C.R. Acad. Sci. Paris, Sciences de la vie / Life Sciences 323 (2000) 99–111

S0764446900001025/FLA

The role of intracellular components in food chain dynamics

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Abstract – The dynamics of a simple food web, including multiple substrates and predator–prey interactions, is studied. An individual-based model is presented that describes the intracellular composition of the biomass of each population with two components: reserves and structural biomass. The model describes the simultaneous uptake of multiple substrates via specific carriers and their assimilation into reserve energy via multiple assimilation pathways. The available energy is used for maintenance and growth. Parameters are estimated by curve-fitting data from the literature under the condition that the elemental balances and the enthalpy balances are met. The proposed model provides an adequate description of the macromolecular composition of biomass. The model is not too complicated to be of use in the study of food webs. The consequences of the presence of intracellular components in a food web on its long-term dynamics are investigated with bifurcation analysis. © 2000 Académie des sciences/ Éditions scientifiques et médicales Elsevier SAS

food chain / bifurcation analysis / continuous culture / ruman bacteria / *Selenomonas ruminantium* / dynamic energy budget model

Résumé – L'importance de composants intracellulaires pour la dynamique des chaînes trophiques. Nous étudions la dynamique d'une chaîne trophique simple comportant divers nutriments et des interactions proie-prédateur. Nous présentons un modèle basé sur l'individu décrivant la composition intracellulaire de la biomasse de chaque population avec deux composantes : les réserves et la biomasse structurelle. Le modèle décrit l'absorption simultanée de multiples substrats qui sont captés selon diverses voies et assimilés sous forme d'énergie de réserve au travers de différentes voies d'assimilation. L'énergie disponible est utilisée pour la maintenance et pour la croissance. Les paramètres sont estimés à partir des données de la littérature en supposant que les bilans des substances et des enthalpies sont équilibrés. Le modèle proposé est suffisamment simplifié pour inclure des modèles de chaînes trophiques. Nous recherchons les conséquences de l'incorporation de composants intracellulaires dans une chaîne trophique sur la dynamique à long terme au moyen d'une analyse de bifurcation. © 2000 Académie des sciences/Éditions scientifiques et médicales Elsevier SAS

chaîne trophique / analyse de bifurcation / bactérie du rumen / culture continu / *Selenomonas ruminantium* / théorie du bilan dynamique de l'énergie / modèle

Version abrégée

Dans la plupart des modèles en microbiologie et en écologie. il est souvent supposé de manière implicite que le grand nombre de composants intracellulaires présents dans les organismes individuels peut être assimilés à un composant unique et que tous les individus de la population sont identiques. Cependant, la composition des microorganismes n'est pas cons-

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tante et les changements de la composition macromoléculaire de la biomasse se déroulant à différents taux de réaction sont susceptibles d'avoir un impact important sur certaines variables comme le rendement et les ATP coûts de production de la biomasse.

Sur la base de la théorie du bilan dynamique de l'énergie (BDE), nous décrivons les variations de la composition en biomasse d'un individu avec deux composantes, les réserves et la biomasse structurelle qui sont considérées comme des constituants chimiques avec une composition fixée. La composition de la biomasse totale peut changer sous l'effet des variations des quantités relatives de réserves et de biomasse structurelle. La théorie du BDE permet d'obtenir un modèle biochimique de flux global simple pour les individus avec des flux d'absorption des substrats pour leur assimilation sous forme de réserves, pour l'utilisation des réserves pour la maintenance ou pour la croissance. La dynamique de la population est représentée par un ensemble de transformations chimiques obéissant à des lois de conservation de la masse et de l'énergie,

Dans cette contribution, nous estimons les paramètres à partir des données (mesurées par Russel en 1986) concernant une population microbienne de *Selenonlonas ruminantium* se nourrissant de deux substrats. Nous faisons l'hypothèse que les substrats sont absorbés de différentes manières et assimilées dans un seul pool de réserve selon diverses voies. Les paramètres du modèle sont estimés en ajustant les courbes de la densité de substrat, des densités de produits, du taux de production de chaleur et de la composition macromoléculaire de la biomasse sous la condition que les bilans de masse et d'énergie sont équilibrés. Cet exemple illustre le fait que la dynamique de la composition de la biomasse macromoléculaire et en élément peut être décrite avec seulement deux composants: les réserves et la biomasse structurelle.

Dans un second exemple, nous considérons en plus un prédateur supérieur qui se nourrit de la population microbienne formant une chaîne trophique simple dans un milieu de culture continu. Le prédateur ingère un substrat (c'est-à-dire sa proie) qui consiste en des quantités variables de deux composants (les réserves et la biomasse structurelle). Le prédateur assimile les composants de la proie selon deux voies d'assimilation possibles. Nous étudions les effets à long terme des variations de la composition de la biomasse sur le comportement de la chaîne trophique au moyen de l'analyse de bifurcation. Les diagrammes de bifurcation présentent des régions où le prédateur supérieur peut coexister de manière permanente avec la proie et les substrats dans un équilibre stable, des régions où l'invasion du prédateur supérieur est impossible et des régions où des cycles limites stables apparaissent.

Ce papier suggère que la dynamique du substrat doit être modélisée explicitement. Ce travail démontre également la nécessité de modéliser le devenir du substrat une fois qu'il a pénétré la cellule, c'est-à-dire de prendre en compte les voies d'assimilation. En conclusion, la modélisation de la composition de la biomasse macromoléculaire, qui est essentielle pour l'utilisation de divers bilans, peut être réalisée d'une manière telle que le modèle qui en résulte nest pas trop compliqué et peut être utilisé pour des modèles de chaînes trophiques.

1. Introduction

Population dynamic processes can be regarded as a class of complex chemical conversions that are subject to energy and mass conservation laws. Such a point of view is usual for microbial populations, because microbiology has many historical links with chemistry, but it is rather unusual for animal populations. Yet, we feel that such a point of view can contribute considerably to the understanding of certain aspects of population biology. The reason that such an attempt has not been undertaken before in animal population biology is probably that the chemistry of metabolism is far from easy, and conservation laws are hard to apply in open systems. Only during the last decade have properties of individuals been included in population dynamical models, in a class of models known as physiologically structured population models. Many ecologists might consider the step to include the chemical basis of metabolism into population biology beyond reach, but we claim that this is feasible on the basis of the dynamic energy budget (DEB) theory [1, 2]. In its most simple formulation, the theory distinguishes two compounds in an organism: reserves and structural biomass, which are both taken to be generalized compounds, i.e. rich mixtures of chemical compounds that do not change in composition. The theory is therefore based on a relaxed version of the concept of homeostasis, and allows changes in the chemical composition of biomass, when the ratio of the reserves and the structural biomass changes because of changes in food availability. This ratio is called the reserve density.

In many classical models in microbiology and ecology [3–8] it is implicitly assumed that the large number of intracellular compounds present in individual organisms can be lumped into one component and furthermore that all individuals in a population are identical and can be lumped together. However, the composition of micro-organisms is not constant, but changes with, for instance, the specific growth rate, see, for example [9–14]. Changes in, for example, the protein content of biomass do have a considerable impact on the ATP costs of the production of biomass [15–18]. Other variables, such

as the yield of biomass, may vary when the intracellular composition of micro-organisms varies [19, 20].

Here we confine ourselves to unstructured populations, where individuals in a population are lumped together into a population reserve mass and a population structural mass. This simplification follows naturally from the DEB theory, in the situation where surface areas of individuals are proportional to their volumes. If an organism does not change in shape during growth, surface area is proportional to volume$^{2/3}$. The DEB theory takes assimilation as proportional to surface area, and maintenance to volume, while changes in the ratio between the two have far reaching implications, which directly lead to patterns in the ontogeny of individuals which structure populations. Changes in the surface area to volume ratio hardly affect the population dynamics in certain situations, for instance when structural biomass divides into two equal parts at a given size. Although structured populations pose no fundamental problems to our approach [1, 2], we focus here on unstructured populations for simplicity's sake.

We will follow the fate of the reserves in terms of energy, and of structural mass in terms of volume. Since the chemical compositions do not change, conversions to moles or weights are straightforward. The two components differ in some basic features. The feeding rate does not depend on the reserve density, only on the structural mass and food availability, and the reserves do not require maintenance. Food is converted into reserves, a process called assimilation, and reserves are used for growth and maintenance. The utilization rate depends in a simple way on the reserve density and the structural mass. Some relevant details of the model are presented in sections 3 and 4 on the model for individuals.

The governing equations for communities consisting of a number of resources and interacting populations form a set of coupled nonlinear ordinary differential equations (ODEs). We use bifurcation theory [21, 22] to analyse these systems. Bifurcation analysis gives information about the long-term dynamic behaviour of nonlinear dynamic systems; that is, the asymptotic stability of equilibria, periodic orbits and chaotic attractors. The structural stability is studied with respect to so-called free or bifurcation parameters. For the chemostat (continuous culture) case these are the control parameters; namely, the dilution rate and the substrate concentration in the reservoir which are set by the experimentalist. If an attractor changes character or disappears completely under slight adjustment of a free parameter, the system is structurally unstable. The point in the bifurcation parameter space where this happens is called a bifurcation point.

In previous papers [23–26] we applied bifurcation analysis to microbial food chains under chemostat conditions. Although each population consists of the two components, it was assumed that the predator digests only the structural biomass component of its prey. This is unrealistic because reserves contribute substantially to the nutritional value of the prey [27]. Thus, decomposition of organisms into two components implies that predators feed on at least two substrates, even if they select a single species of prey. Life on several substrates represents, therefore, a natural situation for DEB modelling. In section 2 we consider the various alternative situations concerning the interaction of two substrates in the uptake process.

Reserve energy can be utilized for growth, i.e. the increase in structural volume, and reserve energy is continuously needed for maintenance of the structural part of the individual. Maintenance exerts a strong influence on the dynamic behaviour at low dilution rates, and energy reserves influence the dynamic behaviour at high dilution rates [26]. Energy reserves allow the individual to survive periods of discontinued substrate availability. Since such circumstances are common in nature, incorporation of reserves adds much to the realism of the model [1]. Study of the dynamic behaviour of models is not only of interest for theoretical ecology, but also for the continuous culture apparatus used in experimental microbiology and for applied microbiology in, for example, waste water treatment plants [26].

In this paper, we aim to analyse the effect of changes in biomass composition on the population dynamics on the basis of two examples. In the first example, section 3, a microbial population of *Selenomonas ruminantium* HD 4, consumes two substrates. The substrates are taken up by different types of carriers and assimilated into reserves via different pathways. Parameters are estimated from data measured by Russell [28] whereby mass and enthalpy balances [29] are taken into account. It appears that the dynamics of the elemental composition and of the macromolecular composition of biomass can be described with just two components: reserves and structural biomass. In the second example, section 4, a top predator which consumes the microbial population is introduced into the first system forming a simple food web in the chemostat environment. The predator ingests one substrate (i.e. the prey) that contains a varying ratio of two components (i.e. reserves and structural biomass). The predator assimilates the components in the prey via two assimilation pathways.

2. Uptake of two substrates

As is widely recognized, two different substrates can be classified as substitutable or complementary. This classification is of use in idealized modelling contexts, such as the DEB model, where one or more substrates are transformed into reserves. We think here of substrates and reserves as generalized compounds, i.e. mixtures of chemical compounds that do not change in composition, and that can be quantified in terms of C-moles. Pure chemical compounds represent a special case of a generalized compound. Two substrates are substitutable if each compound can be chemically converted into reserves, while they are complementary if both substrates are required simultaneously, with a fixed stoicheiometry.

The popular Holling type II (or Michaelis-Menten) models for substrate uptake rest on the mechanism that each

arriving substrate particle blocks the carrier that handles the uptake for a certain period, such that particles that arrive while the carrier is busy are rejected. The number of carriers is treated as given, and can depend on the size of the organism. This mechanism ensures that the uptake rate is a hyperbolic function of the substrate concentration if the intensity of the arrival process is taken to be proportional to the substrate concentration, which applies to a variety of diffusion-based transport models. The maximum uptake is achieved when all carriers are busy all the time.

Two substitutable substrates can compete for access to the same carriers, or they can use different carriers. This results in different uptake rates, which will be briefly discussed.

When a predator feeds on two species of prey, these prey represent competing substrates, because the digestive system of the predator decomposes both prey into the same compounds that pass the gut wall. The carriers in the gut wall do not 'know' from which prey species the compound they can handle originated. An increase in the density of one prey reduces the feeding rate on the other, because of satiation of the predator (via satiation of the carriers). When x_i represents the ratio of the density of prey i and the saturation coefficient for that prey, the scaled functional response of the predator is given by $f = (1 + (1 + x_1 + x_2)^{-1})^{-1}$ if both preys require the same handling time. In micro-organisms the situation is different when specific carriers for each different substrate are present simultaneously. In that case the substrates do not compete, as is discussed in section 3.1.

When two substrates are complementary, the absence of one substrate prevents the uptake of the other; the uptake of a carbon source is futile when a nitrogen source is absent. Empirical evidence frequently indicates that uptake of the most abundant substrate (relative to the needs) is set by the least abundant substrate: the minimum rule. This rule has, however, complex implications in combination with reserves; substrate can be absent in the environment, while growth is not restricted because of the presence of reserves. If the roles of limiting and non-limiting substrates do not switch at the same time for all individuals in the population in a variable environment, it is almost impossible to evaluate population behaviour from individual behaviour. Moreover, sharp switches are not realistic at the molecular level, because of the intrinsic stochasticity of the arrival process.

The dynamics of the synthesizing unit, as recently proposed by Kooijman [30], solves this problem in being close to a minimum model, while avoiding the sharp switches. This is a special case of the standard association/dissociation enzyme kinetics, where the dissociation rates of the substrate–enzyme complexes are taken to be negligibly small. The result is that the scaled functional response is given by $f = (1 + x_1^{-1} + x_2^{-1} - (x_1 + x_2)^{-1})^{-1}$.

The rejection unit represents another special case of standard enzyme kinetics, which is more frequently applied, despite its limited realism. The dynamics results after increasing both the association rates of substrates and enzyme, as well as the dissociation rates of the enzyme–substrate complexes to infinity, such that their ratios remain constant. This results in the scaled functional response $f = (1 + x_1^{-1})^{-1} (1 + x_2^{-1})^{-1}$. This model is frequently referred to as the multiplicative model.

The uptake patterns mentioned above can be modified in various ways, for instance, when the abundances of both substrates are coupled. This occurs, for example, when a predator feeds on a prey that consists of a structural mass and reserves; the structural mass of algae is of limited nutritional value to daphnids because daphnids cannot digest cellulose or photo-pigments, while they grow well on the lipid-rich reserves of algae. If the algal reserves hardly modify the handling time of algae by daphnids, changes in algal reserve density only show up as changes in nutritional value, and do not affect the feeding rate directly. The algal reserves do affect the feeding rate indirectly via the population level, by enhancing the propagation of daphnids. If the reserves extend the handling time by daphnids, algal reserves compete with algal structural biomass, as is discussed in more detail [27].

3. Growth on two substrates

3.1. Individuals

The dynamic energy budget (DEB) theory describes the dynamics of energy and volume on the individual level [1]. We consider the case where two substrates are present with densities X_{01} and X_{02} (trophic level 0), that are taken up by an individual with volume V_1 (trophic level 1). The individual grows in one dimension (filament), so its volume is proportional to the surface area. It is assumed that different types of carriers are present for different substrates. The carriers for substrates X_{01} and X_{02} are characterized by their affinity for a particular substrate (via the saturation coefficients $K_{X_{01},1}$ and $K_{X_{02},1}$) and by a particular number of carriers per surface area (via the maximum ingestion rates $[I_m]_{X_{01},1}$ and $[I_m]_{X_{02},1}$). The ingestion rates of X_{01} and X_{02} are given by

$$I_{X_{0i},1} = [I_m]_{X_{0i},1} f_{X_{0i},1} V_1; \quad f_{X_{0i},1} \stackrel{\text{def}}{=} \frac{X_{0i}}{K_{X_{0i},1} + X_{0i}}; \quad i = 1, 2 \qquad (1)$$

where f is the scaled functional response.

In general, different substrates are assimilated into reserves via different pathways with different assimilation efficiencies. The maximum assimilation rates for substrates X_{01} and X_{02} are $[A_m]_{X_{01},1}$ and $[A_m]_{X_{02},1}$. The assimilation rates for X_{01} and X_{02} are given by

$$A_{X_{0i},1} = I_{X_{0i},1} \frac{[A_m]_{X_{0i},1}}{[I_m]_{X_{0i},1}} = [A_m]_{X_{0i},1} f_{X_{0i},1} V_1; \quad i = 1, 2 \qquad (2)$$

It is assumed that the dynamics of the reserve density is proportional to the reserve density. Both assimilation fluxes add to one pool of reserves:

$$\frac{d[E]_1}{dt} = [A_m]_{X_{01},1}\, f_{X_{01},1} + [A_m]_{X_{02},1}\, f_{X_{02},1} - ([A_m]_{X_{01},1} + [A_m]_{X_{02},1})\, \frac{[E]_1}{[E_m]_1} \quad (3)$$

Reserve energy is continuously needed for maintenance of the integrity of the structural part of biomass. The remaining reserve energy is utilized for growth. The energy fluxes to growth, G_1, and maintenance, M_1, are

$$G_1 = [G]_1\, \frac{dV_1}{dt}; \quad M_1 = [M]_1\, V_1 \quad (4)$$

where $[G]_1$ denotes the volume-specific costs for growth and $[M]_1$ denotes the volume-specific maintenance rate.

The utilization rate of the reserves equals the energy fluxes to maintenance and growth

$$A_{X_{01},1} + A_{X_{02},1} - \frac{dE_1}{dt} = M_1 + G_1 \quad (5)$$

It is convenient to define the specific energy conductances, $v_{X_{01},1}$ and $v_{X_{02},1}$, the maintenance rate coefficient, m_1, the energy investment ratio, g_1, and the scaled energy density, e_1,

$$v_{X_{01},1} \stackrel{\text{def}}{=} \frac{[A_m]_{X_{01},1}}{[E_m]_1}; \qquad v_{X_{02},1} \stackrel{\text{def}}{=} \frac{[A_m]_{X_{02},1}}{[E_m]_1};$$

$$m_1 \stackrel{\text{def}}{=} \frac{[M]_1}{[G]_1}; \quad g_1 \stackrel{\text{def}}{=} \frac{[G]_1}{[E_m]_1}; \quad e_1 \stackrel{\text{def}}{=} \frac{[E]_1}{[E_m]_1}$$

Equation (3) can be written as

$$\frac{de}{dt} = v_{X_{01},1}\, f_{X_{01},1} + v_{X_{02},1}\, f_{X_{02},1} - (v_{X_{01},1} + v_{X_{02},1})\, e_1 \quad (6)$$

Note that equation (6) is a constitutive relation and not a balance equation. Homeostasis of the reserves is preserved because it is assumed that both uptake flows add to the same reserve pool. A consequence of this assumption is that the maximum energy density $[E_m]$ can change with the number and nature of substrates. It appears that $[E_m]$ is not only species-specific but also depends on the composition of food. This is a choice for simplicity. An alternative is that each substrate adds to the maximum energy density via an additional reserve pool. This would lead to considerable complications, because each type of reserve would have a characteristic composition which would be hard to unravel from the available data on biomass composition. Alternative models for the uptake of multiple nutrients have been proposed [31–34].

The growth rate of an individual with volume V_1 follows from equations (5) and (6),

$$\frac{dV_1}{dt} = \frac{(v_{X_{01},1} + v_{X_{02},1})\, e_1 - m_1\, g_1}{e_1 + g_1}\, V_1 \quad (7)$$

Equation (7) shows that when multiple substrates are taken up simultaneously, each substrate contributes to the growth rate.

3.2. Continuous culture

The density of the structural biovolume of population X_1, is the sum of all individual volumes V of the species in trophic level 1, divided by the culture volume V_c. The constitutive relation (6) remains valid on the population level.

Two substrates, with reservoir substrate densities X_{r1} and X_{r2}, are supplied to a continuous culture with dilution rate D. The volume of the continuous culture V_c is kept constant through removal of culture with dilution rate D. The dynamics of continuous culture is given by equations (8) and the constitutive equation (6), and will be referred to as system **1,**

$$\frac{dX_{0i}}{dt} = D(X_{ri} - X_{0i}) - [I_m]_{X_{0i},1}\, f_{X_{0i},1}\, X_1; \quad f_{X_{0i},1} \stackrel{\text{def}}{=} \frac{X_{0i}}{K_{X_{0i},1} + X_{0i}}; \quad i = 1, 2 \quad (8a)$$

$$\frac{dX_1}{dt} = (\mu_1 - D)\, X_1; \quad \mu_1 = \frac{(v_{X_{01},1} + v_{X_{02},1})\, e_1 - m_1\, g_1}{e_1 + g_1} \quad (8b)$$

where μ_1 is the volume-specific population growth rate for a population with structural volume density X_1.

Complete elemental balances can be constructed with the dynamics of products and nutrients. In Hanegraaf [29] it is shown that the rates of product formation or nutrient consumption can be described as weighted sums of the assimilation rates (of substrates X_{01} and X_{02}), the maintenance rate, and the growth rate of the population in trophic level 1.

3.3. Analysis of steady state data

Conversion coefficients are used to convert energy and volume to observable quantities measured routinely in microbial practice. For a population with structural volume density, X_1, and scaled reserve density, e_1, the biomass density on the basis of C_1-moles is

$$W_1 = ([d_{mv}]_{X_1} + [d_{me}]_{E_1}\, e_1)\, X_1 \quad (9)$$

where conversion coefficients $[d_{mv}]_*$ and $[d_{me}]_*$ convert volume and energy of compound * to C_1-moles. Since mass balances are actually elemental balances it is convenient to express compounds in C_1-moles. C_1-moles are converted to dry weights with the C_1-molecular weights of the compounds. The elemental balances, the enthalpy balance and the relation between model parameters and observable quantities are treated in Hanegraaf [29].

In *figure 1* are shown steady state data on *Selenomonas ruminatium* HD 4 in anaerobic continuous culture on rich medium plus glucose, measured by Russell [28]. Note that

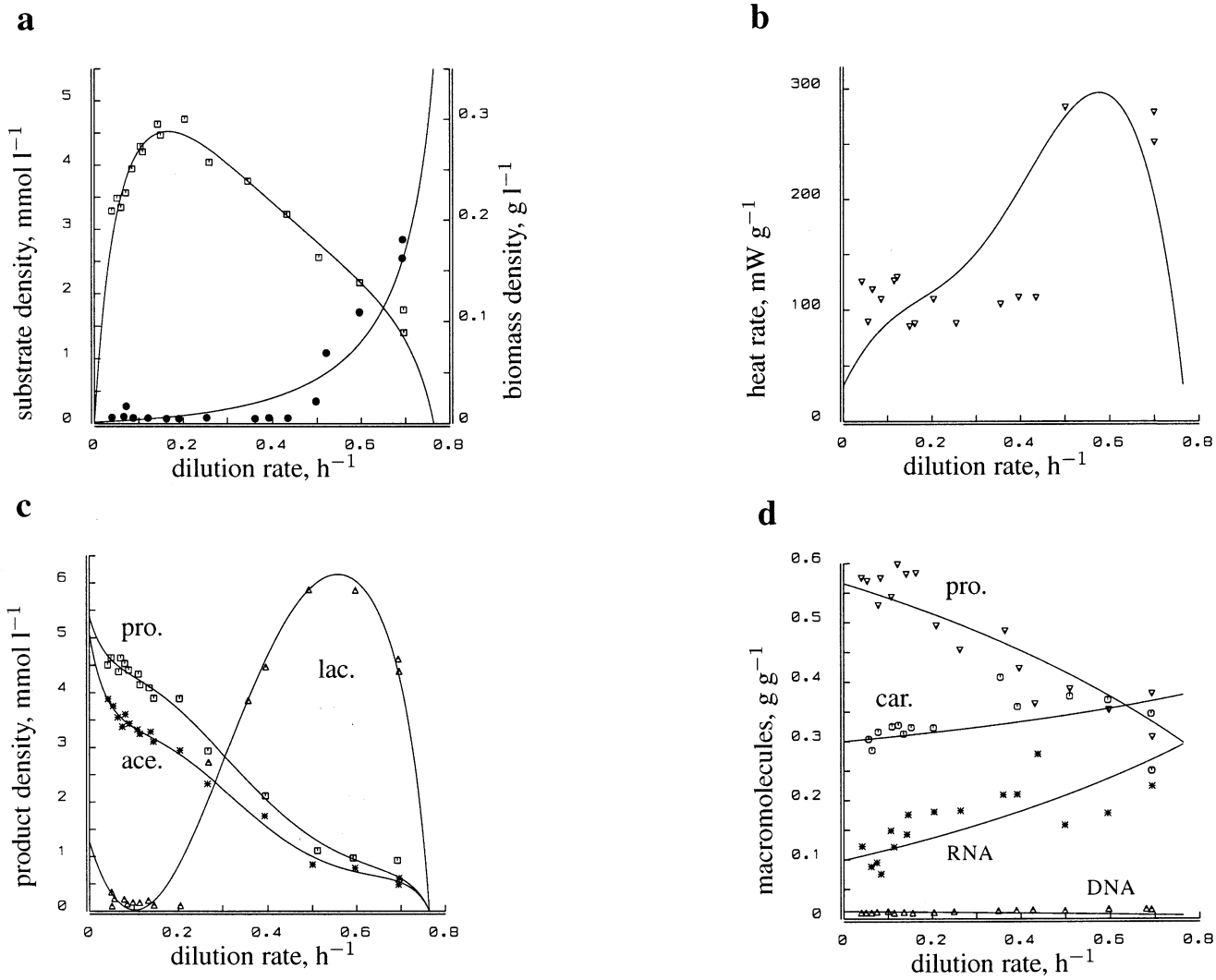

Figure 1. Steady state data from Russell [28], on *Selenomonas ruminantium* HD 4 in anaerobic continuous culture on rich medium, 8 C_1-mmol L^{-1}, plus glucose, 33 C_1-mmol L^{-1}, at 39 °C.
a. Substrate density in the culture, ●, in mmol glucose L^{-1}, and biomass density, □ in g dry weight L^{-1}. **b.** Weight-specific heat rate, ▽, in mW g^{-1}. **c.** Product density of propionic acid, □ acetic acid, *, and lactic acid, Δ, in mmol L^{-1}. **d.** Protein, ▽, carbohydrate, ⊙, RNA, *, and DNA, △, as a fraction of the dry weight, in g g^{-1}. The curves were calculated with the parameters shown in *table I*.

'rich medium' consists of many compounds which are treated in our calculations as one substrate with constant composition. The compounds in rich medium are lumped because the densities of these compounds were not measured. All data in *figure 1* were fitted simultaneously to estimate the parameters in *table I* under the condition that the elemental balances for carbon, hydrogen, oxygen and nitrogen meet, the condition that the enthalpy balance meets and the condition that the sum of the major macromolecules in biomass equals the dry weight of biomass. The curves in *figures 1 and 2* are calculated with the estimated parameters in *table I*.

The values of the energy conductances, the maintenance coefficient and the cost for growth are in the order of magnitude we normally estimate for bacteria [29]. It is convenient to calculate the yields during the estimation procedure. The yields shown in *figure 2.a* are the production rates of the compounds divided by the uptake rate of glucose, $[d_{mv}]_{X_{01}}\,[l_m]_{X_{01},1}\,f_{X_{01},1}\;X_1$. Note that the yield on oxygen in *figure 2.a* should be exactly zero in anaerobic conditions and that this condition had to be relaxed. The time parameters t_{X_1} and t_{E_1} in *table I* are defined as $[d_{mv}]_{X_1}/([d_{mv}]_{X_{01}}\,[l_m]_{X_{01}})$ and $[d_{me}]_{E_1}/([d_{mv}]_{X_{01}}\,[l_m]_{X_{01}})$. At the bottom of *table I* are shown the ratios of the time parameters for protein *p*, RNA *r*, DNA *d*, carbohydrate *c*, lipid *l*, and the time parameters for structural biomass X_1 or reserves E_1. These ratios are sufficient to characterize the constant fractions of the macromolecules that constitute the structural biomass and the reserves, see Hanegraaf [29].

The macromolecular composition of the reserves and the structural biomass is constant while the macromolecular composition of biomass varies when the relative

Table I. Parameters estimated for *Selenomonas ruminantium* HD 4 in anaerobic continuous culture on rich medium (substrate 2, ($[d_{mv}]_{X_{02}} X_{r2} = 8$ C_1-mmol L^{-1}) plus glucose (substrate 1, ($[d_{mv}]_{X_{01}} X_{r1} = 33$ C_1-mmol L^{-1}) at 39 °C *.

$v_{X_{01,1}} = 0.374$ h^{-1}	$v_{X_{02,1}} = 0.730$ h^{-1} (*)	$\frac{[d_{mv}]_{X_{02}}[I_m]_{X_{02,1}}}{[d_{mv}]_{X_{01}}[I_m]_{X_{01,1}}} = 0.250$ $\frac{C_1\text{-mol}}{C_1\text{-mol}}$ (*)
$m_1 = 0.075$ h^{-1}	$g_1 = 0.110$	
$t_{X_1} = 0.070$	$t_{E_1} = 0.281$	$[d_{mv}]_{X_{01}} K_{X_{01,1}} = 15.37$ C_1-mmol L^{-1} (*)
		$[d_{mv}]_{X_{02}} K_{X_{02,1}} = 120.8$ C_1-mmol L^{-1}
$t_{pX_1}/t_{X_1} = 0.606$	$t_{rX_1}/t_{X_1} = 0.070$	$t_{dX_1}/t_{X_1} = 0.011$
$t_{pE}/t_E = 0.077$	$t_{rE}/t_E = 0.423$	$t_{dE}/t_E = 0$ (*)
$t_{cX_1}/t_{X_1} = 0.265$	$t_{\ell X_1}/t_{X_1} = 0.019$ (*)	
$t_{cE}/t_E = 0.453$	$t_{\ell E}/t_E = 0.019$ (*)	

* The parameter set was estimated at $t_E/t_{X_1} = 4$. Fixed parameters are indicated with (*). All data in *figure 1* were fitted simultaneously and the number of estimated parameters per curve is 2.2 (the parameters for the products are not shown (*table 7.4* [29]). The units of the time parameters of compound * are h (C_1-mol*) (C_1-mol glucose)$^{-1}$.

amounts of reserves and structural biomass vary. The macromolecular composition in combination with the C_1-molar enthalpies of the macromolecules [29, 35] provides the enthalpy of structural biomass, $\tilde{\mu}_{X_1} = -530.5$ kJ C_1-mol^{-1}, and that of the reserves, $\tilde{\mu}_E = -490.6$ kJ C_1-mol^{-1}. In practice, it proved to be impossible to estimate these enthalpies directly from data on heat development. The heat development calculated with the C_1-molar enthalpies of the reserves and the structural biomass and the C_1-molar enthalpies of all other compounds is negative at high growth rates in (*figure 7.7* [29]). Therefore, it was assumed that there are systematic errors in the measurements of the product densities. From the macromolecular composition in combination with the elemental composition of the macromolecules [29, 35], we can calculate the elemental composition of the structural biomass, $C_1H_{1.564}O_{0.487}N_{0.206}$, and that of the reserves, $C_1H_{1.393}O_{0.715}N_{0.181}$, which are needed for the elemental balances.

It appears that the many compounds which constitute an organism can be described with just two components (reserves and structural biomass) with invariant compositions. Changes in the densities of the compounds and other properties of biomass can be described by variations of the density of the reserves relative to that of the structural biomass. The description of the macromolecular composition enables the study of dynamic properties of biomass such as the elemental composition, the enthalpy of biomass and the ATP costs of biomass. Next to the elemental balance and the enthalpy balance, NADH, NADPH and ATP balances can be included.

4. Predator–prey interaction

A predator that consumes a prey will assimilate the multitude of compounds present in the prey biomass. The densities of these compounds may vary relative to each other. Above, it was shown that we gain a realistic descrip-

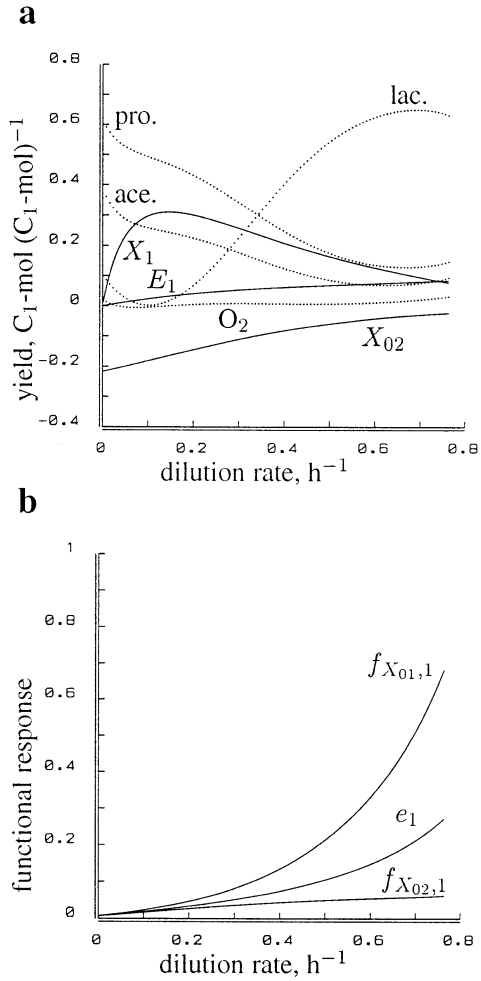

Figure 2. Functional response and yields as functions of the growth rate, for *Selenomonas ruminantium* HD 4 in anaerobic continuous culture on rich medium with glucose.
The functions are calculated with the parameters in *table I*.
a. C_1-molar yield on glucose in C_1-mol (C_1-mol glucose)$^{-1}$ of the reserves, E_1, the structural biomass, X_1, and substrate, X_{02}, (solid curves) and of O_2, propionic acid, acetic acid and lactic acid (dotted curves). The yield of substrate X_{01} (glucose) is –1. **b.** Functional response for glucose, $f_{X_{01,1}}$ and for rich medium, $f_{X_{02,1}}$, and the scaled reserve energy density, e_1.

tion of the composition of biomass when the compounds in biomass are lumped into two components, reserves and structural biomass. The reserves and the structural biomass of the prey can be regarded as two coupled substrates and therefore the derivation of the governing equations is along the same lines as in the previous section.

4.1. Energy and volume of individual predators

The substrate of a predator with volume V_2 is a population of identical organisms that each have volume V_1 and reserve energy volume $e_1\ V_1$. The reserves do not add to the volume of the organisms when it is assumed that the volume of reserves replaces an equal volume of water. The volume density of the prey, X_1, is therefore a measure of the number of organisms per litre, and the functional response for prey is $f_{X_1,2} = X_1 /(K_{X_1,2} + X_1)$.

Prey is ingested via a single uptake mechanism but it is convenient to use the uptake rates of the reserve energy volume and the structural biovolume separately. The rates of uptake of structural biomass and reserve material differ, but are coupled via the equation for W_1 of the prey (9). To find the ingestion rate of structural biomass and reserve of prey, W_1 is converted to structural volume equivalents: $W_1 /[d_{mv}]_{X_1} = X_1 + e_1\ X_1 [d_{me}]_{E_1} /[d_{mv}]_{X_1}$. The maximum ingestion rate of structural volume is $[I_m]_{X_1,2}$ and that of reserve energy volume is $[I_m]_{E_1,2} = [I_m]_{X_1,2}\ [d_{me}]_{E_1} / [d_{mv}]_{X_1}$. Prey is ingested with rate

$$I_{X_1,2} + I_{E_1,2} = [I_m]_{X_1,2}\ f_{X_1,2}\ V_2 + [I_m]_{E_1,2}\ e_1\ f_{X_1,2}\ V_2 = \left(1 + e_1 \frac{[d_{me}]_{E_1}}{[d_{mv}]_{X_1}} \right) [I_m]_{X_1,2}\ f_{X_1,2}\ V_2; \quad f_{X_1,2} = \frac{X_1}{K_{X_1,2} + X_1} \quad (10)$$

Prey is ingested via one transport system, but reserves and structural biomass may be assimilated via two different assimilation pathways with different efficiencies because the compositions of reserves and structural biomass may differ. The maximum assimilation rates for substrates X_1 and E_1 are $[A_m]_{X_1,2}$ and $[A_m]_{E_1,2}$. The assimilation rates for substrates X_1 and E_1 are

$$A_{X_1,2} = I_{X_1,2} \frac{[A_m]_{X_1,2}}{[I_m]_{X_1,2}} = [A_m]_{X_1,2}\ f_{X_1,2}\ V_2;$$

$$A_{E_1,2} = I_{E_1,2} \frac{[A_m]_{E_1,2}}{[I_m]_{E_1,2}} = [A_m]_{E_1,2}\ e_1\ f_{X_1,2}\ V_2 \quad (11)$$

The dynamics of the scaled energy density of the reserves e_2 and the growth rate of an individual with volume V_2 are derived analogous to equations (6) and (7)

$$\frac{\mathrm{d}e_2}{\mathrm{d}t} = (v_{X_1,2} + v_{E_1,2}\ e_1)\ f_{X_1,2} - (v_{X_1,2} + v_{E_1,2})\ e_2 \quad (12)$$

$$\frac{\mathrm{d}V_2}{\mathrm{d}t} = \frac{(v_{X_1,2} + v_{E_1,2})\ e_2 - m_2\ g_2}{e_2 + g_2}\ V_2 \quad (13)$$

4.2. Continuous culture with coupled substrates

A top predator is introduced to continuous culture system **1**, treated in section 3.2, see equations (6) and (8). The top predator preys on the trophic level 1 species, which in turn assimilates two substrates. The dynamics of continuous culture is given by equations (6) and (8a) of system **1**, the constitutive relation in equation (12) and equations (14), and will be referred to as system **2**,

$$\frac{\mathrm{d}X_1}{\mathrm{d}t} = (\mu_1 - D)\ X_1 - [I_m]_{X_1,2}\ f_{X_1,2}\ X_2;$$

$$\mu_1 = \frac{(v_{X_{01},1} + v_{X_{02},1})\ e_1 - m_1\ g_1}{e_1 + g_1} \quad (14a)$$

$$\frac{\mathrm{d}X_2}{\mathrm{d}t} = (\mu_2 - D)\ X_2;\ \mu_2 = \frac{(v_{X_1,2} + v_{E_1,2})\ e_2 - m_2\ g_2}{e_2 + g_2} \quad (14b)$$

In the numerical procedures of the bifurcation analysis presented below, equations for $\mathrm{d}W_1 /\mathrm{d}t$ or $\mathrm{d}X_1 /\mathrm{d}t$ and $\mathrm{d}e_1\ X_1 /\mathrm{d}t$ were used instead of equations (6) and (14a). These sets of equations gave identical numerical results, as expected, but differ in the ease with which bifurcation curves could be continued.

A predator can follow several strategies.

1) The reserves are assimilated with the same efficiency as structural biomass; $v_{X_1,1} = v_{e_1,1}$. One would expect this when the macromolecular composition of the reserves and the structural biomass are identical. In practice, the macromolecular composition of the reserves and the structural biomass can be similar [29] but not equal because the reserves do not contain DNA.

2) The reserves and structural biomass do not have the same composition and are assimilated via different pathways; in general $v_{X_1,1} \neq v_{e_1,1}$.
When the reserve density of the prey fluctuates in an unpredictable way it may be a good strategy to invest little in the assimilation pathway of this substrate, so $v_{e_1,1} < v_{X_1,1}$.
In principle, assimilation of the reserves of the prey can add much to reserve formation of the predator. For example the reserves may contain an efficient source of ATP (e.g. carbohydrates) that can be used to assimilate monomers into macromolecules. This is more efficient than the use of part of the monomers as an ATP source. In this case the strategy may be justified to invest highly in the pathway for the assimilation of the reserves of the prey $v_{e_1,1} > v_{X_1,1}$.

3) The reserves are not assimilated, for example because the reserve density of the prey is very low or the composition of the reserves of the prey is unfavourable.

Bifurcation diagrams for the first and second case are presented below. Bifurcation diagrams for the DEB theory where only the structural volume is assimilated (i.e. item 3 in the enumeration above) are treated in Kooi et al. [26].

4.3. Bifurcation diagrams

Bifurcation analysis gives information about the long-term dynamic behaviour of nonlinear dynamic systems. In the case of food webs the dynamic system consists of

Table II. List of bifurcations, codimension-one curves and codimension-two points.

Bifurcation	Description
	Codim-1 curves
TC	transcritical bifurcation: invasion through boundary equilibrium
T	tangent bifurcation: coalescence of two interior equilibria
	T_- coalescence of a stable and unstable equilibrium
	T_+ coalescence of two unstable equilibria
*H**	Hopf bifurcation: * = – supercritical, * = + subcritical; food chain becomes unstable, origin of stable (supercritical) or unstable (subcritical) limit cycle
	Codim-2 points
M	codimension-two bifurcation point; origin of a tangent bifurcation
L	Bautin bifurcation point; transition from sub- to supercritical Hopf bifurcation
BT	Bogdanov-Takens point, origin of the Hopf bifurcation curve and a global bifurcation curve

(autonomous and nonlinear) differential equations, which model the predator–prey–substrate interactions. These equations determine the time-evolution of the state variables which describe the substrates and populations. The state space has dimension 6: there are two state variables per population (*e* and *V*) and two for the substrates (X_{01} and X_{02}).

Since the equations for continuous culture are bounded (due to conservation laws) their solution stays in a bounded region of the state space. This implies the existence of so-called limit sets, i.e. equilibria, periodic orbits and chaotic attractors. Stability of these limit sets depends on whether the system evolves back to the set after perturbations (i.e. after small changes in the initial conditions). For equilibrium points, for instance, the stability is calculated via linearization of the system and study of the eigenvalues of the so-called Jacobian matrix. This avoids the calculation of the evolution of the system for the infinite number of possible initial perturbations.

In much the same way as we can investigate the stability of an equilibrium point by perturbing the initial conditions, it is possible to inspect the robustness of the system's long-term behaviour by a perturbation of the differential equations via changes in the values of parameters (the so-called bifurcation parameters). The system is said to be structurally stable when for any change of such parameters there is no qualitative change in the long-term behaviour. A qualitative change is for instance the disappearance of the top predator. When the qualitative changes can be characterized near a single point in the state space these bifurcations are called local bifurcations.

Bifurcation analysis studies the structural stability of dynamic systems with respect to the bifurcation parameters [21, 22]. For fixed predator–prey interactions and with fixed vital parameters of the species in the food web, the bifurcation parameters are the environmental parameters. For continuous culture the bifurcation parameters are the dilution rate, *D*, and the density of substrates in the influx, X_{r1} and X_{r2}, which are under control of the experimenter. In *table II* the bifurcation curves and points are listed. Transcritical bifurcation curves mark the boundary of the region where the top predator can permanently coexist with the prey and the substrates. In the region on the other side of the transcritical bifurcation curves the invasion in the food web by the top predator is eventually not successful. Hopf bifurcations mark the boundaries of regions where limit cycles exist.

The bifurcation curves were calculated with LOCBIF [22, 36, 37]. This computer program is an implementation of a continuation technique that facilitates the study of the change of the position of the bifurcation points due to a change of bifurcation parameters. Starting at an equilibrium point, one bifurcation parameter is changed and one point of the bifurcation curve can be found by checking for conditions which are indicative of structural instability with test functions. Starting at this point, the bifurcation curve is continued while test functions which are indicative of higher codimension bifurcation points are checked. To continue Hopf bifurcation curves the test functions evaluate the eigenvalues of the Jacobian in equilibrium points for a zero real part of two conjugate eigenvalues. To continue transcritical bifurcation curves the test functions check if there is one real zero eigenvalue while the abundance of the top predator is zero. The resulting bifurcation diagrams summarize the asymptotic (long-term) behaviour of the top predator in relation to the other trophic levels in the system.

Figure 3.a, b, c gives the bifurcation diagrams for $v_{E_{1,2}} = v_{X_{1,2}} = 0.171\ \mathrm{h}^{-1}$, $v_{E_{1,2}} = 0.051\ \mathrm{h}^{-1}$ and $v_{X_{1,2}} = 0.290\ \mathrm{h}^{-1}$ and $v_{E_{1,2}} = 0.290\ \mathrm{h}^{-1}$ and $v_{X_{1,2}} = 0.051\ \mathrm{h}^{-1}$, respectively (*table III* gives parameter values). For the sake of simplicity we do not vary X_{r1} and X_{r2} independently, but scale X_{r1} and X_{r2} simultaneously with a factor α, which equals one for the values of X_{r1} and X_{r2} in *table I*. The bifurcation parameters are therefore *D* and α, and the bifurcation parameter space has dimension 2. The (estimated) vital parameter values for the prey and the vital parameter values we choose for the top predator are shown in *tables I* and *II*. Since the sum of the energy conductances is constant, $v_{X_{1,2}} + v_{E_{1,2}} = 0.341\ \mathrm{h}^{-1}$, the value of the maximum growth rate of the top predator is $\mu_{m,2} = 0.3\ \mathrm{h}^{-1}$ in all diagrams. The symbols in the figures are explained in *table IV*.

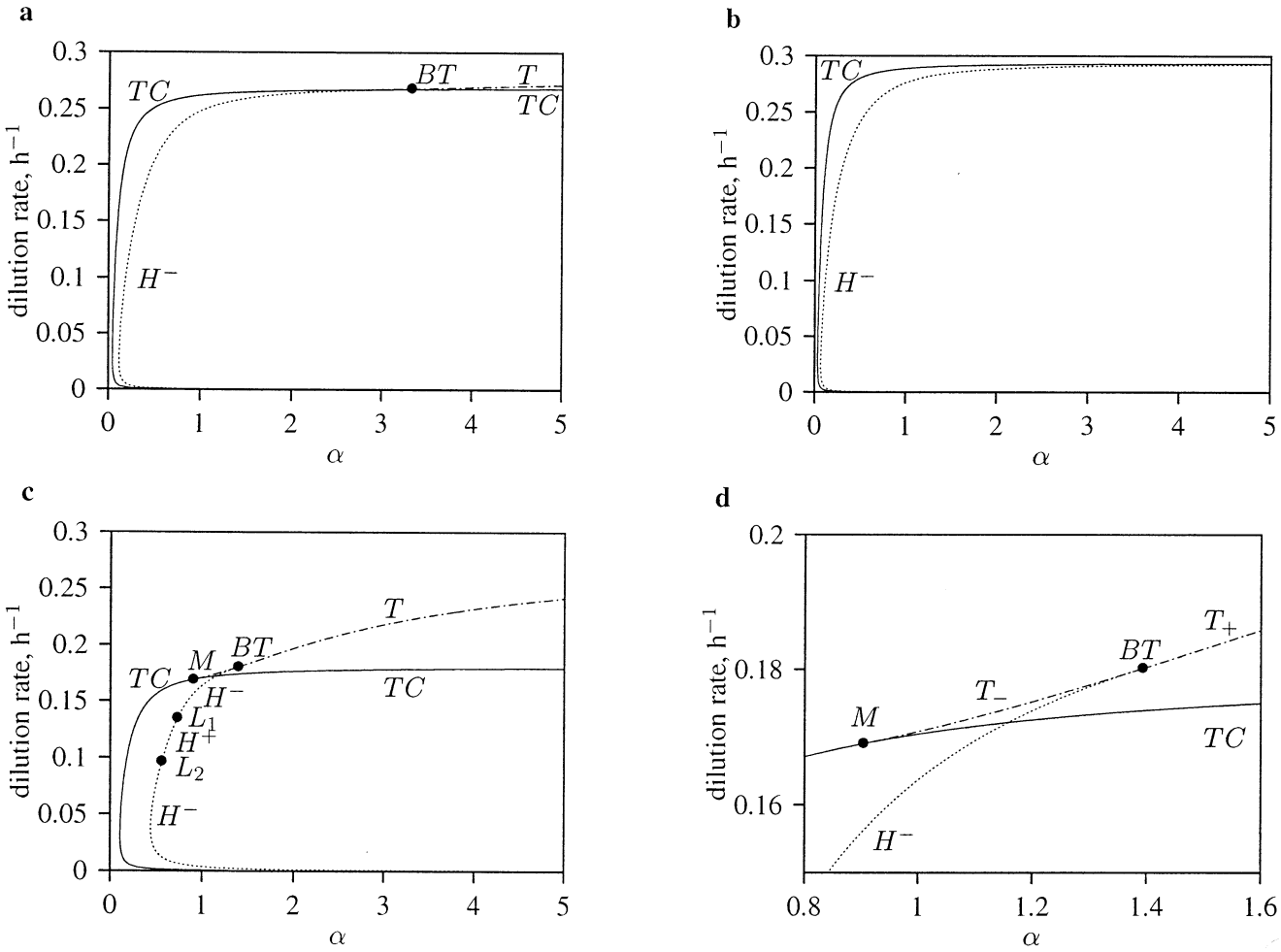

Figure 3. Bifurcation diagrams for the top predator of system **2** (6, 8a, 12, 14).
The bifurcation parameters are the dilution rate and the factor α, where α scales both substrate reservoir densities X_{r1} and X_{r2} simultaneously (α equals one for the values of the reservoir densities shown in *table I*). The maximum growth rate of the predator is $\mu_{m,2} = 0.3$ h^{-1}.
The parameter values for the prey are shown in *table I*.
Apart from the values shown in *table III* the parameter values chosen for the top predator are: **a.** $v_{E_1,2} = v_{X_1,2} = 0.171$ h^{-1}; **b.** $v_{E_1,2} = 0.051$ h^{-1} and $v_{X_1,2} = 0.290$ h^{-1}; **c.** and **d.** $v_{E_1,2} = 0.290$ h^{-1} and $v_{X_1,2} = 0.051$ h^{-1}. In **d.** a detail of **c.** is depicted. The symbols in the figures are explained in *table IV*.

Three regions of interest are found in all bifurcation diagrams. A region where a stable limit cycle exists is present to the right of the Hopf bifurcation curve, *H**. A region where stable equilibria are reached lies between the transcritical bifurcation curve, *TC*, and *H**. A region where the top predator goes to extinction is situated above and to the left-hand side of *TC*. In *figure 3.a, c* a tangent bifurcation curve *T* is present above part of *TC*. Between the tangent bifurcation curve and the transcritical bifurcation curve the top predator does not go to extinction but there are two (interior) steady state solutions where the biovolume of the top predator is positive. At the tangent bifurcation curve these two positive solutions merge.

Codimension-two points are present on the bifurcation curves. Point *BT* is a Bogdanov-Takens point. *Figure 3.d* shows a detail of *3.c* around this point. A subcritical Hopf bifurcation curve H^- originates in this codimension-two bifurcation point. It is well-known that the Bogdanov-Takens point is also the origin of a global homoclinic bifurcation to a saddle equilibrium. This curve lies

Table III. Parameter values chosen for the top predator in system **2** (6, 8a, 12, 14) to calculate the bifurcation curves in *figure 3* *.

$v_{X_1,2} + v_{E_1,2} = 0.341$ h^{-1}	$m_2 = m_1 = 0.075$ h^{-1}	$g_2 = g_1 = 0.110$
$\dfrac{[d_{mv}]_{X_1}[I_m]_{X_1,2}}{[d_{mv}]_{X_{01}}[I_m]_{X_{01},1}} = 0.07\ \dfrac{\text{C}_1\text{-}mol}{\text{C}_1\text{-}mol}$	$\dfrac{[d_{me}]_{E_1}[I_m]_{E_1,2}}{[d_{mv}]_{X_{01}}[I_m]_{X_{01},1}} = 0.28\ \dfrac{\text{C}_1\text{-}mol}{\text{C}_1\text{-}mol}$	$[d_{mv}]_{X_1} K_{X_1,2} = 15$

* The value of the maximum growth rate of the predator is $\mu_{m,2} = 0.3$ h^{-1}. The units of $[d_{mv}]_{X_1} K_{X_1,2}$ are C$_1$-mmol L^{-1}.

Table IV. List of symbols (adapted from [1]) *.

Symbol	Dimension	Interpretation
$A_{*i-1,i}$	$e t^{-1}\, \ell_c^{-3}$	Assimilation rate of substrate $*i-1$ for trophic level i (2)
$[A_m]_{*i-1,i}$	$e \ell_i^{-3}\, t^{-1}$	Volume-specific maximum assimilation rate of substrate $*i-1$ (2)
α	–	Scaling factor for reservoir substrate densities X_{r1} and X_{r2}
$[d_{me}]_*$	$\# \ell_*^{-3}$	Conversion coefficient: C_1-moles of compound * per energy density volume eV of * (9)
$[d_{mv}]_*$	$\# \ell_*^{-3}$	Conversion coefficient: C_1-moles of compound * per volume of * (9)
e_i	–	Scaled energy density (of the reserves): $[E]_i / [E_m]_i$
$[E_i]$	$e \ell_i^{-3}$	Energy density, energy in the reserves per structural volume: E_i / V_i
$[E_m]_i$	$e \ell_i^{-3}$	Maximum energy density
$f_{X_{i-1,i}}$	–	Scaled hyperbolic functional response to substrate X_{i-1} for tropic level i: $X_{i-1} / (K_{X_{i-1,i}} + X_{i-1})$
g_i	–	Energy investment ratio, scaled costs for growth: $[G]_i / [E_m]_i$
G_i	$e t^{-1}\, \ell_c^{-3}$	Energy investment rate, energy rate to growth (4)
$[G]_i$	$e \ell_i^{-3}$	Volume-specific costs for growth (4)
h_*	$e \#^{-1}$	Partial molar enthalpy per C_1-mole of compound *
$I_{*i-1,i}$	$\ell_{i-1}^3\, t^{-1}\, \ell_c^{-3}$	Ingestion (uptake) rate of substrate $*i-1$ for trophic level i (1)
$[I_m]_{*i-1,i}$	$\ell_{i-1}^3\, \ell_i^{-3}\, t^{-1}$	Volume-specific maximum ingestion rate of substrate $*i-1$ (1)
$K_{X_{i-1,i}}$	$\ell_{i-1}^3\, \ell_c^{-3}$	Saturation coefficient of substrate X_{i-1} for trophic level i (1)
m_i	t^{-1}	Maintenance rate coefficient: $[M]_i / [G]_i$
M_i	$e t^{-1}\, \ell_c^{-3}$	Maintenance rate, energy rate to maintenance (4)
$[M]_i$	$e v_i^{-3}\, t^{-1}$	Volume-specific maintenance rate (4)
t	t	Time
t_*	$t \#\#^{-1}$	Time parameter for compound *: $[d_{mv}]_* / ([d_{mv}]_{X_{01}} [I_m]_{X_{01},1})$ or $[d_{me}]_* / ([d_{mv}]_{X_{01}} [I_m]_{X_{01},1})$
V_i	ℓ_i^3	Structural volume
$v_{*i-1,i}$	t^{-1}	Specific energy conductance of substrate $*i-1$: $[A_m]_{*i-1,i} / [E_m]_i$
W_i	$\# \ell_c^{-3}$	Biomass density of the population on the basis of C_1-moles (9)
X_0	$\ell_0^3\, \ell_c^{-3}$	Volume density of substrate
X_i	$\ell_i^3\, \ell_c^{-3}$	Structural volume density of the population
X_r	$\ell_0^3\, \ell_c^{-3}$	Substrate density in continuous culture medium reservoir
μ_i	t^{-1}	Specific growth rate of the population (8b), $\mu_{m,i}$ maximum of μ_i

* Parameters and state variables. Subindex i denotes the trophic level; subindex $*i-1$, i denotes substrate $*i-1$ ingested by trophic level i. The following dimension symbols stand for an abbreviation of the dimension, and differ in meaning from symbols in the symbol column: dimensionless –, number #, time t, energy e, temperature T, mass m, length of environment ℓ_c, length of substrate ℓ_0, length of individual ℓ_i (where $\left(\ell_* = \sqrt[3]{\text{volume of } *} \right)$.

between the tangent J_j^n and the Hopf H^- bifurcation curve. As we move around the BT point the stable limit cycle born through the Hopf bifurcation 'grows' and approaches the saddle equilibrium turning into a homoclinic orbit [22]. A complete description of the dynamics around this point is beyond the scope of this paper, we refer to Kuznetsov [22]. At the so-called Bautin (or generalized Hopf) codimension-two bifurcation points L the Hopf bifurcation curve transits from supercritical H^+ to subcritical H^-. We refer to Kuznetsov [22] for a description of this point. There is a tangent bifurcation curve for interior limit cycles which connects the Bautin bifurcation points L_1 and L_2. This tangent bifurcation curve is not shown in the figures.

In system **1**, equations (6) and (8), the predator in trophic level 2 is not present, and the species in trophic level 1 is the top predator. The maximum growth rate (or maximum dilution rate) for which a steady state can be reached can be calculated analytically:

$$\mu_{m,1} = \frac{(v_{X_{01},1} + v_{X_{02},1})(\beta - m_1 g_1)}{\beta + (v_{X_{01},1} + v_{X_{02},1}) g_1};$$

$$\beta = \frac{v_{X_{01},1} X_{r1}}{X_{r1} + K_{X_{01},1}} + \frac{v_{X_{02},1} X_{r2}}{X_{r2} + K_{X_{02},1}} \quad (15)$$

With the values of X_{r1} and X_{r2} in *table I* we obtain $\mu_{m,1} = 0.764\ h^{-1}$. The transcritical bifurcation curve for system **1**, which marks the boundary of the region where the population can permanently coexist with the substrates, can be calculated with equation (15). These curves are not shown in *figure 3*. In a batch culture there is no depletion due to wash-out and it is assumed that the substates are unlimited available. Then $Xr >> K$, and the maximum growth rate (equation (8b) with $e = 1$) is higher

than that in continuous culture in steady state, because in steady state we have $e < 1$, see *figure 2.b*. In system **2**, the prey can only approximate its maximum growth rate because it is subject to predation besides wash-out for the reactor.

5. Discussion and conclusions

Assumptions in the DEB theory are on the level of the individual because the individual decides how it reacts to its environment even if it is part of a population. Our goals include the study of higher trophic levels, populations, food chains and food webs as well as the study of details on the intracellular level. In this study and in Hanegraaf [29] it is shown that the intracellular composition of micro-organisms can be modelled with the DEB theory and that it allows a detailed analysis of measurements on populations. The macromolecular composition of biomass can be modelled with the two components in the DEB model; reserves and structural biomass. We also showed that the composition of prey influences higher trophic levels.

The parameter set we used for bifurcation analysis is consistent with continuous culture data on the elemental composition and macromolecular composition of biomass, heat development, substrate utilization, biomass and product formation. During parameter estimation the conditions were satisfied that the elemental balances meet, that the enthalpy balance meets and that the sum of the major macromolecules in biomass equals the dry weight of biomass. The parameter set we used for bifurcation analysis is therefore realistic. Modelling of the molecular composition of biomass allows the construction of ATP and NADPH/NADH balances. The balances mentioned in this paragraph can be of use in bifurcation analysis of food-chains. Calculations of trajectories in state space are subject to the restriction that balances have to meet, and therefore the long-term dynamic behaviour and the resulting asymptotic behaviour are restricted by balances.

A consistency criterion formulated in Arditi and Michalski [38] requires that: equations must be invariant under identification of identical species. This property of invariance must be fulfilled by any model used to describe food web dynamics [38], and therefore also for system **1** (6, 8) and system **2** (6, 8a, 12, 14) in the case of identical substrates. Two substrates are identical when all of their properties are identical. In our model substrates have properties and not just different indexes. In system **1** these properties include the molecular formulas and the partial molar enthalpies and in system **2**, in the case that a prey is the substrate, these properties also include the macromolecular composition and the dynamics of formation and utilization. Even when all properties of the substrates are identical, so there is actually one substrate, the predator can decide to ingest the substrate via different carrier systems and/or assimilate it via different assimilation pathways. Such behaviour is not uncommon for micro-organisms: respiro-fermentative bacteria or yeasts assimilate glucose via oxidative and/or fermentative pathways, depending on the growth rate. In [29] it is shown that the model for multiple carriers and multiple pathways describes measurements on a large variety of respiro-fermentative yeasts and bacteria adequately. In [29] also a one-substrate model is tested to data. When a suitable substrate is introduced to a culture of micro-organisms, the micro-organisms will synthesize the appropriate carrier enzymes and the enzymes for the assimilation pathway. Modelling the synthesis of these enzymes involves modulations of the maximum uptake rate and the maximum assimilation rate and can bring about continuous transitions of the models mentioned in this paragraph. Modulations of $[I]_m$ and $[A]_m$ to incolporate adaptation processes are beyond the scope of this paper because of lack of a general theory.

In previous papers we studied bi-trophic food chains with a single substrate at the base [23–26]. The bifurcation diagram appeared similar to that of the so-called Rosenzweig-MacArthur predator–prey model which is often used to describe the dynamics of ecosystems [26, 39, 40]. The substrate–prey–predator can coexist in a stable equilibrium or as a stable limit cycle, a phenomenon associated with the paradox of enrichment [7]. In Kooi et al. [26] it was shown that the introduction of maintenance is important at small values of the dilution rate where it causes the codimension-one curves to approach the X_r-axis asymptotically. The codimension-one curves in *figure 3* approach the α-axis, where α is a factor that scales the reservoir concentrations of both substrates simultaneously. In Kooi et al. [26] it was shown that the introduction of reserves influences the dynamic behaviour at large dilution rates and yields a smaller growth rate than the Marr-Pirt model. The reserves dampen the stochastic effects of small numbers of prey, although this is not apparent from bifurcation diagrams of continuous models. *Figure 3* shows that enrichment of the food chain, by increasing the reservoir densities of substrate, has the result that the top predator almost reaches its maximum growth rate $\mu_{m,2} = 0.3\ \mathrm{h}^{-1}$. The top predator (figure 6 in Kooi et al. [26]) does not assimilate the reserves of prey and does not reach its maximum growth rate, i.e. $0.048\ \mathrm{h}^{-1}$ (equation (11) in [26]). The results obtained in this paper show that the presence of an extra substrate yields more complex dynamic behaviour, including homoclinic orbits. These homoclinic orbits were also obtained for the Bazykin's predator–prey system which adds a quadratic death term for the predator to the Rosenzweig-MacArthur equations [22].

This paper supports the proposition that the dynamics of substrate(s) should be modelled explicitly, as argued before [23, 25]. This paper also shows the merits of modelling the fate of the substrate after it has entered the cell, i.e. modelling the assimilation pathways. It is con-

cluded that modelling the varying macromolecular composition of biomass, which is essential for the application of several balances, can be done in such a way that the resulting model is not too complicated to be of use in the study of food webs.

Acknowledgements: This work was supported by grant number 805.39.751 of NWO-STW. We thank P. Auger for his translation of the abstract and abridged version into French, and F. Kelpin, M. Luger and P. Doucet for helpful discussions and technical support.

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