J. mar. biol. Ass. U.K. (1978) 58, 749–772
Printed in Great Britain

STEADY-STATE ENERGETICS OF A PLANKTONIC HERBIVORE

M. R. DROOP AND J. M. SCOTT
Dunstaffnage Marine Research Laboratory, Oban, Scotland

(Figs. 1–13)

The marine rotifer *Brachionus plicatilis* Müller was grown bacteria-free in a chemostat with the alga *Brachiomonas submarina*, labelled with ^{57}Co vitamin B_{12}, as the limiting nutrient.

State variables were recorded at a number of steady states between zero dilution rate and washout. These were algal input biomass, algal (and faecal) output biomass, rotifer (and gut) output biomass, in terms of carbon, nitrogen and vitamin B_{12}; and input and output dissolved vitamin B_{12}.

From these were calculated input, ingestion, assimilation, excretion and growth rates and the associated transfer efficiencies. Ingestion and assimilation efficiencies were high and not obviously growth rate dependent, whereas the growth efficiency was interpreted as ranging from zero at zero growth rate to a maximum at half maximal growth and back to zero at washout, being the result of the carbon and nitrogen excretion rates, which, finite at zero growth rate, increased with increasing rapidity with increasing growth rate, tending to infinity as the maximum growth rate was approached.

Nitrogen and carbon excretion rates were correlated.

'Slow-adapted and 'fast-adapted' modes of growth were recognized with characteristically different growth and energy parameters.

The reasons for and implications of these findings are discussed.

INTRODUCTION

Our objective is to examine the relation between growth rate and respiration in a steady-state laboratory population of a small planktonic herbivore, a metazoan microbe, so to speak. Microbiologists have long recognized the effect of the requirement for maintenance energy in bacterial economy, but how this requirement is affected by the growth process is not clear (Stouthamer, 1977). The original hypothesis that it was independent of growth rate (Pirt, 1965) is probably not generally tenable.

Brachionus plicatilis Müller, a marine rotifer, can be grown in bacteria-free continuous culture on a single alga as food (Droop, 1976). It offers many of the advantages of a microbe, such as the possibility of maintaining a healthy population in a constant and predetermined rate of growth by control of the rate of food supply, and thus automatically maintaining constant conditions with respect to biomass and ambient food concentration, etc. Both Winberg & Duncan (1971) and Conover & Francis (1973) have discussed the difficulties encountered in the analysis of transient systems such as usually attempted by zoologists. At best, animals taken into the laboratory are in what amounts to a batch culture which is changing all the time and, at worst, are dying more or less slowly.

Suppression of the time element, which is a characteristic of a steady-state system, has two important implications. First, that the influence of the previous history of the

material is largely suppressed and second, a knowledge of rate variables such as feeding, assimilation, growth rate, etc. is obtained directly from easily measured state variables such as nutrient concentration, biomass, etc. Another characteristic of the system as we shall apply it is that, since the population is statistically homogenous and incoherent, i.e. not synchronized, the knowledge obtained is of the population and not of individuals.

Brachionus has been used extensively as food for larvae of plaice (*Pleuronectes platessa*) and of herring (*Clupea harengus*). Some 300 μm in length when full grown, it feeds on the move, taking in particles below 30 μm. Population increase in the wild is parthenogenetic. The thick-walled, sexually produced resting eggs aid overwintering and dispersal, and the sexual process presumably also has a genetic function. The production of sexual eggs is sufficiently rare to be quite without influence in continuous culture.

CONCEPTUAL OUTLINE

The operation of a nutrient-limited chemostat is well documented in the microbiological literature (e.g. Herbert, Elsworth & Telling, 1956; Tempest, 1970). Essentially we have a constant population multiplying at a constant specific rate in a constant volume reactor being fed with fresh medium at the same specific rate. It can be shown that, provided certain conditions are fulfilled, a controlling factor, usually the equilibrium concentration of one of the nutrients, ensures that the two rates are the same. In the system we propose to discuss, the subject is the rotifer and the 'limiting nutrient' the food alga, and we propose to measure the parameters of a number of steady states between zero dilution rate and washout.

The rotifer chemostat reactor can be visualized in terms of a three-compartment model, the compartments being identifiable components of the system upon which

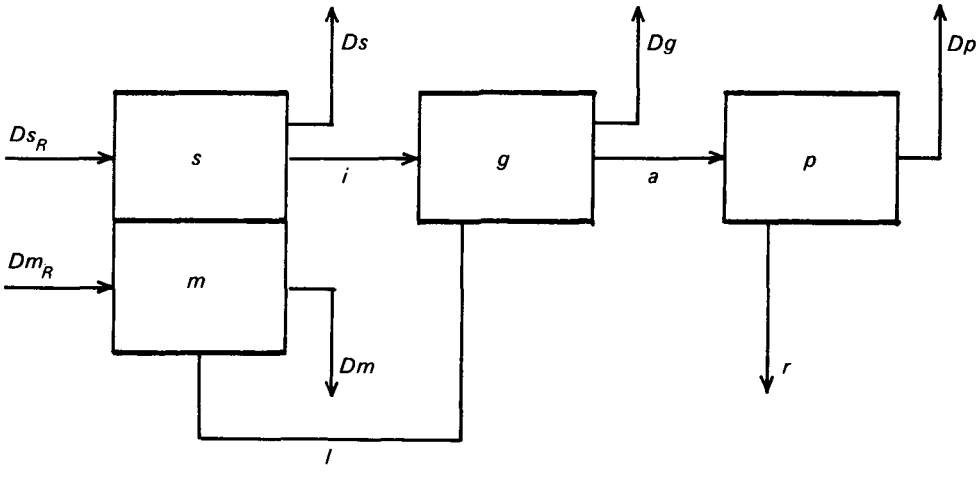

Fig. 1. 3-compartment model of steady-state chemostat in terms of the carbon budget. *State variables*: *s*, algae in reactor; *m*, dissolved carbon in reactor; *g*, gut contents; *p*, rotifer. *Rate variables*: Ds_R, algal input; Dm_R, dissolved carbon input; *Ds* algal output; *Dm*, dissolved carbon output; *i*, net ingestion; *l*, digestion loss; *Dg*, gut output; *a*, assimilation; *Dp*, rotifer output; *r*, respiration.

Table 1. *Primary symbols, mainly state variables*

Symbol	Explanation	Units	Dimensions
D	Dilution rate ($\equiv$ rotifer specific growth rate, μ)	Volumes per day	t^{-1}
μ_a	Net algal growth rate on a carbon or nitrogen basis†	$\log_e$ per day	t^{-1}
s_R	Input algal carbon or nitrogen	μg per ml	ml^{-3}
s	Output algal carbon or nitrogen		
m_R	Input dissolved carbon or nitrogen‡		
m	Output dissolved carbon or nitrogen‡		
g	Gut carbon or nitrogen§		
p	Rotifer carbon or nitrogen		
$s_R^\star$	Input algal vitamin B_{12}	pg per ml	ml^{-3}
$s^\star$	Output algal vitamin B_{12}		
$m_R^\star$	Input dissolved vitamin B_{12}		
$m^\star$	Output dissolved vitamin B_{12}		
$g^\star$	Gut vitamin B_{12}§		
$p^\star$	Rotifer vitamin B_{12}		

† Measured in the absence of animals (see p. 756).
‡ Not measured (see p. 753).
§ See p. 755.

Table 2. *Transfer rates*

N.B. Since Dm and Dm_R could not be measured, a and l were computed by equating ζ and $\zeta^\star$ – see table 4.

	Carbon and nitrogen	Vitamin B_{12}
Cell input	Ds_R	$Ds_R^\star$
Medium input	Dm_R	$Dm_R^\star$
Cell output	Ds	$Ds^\star$
Medium output	Dm	$Dm^\star$
Gut output	Dg	$Dg^\star$
Rotifer output	Dp	$Dp^\star$
Net ingestion	$i = D(s_R - s) + \mu_a s$	$i^\star = D(s_R^\star - s^\star)$
Digestion loss	$l = D(m - m_R)$ $[\equiv i - a - Dg]$	$l^\star = D(m^\star - m_R^\star)$
Assimilation	$a = i - l - Dg$ $[\equiv \zeta^\star i]$	$a^\star = i^\star - l^\star - Dg^\star$
Excretion	$r = a - Dp$	

Table 3. *Conservation equations in terms of transfer rates*

Carbon and nitrogen	Vitamin B_{12}
Algal input (Ds_R) = net ingestion (i) + algal output (Ds)	$Ds_R^\star = i^\star + Ds^\star$
Medium input (Dm_R) + digestion loss (l) = medium output (Dm)	$Dm_R^\star + l^\star = Dm^\star$
Net ingestion (i) = assimilation (a) + digestion loss (l) + gut output (Dg)	$i^\star = a^\star + l^\star + Dg^\star$
Assimilation (a) = rotifer output (Dp) + excretion (r)	$a^\star = Dp^\star$
Algal input (Ds_R) + medium input (Dm_R) = algal output (Ds) + medium output (Dm) + gut output (Dg) + rotifer output (Dp) + excretion (r)	$Ds_R^\star + Dm_R^\star = Ds^\star + Dm^\star + Dg^\star + Dp^\star$

measurements can be made directly (the state variables) and the interconnexions between compartments, the rate variables. The three compartments are, (i) medium (subdivided into particulate and dissolved components), (ii) rotifer gut and (iii) rotifer. The pathway of material transfer to and from the reactor and between compartments within it are shown in Fig. 1. The routes recognized are ingestion (the net transfer of particulate material from the medium to the gut), digestion loss (the net loss to the medium of

dissolved components from the food) and assimilation (the transfer of material across the gut wall). Other routes are inputs to and outputs from the reactor, and excretion (including, in the case of carbon, respiration).

The maintenance of a steady state implies the constancy of all variables, so that Kirchhoff's law applies, namely that the algebraic sum of all the rates of transfer of material to and from each compartment is zero. The transfer rates are shown against the interconnecting arrows in the figure and are listed and expressed in terms of dilution rate and the state variables in Table 2, and the complete set of conservation equations is set out in Table 3. Table 1 lists the state variables with their units of measurement and dimensions.

Table 4. *Transfer efficiencies in terms of transfer rates*

	Carbon and nitrogen	Vitamin B_{12}
Ingestion/input	$\epsilon = \frac{i}{Ds_R}$	$\epsilon^\star = \frac{i^\star}{Ds_R^\star}$
Assimilation/ingestion	$\zeta = \frac{a}{i}$	$\zeta^\star = \frac{a^\star}{i^\star}$
Growth/assimilation	$\eta = \frac{Dp}{a} \left(= \frac{D}{r/p+D}\right)^\ddagger$	$\eta^\star = \frac{Dp^\star}{a^\star} (=1)$
Growth/input	$\epsilon\zeta\eta = \frac{p}{s_R}$	$\epsilon^\star\zeta^\star\eta^\star = \frac{p^\star}{s_R^\star}$

‡ Obtained on substitution of $r+Dp$ for a (see Table 2).

Net ingestion is ingestion less defaecation. Like the rotifer itself one can only distinguish between dissolved and particulate components of the culture medium; consequently, in the measurements, defaecated particulates are lumped with as yet undevoured cells. Similarly, digested material excreted from the gut into solution (i.e. digestion loss) is lumped with the unused solutes and, furthermore, the rate of loss must be net because its measurement must also embrace any uptake from solution.

Growth, respiration and nitrogen excretion on the part of the food alga in the reactor have to be accounted for. μ_a, the net algal specific growth rate on a carbon or nitrogen basis, enters into the expression for carbon and nitrogen ingestion rate in Table 2. In the absence of animals in the chemostat μ_a is given by

$$\mu_a = D\left(1-\frac{s_R}{s}\right), \tag{1}$$

where D is the dilution rate, and s_R and s the algal input and output concentrations.

A transfer efficiency, the efficiency with which material is transferred from one compartment of the model to another, is in effect the ratio of the rate of output to the later compartment to that of the input to the earlier. The factors that affect an efficiency are the outputs not leading to the later compartment, i.e. transfer losses. In a linear and essentially non-cyclic network, such as we have, the overall efficiency is the product of those of the parts, so that we can recognize, for example, the growth/input efficiency as being the product of the ingestion/input, assimilation/ingestion and growth/assimilation efficiencies.

The formulation of these efficiencies in terms of the transfer rates is set out in Table 4. It will be noted that the growth/assimilation efficiency for vitamin B_{12} is taken as unity, since vitamin excretion losses would not be distinguished from digestion losses. For the same reason the loss contributing to the carbon growth/assimilation efficiency is assumed to be entirely respiratory. Losses contributing to the measured ingestion/input efficiencies are feeding inefficiency *per se* and defaecation; those contributing to the measured assimilation/ingestion efficiency are likewise assimilation inefficiency *per se* and the digestion losses.

The overall, i.e. the growth/input, efficiency is of some interest since it is a measure of the use the population is making of its resource; and, in so far as the population in question is representative of a particular link in the food chain, it is the efficiency associated with that link, namely the 'ecological efficiency' (Slobodkin, 1962).

Carbon and nitrogen loss is the sum of excretory and digestion losses, neither of which can be obtained from chemostat state measurements in the absence of reliable and sensitive methods for dissolved carbon and nitrogen and for the gas phase. However, an idea of the probable digestion loss and hence the assimilation efficiency for carbon and nitrogen can be obtained indirectly, with the minimum of assumptions, from a knowledge of an easily measured, conservative component of the food. The excretory loss is then found by difference. ^{57}Co vitamin B_{12} is very suitable for this purpose because it is very easily incorporated into the food alga and is simple to measure; and, moreover, both the alga and the rotifer have a requirement for the vitamin. The simplest assumption and the one that gave the most satisfactory result was that the assimilation efficiency did not vary from nutrient to nutrient. Accordingly, the vitamin B_{12} efficiency was taken as a measure of the carbon and nitrogen efficiencies.

MATERIALS AND METHODS

The 250 ml chemostat developed for this experiment has been fully described (Droop, 1976) and is illustrated in block diagram in Fig. 2. It consists of an algal chemostat operating independently of a rotifer chemostat, the former to provide a food source of constant composition for the latter. Algal cells are automatically metered from the algal reactor as required and added to the medium input line of the rotifer chemostat, the electronic monitoring and control ensuring that the algal count in the input medium remained at a constant predetermined value. The algal reactor was of course illuminated, but all parts of the system downstream of the monitoring point were in darkness and the darkened rotifer reactor was immersed in a water bath at 21 °C.

The food alga was *Brachiomonas submarina* var. *pulsifera* (SMBA strain 44, CCAP strain 7/2a), a chlamydomonad of maximum size 25 μm. A number of isolates of the rotifer were used during the course of the chemostat work as it proved impossible to complete a run without altering the clone. All the isolates originated from Port Erin, I.O.M., though that in use up to the end of 1974 was actually obtained from M.A.F.F., Lowestoft, and those from 1975 onwards from Spain. Isolation and purification of the rotifer was a simple matter of removing single asexual eggs from the parent, washing and subjecting them to an antibiotic mix, as reported for *Philodina*, in a pure culture of the food alga (Droop, 1967).

The culture medium used for both *Brachionus* and *Brachiomonas* in the chemostats was S88 with added thiamine and vitamin B_{12} (Droop, 1968). This is a half-strength artificial sea water, essentially a mineral medium but containing glycylglycine and glycine as pH buffers. The vitamin B_{12} concentration of this medium is 100 pg/ml. The ^{57}Co label (^{57}Co labelled cyanocobalamin C2P, 'high activity for injection' from RCC Amersham, Bucks) was added to the rotifer medium and not to the algal medium. This enabled us to put some 80% of the label into the algae actually destined for the rotifer reactor.

A chemostat run would normally take several months. After sterilization and installation of the apparatus the algal dosimeter was zero set on blank medium (Droop, 1976). The algal chemostat would then be inoculated, set running and brought to equilibrium at a dilution rate of *ca.* 0·5/day. The dosimeter could then be set to deliver a convenient algal concentration (*ca.* 15 μg C/ml) and the rotifer chemostat dilution rate set to a low value. The rotifer could now be introduced into the reactor and the apparatus left to come to equilibrium, and readings could start. Since for each reading it was necessary to withdraw 80 ml from both the input vessel and reactor (both 250 ml) sufficient time had to be left for the vessels to refill and the chemostat to equilibrate before another reading was taken; three residence times ($3/D$ days) were usually sufficient. A reading with abnormally high values of s or s^* would indicate absence of equilibrium and the reading would be discarded.

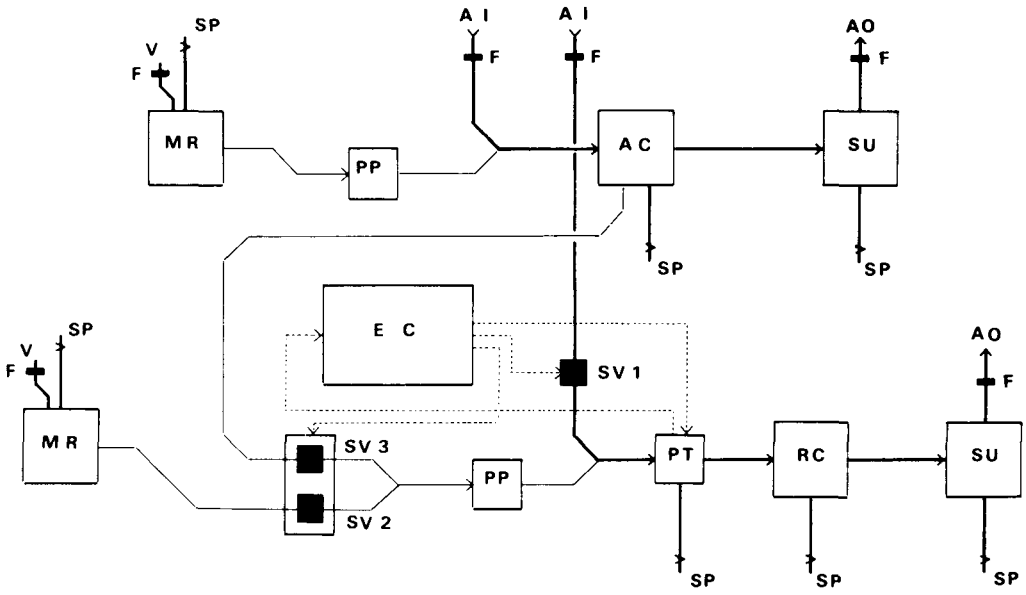

Fig. 2. Rotifer chemostat, block diagram. Vessels MR and SU are 1 l flasks; AC and RC 300 ml spherical long necked flasks. MR, Medium reservoirs; AC, algal reactor; SU, sumps; PP, peristaltic pumps (Watson Marlow); PT, phototransistor assembly and input sampling vessel; SP, sampling ports; RC, rotifer reactor; F, sterilizing air filters; V, vents; AI, air inputs connected to separate pistons of a 'High-Flo' pump; AO, air outlet; SV, Londex solenoid clamp valves; EC, electronic control unit. Dotted lines are electrical connexions; light lines, 0·5 mm bore silicone tubing; heavy lines, 3·2 mm bore silicone tubing. Reprinted with permission from *Proceedings of the 10th European Symposium on Marine Biology, Ostend*, 1975.

Measurement of the input concentrations (s_R, m_R^*, s_R^*) were made on 80 ml samples from a vessel situated between the point of algal addition and the rotifer reactor. Their measurements had to be corrected for water vapour equilibration since the remainder of measurements were made on the reactor. The correction was made on the basis of the total radioactivity per unit volume in each vessel. The tables show the corrected figures.

The scheme of sample manipulation is shown in Fig. 3. First the animals were removed from the 80 ml sample by filtration on a 64 μm stainless steel gauze, then the remainder of the particulate matter (algae and faeces) removed by filtration on GFB glass paper previously rendered free of carbon by pre-heating to 500 °C.

Coulter population volume analyses were performed on sub-samples before filtration. The information obtained from them was however ancillary to the present purpose and will not be discussed in detail.

The micro-filters used (Droop, 1974) enabled the fractions to be placed in the well of an Na I

scintillator and counted without removal from the filter. The vitamin B_{12} in the supernatants was adsorbed on a 2 mm layer of charcoal in the same type of filter and similarly counted. The vitamin concentration was given by

$$\frac{\text{cpm of fraction}}{\text{sum of cpm of all fractions}} \times \text{vit. } B_{12} \text{ concentration of culture medium}$$

and was expressed as pg/ml. Individual counts were never less than 1000 after subtraction of background.

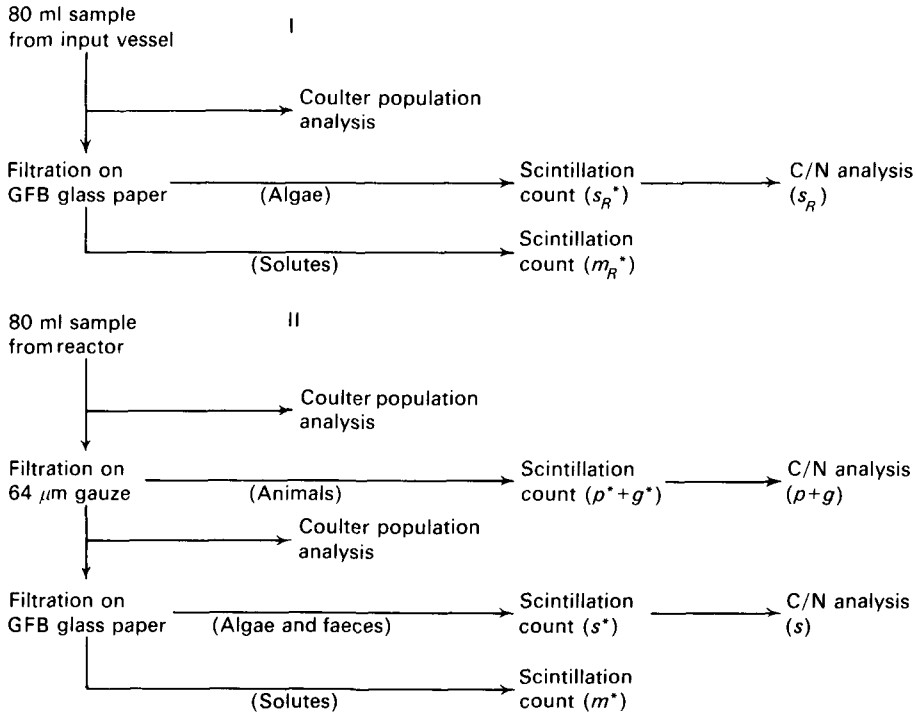

Fig. 3. Schedule of sample manipulations. For explanation of symbols see Table 1.

For the carbon and nitrogen analyses, after the scintillation counts the glass papers were removed from the holders, dried in a desiccator and the carbon and nitrogen content measured in a Perkin Elmer CN Analyser. The carbon and nitrogen figures are expressed as μg/ml.

It was impractical, indeed impossible, to separate the gut contents of a steady-state population whilst retaining the steady state. Gut and animal were therefore routinely measured together and small blanket corrections applied to reduce gut plus animal to animal. To arrive at the value of these corrections it was necessary to purge the animals and measure both the resulting loss of particulate carbon, nitrogen and radioactivity from the animals and the gain to the medium. Purging was effected by replacing culture medium (labelled algal suspension) by a fine suspension of magnesium trisilicate in an artificial sea water free of nitrogen and organic carbon. The rotifers readily ingested the silicate and they could be seen to be free of algae within the hour. Measurements were made on batch cultures and carried out on 50 ml sub-samples of the rotifer/silicate suspension taken both before and after purging and on sub-samples of suspension alone.

The 95% fiducial limits shown in Figs. 4–9 and 12 were obtained conventionally from the variance of the appropriate linear regression. Those in Fig. 11 were derived by a method that involves the combination of probabilities and which is conservative: thus where each estimate is the product of three variables its 94% limits are taken as the product of the upper and product of the lower 98% limit of each of the three component variables, $0{\cdot}98^3$ being approximately 0·94.

The same considerations apply to the calculation of the fiducial limits of the regression coefficients and intercepts quoted in the text.

Except for the correction for gut contents and that for net algal growth in the reactor, both of which were applied as blanket corrections, all computations were performed on unaveraged data from the individual steady states. The limits shown in the figures were drawn by computer.

RESULTS

Net algal growth

Table 5 shows that there was no obvious trend in the net algal growth in the reactor in the absence of animals with alteration of the chemostat dilution rate. The mean figure was −0·034 per day for carbon and −0·033 per day for nitrogen, thus indicating possibly very slight excess of excretion over growth. These figures were used to correct the calculated rotifer net ingestion rates (see Table 2), although in point of fact they were so small that they could have been ignored.

Table 5. *Chemostat steady-states in absence of animals showing net algal growth* μ_a ($\mu_a = D[1 - s_R/s]$)

Date	Dilution rate D (Vols/day)	Algal input s_R (μg/ml)	Algal output s (μg/ml)	Net algal sp. growth rate μ_a ($\log_e$/day)	
		Carbon			
7 Feb. 77	0·296	11·9	9·39	−0·0791	
10 Feb. 77	0·297	10·9	12·3	+0·0338	
17 Mar. 77	0·540	28·0	22·7	−0·126	
14 Mar. 77	0·551	26·9	24·5	−0·0540	Mean −0·0344
28 Mar. 77	0·846	40·4	41·2	+0·0164	
25 Mar. 77	0·886	43·2	41·8	−0·0297	
30 Mar. 77	0·839	40·6	40·5	−0·0021	
		Nitrogen			
7 Feb. 77	0·296	2·72	2·31	−0·0525	
10 Feb. 77	0·297	2·65	3·60	+0·0784	
17 Mar. 77	0·540	5·93	4·78	−0·130	
14 Mar. 77	0·551	5·11	4·53	−0·0705	Mean −0·0334
28 Mar. 77	0·846	8·39	8·07	−0·0335	
25 Mar. 77	0·886	8·93	8·89	−0·00399	
30 Mar. 77	0·839	9·64	9·40	−0·0214	

Rotifer gut contents

The results of the purging experiment, which was performed with six replications in sequence, is shown in Table 6. In order to interpret the data it was necessary to have a figure for nitrogen and carbon excretion during the period of the experiment. The excretion levels assumed ($(r/p)_h$ in Table 7) were based on the means over the whole chemostat experiment. They were, for carbon, 0·051 μg per μg animal carbon per hour and, for nitrogen, 0·062 μg per μg animal nitrogen per hour. It was not possible to control the effective purging time to the minute, but it lay within the limits 45–60 min.

One can think of the unpurged animals as consisting of animal, gut solids and gut solutes, and budget accordingly. In Table 7 the data are the means of the six estimates

of each parameter (calculated by the method indicated). The correction factors required for the chemostat computations are $p/(p+g)$ and $g/(p+g)$.

The 'gut solutes' represent relative digestion losses over one hour and might have been used to estimate the chemostat digestion losses were it not that the latter are too important a part of the chemostat budget to admit reliance on the result of a single, possibly atypical, batch experiment.

Table 6. *Results of purging of batch cultured rotifers*

Rotifer		Medium solids		Medium solutes after purging
Unpurged *A*	Purged *B*	Control blank *C*	After purging *D*	*E*
		Carbon (μg per ml)		
4·203	3·994	0·396	0·234	
4·012	3·391	0·396	0·518	
4·482	3·617	0·480	0·735	
4·065	3·326	0·480	0·990	
3·991	3·112	0·379	0·744	
3·812	3·233	0·379	0·918	
		Nitrogen (μg per ml)		
1·167	0·756	−0·116‡	−0·134‡	
1·061	0·686	−0·116	−0·062	
1·281	0·697	−0·0933	−0·00269	
1·168	0·639	−0·0933	0·0480	
1·080	0·575	−0·0934	0·0131	
1·029	0·859	−0·0934	0·0213	
		Vitamin (cpm per ml)		
443·69	552·10†		1·468	7·000
443·34	471·18		2·755	6·226
508·93	525·32		5·038	3·490
428·02	411·50	Nil	6·593	2·446
436·52	433·03		5·885	6·981
408·47	468·00		0·490	2·706

‡ Negative on account of C/N Analyser blank.
† An untraced systematic error rendered this set useless.

Table 7. *Budget of batch cultured rotifer: relative amounts of carbon, nitrogen and vitamin of unpurged animal*

Fraction	Computation (C and N)	Carbon	Nitrogen	Computation (V)	Vitamin
Animal, $\frac{p}{p+g}$	$\frac{B+(r/p)_h B}{A}$	0·884 (±0·063)	0·662 (±0·116)	$1-\frac{D+E}{A}$	0·981 (±0·007)
Total gut, $\frac{g}{p+g}$	$1-\frac{B+(r/p)_h B}{A}$	0·116 (±0·061)	0·339 (±0·116)	$\frac{D+E}{A}$	0·0192 (±0·0070)
Gut solids	$\frac{D-C}{A}$	0·0677 (±0·0633)	0·0731 (±0·0506)	$\frac{D}{A}$	0·00828 (±0·00601)
Gut solutes	$1-\frac{B+(r/p)_h B}{A}-\frac{D-C}{A}$	0·133 (±0·049)	0·341 (±0·118)	$\frac{E}{A}$	0·0109 (±0·0070)

Data from Table 6. 95 % fiducial limits in parentheses. $(r/p)_h$, relative hourly rates of excretion, estimated from the mean rates over the whole chemostat experiment.

Table 8. *Chemostat steady states: state variables*

Date	Dilution rate (≡ rotifer sp. growth rate) D (Vols/day)	Carbon: Algal input s_R (μg/ml)	Carbon: Algal output s (μg/ml)	Carbon: Rotifer + gut output $p+g$ (μg/ml)	Nitrogen: Algal input s_R (μg/ml)	Nitrogen: Algal output s (μg/ml)	Nitrogen: Rotifer + gut output $p+g$ (μg/ml)	Vitamin B_{12}: Algal input s_R^* (pg/ml)	Vitamin B_{12}: Medium input m_R^* (pg/ml)	Vitamin B_{12}: Algal output s^* (pg/ml)	Vitamin B_{12}: Medium output m^* (pg/ml)	Vitamin B_{12}: Rotifer + gut output p^*+g^* (pg/ml)
11 Sept. 74	0·359	6·45	2·18	0·94	2·34	0·586	0·295	82·3	17·7	26·1	26·1	47·8
20 Nov. 74	0·467	6·02	1·33	2·30	0·505	0·210	0·083	78·2	21·8	4·66	48·1	47·2
27 Nov. 74	0·225	4·83	1·17	1·74	(0·686	0·551	0·451)	82·1	17·9	3·68	54·5	41·9
4 Dec. 74	0·286	10·0	1·92	4·00	1·52	0·675	0·472	86·8	13·2	3·97	49·6	46·4
16 Dec. 74	0·585	8·41	1·42	2·80	2·16	0·547	0·656	87·7	12·3	4·32	37·1	58·6
23 Dec. 74	0·583	12·4	1·57	2·64	10·3	3·83	0·673	87·8	12·2	5·15	35·5	59·4
30 Dec. 74	0·612	13·3	1·63	2·44	1·94	1·23	0·461	93·8	6·24	5·71	33·9	60·3
10 Jan. 75	0·466	7·26	1·19	3·30	(1·54	0·463	0·485)	89·2	10·8	5·36	31·7	62·9
20 Jan. 75	0·494	12·2	1·87	2·36	3·24	2·86	0·367	93·4	6·56	5·89	42·8	51·1
3 Feb. 75	0·441	9·79	1·19	2·98	2·22	0·619	0·574	78·0	22·0	4·68	30·4	64·9
30 May 75	0·324	28·4	2·02	9·50	6·10	0·469	1·51	81·3	18·7	3·91	30·7	65·4
4 June 75	0·313	34·7	2·62	7·70	3·28	0·827	1·60	84·3	15·7	4·95	28·0	66·8
9 June 75	0·321	37·8	2·16	10·7	8·58	0·864	2·24	89·5	10·5	3·27	26·6	70·1
2 Sept. 75	0·413	10·7	1·76	2·35	2·42	0·734	0·631	84·7	15·3	9·11	40·1	50·5
10 Sept. 75	0·661	31·4	6·92	4·78	7·83	1·95	1·05	85·2	14·8	22·7	24·7	52·7
18 Sept. 75	0·665	24·9	5·18	6·68	8·25	2·12	1·82	87·6	12·4	6·16	32·1	61·6
23 Sept. 75	0·696	16·9	3·79	5·91	4·04	1·64	1·40	89·0	11·0	6·68	26·4	66·8
29 Sept. 75	0·750	26·4	9·42	5·77	11·7	2·82	1·52	92·3	7·65	23·6	23·2	53·1
3 Oct. 75	0·753	27·5	9·17	5·52	4·95	1·90	1·06	91·2	8·76	32·9	19·4	47·4
10 Oct. 75	0·751	32·1	8·23	3·80	8·26	2·24	0·922	91·2	8·76	33·8	26·1	40·0
10 Nov. 75	0·411	24·7	3·78	4·79	6·71	0·903	1·14	83·7	16·3	21·0	32·2	46·4
12 Apr. 76	0·249	17·5	3·37	2·58	7·10	1·54	0·535	93·3	6·72	20·7	40·2	39·1
20 Apr. 76	0·255	14·7	2·44	2·83	5·50	1·18	0·815	87·6	12·4	16·1	31·5	52·3
27 Apr. 76	0·242	15·5	3·24	1·72	6·26	1·46	0·390	85·8	14·2	21·0	42·3	36·7
11 May 76	0·538	16·1	3·12	3·00	5·30	1·49	0·767	85·7	14·3	19·7	38·1	42·1
17 May 76	0·539	14·1	2·17	3·62	5·24	1·15	0·869	86·9	13·1	11·3	35·1	53·3
24 May 76	0·710	16·4	2·43	3·28	5·16	1·03	0·854	86·7	13·3	12·9	36·0	51·0
28 May 76	0·723	22·1	3·68	3·54	8·51	1·69	0·939	81·9	18·1	16·4	27·9	55·6
7 June 76	0·839	17·9	2·83	3·23	6·08	1·10	0·854	87·2	12·8	10·1	29·2	60·7
11 June 76	0·792	25·4	2·01	3·13	7·65	0·317	0·685	84·0	16·0	13·4	24·2	62·3
22 June 76	0·809	16·5	1·35	2·40	4·92	0·334	0·584	86·1	13·9	12·2	32·2	55·6

The nitrogen figures in parentheses on computation produced negative excretion rates.

Chemostat steady states

Some 30 steady states were recorded over a period of two years and used in the analyses. As mentioned earlier, it was not possible to complete a run with a single clone since continued indefinite parthenogenesis eventually resulted in physiological changes in the population. Carbon and nitrogen measurements commenced in September 1974 but the clone in use was by then over 4 years old. Between 9th September and 20th November we observed what appeared to be a sudden loss of vigour. A fresh isolation was made in early January 1975 and the experiment continued. A further isolation was made in the summer and again early in 1976. Analysis revealed that the steady states recorded in November and December 1974 with the first clone were statistically distinct from the remainder. We refer to those as 'slow-adapted' and to the remainder as 'fast-adapted' and we keep the two populations apart in our subsequent handling of the data. The state variables for all the steady states are set out in Table 8. All calculations used to derive the various rates and efficiencies, etc. are based solely on these data and performed strictly according to the formulations set out in Tables 2, 3 and 4.

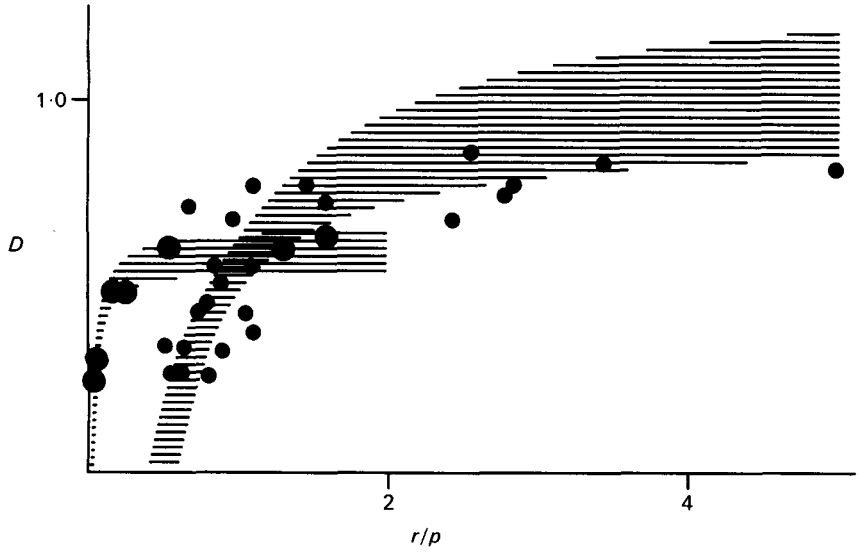

Fig. 4. Plot of dilution rate (D) vs specific rate of respiration r/p. Small circles, 'fast-adapted'; large circles, 'slow-adapted'. Shaded areas, 95 % fiducial limits estimated from the statistics of regression of p/r (carbon) on D.

Excretion of carbon

An inspection of Fig. 4, in which dilution rate is shown plotted against relative rates of respiration, suggests a rectangular hyperbola with asymtotes $D = D'_m$ and $r/p = 0$, passing through the point $D = 0$, $r/p = (r/p)_0$. The equation is

$$D = D'_m \left(1 - \frac{(r/p)_0}{r/p}\right). \tag{2}$$

$(r/p)_0$ we interpret as the zero maintenance rate (the rate when growth rate is zero) and D'_m as the dilution rate corresponding to the growth rate requiring the respiratory expenditure of all the energy assimilated for its achievement.

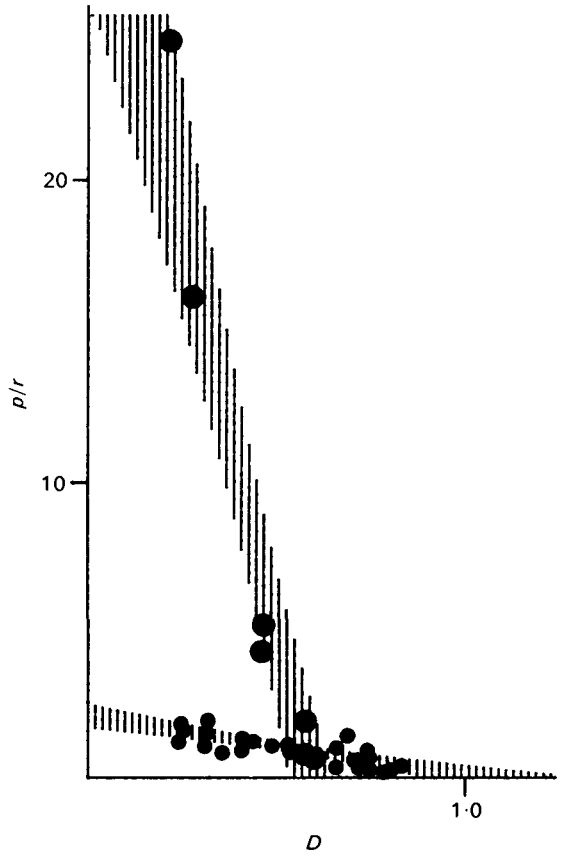

Fig. 5. Regression of p/r on D (carbon). Large circles 'slow-adapted'; small circles, 'fast-adapted'. Shaded areas, 95 % fiducial limits.

A plot of p/r vs D should be linear with negative slope if the equation fits the data

$$\frac{p}{r} = \left(\frac{p}{r}\right)_0 - D\frac{(p/r)_0}{D'_m}. \tag{3}$$

The intercepts are at $D = D'_m$ and $p/r = (p/r)_0$. p/r is the dependent variable in the experiment. It should be noted however that D enters into the calculation of r (see Table 2), so that from a statistical point of view abscissa and ordinate are not entirely independent. In fact we are regressing a modification of the reciprocal of D on D. This plot is shown in Fig. 5. The points do appear to follow a reasonably linear course so that we feel justified in using the equation to describe the relationship. The difference between the fast- and slow-adapted populations is very striking and needs no statistical

analysis to point it out. The regression analyses are however set out in Table 9. The normal, fast-adapted animals would appear to have a maximum growth rate of D'_m = 1·05 per day (96 % fiducial limits ± 0·18) and a zero maintenance rate of 0·49 g C per g animal C per day (95 % limits 0·04), whereas for the slow-adapted animals the maximum growth rate is 0·65 per day (96 % limits ± 0·06) and the zero maintenance rate 0·029 g C per g animal C per day (95 % limits 0·005). However, since total loss is the sum of digestion and respiratory losses and since the former was derived in a somewhat arbitrary manner, the calculated zero maintenance rates quoted are also arbitrary to the same degree.

Table 9. *Linear regression analyses: statistics of the equation* $y = bx + a$.

	Statistical parameters							
Regression	a	b	r	σ^2	$\bar{x}$	$\bar{y}$	σ_x^2	N
Carbon			Fast-adapted					
ϵ on D	0·848	−0·0699	−0·162	0·00785	0·544	0·810	0·954	24
p/r on D	2·06	−1·96	−0·794	0·0979	0·544	0·990	0·954	24
Nitrogen								
ϵ on D	0·759	0·00874	0·0189	0·00964	0·546	0·764	0·952	23
p/r on D	1·61	−1·65	−0·447	0·494	0·546	0·704	0·952	23
Vitamin								
ϵ^* on D	0·878	−0·0914	−0·185	0·0102	0·544	0·828	0·954	24
ζ^* on D	0·673	0·124	0·262	0·00913	0·544	0·741	0·954	24
p^*/s^*_R on D	0·594	0·0352	0·0606	0·0146	0·544	0·613	0·954	24
r/p (C) on r/p (N)	0·207	0·465	0·945	0·147	2·78	5·19	119·0	23
Carbon			Slow-adapted					
ϵ on D	0·610	0·300	0·851	0·000959	0·461	0·808	0·139	7
p/r on D	33·9	−57·3	−0·967	6·30	0·461	7·50	0·139	7
Nitrogen								
ϵ on D	0·561	0·0136	0·0117	0·0253	0·500	0·568	0·0747	6
p/r on D	14·2	−22·5	−0·745	7·56	0·500	2·97	0·0747	6
Vitamin								
ϵ^* on D	0·961	−0·0321	−0·715	0·0000275	0·461	0·947	0·139	7
ζ^* on D	0·438	0·449	0·852	0·00212	0·461	0·644	0·139	7
p^*/s^*_R on D	0·422	0·405	0·849	0·00177	0·461	0·609	0·139	7
r/p (C) on r/p (N)	0·458	0·148	0·439	0·416	1·35	0·658	29·2	6

The parameters of these two regressions have been used to calculate and draw the fiducial limits in Figs. 4, 5, 11 and 12.

Excretion of nitrogen

The nitrogen data were even more scattered than those for carbon, two of the steady states being so wild as to be unusable. The latter are shown in parentheses in Table 8. An inspection of the p/r on D regressions for nitrogen (Table 9), reveals low correlation coefficients, so that neither r/p nor the growth/assimilation efficiency (η) for nitrogen can be calculated with any certainty and little inference can be drawn respecting the variation with growth rate. In spite of this, the slopes and the y intercepts of the p/r on D regressions for the fast and slow populations differ significantly although the x intercepts do not. Thus there is some evidence that the two populations differ in their nitrogen as in their carbon metabolism. The zero maintenance rate (nitrogen) is 0·61 (± 0·11) g N

per g animal N per day for the fast animals and 0·070 (± 0·004) g N per g animal N per day for the slow animals. The 96 % fiducial limits for D'_m are 1·34, 0·686 and 0·88, 0·49 per day respectively for the fast and slow populations.

Further evidence that nitrogen and carbon were behaving in a somewhat similar manner was that for the fast-adapted animals there was a marked correlation between the rate of respiration and rate of nitrogen excretion. This is shown in Fig. 6. The correlation coefficient is 0·94. The slope of the major axis is 0·48, indicating that relatively twice as much nitrogen is excreted as carbon.

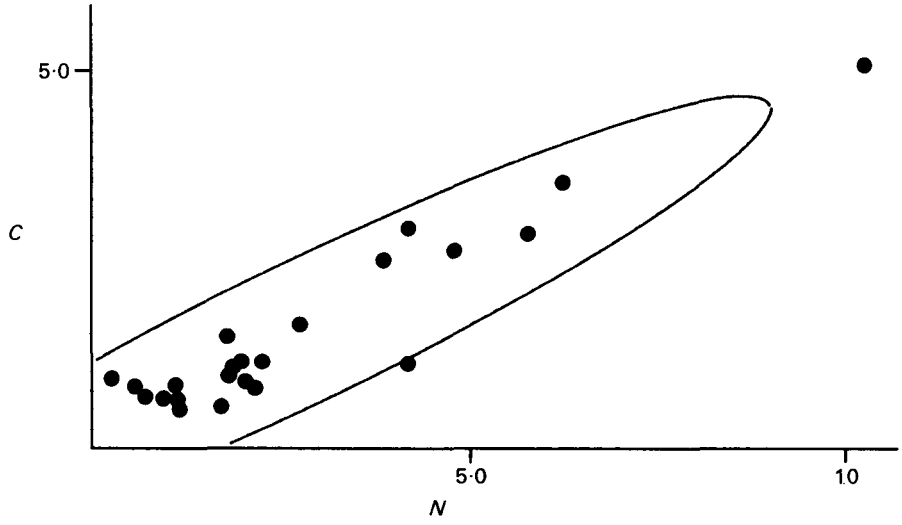

Fig. 6. Correlation of r/p (carbon) and r/p (nitrogen) for 'fast-adapted' animals, showing 95 % confidence contour.

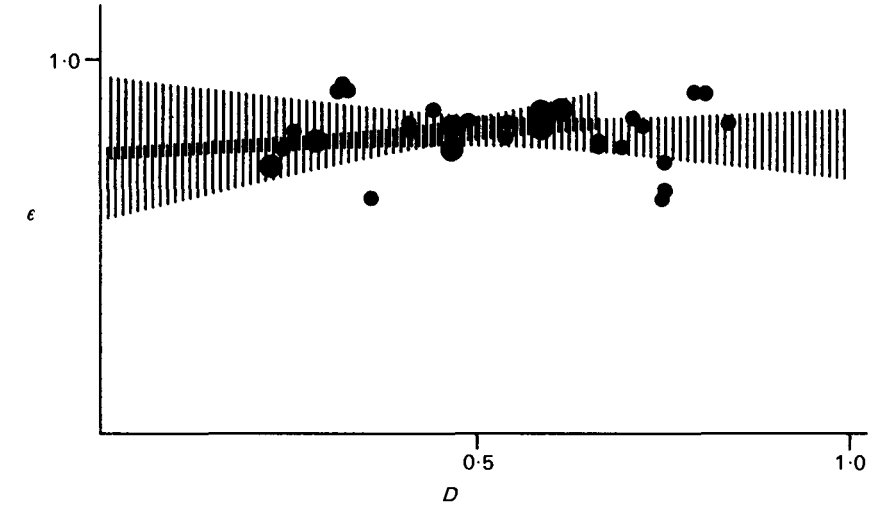

Fig. 7. Ingestion/input efficiency for carbon: regression of ϵ on D. See Fig. 5 for key.

The inference to be drawn from the above is that the hyperbolic relation we found with carbon also exists between the rate of nitrogen excretion and growth rate, even though the nitrogen data taken alone would scarcely warrant such a conclusion.

Transfer efficiencies

Neither the ingestion/input (ϵ) efficiencies for carbon, nitrogen and vitamin B_{12}, nor the assimilation/ingestion efficiency (ζ^*) for the vitamin show any obvious dependence on growth rate, except possibly the carbon ingestion/input efficiency for the slow-adapted animals and the two vitamin assimilation/ingestion efficiencies (Figs. 7, 8 and 9). In the absence of evidence to the contrary we have assumed linear regression analysis to

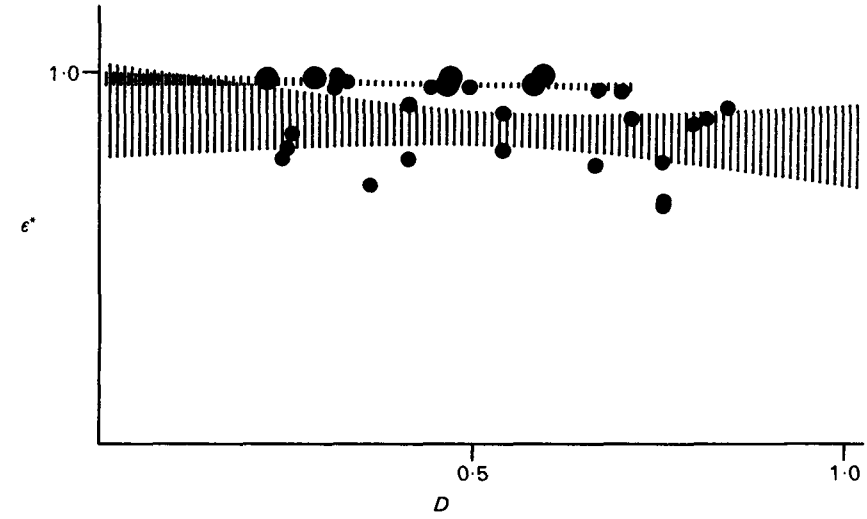

Fig. 8. Ingestion/input efficiency for vitamin B_{12}: regression of ϵ^* on D. See Fig. 5 for key.

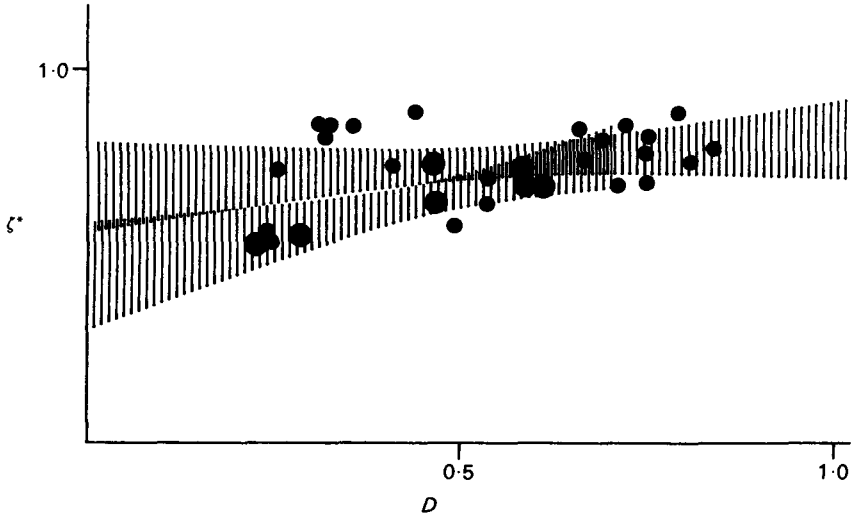

Fig. 9. Assimilation/ingestion efficiency for vitamin B_{12}: regression of ζ^* on D. See Fig. 5 for key.

be appropriate and have used the relevant regression parameters for drawing the fiducial limits in these figures and in Fig. 12.

If there really is no dependence on growth rate then it is permissible to characterize these efficiencies by the means over the whole experiment. These are set out in Table 10. The mean appears to be between 0·75 and 0·85 for both efficiencies. Departures from this mean relate to relative differences in the digestion loss. Thus high ingestion and low assimilation efficiency as in the vitamin figure for slow-adapted animals goes with a high digestion loss, whereas low ingestion and high assimilation efficiency, as in the nitrogen figures for the same animals goes with a low digestion loss. Digestion losses are presumably high when the digestion rate is higher than the assimilation rate. These conclusions of course follow from the theoretical basis of our calculations and are therefore foregone. The ingestion efficiency is directly measured, whereas the assimilation efficiency for carbon and nitrogen is based on the vitamin figures.

Table 10. *Mean efficiencies, ignoring possible growth rate dependence*

	Carbon	Nitrogen	Vitamin
		Fast-adapted	
Ingestion/input (ϵ)	0·810 (±0·037)	0·764 (±0·041)	0·828 (±0·042)
Assimilation/ingestion (ζ)		As under Vitamin	0·741 (±0·041)
Growth/assimilation (η)	0·304 (±0·037)	0·217 (±0·052)	1·0
Overall ($\epsilon\zeta\eta$)	0·181 (±0·025)	0·119 (±0·027)	0·613 (±0·050)
		Slow-adapted	
Ingestion/input (ϵ)	0·808 (±0·048)	0·568 (±0·142)	0·947 (±0·006)
Assimilation/ingestion (ζ)		As under Vitamin	0·644 (±0·072)
Growth/assimilation (η)	0·591 (±0·206)	0·446 (±0·243)	1·0
Overall ($\epsilon\zeta\eta$)	0·293 (±0·078)	0·194 (±0·109)	0·609 (±0·065)

95% fiducial limits in parentheses

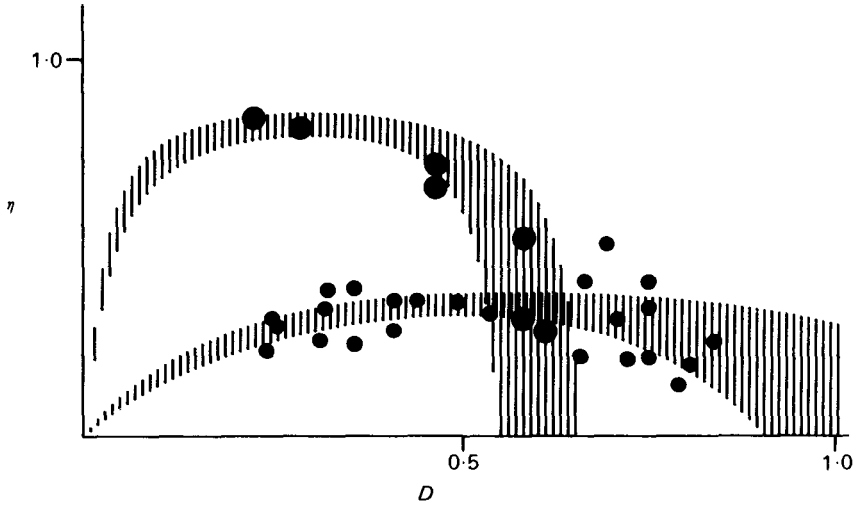

Fig. 10. Growth/assimilation efficiency for carbon: plot of η vs D. See Fig. 4 for key.

The growth/assimilation efficiency for nitrogen presents difficulties since, as we mentioned, it cannot reliably be related to growth rate except indirectly through the suspicion that the nitrogen and carbon excretions correlate. However, for the sake of a figure to quote, the mean growth efficiencies over the whole experiment are also entered in Table 10. We note that slow-adapted animals show higher efficiencies than fast-adapted animals. This we expect in the case of carbon but we have it with nitrogen also. The vitamin B_{12} growth/assimilation efficiency is unity by definition; that for nitrogen is lower than that for carbon in both slow- and fast-adapted populations, but this is to be expected since the rate of nitrogen excretion was on average double that of the respiratory rate.

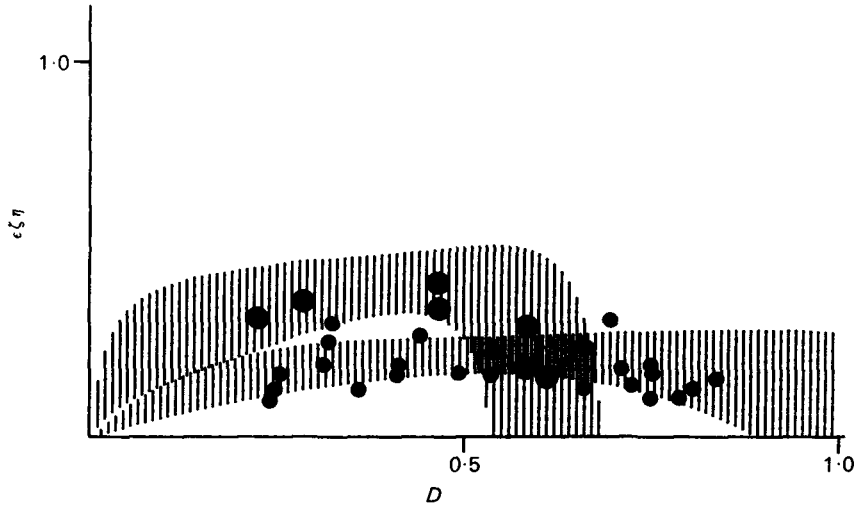

Fig. 11. Growth/input efficiency for carbon: plot of p/s_R on D. Small circles, 'fast-adapted'; large circles, 'slow-adapted'. Shaded areas, 94% limits estimated from the statistics of the three regressions p/r, ϵ and ζ^* on D.

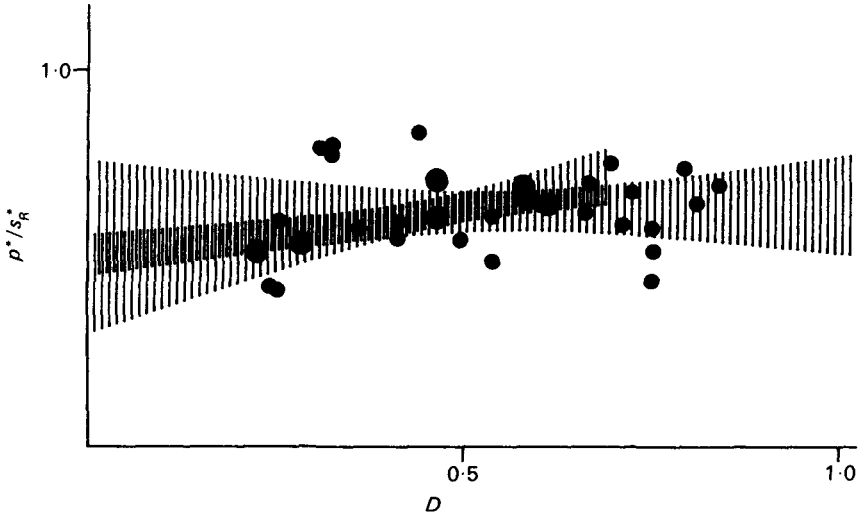

Fig. 12. Growth/input efficiency for vitamin B_{12}: plot of p^*/s_R^* on D. See Fig. 5 for key.

The relation between growth rate and the growth/assimilation efficiency for carbon is shown in Fig. 10. The curve calculated by equation (3) is symmetrical between zero growth rate and D'_m. The theoretical maximum is at $\frac{1}{2} D'_m$ and has a value of 0·93 ($\pm$ *ca.* 0·03) for the slow-adapted animals and 0·34 ($\pm$ *ca.* 0·03) for the fast-adapted animals.

The overall, i.e. the growth/input efficiency ($\epsilon\zeta\eta$) is the product of the three individual

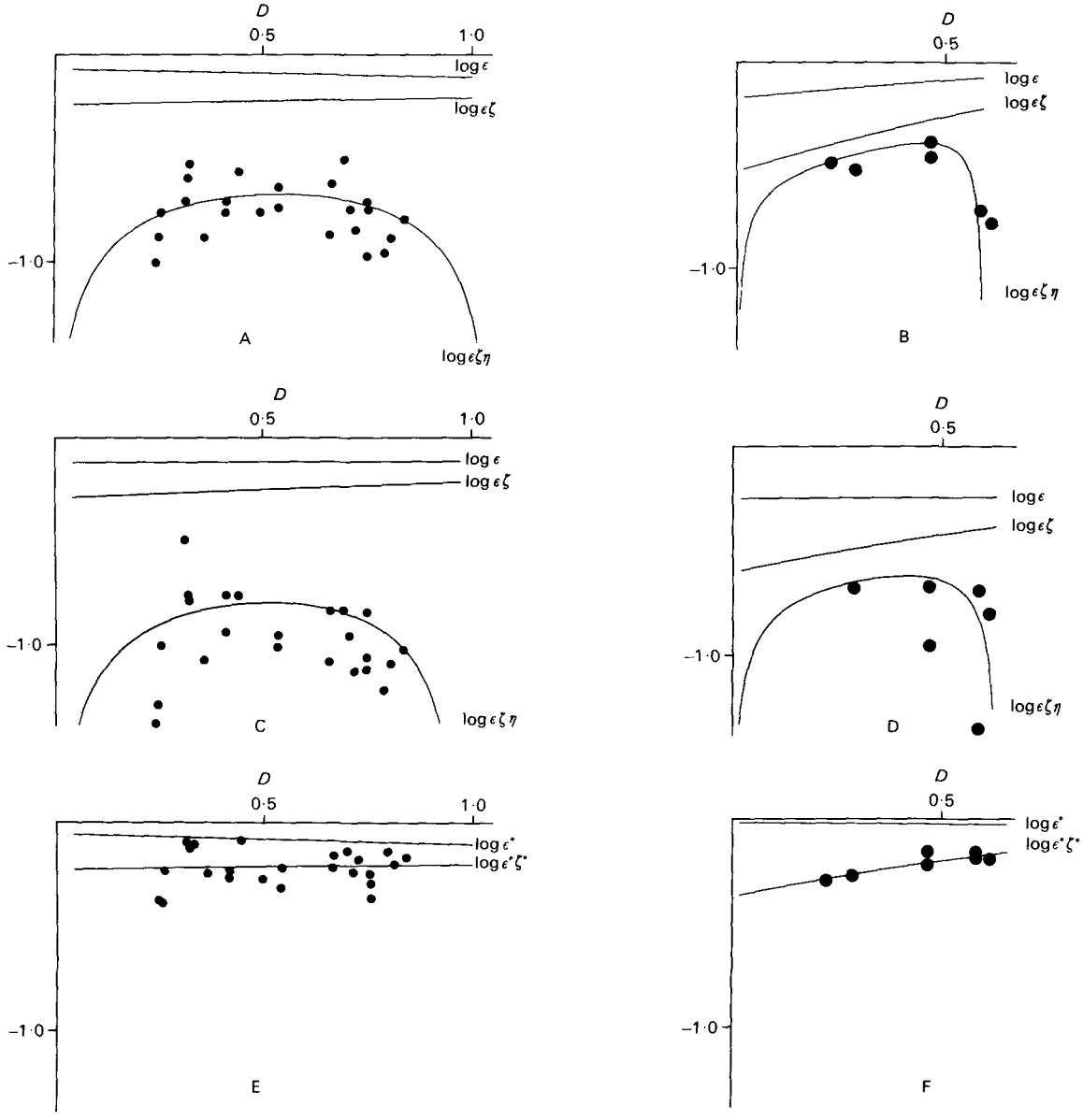

Fig. 13. Cumulative log efficiency curves (means only), log ϵ, log $\epsilon\zeta$, log $\epsilon\zeta\eta$, calculated from the regressions p/r, ϵ and ζ^* on D. The points, log growth/input efficiency data (log p/s_R). (A) Carbon, fast-adapted. (B) Carbon, slow-adapted. (C) Nitrogen, fast-adapted. (D) Nitrogen, slow-adapted. (E) Vitamin, fast-adapted. (F) Vitamin, slow-adapated.

efficiencies. The means are entered in Table 10. The carbon data plotted against growth rate are shown in Fig. 11. The carbon curves generally follow the growth/assimilation efficiency curve (Fig. 10) but are naturally lower and the maxima are displaced from the mid points by the slight slopes of the ingestion and assimilation efficiency regressions. The maxima are 0·22 ($\pm$ *ca.* 0·06) and 0·38 ($\pm$ *ca.* 0·10) respectively for the fast- and slow-adapted animals at $D = 0{\cdot}51$ and 0·40 respectively. The wide spread in the 94% fiducial limits is due in large part to the assimilation/ingestion efficiency error. The theoretical curves are flattened, so that fall-off in efficiency is only likely to be important at the two extremes of growth rate. Indeed, if the two extremes are avoided the overall efficiency is likely always to be more accurately characterized by taking the straight mean.

The corresponding data for vitamin B_{12} are shown in Fig. 12. They are not to related growth rate and are higher than the carbon and nitrogen figures.

Table 11. *Negative correlation coefficients of the regression p/r on D, adopting various assumptions in the calculation of ζ*

	Assumption			
	(1)	(2)	(3)	(4)
	$l/p = 0$	$l/p = n$	$l/p = n\, l^*/p^*$	$\zeta = \zeta^*$
Fast-adapted	0·696	< 0·723	< 0·724	0·794
Slow-adapted	0·978	< 0·937	< 0·945	0·967

The calculated cumulative log efficiencies (Fig. 13 A–F) show clearly where the transfer losses occur. In this presentation the space between the lines is proportional to the logarithm of the loss. It is interesting that the carbon and nitrogen curves, except at the extremes of growth rate, show proportionally more loss by the slow-adapted animals in ingestion and assimilation than in excretion, whereas with the fast-adapted animals by far the greater portion of the loss at all growth rates is accounted for by excretion. But then, very low excretion rates were recorded for the slow animals.

DISCUSSION

Criteria of alternative assumptions involved in the calculation of carbon and nitrogen assimilation efficiencies

There was a choice of the following assumptions: (i) digestion losses (l/p) zero; (ii) digestion losses finite and constant; (iii) digestion losses proportional to the vitamin digestion losses; (iv) assimilation efficiencies (ζ) equal to the vitamin efficiencies (ζ^*). The calculations were carried out incorporating each assumption in turn and the correlation coefficients of the p/r on D regressions compared. Those for carbon are shown in Table 11. Assumptions (i) and (iv) produced the highest correlation coefficients. Assumption (iv) was chosen as being marginally the better and moreover the more likely in view of the magnitude of digestion losses suggested by the purging experiment (Table 7).

Incidentally, since the best assumption was chosen on the basis of the fit of the linear equation (3) and particularly since this plot gave a more even distribution of the vari-

ances than the curvilinear r/p on D plot, we felt it more appropriate to base all the subsequent calculations on the statistics of equation (3) rather than attempt curvilinear analyses for the fiducial limits of r/p, ζ, and $\epsilon\zeta\eta$.

Transfer efficiencies

The carbon transfer efficiencies recorded in our experiment are not directly comparable to any previously published, either for rotifers or any other animals. First, because they refer to incoherent, exponentially growing, steady-state populations rather than individuals or groups of individuals in a particular developmental stage, and second, because they are related to population growth rate, a factor that is not taken into account in the literature. Nevertheless it may be useful to make a few comparisons, if only to highlight the difference of approach.

Doohan (1973) assumed that the (carbon) ingestion efficiency in *B. plicatilis* to be unity for moderate and low food concentrations, whereas our work indicates that it may be as low as 0·8. *Brachionus* is difficult to observe feeding because it does so on the move, but *Philodina*, a bdelloid plankton feeder, feeds while at rest and can be seen to be quite inefficient; by no means all the cells that reach the mouth find their way to the mastix. On the other hand, it will be recalled, our calculations of this efficiency in *Brachionus* would include losses due to defaecation. Assuming, for the sake of argument, that defaecation losses do wholly account for our ingestion/input efficiency, then a maximum defaecation rate of 20% of the gross ingestion rate would be indicated. The comparable figure from Doohan (assuming in her case that defaecation losses wholly account for the assimilation efficiency) is 80%. The difference may be due to the fact that food was being presented at a much higher level than in our experiment, and the food organism was different; but this 80% would also include digestion losses, which Doohan did not consider separately. Other comparable data on defaecation are 70–80% for the water flea *Daphnia pulex* (Richman, 1958) and 9% for the carnivorous marine amphipod *Calliopius laeviusculus* (Dagg, 1976).

The assimilation/ingestion efficiencies, 0·64–0·74, recorded by us are among the highest reported: 0·56–0·91 for damsel fly larvae (Lawton, 1970), 0·91 for *Calliopius* (Dagg, 1976), 0·21–0·78 for *Brachionus calyciflorus* (Galkovskaya, 1963), 0·19 for *B. plicatilis* (Doohan, 1973), 0·16–0·22 for *Asplanchna*, a carnivorous rotifer (Sorokin & Mordukhay-Boltovskaya, 1962) and 0·28–0·36 for *Leptopterna*, a plant-sucking mirid bug (McNeill, 1970). Some of this variety is certainly due to differences in defining the efficiency. For example, for Dagg (1976) it is the ratio of food taken in less faeces to food taken in, and for Doohan (1973) the ratio of the sum of the respiration and egg production rates to ingestion rate, while for us, since faecal loss affects our ingestion, but not our assimilation efficiency, it is essentially the ratio of the rate of net food intake less that lost to solution by digestion to that of the net food intake. Dagg (1976) recognized 'leakage products' as 'those potentially useful metabolites which are lost to organisms through some inefficiency of the conservation mechanism'. This definition would cover our digestion loss so our figure of 32% digestion loss is directly comparable to Dagg's 9% 'leakage'. Doohan's use of a respirable marker in assimilation experiments lasting 48 h

may partly account for her low estimates. Our results show that *Brachionus* can respire as much as five times its body carbon per day.

Winberg & Duncan (1971) list 22 published measurements of growth/assimilation efficiency for animals ranging from the chicken to *Paramecium*. The mean of this table is 0·44 and the standard deviation 0·13. Our carbon values for fast-adapted *Brachionus* lie comfortably within this range, but the high efficiency achieved by the slow-adapted animals takes them well outside it.

The values recorded by us for the overall efficiency (mean 0·18 and 0·28 respectively) are high compared with that reported by Doohan (1973) for the same animal, namely 0·11. But the figures are not really comparable; ours refer to the whole of growing populations while Doohan's refer to egg production by adult females. Our figures are also high compared to Lasker's for *Euphausia pacifica* (0·11) (Corner, Cowey & Marshall, 1967) and with those for *Hydra* and *Daphnia* discussed by Slobodkin (1962), 0·17 and 0·13 respectively. Slobodkin took into account the effect of the rate of removal of (predation on) the population on efficiency, but did not relate this to the rate of growth. Our data are representative of a situation in which removal is unselective and equal to the rate of growth.

Our nitrogen data are probably not sufficiently reliable to warrant detailed comparisons. Suffice to note that Corner *et al.* (1967) gave 0·38 for overall efficiency in nitrogen utilization during the life of *Calanus finmarchicus*. Our means for the fast- and slow-adapted *Brachionus* were respectively 0·15 and 0·29.

The source of the respiratory loss

The most striking result of our experiment has been the asymptotic shape of the relation between growth rate and respiration. A maintenance respiratory rate, which was to be expected, had imposed upon it a further rate that appeared to be growth rate dependent. Considering respiration as a function essential to life and growth it is possible to envisage the actual rate of respiration as that necessary to support a particular rate of growth, the limit being the point at which all the carbon taken in is respired.

It is conceivable but we think unlikely, that we are observing here an artifact of continuous culture. One might suppose the structure of a continuously growing population changes with changing growth rate. Certainly, fast growing *Brachionus* reach maturity and reproduce earlier than do slow growing animals. This conclusion follows from the fact that immature amictic females contain a constant and limited number of egg nuclei (eight in most clones). It is possible therefore to calculate the time taken for the females to grow and mature from the growth rate. However, in the chemostat the mean time from hatching to laying the nth egg is

$$t_n = \frac{\log_e n}{D} \tag{6}$$

while the mean age is $1/D$ and the mean number of eggs laid per rotifer before washout is e, i.e. 2·72. (The time for eggs to hatch after release is very short and can be ignored.) Thus, although the mean age of a sampled population varies inversely with dilution

rate, its maturity is constant. To explain the respiration curve by age alone, one would have to postulate the absurdity that respiration per unit biomass is infinitely high in young animals and only reaches measurable proportions after the first day ($1/D'_m = 1{\cdot}1$ days). It is difficult to see how the animals would survive the first day. On the other hand age *per se* may have something to contribute.

Apart from the chemical energy demand presumably associated with growth (including assimilation, internal transport and metabolism), energy may have to be expended in capturing the food. All this energy is manifest as respiratory loss. The question is, can we either *a priori* or on experimental evidence apportion this growth rate dependent demand between the biochemical and mechanical functions of the animal? The answer is almost certainly no, but we can examine the consequences of assuming the two extremes, namely all the growth rate dependent demand being (i) mechanical or (ii) biochemical.

In order to grow faster the animal has to take in food at a faster rate, but in order to do this it has to sweep a greater volume of water. This is the model generally assumed for filter-feeding zooplankton. Assuming that intake of food is proportional to the velocity of the currents generated then it follows that the power requirement is proportional to the cube of the rate of intake. Since rate of respiration has the dimensions of a power one might expect the respiratory rate to vary as the cube of the growth rate.

However, neither the plot of D^3 vs r/p nor D^3 vs r nor D vs $\sqrt[3]{r/p}$ eliminated the curvature associated with the plot of D vs r/p (Fig. 4), and we have to conclude that there is no evidence of an entirely mechanical growth rate dependent power demand.

A state of affairs in which the demand is entirely biochemical is encountered among those microbes in which substrate and organism are brought together by a process of diffusion. In *Brachionus* one would have to assume that the ciliary currents do not vary with growth rate. Although *Brachionus* feeds on the move and its feeding is analogous to that of a true filter feeder, the effect of the laws governing the intake of food is modified by the fact that the rotifer in our experiment was living in a vigorously stirred environment. If mixing in the reactor were perfect no advantage would accrue from swimming, and we would have a state of affairs in which organism and food were brought together by externally supplied energy. The rate of feeding would then be governed entirely by the concentration of food. As a matter of fact, the concentration of food is in any case the stimulus governing the feeding rate whether feeding is passive or active: if it were not so chemostat operation would not be possible. Therefore, since the cube law does not apparently operate and since there is no clear evidence of faster swimming with faster growth rate it is probably expedient to conclude that the major part of the growth rate dependent power demand originates in the chemical reactions leading to growth and not in prey capture.

On the other hand, were this the case one would expect to find evidence of a similar growth rate dependent power demand in the microbial literature. Although it is now accepted that part of the maintenance requirement may be growth rate dependent (Stouthamer, 1977) there appears yet to be no clear pattern. Tempest & Herbert (1965), however, published one curve that is suggestive of a pattern similar to ours; this was

for the rate of fermentative CO_2 production by the yeast *Torula utilis* in anaerobic glucose-limited growth. With aerobic cultures, however, the demand was merely proportional to the growth rate.

Slow- and fast-adapted growth

We do not know whether or not the change from 'fast' to 'slow' adapted growth is reverslble and we do not know the stimulus that led to the change nor whether it was external or internal in origin, though we suspect the latter. Indefinite parthenogenetic multiplication may well eventually result in loss of functions, as it does in *Paramecium* prevented from undergoing conjugation. On the other hand, there are precedents for reversible changes in other organisms. In some algal populations, for instance, the change from 'fast' to 'slow' modes of growth is reversible (Droop, 1974); moreover, the advantages and disadvantages conferred are somewhat analogous.

One could assess competitive advantage in this context as the ratio of the growth and respiration rates. Thus in Fig. 4 the critical point, the intersection of the two curves, is in the region $D = 0{\cdot}6$, $r/p = 1{\cdot}2$. In the domain between these co-ordinates and the origin, 'slow-adapted' animals are more efficient and would outgrow 'fast-adapted' animals, while in the domain above the position is reversed and the 'fast-adapted' animals would win. Since concentration of food is the variable governing the rate of food intake and hence both growth and respiration rates, one can conclude that when food is scarce the 'slow-adapted' animals are more fit than the 'fast-adapted'. The 'slow-adapted' mode is thus seen as a mechanism for making the best use of scarce food, perhaps a kind of aestivation. Unfortunately in our chemostat experiment the s values were too low for any trend to show above the noise. The mean value for s throughout the experiment was 3·13 μg C per ml ($\equiv$ *ca.* 15000 *Brachiomonas* cells per ml) and it is safe to conclude that the food concentration corresponding to the critical point is not far from this figure. Batch feeding experiments are usually carried out with much higher food concentrations than this. For instance Doohan (1973) used 350000–500000 *Dunaliella* cells per ml (i.e. *ca.* 14–20 μg C per ml).

Concluding remarks

A steady-state chemostat offers limited analogy with the real world although it is tempting to view it as a miniature ecosystem. On the other hand, the artificiality of the system is largely overt, so that the information obtained can be related in a simple manner directly to the organism, and in principle is applicable in any other system, provided of course sufficient information regarding the latter is to hand. The same cannot be said of batch and other 'more natural', though none the less as artificial, laboratory experiments where the time element and previous history of the material introduce imponderables.

At its best, a dissection of the life history for the purpose of energetics provides an insight into the biology of a species that our approach lacks, but may be somewhat less valuable from the point of view of the energetics of an ecosystem. Our findings suggest that the relation between population growth rate and power demand could be

of great importance in the wider context of energy flow. Moreover, although the steady-state chemostat is a cumbersome tool, it is possibly the only, or at least the most direct, way of studying this relation.

REFERENCES

CONOVER, R. J. & FRANCIS, V., 1973. The use of radioactive isotopes to measure the transfer of materials in aquatic food chains. *Marine Biology*, **18**, 272–283.

CORNER, E. D. S., COWEY, C. B. & MARSHALL, S. M., 1967. On the nutrition and metabolism of zooplankton. V. Feeding efficiency of *Calanus finmarchicus*. *Journal of the Marine Biological Association of the United Kingdom*, **47**, 259–270.

DAGG, M., 1976. Complete carbon and nitrogen budgets for the carnivorous amphipod *Calliopius laeviusculus* (Krøyer). *Internationale Revue der gesamten Hydrobiologie u Hydrographie*, **61**, 297–357.

DOOHAN, M., 1973. An energy budget for adult *Brachionus plicatilis* Müller (Rotatoria). *Oecologia*, **13**, 351–362.

DROOP, M. R., 1967. A procedure for routine purification of algal cultures with antibiotics. *British Phycological Bulletin*, **3**, 295–297.

DROOP, M. R., 1968. Vitamin B_{12} and marine ecology. IV. The kinetics of uptake growth and inhibition in *Monochrysis lutheri*. *Journal of the Marine Biological Association of the United Kingdom*, **48**, 689–733.

DROOP, M. R., 1974. The nutrient status of algal cells in continuous culture. *Journal of the Marine Biological Association of the United Kingdom*, **54**, 825–855.

DROOP, M. R., 1976. The chemostat in mariculture. In *Proceedings of the Tenth European Marine Biology Symposium*, Ostend, 1975, vol. 1, pp. 71–93.

GALKOVSKAYA, G. A., 1963. Utilization of food for growth and conditions for maximum production of the rotifer *Brachionus calyciflorus* Pallas. *Zoologicheskiĭ zhurnal*, **42**, 506–512. [Russian.] [Translation by Fish. Res. Bd Can. No. 997.]

HERBERT, D., ELSWORTH, R. & TELLING, R. C., 1956. The continuous culture of bacteria; a theoretical and experimental study. *Journal of General Microbiology*, **14**, 601–622.

LAWTON, J. H., 1970. Feeding and food energy assimilation in larvae of the damsel fly *Pyrrhosoma nymphula* (Sulz.) (Odonata: Zygoptera). *Journal of Animal Ecology*, **39**, 669–689.

MCNEILL, S., 1970. The energetics of a population of *Leptopterna dolabrata* (Heteroptera: Miridae). *Journal of Animal Ecology*, **40**, 127–141.

PIRT, S. J., 1965. The maintenance energy of bacteria in growing cultures. *Proceedings of the Royal Society* (B), **163**, 224–231.

RICHMAN, S., 1958. The transformation of energy by *Daphnia pulex*. *Ecological Monographs*, **28**, 273–291.

SLOBODKIN, L. B., 1962. Energy in animal ecology. In *Advances in Ecological Research*, vol. 1 (ed. J. B. Cragg), pp. 69–101. London and New York: Academic Press.

SOROKIN, Y. I. & MORDUKHAY-BOLTOVSKAYA, E. D., 1962. A ^{14}C study of rotifer *Asplanchna* feeding. [Russian.] *Byulleten' Instituta biologii vodokhranilishch*, **12**, 17–20.

STOUTHAMER, A. H., 1977. Energetic aspects of the growth of micro-organisms. *Symposia of the Society for General Microbiology*, **27**, 285–315.

TEMPEST, D. W., 1970. The continuous cultivation of micro-organisms. 1. Theory of the chemostat. In *Methods in Microbiology*, vol. 2 (ed. J. R. Norris and D. W. Ribbons), pp. 259–276. London and New York: Academic Press.

TEMPEST, D. W. & HERBERT, D., 1965. Effect of dilution rate and growth-limiting substrate on the metabolic activity of *Torula utilis* cultures. *Journal of General Microbiology*, **41**, 143–150.

WINBERG, G. G. & DUNCAN, A., 1971. *Methods for the Estimation of Aquatic Animals*. 175 pp. London and New York: Academic Press.