With the exception of the early work by Richman (1958) and Schindler (1968), virtually no one has simultaneously measured assimilation and respiration. Controlled interspecific comparisons are even rarer. Burns (1969), Downing (1981), and DeMott (1982) provide some information on clearance rates, but almost no comparative data are available for assimilation or respiration—the two energetic subcomponents that ultimately determine the net energy intake of individuals.

This lack of data has not discouraged several individuals from combining size-spe-

cific functions of clearance, assimilation, and respiration rates from various sources into general models of size-specific net energy intake (Threlkeld 1976; Hall et al. 1976; Lampert 1977a; Lynch 1977). Such models have been used to generate expected patterns of size-specific variation in competitive ability, vulnerability to starvation, and life history expression and evolution. The accuracy of the predictions of these models is obviously a function of the validity of the assumptions that interspecific variation in the size specificity of energetics is not great and that the pooling of data from different studies (often with very different experimental settings) is not of major consequence. A third problem is the possibility that differences between short term assimilation and respiration measurements may not in fact be reflected in realized growth and reproduction.

In order to examine these problems we raised four species of *Daphnia* in comparable environments and measured their size-specific rates of feeding, assimilation, respiration, growth, and reproduction. Our results reveal that the complexities addressed in the previous paragraph are indeed real and that future work in this area should proceed with much more attention to detail than it has in the past. We thank B. Heckt and K. Spitze for assistance, and W. DeMott and J. Jacobs for helpful comments.

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Table 1. Composition of algal medium used in this study. Double-distilled water is used throughout.

Algal medium recipe	
CaCl <sub>2</sub> ·2H <sub>2</sub> O	36.8 mg liter <sup>-1</sup>
MgSO <sub>4</sub> ·7H <sub>2</sub> O	37.0 mg liter <sup>-1</sup>
KHCO <sub>3</sub>	15.0 mg liter <sup>-1</sup>
K <sub>2</sub> HPO <sub>4</sub>	8.7 mg liter <sup>-1</sup>
NaNO <sub>3</sub>	85.0 mg liter <sup>-1</sup>
Na <sub>2</sub> SiO <sub>3</sub> ·9H <sub>2</sub> O	28.4 mg liter <sup>-1</sup>
FeCl <sub>3</sub> ·6H <sub>2</sub> O	1.1 mg liter <sup>-1</sup>
Trace element solution*	0.3 ml liter <sup>-1</sup>
After autoclaving	
Biotin	0.1 mg liter <sup>-1</sup>
Vitamin solution†	0.3 ml liter <sup>-1</sup>

\* In 100 ml of distilled water dissolve 5.00 g of EDTA, 2.20 g of ZnSO<sub>4</sub>·7H<sub>2</sub>O, 1.14 g of H<sub>3</sub>BO<sub>3</sub>, 0.51 g of MnCl<sub>2</sub>·4H<sub>2</sub>O, 0.49 g of FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.16 g of CoCl<sub>2</sub>·6H<sub>2</sub>O, 0.16 g of CuSO<sub>4</sub>·5H<sub>2</sub>O, and 0.11 g of (NH<sub>4</sub>)<sub>2</sub>MoO<sub>7</sub>·4H<sub>2</sub>O. Heat mixture to 100°C and dissolve by bringing to pH 6 with KOH. (After Levine and Ebersold 1958.)

† To 1 liter of distilled water add 7.00 g of calcium pantothenate, 0.0003 g of vitamin B<sub>12</sub>, 0.60 g of thiamin, 0.40 g of riboflavin, 1.30 g of nicotinamide, 3.30 g of folic acid, 0.30 g of putrescine, 5.00 g of choline, and 11.00 g of inositol. Store frozen. Mixture will not dissolve until diluted in medium.

## Methods

The four species of *Daphnia* used (*Daphnia ambigua*, *Daphnia galeata mendotae*, *Daphnia parvula*, and *Daphnia pulex*) have been maintained in the laboratory for more than 2 years as single clones extracted from east central Illinois lakes and ponds. The taxonomic status of *D. pulex* was verified by the criteria of Brandlova et al. (1972) and Schwartz et al. (1985). To avoid problems with intraspecific variation and clonal succession in stock cultures we used a single clone of each species. The ingestion, assimilation, and respiration rates that we report were determined in Plön, West Germany, in 1982; the size-specific growth and reproductive rates for the same clones were measured earlier in Champaign, Illinois. One of us (M.L.) oversaw the design of both types of experiment to maintain as much continuity in the environmental setting of the animals as possible. The only difference in treatment protocol is that life table animals were reared individually in beakers, while animals used for physiological rate measures were reared in mass culture but with about the same volume of medium per individual.

Throughout the study, the food supply consisted of a mixture of 80,000 *Scenedesmus dimorphus* and 16,000 *Chlamydomo-*

Table 2. Composition of zooplankton medium used in this study. Double-distilled water is used throughout. We do not autoclave the medium, but produce it in 20-liter quantities stored at 4°C for no more than 7 days.

Zooplankton medium recipe	
KCl	50.0 mg liter <sup>-1</sup>
MgSO <sub>4</sub> ·7H <sub>2</sub> O	40.0 mg liter <sup>-1</sup>
CaCl <sub>2</sub> ·2H <sub>2</sub> O	26.5 mg liter <sup>-1</sup>
K <sub>2</sub> HPO <sub>4</sub>	6.0 mg liter <sup>-1</sup>
KH <sub>2</sub> PO <sub>4</sub>	6.0 mg liter <sup>-1</sup>
NaNO <sub>3</sub>	50.0 mg liter <sup>-1</sup>
NaSiO <sub>3</sub> ·9H <sub>2</sub> O	20.0 mg liter <sup>-1</sup>
FeCl <sub>3</sub> ·6H <sub>2</sub> O	1.1 mg liter <sup>-1</sup>
Biotin	0.1 mg liter <sup>-1</sup>
Vitamin solution (Table 1)	0.3 ml liter <sup>-1</sup>

*nas reinhardtii* cells ml<sup>-1</sup>. The carbon content cell<sup>-1</sup> for the two species are 0.86 and  $5.33 \times 10^{-5}$   $\mu\text{g}$ , yielding a total food concentration of 1.54  $\mu\text{g C ml}^{-1}$ . Pure cultures were initiated weekly so that the food supply was always 7–14 days old. Preparation of the food supply required removal of cells from the algal medium by centrifugation followed by their resuspension and dilution in zooplankton medium to the appropriate density; cell densities were determined by hemacytometer counts in Champaign and spectrophotometric curves (fitted to cell counts) in Plön. As several investigators have had difficulties in growing cladocerans on artificial media, and as this paper will be the first in a series on cladoceran life histories, we provide the recipes for our media in Tables 1 and 2. These algal and zooplankton media are similar to those of Guillard and Lorenzen (1972) and Murphy (1970), and using them we have successfully raised more than 12 species of *Daphnia*, *Ceriodaphnia*, *Bosmina*, *Scapholeberis*, and *Diaphanosoma*.

The animals were kept at  $20 \pm 1^\circ\text{C}$  on a 12 : 12 light/dark schedule. For the life table experiments, animals were raised individually in 40 ml of medium in 50-ml glass beakers inside of clear plastic boxes to prevent evaporation; those in Plön were mass-cultured in 1.2-liter glass bottles circulated on a plankton wheel. In both cases, the food supply was changed every other day. Finally, to minimize complications that could arise from maternal effects (Lynch and En-

nis 1983), all individuals in our experiments were taken from the third consecutive generation raised on the controlled food supply.

**Ingestion and assimilation**—Radiotracer methods were used to measure ingestion and carbon assimilation rates. The day before an experiment a small quantity of both algal species was centrifuged, resuspended in 100–150 ml of fresh algal medium, and incubated with either 50  $\mu\text{Ci}$  [ $^{14}\text{C}$ ]sodium bicarbonate or 100  $\mu\text{Ci}$  carrier-free  $^{32}\text{P}$  overnight on a shaking table under fluorescent light. The next morning the algae were centrifuged again, resuspended in fresh zooplankton medium, and diluted to the appropriate concentration.

Before we ran the main experiments, we had to check whether the animals differentially selected or digested the two algae. We tested for differential feeding with the dual-labeling technique (Lampert 1974) with mixtures of algae labeled with  $^{32}\text{P}$  and  $^{14}\text{C}$  (Lampert 1981). To make sure that there was no systematic error introduced by the dual-labeling method, we tested both combinations, once providing the animals with  $^{32}\text{P}$ -labeled *Scenedesmus* and  $^{14}\text{C}$ -labeled *Chlamydomonas* and the second time with  $^{14}\text{C}$ -labeled *Scenedesmus* and  $^{32}\text{P}$ -labeled *Chlamydomonas*. Jacobs' (1974) coefficient of selection,  $D$ , was calculated as a measure of selectivity. It has limits of  $-1$  and  $+1$ ; in this case  $D = -1$  indicates exclusive feeding on *Chlamydomonas*,  $+1$  exclusive feeding on *Scenedesmus*, and  $0$  nonselective feeding. Although in this preliminary experiment we used *Daphnia* ranging from 1.0 to 2.5 mm long, we found no trend in selectivity for animals of different size, and all were pooled in the final analysis of data. Because  $\bar{D} \pm 95\% \text{ C.L.} = -0.009 \pm 0.033$  ( $N = 18$ ) for the treatment of  $^{32}\text{P}$ -labeled *Scenedesmus* and  $^{14}\text{C}$ -labeled *Chlamydomonas*, and  $-0.004 \pm 0.049$  ( $N = 22$ ) for the reverse treatment, we assume that both components of the food supply are ingested with equal efficiency. Similar results have been reported for *Daphnia rosea* (DeMott 1982).

To test for digestibility differences between the two foods, we did assimilation experiments as described below, but with pure suspensions of *Scenedesmus* and

*Chlamydomonas* instead of the mixture, and only with adults about 2 mm long. Dividing these assimilation rates by the ingestion rates of animals 2 mm long, we got similar assimilation efficiencies: 72.8% for *Chlamydomonas* and 65.8% for *Scenedesmus*.

Even though the two algae appear to be close in ingestibility and digestibility, as a further precaution we labeled both with  $^{14}\text{C}$  before our main experiments. The radioactivity of the stocks was checked before they were diluted, and the specific activity ( $\text{dpm mg}^{-1} \text{ C}$ ) of both algae was adjusted to the same value by diluting the suspension of higher specific activity with unlabeled cells. Identical specific activities of the two food components ensured that true carbon ingestion and assimilation rates would be estimated regardless of differential ingestion or digestion.

Feeding experiments were done in 250-ml glass beakers with animals from stock cultures that were preadapted to the experimental conditions. The animals were quickly concentrated in 5 ml of unlabeled food suspension and transferred into the labeled food. While they were feeding, three subsamples of 2 ml each were taken from the beaker and filtered through membrane filters to determine the radioactivity of the suspension. To avoid defecation of label, we poured the animals through a sieve after 8 min, immediately narcotized them in soda water, flushed them into a Petri dish, and killed them with a few drops of Formalin. To further avoid losses of label (Holtby and Knoechel 1981; Gulati et al. 1982), we immediately measured the daphnids under a dissecting scope and transferred them singly into scintillation vials by forceps. To keep the handling time short, we repeated every experiment several times with a limited number of daphnids (about 15).

Carbon assimilation rates were measured according to Lampert (1977a). This method eliminates the need for clearing the gut after feeding labeled food and thus avoids losses of tracer from the "metabolic" pool (Lampert 1975). Two subsamples of daphnids were fed labeled food for 1 and 3 h in stoppered 1.2-liter glass bottles submerged in a temperature-controlled ( $20^\circ \pm 0.1^\circ\text{C}$ ) water bath. To avoid significant reduction of the

algal concentration by grazing, we used only a small number of animals (~15) per bottle but set up replicate bottles. At the end of the feeding period the animals were harvested as above.

Vials of individual daphnids were kept overnight with 0.3 ml of a tissue solubilizer at 60°C, cooled, and filled with 5 ml of a toluene-based scintillation cocktail. The samples were counted in a liquid scintillation counter for 10 min with dpm correction by external standardization. Membrane filters were dissolved in a special cocktail (Filter Count, Packard) before LSC counting.

Individual ingestion rates ( $\mu\text{g C ind}^{-1} \text{h}^{-1}$ ) were calculated from the specific activity of the algae (dpm  $\mu\text{g}^{-1} \text{C}$ ), the radioactivity of the animals (dpm  $\text{ind}^{-1}$ ), and the feeding time (min). Each series of experiments yielded values for animals of different sizes which were then subjected to a log-log regression to define the size-specific function.

The estimation of unbiased assimilation rates requires information on the amount of  $\text{CO}_2$  respired by the animals during an experiment. Following the protocol of Lampert (1975, 1977a), we found in our preliminary assimilation experiments (above) that losses of tracer through respiration were nearly identical for the two species of algae: *Scenedesmus*, 15.3 and 23.9%, and *Chlamydomonas*, 15.3 and 25.8%, at 1 and 3 h. Since these values are also very similar to those calculated from the equation given by Lampert (1977a), we did not measure respiratory losses of  $^{14}\text{C}$  in the succeeding experiments but used that equation for correction of assimilation rates instead.

Our final computational procedure for the size-specific assimilation rate function is a slight departure from that introduced by Lampert (1977a). In that case, individual logarithmic regressions were performed on the 1- and 3-h carbon uptake values uncorrected for respiration losses, and then a final allometric regression was performed on certain size-specific values determined from the preliminary regressions after correcting for respiration, subtracting the 1-h value from the 3-h value and dividing by 2. An undesirable property of this protocol is that the arbitrary choice of sizes used in the final

regression obviates any objective statistical analysis of the data. To avoid this problem, we corrected each individual data point for respiration losses before performing the preliminary regressions, and then, after verifying homogeneity of residual variances, tested for significant differences between the 1- and 3-h slopes (Snedecor and Cochran 1973). Because the differences were insignificant in all cases, we used the pooled slope to redefine the 1- and 3-h intercepts under the assumption of equal slopes. The final size-specific function ( $\mu\text{g C assimilated ind}^{-1} \text{h}^{-1}$  vs.  $\mu\text{g dry wt of animal}$ ) was then determined by dividing the difference between the 3- and 1-h functions by 2.

*Respiration*—Since the respiratory rate of *Daphnia* may be influenced considerably by the quantity of food (Porter et al. 1982; Lampert and Bohrer 1984), it is essential to measure the respiration of the animals in their normal food suspension. We selected the "closed bottle" method. We put 10–100 daphnids of about the same size into ground-glass-stoppered bottles of calibrated volume close to 30 ml with fresh food suspension as the medium. We filled three to five control bottles with the same suspension but without animals. All bottles were incubated at  $20^\circ \pm 0.1^\circ\text{C}$  in a water bath under subdued light.

Oxygen concentrations in the bottles were determined initially and after 4 h by injecting 2-ml subsamples into a temperature-controlled water-jacketed electrode chamber of a Radiometer PMH 72 oxygen analyzer (Weider and Lampert 1985). The decrease of  $\text{PO}_2$  during 4 h was usually in the range of 10% and never >20%. At the end of an experiment, the contents of the bottles were poured through a sieve and the animals preserved. All daphnids were measured under a dissecting scope and the egg/embryo numbers recorded.

There are two reasons to correct the rates of oxygen uptake in the experimental bottles. First, the oxygen uptake in bottles containing adults will tend to be inflated if eggs or embryos are present. We removed this bias by performing preliminary size-specific regressions for subadult size classes, assuming the average egg/embryo mass to be equal to the average mass of fresh eggs and first

instar individuals, and determined their individual oxygen consumption by extrapolating the regression. Total egg/embryo respiration was then estimated by multiplying by the total number of eggs, embryos, and first instar animals counted in our preserved samples, and this source of oxygen uptake removed by elevating the final oxygen concentration in the animal bottles accordingly. Although the assumption of equal egg, embryo, and newborn respiration may not be precisely true, any violation of it would be of little consequence since this correction had a minor effect on our final computation.

Second, the grazing activity of the animals may result in some alteration of the background respiration relative to that in the controls. Under the assumptions that the animals remove all respiring particles from the medium with equal efficiency and that their clearance rates remain constant for the duration of the experiment, an approximate weighting factor for the background respiration can be determined as follows. At any time  $t$  during an experiment, the rate of background respiration will be

$$R_{B,t} = R_{B,0} \exp(-fNt)$$

where  $R_{B,0}$  is the control rate of respiration,  $f$  is the clearance rate per individual, and  $N$  is the number of animals in the bottle. The average background rate of respiration in an experimental bottle is

$$\bar{R}_B = \frac{\int R_{B,t} dt}{t}$$

Performing this integration and dividing by  $R_{B,0}$  gives the proportion of control respiration expected to have occurred in the experimental bottle.

$$\frac{\bar{R}_B}{R_{B,0}} = \frac{1}{fNt'} \times [1 - \exp(-fNt')]$$

where  $t'$  is the duration of the experiment. We calculated this factor for each of our respiration experiments and multiplied it by the control respiration to estimate the background respiration in our experimental bottles.  $f$  was determined from the size-specific feeding rate function and the mean size of the animals in an experiment, and  $N$  from the number of preserved animals.

For comparison with rates of carbon as-

Table 3. Least-squares regressions of body weight ( $\mu\text{g}$  dry wt) on length (mm) and mean dry weights ( $\mu\text{g}$ ) of fresh eggs. Lengths of animals were measured from the base of the tail spine to the top of the eyespot in *Daphnia ambigua*, *Daphnia galeata mendotae*, and *Daphnia parvula*, and to the top of the head in *Daphnia pulex*.

	Body weight-length regression	Egg wt
<i>D. ambigua</i>	$B = 5.740L^{2.310}$	0.851
<i>D. galeata mendotae</i>	$B = 5.480L^{2.200}$	1.415
<i>D. parvula</i>	$B = 4.740L^{2.190}$	0.986
<i>D. pulex</i>	$B = 10.674L^{2.093}$	2.110

similation, oxygen consumption rates need to be converted to carbon loss. After correcting the individual rates of oxygen uptake for background egg/embryo and nondaphnid respiration, we converted them to carbon equivalents with the factor  $0.3753 \mu\text{g C } \mu\text{g}^{-1} \text{O}_2$ , under the assumption that the respiratory quotient for well fed animals is about equal to 1.0 (Lampert and Bohrer 1984). The final size-specific functions for respiration were then determined by log-log regression of all the data.

*Direct estimation of net carbon intake*—We directly measured the net carbon intake of different sized individuals by monitoring 50–100 individuals of each species throughout their lives. Each animal was examined daily under a microscope for growth (length), survival, and reproduction (number of eggs carried, progeny released). To determine the investment that individuals make in growth, we estimated length-dry weight regressions for each species by drying 30–40 single individuals of a variety of sizes at  $60^\circ\text{C}$  for 24 h and then weighing them to the nearest  $0.1 \mu\text{g}$  with a Mettler M22 electrobalance. The adult females used in the estimation of the length-weight relation included only those that had recently emptied their ovaries by producing a new clutch, and the clutch was removed before weighing to ensure that estimated rates of investment in growth were not biased by changes in ovary or progeny weights. Least-squares linear regressions of the logarithmic transformations (Table 3) are all highly significant ( $P < 0.0001$ ). The dry weight invested in offspring was determined for each species by weighing 4–5 batches of 5–15 freshly laid eggs (Table 3).

Table 4. *F*-test comparisons between species for slopes and intercepts of the size-specific ingestion and assimilation rate functions. *Daphnia ambigua*—*amb*; *Daphnia galeata mendotae*—*gal*; *Daphnia parvula*—*par*; *Daphnia pulex*—*pul*. NS indicates that the null hypothesis cannot be rejected at the 0.05 level.

	<i>amb-gal</i>	<i>amb-par</i>	<i>amb-pul</i>	<i>gal-par</i>	<i>gal-pul</i>	<i>par-pul</i>
Ingestion						
Slope						
<i>F</i>	8.514	0.008	4.294	4.850	0.532	2.097
df	1,122	1,76	1,146	1,116	1,186	1,140
<i>P</i>	<0.005	NS	<0.05*	<0.05*	NS	NS
Intercept						
<i>F</i>	16.597	22.958	63.075	71.271	237.651	21.688
df	1,123	1,77	1,147	1,117	1,187	1,141
<i>P</i>	<0.001	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Assimilation						
Slope						
<i>F</i>	2.458	1.772	12.449	0.025	4.525	4.348
df	1,178	1,167	1,164	1,191	1,188	1,177
<i>P</i>	NS	NS	<0.001*	NS	<0.05	<0.05
Intercept						
<i>F</i>	2.048	201.675	115.579	98.669	120.251	0.390
df	1,179	1,168	1,165	1,192	1,189	1,178
<i>P</i>	NS	<0.001*	<0.001*	<0.001	<0.001	NS

\* The residual variances of the two regressions were heterogeneous and the *F*-values may be inflated.

A very small number of *D. pulex* produced an ephippium (resting egg) during the experiment; the dry weights ( $\mu\text{g}$ ) of these were related to the length (*L*) of the female by the logarithmic regression  $E_p W (\mu\text{g}) = 2.90L^{2.60}$  ( $P < 0.001$ ). Ephippial production always followed a barren instar; therefore, in the following analyses we assume that the weight of an ephippium represents an investment in reproduction accumulated over the previous two instars.

The dry weight investment ( $\mu\text{g d}^{-1}$ ) that an individual makes in growth is

$$G(L_o) = \frac{\alpha_1(L_o^{\alpha_2} - L_D^{\alpha_2})}{D}$$

where  $L_o$  and  $L_D$  are the lengths of an individual in two adjacent instars,  $D$  is the instar duration (days), and  $\alpha_1$  and  $\alpha_2$  are the coefficient and exponent of the length-weight regression. Investment in reproduction ( $\mu\text{g d}^{-1}$ ) is  $R = 0$  if no clutches or ephippia are produced in the following two instars, is

$$R = C \times EW/D$$

(where  $C$  is the size of the clutch in the following instar and  $EW$  is the dry weight  $\text{egg}^{-1}$ ) if a clutch is carried in the following instar, or is

$$R = E_p W(L)/2D$$

when an ephippium is carried in one of the two following instars by a mother of length  $L$ . These data were calculated for each instar of every animal and pooled into 0.1-mm size classes. The dry weight sum, ( $G + R$ ), for each size class was then converted to net carbon intake ( $\mu\text{g C ind}^{-1} \text{d}^{-1}$ ) by multiplying by 0.42, a conversion factor empirically derived by Lemcke and Lampert (1975) for *Daphnia pulex* that we assume to apply equally to all stages.

### Results

The size-specific ingestion rate functions are illustrated in Fig. 1. Although the residual variances of the regressions tended to be heterogeneous, the magnitude of the *F* values in the comparisons of intercepts for the different species leaves little doubt that they are all significantly different (Table 4). The exponents of the power functions were less variable (all close to 1), although those for *D. ambigua* and *D. galeata mendotae* were clearly different (Table 4).

Significant differences in the size-specific assimilation rate functions were also detected for all interspecific comparisons ex-

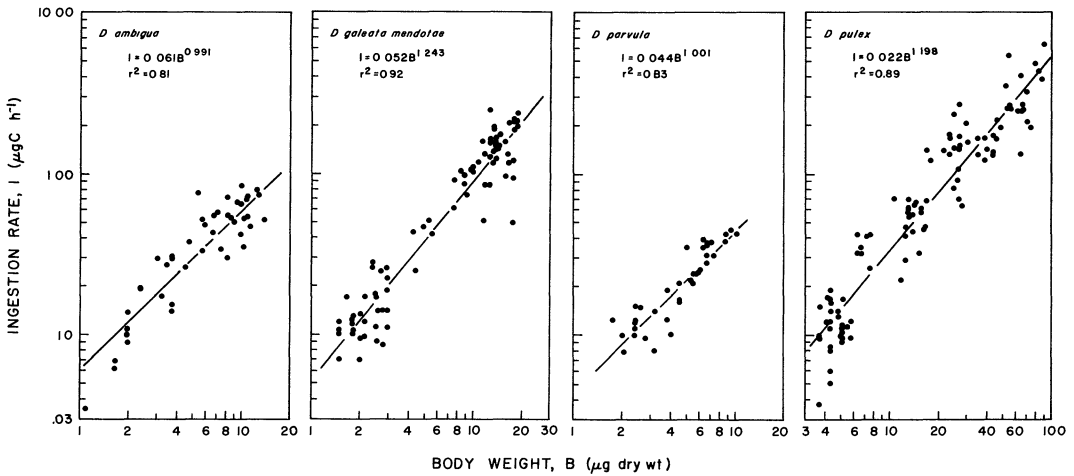


Fig. 1. Size-specific ingestion rate functions for the four species.

cept *D. ambigua*-*D. galeata mendotae* (Fig. 2, Table 4). However, as in the case of ingestion rates, differences between slopes tended to be less significant than those between intercepts. The ranking of intercepts was the same as in the case of ingestion rates (*D. ambigua* > *D. galeata mendotae* > *D. parvula* > *D. pulex*).

A comparison of the regressions for ingestion and 1-h assimilation rates (Fig. 2) suggests that the former may not be reliable. Although all species had nearly identical slopes for assimilated carbon at 1 and 3 h, the slopes for the ingestion rate regressions were consistently higher (in the case of *D. galeata mendotae* significantly so,  $F_{1,129} =$

9.576;  $P < 0.001$ ). The smallest instars of *D. ambigua*, *D. galeata mendotae*, and *D. pulex* assimilated slightly more carbon during their first hour than can be accounted for by their ingestion rates measured during the first 8 min.

The simplest explanation for these disparities is that during the first few minutes in their radiolabeled food supply the animals feed at something less than their normal rate, and that small animals are affected to a greater extent than large ones. The mechanical disturbance of moving the animals or their brief crowding before the radio-labeled food is added may be responsible for such a temporary depression. Alternatively,

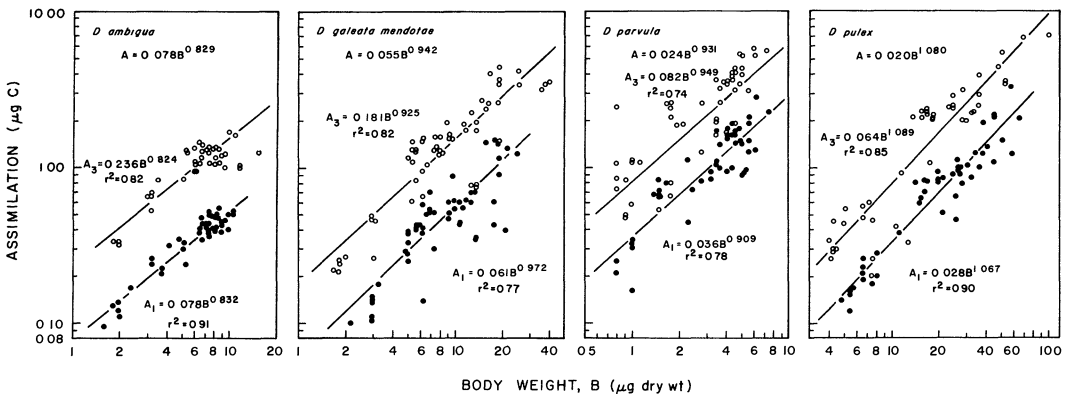


Fig. 2. Size-specific functions for 1- and 3-h carbon uptake values corrected for losses by respiration ( $A_1$  and  $A_3$ ). Inset functions,  $A$  ( $\mu\text{g C d}^{-1}$ ), are the final assimilation rate functions calculated by pooling slopes from  $A_1$  and  $A_3$  as described in the text.

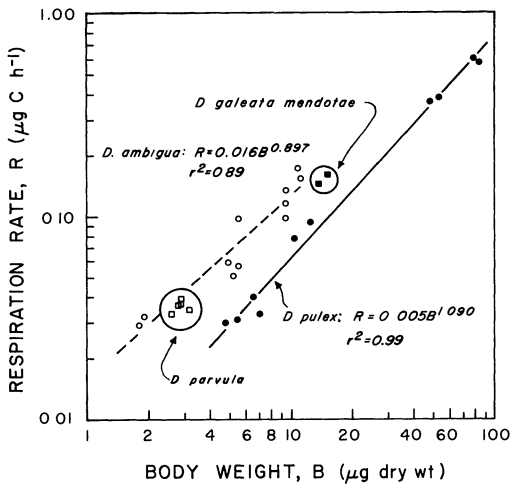


Fig. 3. Size-specific respiration rate functions for *Daphnia ambigua* and *Daphnia pulex*, and single points for *Daphnia galeata mendotae* and *Daphnia parvula*.

despite rapid processing, there might be some loss of label in short term measurements that is more pronounced in small animals. Since reports of assimilation efficiencies >100% occasionally appear in the literature (Porter et al. 1982), the inaccuracy of short term measures of ingestion rate may be a general problem.

We have data only for *D. ambigua* and *D. pulex* sufficient to determine the size-specific respiration rate functions (Fig. 3). Although the slopes of the regressions were not quite significantly different ( $F_{1,17} = 3.474$ ;  $0.10 < P < 0.05$ ), the elevation of the *D. ambigua* regression was about three times that for *D. pulex* ( $F_{1,18} = 57.233$ ;  $P < 0.001$ ). Thus, under the experimental conditions *D. ambigua* respire at about three times the rate of *D. pulex* of similar mass. The few data points for *D. galeata mendotae* and *D. parvula* suggest that their respiration rates are closest to that of *D. ambigua*.

In theory, once two additional outputs of carbon (organic carbon excretion and molts) are accounted for, it should be possible to predict the size-specific patterns of net production (growth + reproduction) from size-specific physiological functions. Unfortunately, it is impossible to measure the organic carbon excreted by feeding animals because they also produce dissolved organic

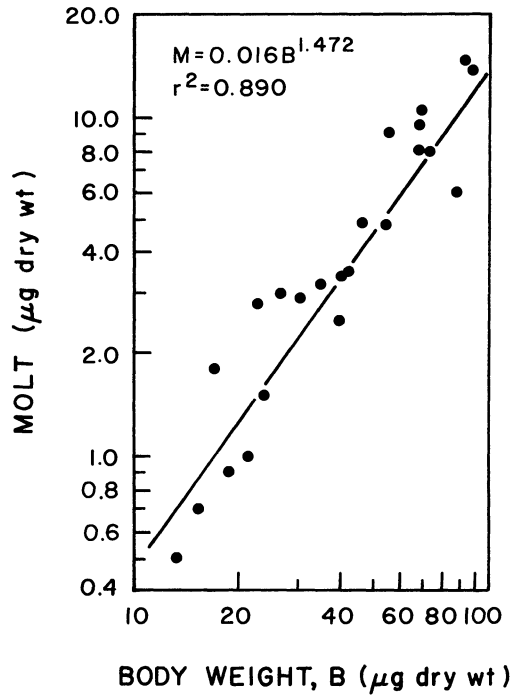


Fig. 4. Allometric function for molt weight in *Daphnia pulex*.

carbon by damaging food particles before ingestion. We can however gain some insight into the problem by measuring both organic carbon excretion and  $\text{CO}_2$  release by radio-labeled animals that are not fed during an experiment. Such data have been gathered by Lampert (unpubl. data), using large *Daphnia magna* adults. The ratio of organic carbon to  $\text{CO}_2$ -carbon released ( $\pm$ SD),  $0.169 \pm 0.017$  ( $N = 15$ ), is very consistent in these experiments, but we are unable to say whether it is a function of body size.

Because of the difficulty of completely recovering the molts of small individuals, we could obtain this information only for the large *D. pulex* (Fig. 4). The slope ( $\pm$ SE) of this allometric function,  $1.472 \pm 0.113$ , is highly significant and indicates that increases in size are accompanied by substantial increases in the cost of producing exoskeletal material—a price of the crustacean mode of growth.

Under the additional assumptions of 2.5 days per instar at  $20^\circ\text{C}$  (approximately as observed) and 42% of the molt dry weight

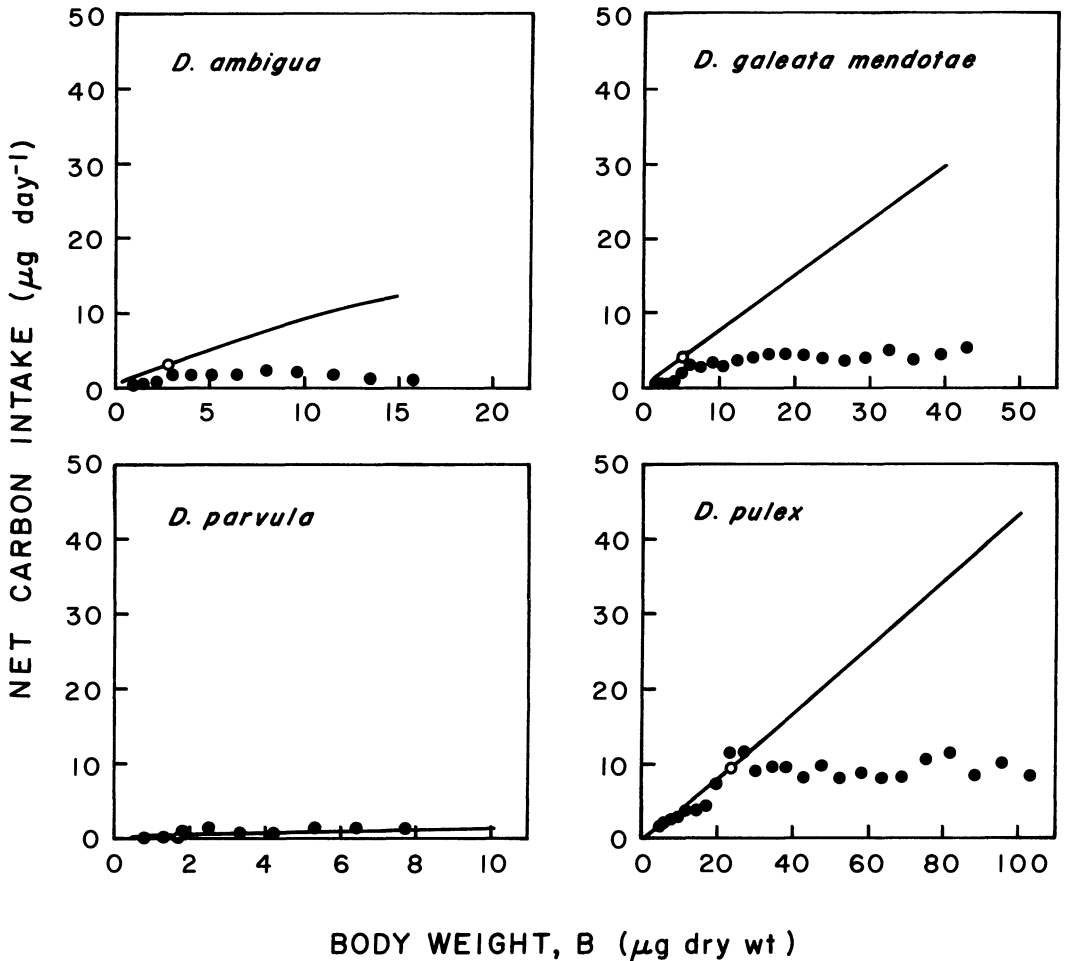


Fig. 5. Predicted size-specific patterns of net carbon intake determined from size-specific physiological functions compared with direct measures determined from life table analyses. Open circles denote the size at first reproductive investment, i.e. the mean size of individuals in the instar just before production of the first clutch.

as carbon, the daily net carbon intake is predicted to be

$$F(B) \approx 24[A(B) - 1.169R(B) - 0.007M(B)].$$

The coefficient, 0.007, is from  $(2.5 \text{ d molt}^{-1} \times 24 \text{ h d}^{-1})^{-1}$ . Assuming that  $M(B)$  in Fig. 4 is representative of all the species and that the respiration rates of *D. galeata* and *D. parvula* may be approximated by that of *D. ambigua*, we obtain the four  $F(B)$  functions in Fig. 5. Because  $M(B)$  has only a minor influence on  $F(B)$  and the slopes of  $A(B)$  and  $R(B)$  are very close to 1.0, each of the  $F(B)$  functions is nearly linear. The directly measured net rates of carbon intake

are also presented in Fig. 5. In the case of *D. parvula* the predicted and observed values of  $F(B)$  are very similar, as they are for prereproductive *D. pulex*. But the  $F(B)$  estimates derived from physiological data greatly overestimate the observed adult values for *D. ambigua*, *D. galeata mendotae*, and *D. pulex*. Because the direct size-specific estimates become approximately constant almost immediately following maturity, the discrepancy increases with body size.

The upper limits to our observed  $F(B)$  functions are not contrary to the commonly observed increase of clutch with body size.

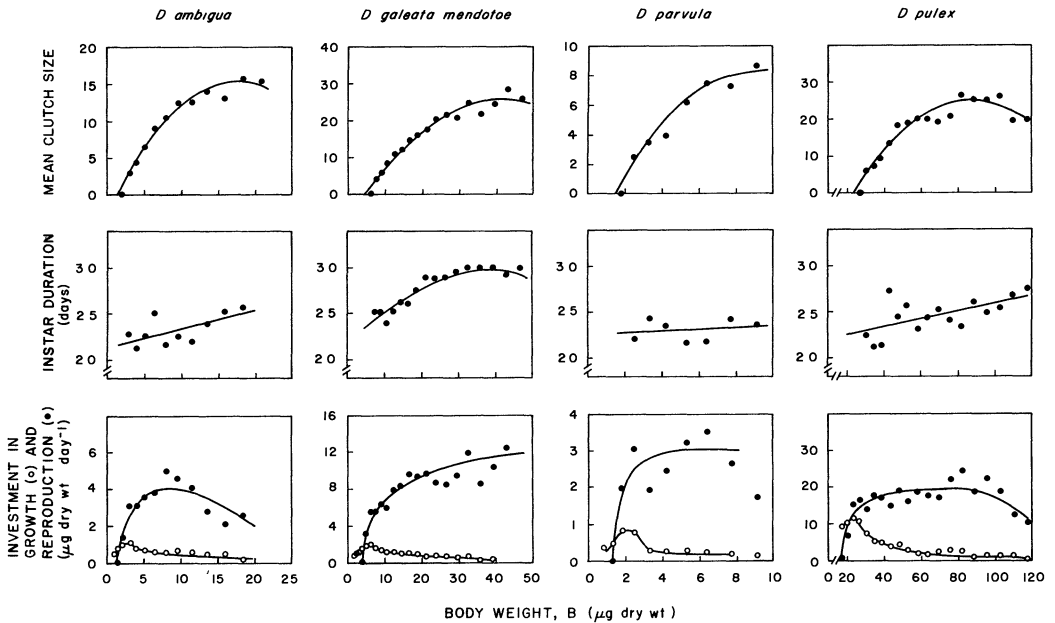


Fig. 6. Mean size-specific clutch sizes, instar durations, and rates of investment in growth and reproduction determined from the life table analyses. Solid lines for mean clutch sizes and instar durations are fitted polynomial regressions; all are significant ( $P \leq 0.05$ ) except that for *Daphnia parvula* instar durations. Curves in lowest panels were fit visually.

All four species exhibited a strong positive association of clutch size and body size, but, except for *D. parvula*, this tended to be offset by longer instar durations in larger animals (Fig. 6); i.e. the energy that goes into the clutches of large animals is accumulated over a longer period than that for small animals. In addition, the relatively low reproductive investment of small animals tends to be balanced by their high investment in growth (Fig. 6).

The amount of carbon "missing" for these three species is substantial. For an intermediate-sized *D. ambigua* of 9  $\mu\text{g}$  dry wt,  $\sim 19$  additional eggs per clutch are required to account for the discrepancy between observed and expected  $F(B)$ , and for a 25- $\mu\text{g}$  *D. galeata mendotae* and 60- $\mu\text{g}$  *D. pulex* the discrepancies are equivalent to  $\sim 26$  and  $\sim 18$  eggs. In each of these cases the number of eggs "missing" is about equal to the maximum observed clutch size (Fig. 6); i.e. the physiological data result in clutch size predictions that are roughly double what the animals are capable of producing in the experimental setting.

A possible explanation for the missing carbon is that because of the depressive effects of grazing in a small volume of water unrenewed for 2 days, the adult *Daphnia* in the life table beakers were exposed to a lower average food concentration and hence assimilated carbon at lower rates than individuals in the short term assimilation rate experiments. A simple experiment demonstrated the potential importance of this problem, at least for large *D. pulex*. Following our life table procedures, we started with beakers of 40 ml of fresh  $^{14}\text{C}$ -labeled food. At 0, 24, and 48 h a single 2.0-mm *D. pulex* was allowed to feed for 3 h in each beaker and then removed for scintillation counting. Control beakers had not been grazed before *Daphnia* was added, but a single individual was always present in each experimental beaker to simulate the normal 48-h grazing period. The net 3-h accumulations of carbon ( $\mu\text{g ind}^{-1} \pm \text{SD}$ ,  $N = 8$ ) at 0, 24, and 48 h in the controls were  $3.86 \pm 1.03$ ,  $2.50 \pm 0.80$ , and  $2.41 \pm 0.90$ , and in the experimental beakers after 24 and 48 h were  $1.23 \pm 0.53$  and  $0.27 \pm 0.20$ . Clearly these

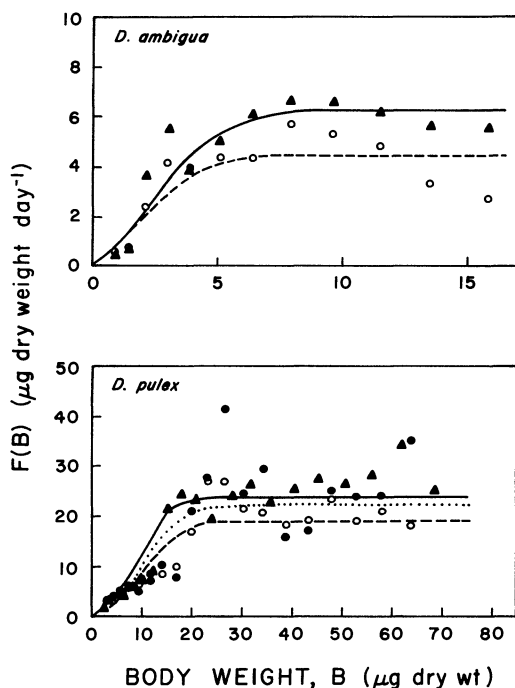


Fig. 7. Size-specific rates of net incorporation of dry weight (growth + reproduction) directly measured from life table analyses of *Daphnia ambigua* and *Daphnia pulex*. Open circles and dashed lines denote values for standard  $1.54 \mu\text{g C liter}^{-1}$  food, triangles and solid lines  $3.08 \mu\text{g C liter}^{-1}$  food, both in 40 ml of fresh food supplied to the animal on alternate days. Closed circles and dotted line for *D. pulex* denote values for animals grown on  $1.54 \mu\text{g C liter}^{-1}$  food, but with 200 ml of fresh food supplied to each animal daily. Curves are fitted by a nonlinear least-squares procedure to a sigmoid function.

animals were significantly depressing their food supply over a 48-h cycle.

Nevertheless, the observed patterns of  $F(B)$  do not seem to be greatly influenced by either food limitation or a container effect. To test for this, we grew both *D. ambigua* and *D. pulex* at double the previous food concentration but under otherwise identical conditions (Fig. 7). In addition, a cohort of 20 *D. pulex* was grown as single individuals in 250-ml beakers with 200 ml of  $1.54 \mu\text{g C liter}^{-1}$  food replenished every 24 h (these animals were provided with  $10\times$  as much fresh medium as in the standard experiment). The data were fit by a nonlinear least-squares procedure to the two-parameter sigmoid model,

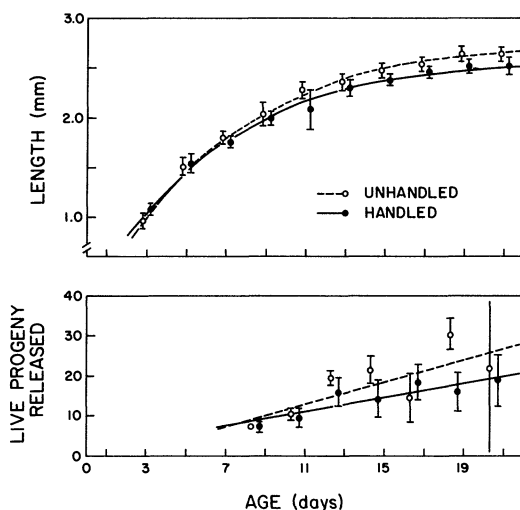


Fig. 8. Comparison of rates of growth and reproduction (mean number of progeny released per mother per 2 days) for handled and unhandled animals. Growth curves were estimated by nonlinear least-squares fit of the function  $L = L_{\max}[1 - \exp(-aT)]$ , where  $L_{\max}$  is asymptotic length,  $T$  is age, and  $a$  is a fitted constant that determines the slope of the curve. Both regressions are highly significant;  $L_{\max}$  and  $a$  are 2.58 and 0.17 for handled animals and 2.77 and 0.15 for unhandled animals. Linear regressions for both reproductive functions are significant (handled:  $r = 0.78$ ,  $P < 0.01$ ; unhandled:  $r = 0.60$ ,  $P < 0.05$ ). Bars on the points are 2 SE.

$$F(B) = \frac{F_{\max} + 1}{1 + F_{\max} \exp(-aB)} - 1; \quad (1)$$

all regressions were highly significant.

Doubling of the food supply did result in an elevation of  $F_{\max}$  in both cases;  $F_{\max} \pm 2$  SE for normal and double food is  $4.42 \pm 1.10$  and  $6.27 \pm 1.67$  for *D. ambigua* and  $19.34 \pm 3.53$  and  $23.98 \pm 4.84$  for *D. pulex*. However the change was not great, certainly not great enough to account for the discrepancies in Fig. 5.  $F_{\max}$  for *D. pulex* grown in 200 ml ( $22.37 \pm 6.34$ ) was between the two values in 40 ml. In all cases, the shape of  $F(B)$  was retained; elevating the food supply did not eliminate the plateau.

We performed a final experiment to determine the repercussions of our daily handling of the animals for the measurement of individual growth and reproduction. A large cohort of "unhandled" *D. pulex* was maintained by our standard protocol except that individuals were transferred gently to

Table 5. Size-specific respiration functions for cladocerans derived from the literature, of the form  $R(B) = aB^b$  where  $R$  has units of  $\mu\text{g C ind}^{-1} \text{h}^{-1}$  and  $B$  is body size in  $\mu\text{g dry wt}$ .

	Source	$a$	$b$
<i>Daphnia ambigua</i>	Armitage and Lei 1979	0.008	0.51
	This study	0.016	0.90
<i>Daphnia magna</i>	Kersting and Leeuw-Leegwater 1976	0.008	0.82
	Schindler 1968	0.004	0.99
<i>Daphnia pulex</i>	Buikema 1972	0.011	0.78
	Richman 1958	0.007	0.81
	This study	0.005	1.09
<i>Simocephalus exspinosus</i>	Obreshkove 1930	0.006	0.84
<i>Simocephalus vetulus</i>	Ivanova and Klekowski 1972	0.007	0.82

new medium every other day with a wide-bore pipette rather than being examined daily under the microscope. Newborns produced by the unhandled mothers were counted visually and removed by pipette each day (as in the case of our normally handled animals). Growth of the unhandled animals was estimated every other day for 3 weeks by permanently removing 10–12 randomly selected individuals from the cohort and measuring them. A parallel cohort of 25 normally treated animals served as a control.

The estimated growth functions of the two groups of animals were quite similar (Fig. 8), although the asymptote for the growth curve of the unhandled animals ( $\bar{X} \pm 2 \text{ SE} = 2.77 \pm 0.06$ ) as determined by nonlinear least-squares analysis appears to be significantly higher than that of the regularly measured group ( $2.59 \pm 0.08$ ). For the first half of the experiment (when the animals might be expected to be maximally sensitive to handling effects), however, the mean lengths of the two groups were extremely similar; on days 3 and 5, the mean length of the handled animals actually exceeded that of the unhandled ones. Since the cumulative points of the growth function of the handled cohort will be especially sensitive to the chance elimination of deviant animals at earlier points in the sampling schedule, it is possible that the slight divergence between the two growth curves late in the experiment is largely due to sampling error. In any event, since the growth function of unhandled animals asymptotes at about the same age as that of the controls, and since growth accounts for only a minor portion of the adult energy budget (Fig. 6),

a handling effect on growth cannot account for the plateau in  $F(B)$  in *D. pulex*.

Routine handling does not appear to have a major effect on investment in reproduction either. There is a significant linear relation between the rate of production of live progeny and age in both cohorts, but although the slope is slightly higher for the unhandled animals (Fig. 8), it is not significantly so ( $F_{1,10} = 0.773$ ). The elevations of the two regressions are not significantly different ( $F_{1,11} = 2.682$ ).

We therefore conclude that the general shape of our observed  $F(B)$  functions is not an artifact of the laboratory setting. (We acknowledge that the choice of a sigmoidal model over a hyperbolic or rectilinear one is somewhat subjective, but note that the qualitative results of all three models are similar.) Several other estimated  $F(B)$  functions for species of *Daphnia* grown under a variety of conditions exhibit shapes similar to those that we report here (Lampert 1977c; Lynch 1980a,b). It appears that one or more parameters of the metabolic model or the structure of the model itself must be in error. The problem can hardly be with the molt weight estimates, since  $M(B)$  has a negligible effect on  $F(B)$ . Moreover, our size-specific functions for respiration rates are not greatly different from others that can be derived from the literature (Table 5); clearly they do not appear to be gross underestimates. Even if our estimates of  $R(B)$  were off by as much as 100%, the effect on the calculated  $F(B)$  functions would be small since the measured assimilation rates are roughly an order of magnitude larger than those for respiration. Finally, our assimilation rate estimates do not seem unreason-

able when compared with those previously reported (Lampert 1977b).

### Discussion

Although the discrepancy between the "physiological" and the "direct" determinations of  $F(B)$  cannot be explained by our data, several factors may be involved. First, the physiological model may overestimate the real production because the assimilation rate is too high or the respiration rate is too low or both. Since the respiration rate has only a minor effect on the outcome, an overestimation of the assimilation rate seems most likely. This might result from inhomogeneous labeling of the algae. If, for example, the carbohydrate pool of our algae had a higher specific activity than the rest of the cell and if carbohydrates are better assimilated, the assimilation rate would be overestimated. The assimilation efficiencies of more than 100% calculated for small animals may provide evidence that such an error is involved.

On the other hand, certain container effects such as the settling of algae cannot be excluded as an influence in our direct estimates. Even though our additional experiments indicate that the container effect is not large, such an effect would probably result in underestimation of the production rate. Since these two possible errors work in different directions, their joint operation might result in a large discrepancy between the physiological and the direct determinations of  $F(B)$ .

The metabolic model may also not accurately predict directly observed net carbon uptake because it fails to account for diel variation in the metabolic parameters. Diel cycles in ingestion rates with up to 10-fold differences between low and high phases are characteristic of *Daphnia* (Chisholm et al. 1975; Haney and Hall 1975; Starkweather 1975, 1978). Moreover, the amplitude of the oscillation increases dramatically with body size from an almost negligible value for newborns (Haney and Hall 1975). If we assume that the animals whose assimilation rates we measured tended to be in their high phase of activity, this provides a possible explanation for the tendency of our metabolic model to increas-

ingly overestimate net carbon intake with body size. The primary difficulty with this interpretation is that *Daphnia* most frequently exhibit feeding peaks in the dark (Starkweather 1983).

Although the deviations were not as large as in this study, Lampert (1977a,b) also encountered problems in fitting observed production data to physiological expectations. In his case, the predictions of the physiological model were too high for very small and very large *D. pulicaria* but quite accurate for intermediate size classes. The reason for the inaccuracy for large animals was the same as given here; while the metabolic data predicted a linear increase in  $F(B)$ , the direct measures indicated a plateau in  $F(B)$  following maturity.

An analysis similar to ours has been performed with another clone of *D. pulex* by Paloheimo et al. (1982). As in our study, they found very good agreement between observed juvenile growth rates and those predicted from metabolic data, but contrary to our results and to those of Lampert (1977a,b), their observed investments in reproduction averaged ~50% greater than predicted from physiological data. Like us, Paloheimo et al. (1982) failed to account for diel variation in the metabolic parameters, and they may have measured assimilation during a phase of low adult activity. In any event, their point estimates of assimilation are clearly too low, since they neglected  $^{14}\text{C}$  assimilated and subsequently respired before measurement.

None of these studies provides much support for the use of short term physiological rate data to project the productivity of populations or the growth and reproduction in individuals. Indeed, if diel metabolic cycles are a major reason for our inability to predict  $F(B)$ , then the construction of a general and quantitatively accurate production model on a physiological basis will require a tremendous amount of additional research since metabolic cycles are subject to environmental modification. This does not mean that short term determinations of metabolic rates should be abandoned. Physiological information is absolutely essential for the elucidation of the mechanistic basis of genetic and environmental variation in

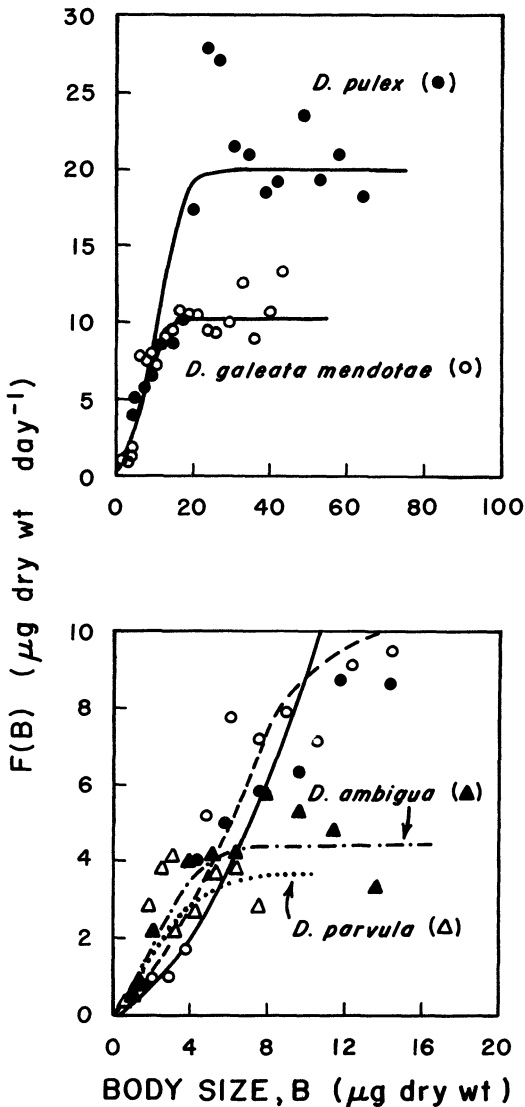


Fig. 9. Observed size-specific patterns of net incorporation of dry weight for four species of *Daphnia*. Lines fitted to Eq. 1 in text by nonlinear least-squares regression. Note change of scale and inclusion of data for large species in lower panel.

net energy intake. When such data are obtained under carefully controlled conditions, they can help clarify the bases of nutritional variation between food types (ingestibility, assimilability, toxicity) as well as the consequences of physical and chemical changes in the environmental setting (Arnold 1971; Porter and Orcutt 1980; Lampert 1977b, 1981). Despite the limi-

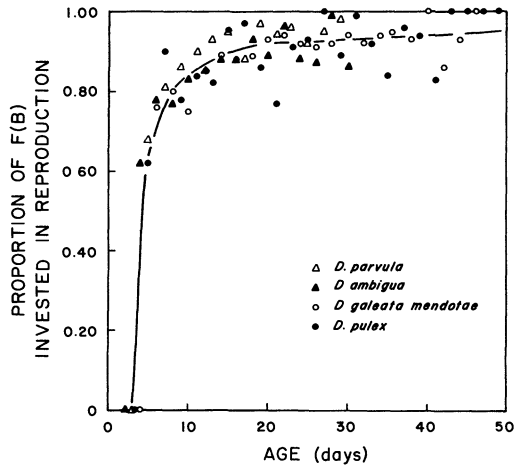


Fig. 10. Age-specific pattern of investment of energy in reproduction for *Daphnia*. Curve fit visually.

tations of our metabolic data, they clearly indicate significant physiological differences among *Daphnia* species. Extrapolation of such data across species should therefore be avoided.

Earlier experiments by Lampert (1977a) and our inability to detect heterogeneity in residual variances or significant differences between the slopes of 1- and 3-h size-specific assimilation functions provide strong support for the continued use of the difference technique. Many techniques, none without limitations, are now available for measuring respiration (Lampert 1984). We chose a bottle technique to minimize disturbance to the animals through mechanical stimulation, crowding, or depression of the food supply. It remains to be seen whether variation in respirometry technique results in significant differences in size-specific functions, but it is known that the metabolic rate is influenced by crowding (Zeiss 1963), food level (Porter et al. 1982), and degree of acclimation to temperature (Armitage and Lei 1979) and light (Buikema 1972).

Our results indicate that the standard practice of measuring ingestion rates over only a few minutes may yield biased estimates if the animals are disturbed before the experiment. To avoid such bias, we should measure ingestion rates over as long a period as possible (Rigler 1961), but radioisotope techniques necessitate short exper-

imental durations because of the short gut retention times of zooplankton. Direct observation of ingestion is obviously the best approach but is not practical. Investigators requiring very accurate feeding rate estimates might well consider the use of a steady state technique similar in principle to flow-through respirometry.

If one is primarily interested in the end-product of anabolism and catabolism,  $F(B)$ , the shortest and most accurate route to it is by direct measurement. An understanding of the phylogenetic pattern of diversification of  $F(B)$  as well as of its environmental dependence is of both practical and theoretical interest.  $F(B)$  is a function of the compatibility of an organism's foraging morphology and behavior, the availability and quality of food, and the suitability of the physical environment. It is, therefore, an important measure of fitness in an ecological sense (Lynch 1980a). When combined with information on the schedules of age-specific allocation to reproduction and size-specific survivorship,  $F(B)$  can be used to project the future growth of a population. It is also an essential ingredient of a mechanistic life history theory that can be used in the analysis of the evolution of sizes at birth and maturity (Lynch 1980b).

Our direct estimates of  $F(B)$  have revealed two strikingly conservative elements of the *Daphnia* life history that, if general, will greatly facilitate efforts to analyze the phylogenetic diversification of life histories in this genus. First, despite major differences in allometric physiological functions as well as length-weight relations, each species exhibits an  $F(B)$  function that increases monotonically with size until maturity and then remains roughly constant (Fig. 9). Since the net energy intake of pre-reproductive individuals of all four species is quite similar, significant interspecific differences in  $F(B)$  only begin to emerge after maturation, and then there is an orderly pattern. Because  $F(B)$  approaches an asymptote following maturation, maximum  $F(B)$  and the size at which it is attained increase with size at maturity.

Second, our results suggest that, for a specific food concentration, the age-specific pattern of resource allocation to growth and

reproduction is nearly invariant between *Daphnia* species (Fig. 10). Despite major differences in the sizes at birth and maturity, species covary in a pattern that results in very little interspecific variation in the age at first reproductive investment. Once this age has been reached, the subsequent energy allocation pattern appears to be fixed.

These results suggest that life history evolution among *Daphnia* may be strongly regulated by one key character—size at maturity. Why should the future adult energy budget be so tightly constrained by the size at first reproductive investment? One possibility is that after maturation, allometric growth of structures involved in energy acquisition is approximately balanced by growth of tissues requiring energy input for maintenance. The exoskeleton is an example of an anatomical feature not involved in energy intake that increases with body mass at a disproportionately high rate in *Daphnia* (Fig. 4). Alternatively, it is possible that hormonal or other subcellular changes accompanying the maturation process place an absolute limit on the rate at which assimilated material can be converted into new biomass.

Although large *Daphnia* species do have greater energetic capabilities than smaller ones, as predicted by considerable theory (Brooks and Dodson 1965; Hall et al. 1976), there seems to be little energetic advantage for an individual to grow beyond its size at maturity. Nevertheless, all species of *Daphnia* show indeterminate growth. The possibility that continuous growth is a necessary consequence of the molt process seems unlikely since *Daphnia* can successfully molt to reduced carapace sizes. However, the growth schedule of a *Daphnia* will be a primary determinant of its survivorship schedule since its mortality sources are highly size selective (Lynch 1983). An inevitable consequence of indeterminate growth is a reduction throughout life in vulnerability to invertebrate predators accompanied by an increase in vulnerability to vertebrates (Lynch 1980a). This suggests the possibility that even though *Daphnia* species now exist in a diversity of environments with and without both types of predators, the members of the extant lineage either share a com-

mon ancestor for which invertebrate predation was a very powerful selective agent or are all currently experiencing the greatest response to selection in environments dominated by invertebrate predators.

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