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RESEARCH ON THE PHYSIOLOGICAL BASIS OF POPULATION DYNAMICS IN RELATION TO ECOTOXICOLOGY

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RESEARCH ON THE PHYSIOLOGICAL BASIS OF POPULATION DYNAMICS IN RELATION TO ECOTOXICOLOGY

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SUMMARY

One of the main obstacles that hampers ecotoxicology is the poor insight into the relationship between physiological and population dynamics. The role of laboratory experiments, modeling, mathematical analysis and computer simulation studies is discussed in research aiming at this relation. Energy and nutrient budgets of organisms are found to be of vital importance. This paper evaluates the progress that has been made in concrete efforts to work out energy and nutrient budgets for simple freshwater plankton systems stressed by toxic chemicals with different modes of action.

KEYWORDS

Energy & nutrient cycles; Individual vs population behaviour; Mode of action.

INTRODUCTION

This paper critically surveys the aims, principles and outlooks in fundamental research on ecotoxicology. It is based on seven years of experience with a multi-disciplinary program within the Netherlands Organization for applied scientific Research TNO (MT-TNO) in collaboration with the Free University of Amsterdam (VUA) and, for some specific aspects, with other scientific institutions, in particular with the Institute for Theoretical Biology of the University of Leiden, the Centre for Mathematics and Computer Science in Amsterdam and the Institute for Microbiology and Plantphysiology of the University of Bergen (Norway).
The research evaluated was financially supported by the Ministry of Housing, Physical Planning and Environment, The Ministry of Transport and Public Works and Shell International Research B.V. The results mentioned in this paper are included or will be included in more technically inclined papers presented elsewhere (see references), together with their full scientific responsibilities.

AIM

Hazard evaluations and risk assesments of pollution events include a wide variety of economic, social, medical and environmental aspects. When put into quantitative terms, these amount to a definition of a loss function that, among other things, weights the difference between blank and stressed behaviour of ecosystems. Elements of this behaviour should include the probability that any population considered goes extinct and the vulnerability of populations towards harvesting and changes in the environment that are not (completely) human-controlled.

Owing to the lack of an appropriate frame of reference, risk assessments do generally not include these types of behavioural elements. Most are still based on LC50 values derived from one-species toxicity tests, to which some safety factor is applied (see Kooijman, 1987a, for an attempt to include at least some biology in such safety factors). During the last decade, many multi-species toxicity tests have been developed to supplement results routinely obtained from one-species tests (see Cairns, 1985, for an evaluation). Our aim is not to propose new multi-species toxicity tests, but to contribute to the development of ecotoxicology as a part of science.

In an attempt to separate the scientific part in hazard evaluations from other aspects, we see rather a restricted principal task for ecotoxicology, namely to give a description of the effects or the expected effects of a xenobiotic if released into the environment. Such a description can be split quite naturally into two parts:

The first part is a description of the (geo)physical transport of the xenobiotic compound through the environment and of its chemical transformations. These transformations involve degradation processes like oxidation, photo-degradation, microbial decomposition, speciation etc.

The second part is a description of the ecological effects given time and space inhomogeneous concentrations of the xenobiotic (and its metabolites).

If we are dealing with a temporary and local pollution event involving a mineralizing organic pollutant, the first part seems to be the most important. In that case the environment will eventually recover completely. If we are not dealing with such a pollution event, but with one that involves pollutants that do not mineralize and/or the pollution is not local or temporary, the second part also becomes important. The environment may not completely recover in this case, so one should consider actions to reduce possible effects. Adequate methods are now available for the description of transportation and transformation processes (see e.g. Thomann & Mueller, 1986). This does not hold for the ecological effects. Even in blank situations little is known about the dynamical behaviour of ecosystems, because such knowledge should rest on theories on population dynamics. Existing theories on population dynamics do hardly account for properties of individuals however. They seek explanations for population dynamics at population level itself, see e.g. Emlen, 1984. This deficit is especially felt in ecotoxicology because toxic agents operate on individuals, not on populations directly.

There are in fact only three ecologically relevant effects on individuals, those on survival probability, on reproduction rate and timing and on the genome. Almost all other sublethal effects of xenobiotics however eventually show up as effects on reproduction, due to the preservation law for energy (see further on).

Therefore present-day ecology provides too weak a basis to support ecotoxicology.

Our work aims at development of a conceptual frame-work that can accommodate the description of ecological effects of toxic chemicals. This introduction describes some of the ideas and principles we used and developed to make some steps towards this aim.

ECOSYSTEMS

The core of an ecosystem, be it terrestrial or aquatic, consists of producers and consumers. See Fig. 1. So there are two types of cycles, the (open) energy cycle and the (partly closed) nutrient cycles.

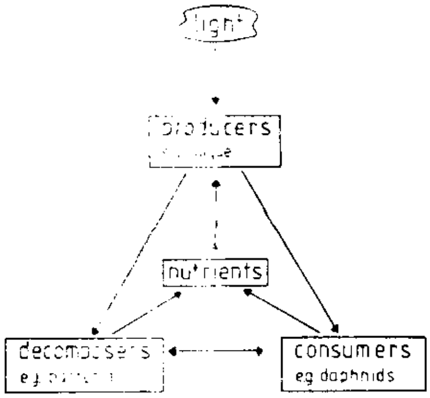

Fig. 1. The core of a ecosystem, in which there exists an energy cycle and nutrient cycles.

Energy is usually caught in as light (by plants including algae) or derived from minerals (see Stouthamer, 1986). It is stored as lipids, carbohydrates and proteins (which partially serve also as nutrient stores). Nutrients are defined as chemicals required by the organism that it is unable the synthezise itself.

In the consumers, the two types of cycles are rather tightly coupled (eg. It is possible to grow daphnids on algae only. These algae contain the required energy as well as the nutrients). In the producers, the cycles are uncoupled, i.e. the organisms have to obtain energy and nutrients separately. If one of them falls short, they cannot grow (see e.g. Droop et al, 1982). The decomposers, i.e. bacteria and fungi, have in this respect, a position between the producers and the consumers (see Bosatta & Berendse, 1984, Bosatta & Agren, 1985, for recent model formulations). An ecosystem can, in principle, do without consumers but not without producers or decomposers.

This is a somewhat idealised schematic representation of ecosystems. In real-world ecosystems, the microbial decomposers are assisted in their function of liberating nutrients from their organic matrix by a variety of species of animals that are not specialized to do this. There also exist different types and strengths of linkages (eg. corals and lichens) that are hard to classify into one of the three categories.

So far, this view of ecosystems is widely accepted among ecologists. Disagreement comes to the surface when the factors are considered that actually control the rate of processes within cycles. Attention has concentrated too much on the consumers, which consist of a bewildering variety of species feeding on producers, decomposers and on each other. Although the abundance of consumers is limited by their food supply, the limitation may be operative only during a short period of the year or life-span of the organism. This is due to fluctuations in food supply, induced by fluctuations of chemical and physical controls of the system. Spatial inhomogenities of organism numbers and food availability, likewise, complicate the analysis of population dynamics enormously. All habitats are patchy and organisms migrate from one patch to another. Although ecosystems as a whole are stable (i.e. develop slowly), patches themselves can be highly unstable.

In view of the emphasis on the analysis of stability in models for population dynamics, a principle in present-day model building seems to be that a model is only an acceptable model if it is stable. This assumption is not biologically accurate.

Many ecological textbooks present a rather static view of ecosystems. The systems are assumed to have a constant mean composition, the so-called structure, around which there is some variation (which might be considerable). This leads to a concept of ecosystem function based on rates of processes. If we conceive of ecoxystems in a more dynamic way, by emphasizing continuous structure change, the function is more difficult to define because it is tightly linked to the dynamics of the structure. If function stands for a lumped variable like total oxygen production or consumption, the frequently cited statement that structure is more sensitive to pollution than function is self-evident.

STRATEGY

The basic philosophy behind our research is schematically given in table 1.

TABLE 1 Research strategy.

		literature study	experiments	modeling	mathematical analysis	computer simulation
1. Stationary states						
1.1	Blank situation					
	- Give descriptions of budgets for individuals (energy, nutrient).	x	x	x		
	- Derive properties of population dynamics in simple environments.		x		x	x
	- Simplify the models by stripping the details.			x	x	
	- Integrate models into models for ecosystems (competition, predation)				x	x
1.2	Stressed situation					
	- Identify mode of action.	x	x			
	- Describe effects quantitatively at the level of the individual.		x	x		
	- Evaluate effects on pop. dynamics.		x		x	x
	- Evaluate effects on ecosystems.				x	x
2. Transient states						
	- Adaptation & selection for resistance.					
	- Recolonisation by migration.					

Although the transient states of adaptation and selection for resistance towards the xenobiotic and of recovery by immigration from unpolluted areas are crucial for ecotoxicology, their significance will not be understood as long as we do not understand the stationary states. Therefore we decided first to consider stationary states, in which the xenobiotic has a constant concentration in time and space, such that the population does not become extinct within a few generations (i.e. the xenobiotic is sublethal for the population). We also chose to start with very simple environments to localize the more persistent problems in the analysis of population dynamics.

The aim of the project is to produce models that have parameters that can be interpreted physiologically. This is important because the xenobiotic changes the physiology of the organisms. This change shows up as a change in one or more parameters of the model that describes the physiology of the individual in the blank situation. (This assumption is justified because we are considering stationary situations. In transient states we need additional state variables like the tissue concentration of the toxicant to model accumulation and effects. It has been shown in Zonneveld & Kooijman (1987) that the effects of light regime and parasites on the energy budget of the host could also be described as an effect on parameters. This circumstance greatly simplifies the evaluation of the ecological implications of changes in energy budgets.) Because the parameters of the model that describe the population dynamics are functions of the individual-model, the effect of the xenobiotic can be readily evaluated at the level of the population, given its effects on individuals.

It is important to realize that, from a mathematical point of view, the individual-model completely specifies the population dynamics and the set of models that do so for each species present in the ecosystem completely specifies the behaviour of the ecosystem. Therefore due attention has been given to individual-models. So, given the set of individual-models, we in fact have an ecosystem-model as well. The problem lies in extracting useful information from such a model. The methodology behind it is the kernel of the present project and will be discussed at several places below.

The mathematics that is involved in relating the individual-model to the population level are inadequate to meet the needs of ecologists. Not only are there no ready-to-use results available, but the frame-work within which such results have to be found is completely lacking. Because mathematical analysis is vital in this respect, we encouraged mathematicians to develop such a frame-work and to get scientists with similar needs involved. As a result an intensive cooperation was developed with institutes in several countries and a book was written that presents the state of the art (Metz & Diekmann, 1986).

We developed computer software to simulate population dynamics in order to study phenomena at population level. We do not see this type of study as an alternative for mathematical analysis, but as a useful additional source of information. An important reason is that in computer simulation studies it is hard to determine to what extent the results depend on the specific features of the model and on the values of the parameters that have been chosen. Mathematicians usually keep their analyses as qualitative as possible and limit reliance on specific model formulations. We also stimulated research on methods to improve the efficiency of algorithms for the simulation of population dynamics. As a result, de Roos (University of Leiden, see e.g. de Roos, 1987) is now working on these methods, in the general case that the individual-model is not yet specified.

After developing useful characterizations of population dynamics, our strategy is that one should first strip details stepwise from the population-models, before integrating them into a model for ecosystem dynamics. Models with more than, say, ten parameters rarely contribute much to our understanding of the dynamics at a higher level of integration and therefore have little predictive power. The stripping is not expected to be uniquely defined. One should do it in different ways for different purposes. It would be unwise to produce the stripped version as an initial modelling exercise because in that case it will not be obvious how the parameters relate to the individual level (precisely for this reason existing population dynamical theories are of little help) and it will not be obvious how to relate different stripped models, which might all have their own relevance.

We recognize that it is useless to wonder whether a model is right or wrong, because in the end all models are wrong. One should wonder if a model is useful or not. Because the problems in ecotoxicology are often more concrete than in ecology, ecotoxicology can have quite an impact on ecology. Another argument not to model population dynamics directly is that it tends to be much more erratic than the physiological behaviour of individuals. This complicates the choice of appropriate assumptions at this level. The process of stepwise stripping of details is related to the concept of the hierarchical structure of ecosystems, as has been discussed recently by O'Neill et al (1986). We think however that one should work top down as well as bottom up in order to understand ecosystem dynamics.

Apart from the fact that hazard evaluations inevitably depend on model formulations, the attempt to build models in combination with an experimental program has shown to be a very powerful combination. These attempts not only have a strong organizing effect on the type of experiments to be done, but they also reveal that experimental findings can be interpreted in other ways. (Oxygen consumption rate, for instance, is usually interpreted as a measure of routine metabolic rate. On the basis of the model formulations it appeared however that energy expenditure on growth might not be negligible.) This is possible because the abstract concepts we have in mind rarely allow for direct measurement. These two aspects of model building are perhaps the most useful to science. We also had the experience that model building can be valuable for mathematics. Some of the mathematical analyses developed so far proved to be less general than they were held to be before the modelling effort. So, there is a stimulating effect towards mathematics in choosing proper topologies and in generating new collections of mathematical problems. In this respect, there is two-way rather than one-way traffic. (Mathematicians involved in the collaboration do not see themselves as servants of science.)

We recognize that this research strategy is very ambitious and that important progress may only be expected in the long term. We do believe that ecology needs a stronger foundation that has to be found in what is called physiological ecology (rather than in pressing further on unstructured populations, see e.g. Ginzburg, 1986). The idea is not to model ecosystem dynamics in detail, because it involves too many factors, but to evaluate as detailed as possible what might be expected on the basis of first principles and exploiting all the constraints set by chemistry and physics on ecosystem dynamics. In comparing actual with expected behaviour, we will come to appreciate the immense variety of subtle relations nature reluctantly reveals. We do not think that it is very helpful to start with these relations in attempts to understand ecosystem dynamics, because this will only lead to the conclusion that ecosystems are so complex that they cannot be understood.

EXPERIMENTAL BASIS

In order to develop concepts that are useful to characterize xenobiotically induced changes in the dynamics of an ecosystem, we selected, as a start, the fresh water pelagic habitat to study in more detail. The reasons were that here temporal and spatial fluctuations are relatively less pronounced, and that this type of habitat is relatively easy to mimic in the laboratory. Test species were selected on the basis of various criteria. It must be easy to culture them in stress-free conditions during the whole life cycle, the generation time should not be too long, because this complicates population dynamical studies, nor too short, because this goes with small body size, which impedes observations at the individual level. Lastly they must be representative and toxicity data concerning them must be available. The OECD guidelines for testing the aquatic toxicity of compounds prescribe the green algae *Selenastrum capricornutum* and *Scenedesmus subspicatus*, the micro-crustacean *Daphnia magna*, and one or more fish species (OECD, 1981).

Partly inspired by the work of Slobodkin (1962) and the extensive experience accumulated within TNO (see TNO, 1980), we selected the waterflea *Daphnia magna* as the main zooplankton test organism. (It matures in 7 days and has an expected life span of some 60 days at 20°C). Because body size is a key factor that is closely linked with a wide variety of physiological variables, we also chose the smaller daphnid *Ceriodaphnia reticulata* (whose maximum body length is 1 mm as opposed to 5.5 mm for *Daphnia magna*). Although this species has been proposed as a test animal in toxicity testing, much less toxicological information is available as compared to *Daphnia magna* (Norberg & Mount, 1985). Various species of algae were selected, viz. the green algae *Chlorella pyrenoidosa* (the usual food species of home-bred daphnids, see OECD, 1981), *Selenastrum capricornutum* and *Scenedesmus subspicatus* (the standard species used in toxicity tests with algae, see OECD, 1984), *Chlamydomonas reinhardii* (which is readily used in studies with synchronous cultures, see Lien & Knutsen, 1974), and the cyanobacterium *Microcystis aeruginosa* (which, unlike the green algae, is not eaten by daphnids). Although being a pest in Holland, the latter species has been found to be very sensitive to most chemicals (Slooff & Canton, 1983). No attempt was made to identify the other bacterial species which happen to live in and on daphnids and in their environment. (Daphnids, like almost all other animals require a healthy gut flora to digest the cellulose of the cell walls of the algae. One of the hard experimental problems has been to determine the nutritional value of the bacteria in the water for the daphnids.)

The following xenobiotic compounds were used to perturbate the physiological specifications of the individuals : cadmium chloride, potassium dichromate, sodium metavanadate, 3,4-dichloroaniline, 2,4-dinitrophenol and tetrapropylenebenzenesulphonate (TPBS).(The use of the word *perturbate* acknowledges that ecotoxicology should lead to better ecological insight besides providing a basis for hazard evaluations.) These xenobiotics were selected because of their different modes of action. Cadmium is one of the most studied heavy metals in ecotoxicology, and a broad range of effects have been described. These include effects on energy metabolism and on membrane functions (Giesy et al 1983, Ahlers & Rosick, 1986). Chromate is well known for its mutagenic activity (Flora et al, 1984). In algae it affects the population growth rate (Kooijman et al, 1983) and in daphnids the survival probability (Kooijman, 1985). Vanadium interacts with DNA (Sabbioni et al, 1983), and it has been shown to affect routine metabolism and reproduction in daphnids (Kooijman, 1985). The ecotoxicological effects of 3,4-dichloroaniline have been studied extensively by TNO (Adema & Vink, 1981, Adema et al, 1983, Hooftman & Vink, 1980, Kuiper & Hanstveit, 1984). It reacts with organic material, especially with that containing amino groups (Still & Herrett, 1976) and affects reproduction in daphnids (Kooijman, 1985). In mesocosms, this chemical has been found to be less toxic than could be expected on the basis of single species tests. 2,4-Dinitrophenol is the classical uncoupler of oxidative phosphorylation and inhibits photosystem I in chloroplasts (Corbett, 1974). TPBS is a detergent which affects cell membranes unspecifically (e.g. fish gills, Abel, 1974). Its maximum non-effective concentration has been found to be almost the same for a wide range of test species (Adema et al, 1983).

For the purpose of formulation and testing of assumptions of the individual-model and for determining the mode of action of the compounds, most experiments have been done with individuals as opposed to populations. The type of measurements we made on individual daphnids were: ingestion rate, gut residence time, oxygen consumption rates, length increase, reproduction rate and timing and, for cohorts, survival probability. Modifying experimentally controlled variables were temperature (20°C as a standard), food density and concentration of toxic compound. (Food density has proved to be a key factor determining all the variables measured.) For cohorts of algae whose cell cycles had been synchronized (see Norland et al, 1978 and Lien & Knutsen, 1979), we measured cellular volume increase, onset of cell and nucleus division (with a technique described in Porter and Feig, 1980), chlorophyll content, number of daughter cells (*Chlamydomonas* does not usually divide into more than two daughter cells), and mortality (with a technique described in Crippen & Perrier, 1974). The synchronization is obtained by a light-dark regime under optimal conditions of nutrient, light intensity and temperature. Here the chemical composition of the media rather than the food density was a modifying variable.

Because experiments with animal populations are very laborious, while their results tend to be rather erratic, studies of effects of xenobiotics on zooplankton populations are relatively scarce. For examples of such studies see Halbach et al (1983), Kersting (1984), van Leeuwen et al (1985, 1986, 1987). Studies of pollutant effects on freshwater plankton communities are, on the contrary, rather common, as exemplified by the work of Woltering (1983). Up until now, however they have brought little quantitative insight into the longterm effects of pollutants on ecosystems (Steel, 1979). The type of measurements on populations of daphnids we did were total numbers, length frequency distributions (by means of an automatic image analyser) and production of ephippial eggs. The modifying variables were concentration of toxic compound and food supply rate rather than food density (which is controlled by the population itself).

In contrast with zooplankton, studies of effects on populations of algae are more common (see e.g. Kooijman et al, 1983) than on individuals. We used batch as well as continuous cultures to investigate phenomena that are relevant for the population dynamics of the algae. The cultures were limited by nitrate or phosphate (Rhee, 1980). The effects on cell numbers, cell size distribution, mortality and nutrient utilization have been studied in relation to growth rate and yield.

INDIVIDUAL-MODEL

The basic strategy we followed for the development of an individual-model was to produce a collection of assumptions, partly based on ideas about physical mechanisms, partly on Occams razor (everything is extremely simple unless it proves to be more complex) and partly on empirical data (in this order of preference), that specifies the individual-model completely. These assumptions were subsequently tested against experimental results, which are also used to estimate the parameter values of the model (see Kooijman, 1986a). We avoided assumptions concerning processes such as energy partition that are difficult to test experimentally. We preferred to exploit the black box approach in such cases.

If the assumptions proved to be far from experimental reality or if the assumptions gave rise to too complex a model, they were adjusted. Minor deviations from assumptions were accepted. After all, assumptions, like models, necessarily are idealizations that should not be pushed to the extreme. Assumptions can be considered as the smallest possible sub-models. We in fact consider the list of assumptions to be a summary of what can be called 'scientific insight' into a problem. In multi-disciplinary research this is a key-stone in communication. So the formation of a list of assumptions has two main inputs: experimental findings and model building.

The principles we used in model building were (in order of importance):

- The model should have a sound system theoretical foundation, that is, it should clearly specify the state variables and it must be compatible with preservation laws of mass and energy. (We shall call this type of model 'budgets', although budgets given in the ecological literature are usually of the static type, rather than the dynamic type considered here.)
- It should have parameters with well defined dimensions, not with dimensions depending on parameter values themselves (like for instance in allometric functions).
- It should be accurate enough to allow for experimental testing, but not too specific for the organisms involved because there are thousands of species in an ecosystem and if we have to model them all, we will never understand any ecosystem.
- It should be detailed enough to differentiate between different modes of action of a xenobiotic, but not so detailed that it hampers studies at the population level.

The concept of state variables is very important. The value of the state variables, together with the equations describing their changes and the input to the system, completely specify the path the system will follow. Besides parameters, the equations only contain the state variables and environmental inputs. The system is here the individual operating in its environment.

We found it useful to build the individual-model for one species first and then to generalize (by eliminating some specific assumptions) to other species. This guarantees that the model is consistent and has at least a minimal relevance (in case one should have to build other types of models for other species). It proved to be quite possible to generalize and extend the model to other species and situations (see Kooijman 1986b,c,d), but whether this also holds for the population level and for ecosystem level is still an open question because minor deviations at individual level might show up as large deviations at the ecosystem level, so requiring independent testing.(If the higher levels of organization are not robust with respect to small deviations at low levels, it is hard to see any useful persective for ecology and so for ecotoxicology.)

Model building as described here is more than a technical exercise that a biologist could leave to a specialist. The way a model is built by making choices with respect to the degree of accuracy and the amount of detail, is tightly linked with the research strategy and involves subjective elements, which can be called "scientific intuition".

BUDGETS

An unifying approach to model the physiological behaviour of organisms is to consider the way in which they use nutrients and energy for production processes. (See Stanier et al,1986, for an introduction to cell energetics.) For this purpose we have chosen the state variables age, because for reasons that are not well understood, all multicellular organisms suffer from an aging process, size, because size appears to be the principal factor in input/output relations, and storage, because organisms tend to regulate their internal environment as independently as possible from their surroundings and so smooth out fluctuations.

The concept of storage as a way to integrate recent food history turns out to be unifying between different attemps to model microbial populations (see Kooijman, 1986a), it is a key-concept in relating physiological behaviour of widely different species of animal (see Kooijman, 1986b,c,d), and it can resolve some problems in the mathematical analysis of population dynamics without storage (see Thieme, 1986). At the population level the main function is to delay and reduce the reaction of predator populations to fluctuations in prey populations. Since relative storage capacity tends to increase with body size of a species, and top-carnivores in food webs tend to have the larger body sizes, the significance of storage generally depends on the position in the food web as a general rule. Literature on bacteria (Parnas & Cohen, 1976, and Dawes, 1985) and algae (Collos, 1986) indicate however that the storage concept is also essential to understand the ecological functions of microbes. The introduction of the storage concept represents a further step in modeling individuals with respect to the model given in Kooijman & Metz (1984). This model has been primarily designed for situations of constant food density. The models are identical if one reduces the maximum storage capacity to zero. Although the model with storage has one state variable more, it has less parameters because some parameters in the model without storage could be written as functions of the parameters in the model with storage. This is quite remarkable, because the introduction of new state variables generally increases the number of parameters.

Algae & bacteria

A simplified diagram for the uptake and use of nutrients and energy is given in Fig.2. (See Dawes, 1986, and Gayford, 1986, for an introduction to microbial energetics.) If only one kind of nutrient or only energy is rate limiting, and if uptake is proportional to cell volume (as opposed to surface area as has been assumed for animals and which might be just as reasonable for microbes), then the storage dynamics follow a simple first order differential equation and population dynamics only depend on the sum of the cell sizes and the sum of the storages and not on size and storage frequency distributions. In this case, the dynamics of structured populations is reduced to that of an unstructured one. This simplifies relations between the individual and the population level, but might complicate the relation between the individual level and lower levels because many biochemical reactions are coupled to membrane surfaces. At constant substrate (i.e. food) densities, cells should then grow exponentially. The literature on experimental results is rather divided in this respect.

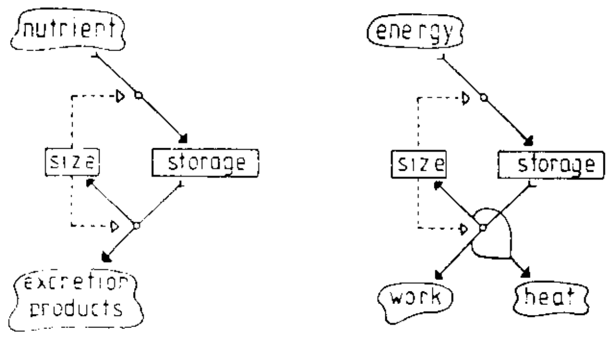

Fig. 2. Simplified diagram for the uptake and use of nutrients and energy in micro-organisms. Division is assumed to occur after the attainment of a certain size.

nutrient or energy flow
information flow
decision valve

From this model it follows that in nutrient limited batch cultures, i.e. cultures with a fixed total amount of nutrient in the medium plus cells, biomass approaches its yield value approximately logistically after an exponential growth phase, which has been frequently observed in experiments. In principle, it is possible to disentangle the uptake and the storage use rates from this type of population growth curve . This opens the possibility to trace the effects of toxic chemicals in more detail. Van der Waal (MT-TNO) has been testing the applicability of the idea and will compare the results with those of synchronous cultures, i.e. cultures in which the cell cycles have been synchronised by means of an appropriate light and dark regime.

A principal problem in modeling the decomposition process is the wide range of mineralization rates amongst the different components of the non-living biological material and the changes in physiological characteristics of bacteria during the subsequent stages of the decomposition process (see Carpenter, 1981, for some model formulations). Results of van Verseveld and Stouthamer (VUA) on oxygen consumption rates in energy limited recycling fermentors indicate that energy budgets at high and low input levels differ qualitatively (see e.g. van Verseveld et al , 1986). This phenomenon, the cell cycle dependence of the budgets and the links between energy and nutrient cycles will be subjects for further research in the context of the present study by Evers (MT-TNO/VUA) because these processes qualitatively determine the dynamics of ecosystems.

<u>Animals</u>

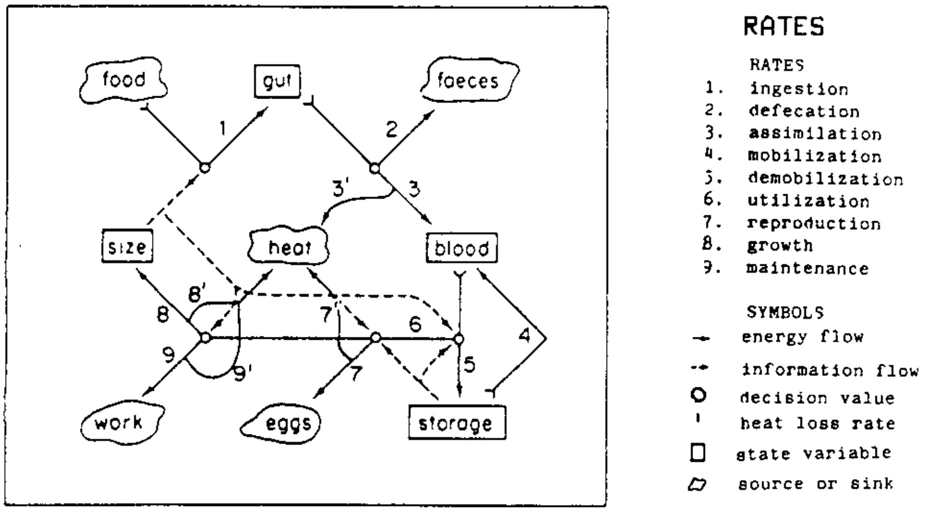

Fig. 3. Simplified diagram for the uptake and use of energy in female animals. Three size stages are assumed: egg, which takes no food, juvenile, which does not reproduce and adult, which does reproduce.

Because energy and nutrient budgets in practice are tightly linked in animals, the approach has been to restrict the study to energy budgets, assuming that nutrients are not rate-limiting. A simplified diagram for uptake and use is given in Fig. 3. (For a general introduction to animal energetics, see Brafield & Llewellyn, 1982.) The relaxation times of the state variables gut and blood are assumed to be small with respect to storage. As a first approximation these variables were disregarded.

The assumptions from which the equations for the change in the state variables size and storage had been derived have been tested experimentally with the waterflea <u>Daphnia magna</u> (work by Ms de Ruiter, Ms Kauffman and Evers, MT-TNO. See Kooijman, 1986a, Evers & Kooijman, 1987). The assumption that eggs do not eat but otherwise use energy in the same way as juveniles do, was used to predict egg development. The prediction was tested by data on snails, fish and birds from the literature (see Zonneveld & Kooijman, 1987, Kooijman, 1986b). For this purpose and for the comparison of parameter values, the model has been extended to include endothermic animals, i.e. animals that use a considerable amount of energy for the purpose of heating the body.

A rather specific feature of the model is that if and only if food density is constant or high, growth is of the von Bertalanffy type (i.e. growth equals a weighted difference between a surface area and a volume). To examine the generality of this assumption the literature was studied extensively to evaluate goodness of fit and to compare parameter values with predictions based on body size scaling relations. Although widely different species have been included, the fit has been found to be quite satisfactory (see Kooijman 1986d). Besides size, temperature proved to be the principal factor that determines the parameter values in the Arrhenius type of way (i.e. the logarithm of a rate depends linearly on the inverse of the absolute temperature. See e.g. Glasstone et al, 1941). This leaves species diversity as only a relatively minor factor.

Since maximum attainable size can be expressed as a compound parameter in this model, there exists a systematic relation between the maximum size a particular species can attain and parameter values, which can be based mainly on reasons of consistency. The body size scaling of some twenty physiological variables that can be written as functions of the parameters of the model were correctly predicted in this way (see Kooijman, 1986c). This indicates that the global features of the model have a rather general applicability. An interesting consequence of the scaling relations, which tie all parameters to the maximum size, is that in modeling food webs, we only have to deal with one parameter per species. So, as a first approximation, the body size distribution of the species involved contains sufficient information.

Possibilities are considered (by Doucet, VUA) to model (hormonal) control systems of energy reserves in more detail and relate this to experimental results and to the present non-detailed model. A very appropriate subject for this purpose is the freshwater pond snail *Lymnaea stagnalis*, because a lot of relevant physiological results has already been obtained by the research group of Joosse (VUA). (Experimental results confirm so far the applicability of the model to *Lymnaea*, see Zonneveld & Kooijman, 1987, VUA.)
The aging process is neatly described by the Weibull distribution (see e.g. Cox & Oakes, 1984) for life times in *Daphnia* and *Lymnaea* (see Slob & Janse, 1986). The time parameter appears to depend on the temperature in the same way as has been found for the other rate parameters. The non-rate parameters do not seem to depend on temperature. (The ultimate size for instance proves to be proportional to a quotient of two rate parameters. This size nevertheless seems to be independent of temperature, which follows from the mechanism mentioned). This indicates that, at least within one species, the temperature primarily operates on the time-axis only. Some coupling was found between the aging process and the food input level, but the qualitative and quantitative nature is not yet sufficiently known to be modeled adequately. Possibilities are considered (by Janse, VUA) to study the aging process and its links with the input level in *Lymnaea* and to relate the survival probability to changes in particular cells of the nervous system.

TOXIC EFFECTS

From routine toxicity experiments with daphnids, reproduction is known to be a very sensitive sublethal parameter. At low concentrations with respect to the chronic LC50, the number of young generally tends to exceed the number in the blank, rather than the opposite way around. This seems to be almost independent from the chemical nature of the toxic compound. The interpretation of this result is far from obvious however. If food density is less than very high (in standard toxicity tests food density is usually very high), this stimulation of reproduction does not occur. For the time being, our interpretation is that high food densities give rise to secondary stress, depending on the state of the algal cultures that serve as food, by the production of polysaccharides by the algae or by the stimulation of growth of bacteria on the elevated concentration of dissolved organic carbon in the water affecting the health of the daphnids. (See Schoenberg, 1985, for a discussion of effects of high food levels and Halbach & Halbach-Keup, 1974, for allelochemical effects of *Chlorella* on *Brachionus*. These considerations show the importance of cultures being stress free.) The toxic substance then suppresses this secondary stress by affecting the algae and/or bacteria sublethally.
An appropriate way to model adverse effects of toxic chemicals on reproduction exclusively is to introduce an egg-survival probability being less than one (or at least less than that in the blank). In this way we can create an energy sink at the right place in the budget model. If the effect is e.g. an increase in the parameter value for the routine metabolism, e.g. because the animal requires energy to fight off adverse effects, the reproduction rate will also decrease (due to the preservation law for energy) and the animal will remain smaller. Although effects on routine metabolism are quite likely, size measured as (cubed) length proves not to be a very sensitive parameter however. This means that the animal reacts, within the model, by channelling more energy into the maintenance process, and so decreasing the energy invested into the reproduction process. In this way different modes of action of the toxic compound result in roughly similar, or even exactly identical changes in the physiology as relevant for population dynamics (for microbial populations one requires almost noise-free data to detect differences in mode of action at population level, see Kooijman et al, 1983). So a lot of information about the exact mode of action of the toxic compound is lost at higher levels of organization. This is quite an encouraging observation, because at detailed biochemical level no two toxic chemicals have exactly the same mode of action (which happen also to depend on the concentration and exposition time), and there are thousands of chemicals requiring urgent consideration.

POPULATION DYNAMICS

As mathematical analysis does not yet seem feasible for population dynamics on the basis of more or less realistic individual-models (see Thieme, 1986 , Diekmann et al, 1984, and Metz & Diekmann, 1986 for evaluations of some basic problems), computer simulation runs have been used to study characteristics of population dynamics. The remarks below refer to preliminary results (see Kooijman et al, 1987), with straightforward (and so rather inefficient) methods.

One species populations

From one-species population dynamics, many salient features can be deduced, which are of importance for the behaviour of ecosystems, where food input is independent of population size. At constant food supply and without predation effects, the simulated population fluctuates strongly, due to a synchronization of life-cycles of individuals. The synchronization of sizes is due to small individuals having the advantage in their scope for growth over large ones. The synchronization of ages is due to the new generation outcompeting the old one shortly after reaching the adult size, at which all individuals start to reproduce simultaneously (due to the synchronization of sizes). The rate of growth and therefore the generation time is primarily determined by thinning of the population due to aging which leaves more food per remaining individual. (This makes one wonder which properties of models at individual level actually determine population dynamics. A lot of information seems to get lost, but which and under which circumstances?) The timing of the reproduction prevents overlap of generations. Apart from the model structure this is a consequence of the model being deterministic. Young within one brood are identical copies of each other.

In *Daphnia* the reproduction cycle is coupled to the moulting cycle. The mother accumulates energy invested in reproduction during the intermoult period and converts it into eggs at the end of this period. It is possible to fill a bottle with water, add some algae and daphnids, close it and observe the population of daphnids for some two years if one places the bottle in a window. In experiments like this, there is some synchronization of sizes, and the population is fluctuating rather then constant, but not as extremely as predicted by the deterministic model.

In order to make the model more realistic, an important question is: How much variation in parameter values between individuals is necessary to reduce this extreme synchronization of life-cycles? The preliminary answer seems to be: just a little bit!

The introduction of new stochastic elements at population level in this way leaves the model deterministic at individual level (apart from the little understood aging process). An interesting feature of this approach is that by allowing parameter values of daughters to depend (in a sloppy way) on those of their mothers, we can study population genetical problems in a more realistic way. Because fluctuations seem to be a structural property of the model, the gradual change of parameter values by selection pressure will speed up and slacken with the respective decreasing and increasing of population size. The stochastic perturbation of parameters linked in the way found in the study on body size scaling relations, seems, for reasons of consistency, to be more attractive than just taking a single parameter. As a first choice we multiplied the relevant parameters by the appropriate (integral) powers of a log-normally distributed random variable with mean 1 and specified (small) variation coefficient and studied characteristics of population dynamics, like total numbers and total volume (i.e."biomass"), as a function of this variation coefficient.(This makes it impossible, however, to work with cohorts of similar individuals and tries both computer memory and running time). The preliminary results indicate that the variation coefficient of the population characteristics rapidly decreased towards some asymptote for increasing variation coefficient of the individual parameters. In other words: Variability between individuals works out stabilizing at population level. (This is consistent with the old ecological literature before the appearance of linear differential equations into population dynamical theories, but it is in agreement with modern theories.)

Another obvious way to introduce stochastic elements into the population-models is in the feeding behaviour of the individuals. Preliminary results indicate however that this model has a strong capacity to smooth out this type of noise. An argument to perturbate parameter values is that growth curves of single individuals closely follow a von Bertalanffy one at constant food density, but with slighly different parameters.

If we average over a population cycle in stable situations, the mean biomass is a constant by definition and the production of the population is in their faeces and in dead animals. In the study of the behaviour of the ecosystem, these production processes are very important because they link the population with the decomposing compartment. Predation pressure, introduced by a (size independent) constant predation probability rate per individual, removes the population from food limited conditions and reduces fluctuations: reproduction is no longer synchronized between individuals and generations do overlap.

In situations of food limitation, a significant reduction of the reproduction by some toxic agent seems to have little effect at population level. The principal factor determining the size of the standing crop is food availability, not the toxic compound. Decreasing the reproduction below some threshold, the population rapidly goes extinct. So toxic effects are masked in populations controlled by food limitation. These effects become obvious as soon as the condition of food limitation is temporarily lifted (for instance due to some commercial harvesting program or by more natural causes). It has been shown in Kooijman & Metz (1986) that, depending on the mode of action of the toxic compound, the effect of the toxic compound is particularly felt in situations of low population growth rates. These important results of the simulation studies and analyses indicate that it might be difficult to interpret observations on concrete pollution events in real ecosystems: The pollution situation might be worse than can be concluded from observations on standing crops. It is one of the mechanisms by which the microcosm type of experiments are generally not found to be as sensitive as might be expected on the basis of single-species tests (see Kooijman, 1985 and 1987).

In modern ecology research is directed to finding constraints for which observed traits of individuals are optimal. One such trait is producing a large number of small eggs as opposed to a small number of large eggs. Until recently the only optimal trait seemed to be the production of an infinite number of zero-sized eggs. The study on egg development on basis of the present model revealed, however, that animals can gain a lot of time by producing large eggs in order to attain a certain size, e.g. the size required for being able to take part in the migration process. Since the size of eggs is for many species typically larger than zero, non-zero-sized eggs are the optimal trait. For this trait food inputs that are not homogeneous in space as well as in time provide the necessary constraint. If true, and if the optimality considerations make sense, real world population dynamics seems to be a very complicated process to study. The stability of large scale systems then is a result of a rapidly changing mosaic of highly unstable small scale systems, between which there exists migratory exchange.

A large number of species closely follow a von Bertalanffy growth curve on the basis of individuals taken from the field (Kooijman, 1986d). Within the present model, this is only possible if food density is constant or, what is more likely, if it is high with respect to the saturation constant. This means that the animals did not suffer from food limitation during the growth process, due to a strategic synchronization of the reproduction process by the parents with predictable fluctuations in food density. This provides another indication that food densities are usually not constant and that the notion of a fluctuating food density might be essential for understanding actual population dynamics.

Two-species populations

Two types of two-species populations are of particular interest: Situations in which two species compete for food and predator-prey systems. A well-known result for unstructured population dynamics says that under not too restrictive conditions a requirement for stable systems is that the number of species of predators should be less than the number of prey species, at least in situations of constant food supply input to the prey species. Under some very special conditions with fluctuating food inputs stable systems are possible in which the number of predator species is greater than the number of prey species. The conditions for stability of systems with more predator species than prey species are probably less restrictive for populations structured by the present model in situations of time-inhomogeneous food inputs when species differ in their maximum attainable size (as they usually do). It can be shown that small sized species beat large sized species in their competition for food at low (constant) food inputs, but that large sized species are better equipped to survive periods without food. Fluctuating food inputs might therefore select for both species alternately. In view of the complexity of actual food webs, it is hard to judge the practical significance of these type of results.

Long-term coexistence of species is one of the central topics in studies on population dynamics, which is still poorly understood. Some biologists think that xenobiotics primarily affect species diversity. In this context it is relevant to study the effect of chemicals on the disappearance rate of competing species. So not only stationary situations but also transient ones are of interest as it is by no means obvious that in the real world subpopulations are stable or should be stable.

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