Population dynamics of rotifers and its consequences for ecotoxicology

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Keywords: *Brachionus*, life tables, population dynamics, ecotoxicology, models, computer simulations

Editor's note and acknowledgement

The paper 'Population dynamics of rotifers and its consequences for ecotoxicology' was presented by Professor Udo Halbach as an invited review during the Third International Symposium on Rotifers at Uppsala, in 1982. Udo's untimely death, early in 1983, prevented him from submitting a final manuscript for the proceedings of that meeting. Because of the importance of this paper, I was encouraged by several rotifer workers to try to publish it posthumously. Dr Gisela Halbach, the late Professor Halbach's wife, immediately agreed to this idea, and performed part of the editing of the text herself. Very special thanks are, however, due to Professor Jurgen Jacobs, Zoologisches Institut der Universität München, former teacher of Udo Halbach, for revising the manuscript, adding reviewer's comments, and improving the figures. Without his generous contribution, the last work of his first student would have been lost for the scientific community.

H. Dumont

Abstract

Population dynamics of the rotifers *Brachionus rubens* and *B. calyciflorus* were studied in the field and analysed by multivariate statistical techniques. There were some correlations, but they are difficult to interpret. Laboratory cultures under various controlled constant conditions showed sigmoid growth curves with subsequent oscillations around an equilibrium. Its level is determined by food quantity; frequencies and amplitudes of the oscillations increase with temperature. The growth curves are described by deterministic models (logistic growth function with one or two time lags) and by stochastic models (SIMULA). Both give simulations which are close to the experimental curves, even in the case of changing temperatures with variable time lags. A third descriptive model is proposed which permits predictions without causal analysis, on the basis of statistical properties. It is used for the development of a highly sensitive bioassay in ecotoxicology. Changes in population density reflect minute sublethal effects of toxic substances such as statistical reductions in lifespan and fertility of the individuals. Experiments suggest chronic sublethal effects which may develop over many generations: adaptation to pentachlorophenol and accumulation of detrimental effects of 4-chloroanilin. Phenol is more toxic at low than at high concentrations. Application of these findings to field conditions is discussed.

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Introduction

Models of population dynamics can be used to describe the patterns of population densities in space and time. Rotifers of the genus *Brachionus*, especially *B. calyciflorus* Pallas and *B. rubens* Ehrenberg, are ideal research tools: They are among the smallest metazoans, and they are cosmopolitan and common in both eutrophic and mesotrophic ponds and lakes. They can be easily cultured under controlled conditions in the laboratory, and they have a very short generation time. Genetically pure lines are easily reared as their normal reproduction is parthenogenetic. Resting (diapause) eggs can be stored for long periods and even sent by mail. Finally, cell constancy and lack of mitoses after hatching result in relatively deterministic life history patterns which, together with a simple behavioural repertoire, invites mathematical treatment and the use of computer simulations to describe their population dynamics.

Intensive studies on the population dynamics of these animals began many years ago (Edmondson, 1957; Erman, 1962a, b; Galkovskaja, 1963; Girke, 1976; Halbach, 1970b, 1972a, b, 1973a, 1978a, b, 1979; Halbach & Burkhardt, 1972; Halbach & Halbach-Keup, 1974; Seitz & Halbach, 1973). These studies, including the modelling and computer-simulations of the population dynamics, stimulated the discussion of their heuristic features (Halbach, 1979). Conceptional models as well as descriptive ones suggested the use of *Brachionus* population dynamics as a bioassay for aquatic ecotoxicology (Halbach, 1981, 1983a, b; Halbach, Siebert & Westermayer, 1983). Minute chronic effects, which are sublethal and hardly noticable at the level of the individual, may be amplified through thousands of animals leading to changes in the population dynamics which can be detected by statistical analysis (Wissel *et al.*, 1980; Beuter *et. al.*, 1981). By applying correlation functions and Fourier-transformations even small statistical reductions of vitality (decreased life expectations and fertility) can be uncovered. Sometimes detrimental effects accumulate (e.g. with 4-chloroanilin) or adaptations occur over many generations (e.g. with pentachlorophenol). These concentration-dependent responses need intensive investigations and discussion (Halbach, Siebert & Westermayer, 1983). Standard tests should be developed which can be used as an ecotoxicological bioassay. Transfer of the laboratory results to field conditions using controlled ponds and plastic bag exposures is now in progress (Fleckner, 1979; Selgert, 1982).

Materials and methods

Brachionus calyciflorus (strain T3/II) was isolated from fishpond T3 at Winterhausen near Würzburg in July 1968. *Brachionus rubens* (strain N12/III) was isolated from the Nidda-pond N 12 near Frankfurt in August 1979. Both strains were started from single females and have been cloned repeatedly.

The animals were cultured in synthetic inorganic freshwater and fed with the chlorococcal green alga *Monoraphidium minutum* (Nägeli) Komáková-Legnerová which was reared under sterile conditions. The composition of the culture media, details of culture conditions and handling techniques have been described previously (Halbach & Halbach-Keup, 1974). For the laboratory experiments the rotifer medium was renewed every 24 h. For determining life table data we used individual cultures in 2-ml tubes, for population dynamics 20-ml tubes.

Food 'dose' was 10^6 cells $\cdot$ $ml^{-1} \cdot d^{-1}$. Algae were centrifuged (5 min. at 6 g) and resuspended in fresh rotifer medium before use. Their density was determined nephelometrically with a photometer at 680 nm.

The experiments were made in darkness using climate-controlled chambers at 15 °C and 20 °C (ca. 1 °C each). Counts of population density were made during transfer by micropipettes. Filtration rates were determined with 32-P labeled algae, using Cerenkov counting of the radioactivity of living animals so that the filtration rate could be measured repeatedly during the life of an organism (Haney *et al.*, 1983; Brauer, 1983).

Respiration rates were measured on isolated specimens using a Cartesian diver method (Leimeroth, 1980a, b). Statistical analyses were performed with PDP 12/84, simulations with UNIVAC 76/3. For numerical integration of the differential equations we used the FORTRAN-based algorithm CSMP including RUNGE-KUTTA-methods with automatically varying step width to minimize integration errors. For stochastic simulations the ALGOL-based SIMULA was applied.

Field studies were carried out with a 2-liter RUTTNER-sampler in 15 small ponds of the Würzburg Botanical Garden as well as in three fish ponds near Winterhausen close to the Main river (Halbach, 1970a, b, 1972a). Samples were counted under an inverted microscope. We used a census frequency of three days because at longer intervals important information may be lost (e.g. exact timing and size of population maxima and minima). Shorter sampling intervals were unnecessary since additional information would be hidden by the 'noise' of sampling and counting errors (see Hal-

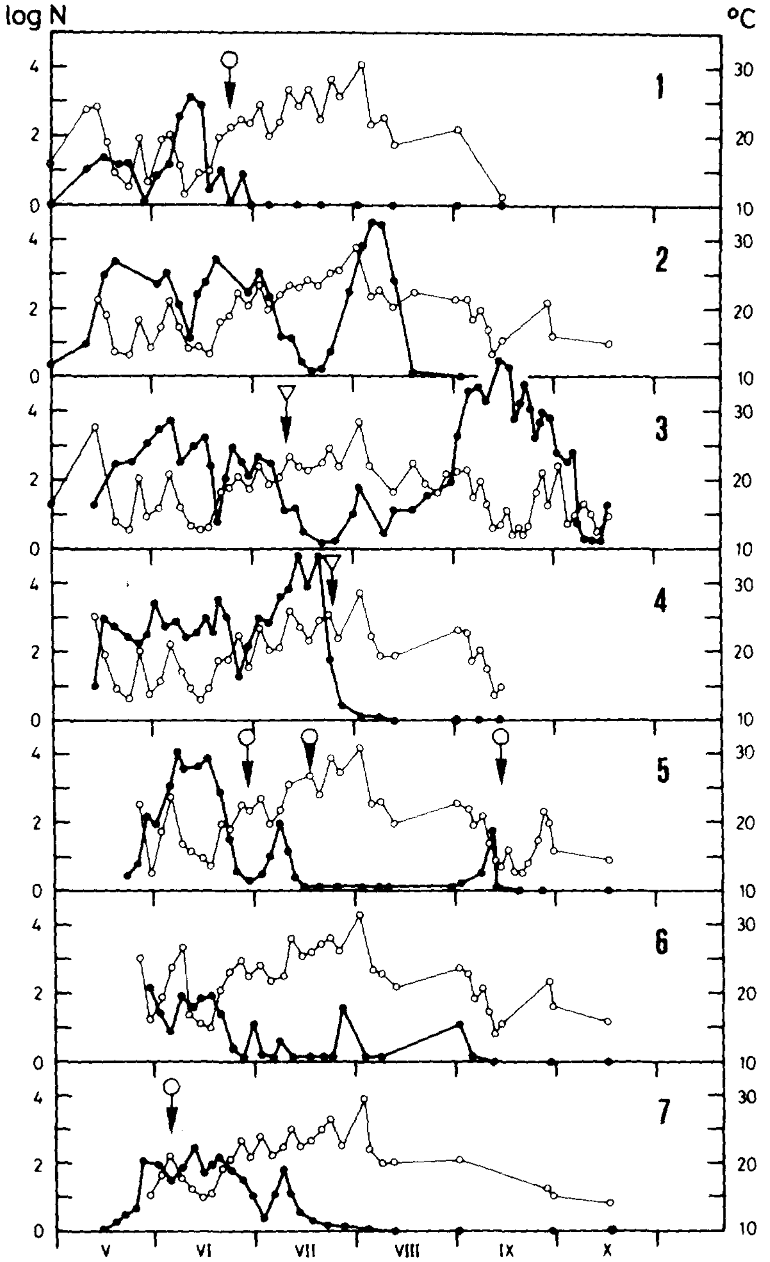

Fig. 1. Population dynamics of *Brachionus calyciflorus* (solid symbols, thick line) and changes in temperature (open symbols, thin line) in 7 ponds of the Botanical Garden of the University of Würzburg during summer 1967. Triangles indicate the occurrence of predators (more than 10^2 *Asplanchna brightwelli* per liter), circles the occurrence of competitors (more than 10^3 *Brachionus rubens* per liter). Note the very irregular fluctuations without any obvious correlations (Halbach, 1970b).

bach, 1979, Fig. 4).

Qualitative and quantitative chemical analyses were made with a GCMS.

Results and discussion

The changes of rotifer populations in 7 basins of the Botanical Garden of the University of Würzburg during one season are presented in Fig. 1. Very strong and irregular fluctuations are evident but there is no apparent correlation or synchrony among the curves of different basins suggesting that they were completely independent. For example, in July a maximum of population density occurred in pond 4 while the neighbouring ponds 2 and 3 exhibited minima. This lack of correlation is confirmed by a plot of population density against temperature (Halbach, 1970b, Fig. 10) in which no obvious pattern is recognizable. Subtle dependences hidden by time lags may still be uncovered by cross-correlations (Halbach, in prep.). There were, however, significant correlations between temperature and certain population dynamics parameters: The absolute value of the slope of the population curves (i.e. the absolute growth rate) was positively correlated with temperature (Halbach, 1979, Fig. 7). Also the fluctuations increased with increasing temperature and were greater in the field than in experiments, the causes for this difference are not yet understood.

Another ecological factor of particular importance is food (Edmondson, 1957; Halbach, 1972b; Halbach & Halbach-Keup, 1974; Scott, 1983). It is well known from laboratory experiments that suspension feeding rotifers selectively feed on algae (Erman, 1962a, b; Edmondson, 1965; Gilbert & Starkweather, 1981; Starkweather & Gilbert, 1981; Starkweather & Kellar, 1983; Scott, 1983). The animals select not only by size but also by other (e.g. chemical) features (Edmondson, 1957). The selection process is not easily observed under the microscope because the filtering process is very rapid and the gut contents consist of masticated algae which are no longer identifiable (Rezvoj, 1926). However, statistical analysis of the biomass of algal groups and population parameters can yield useful information (Halbach, 1972a). A positive correlation should exist if algae as food regulate growth rates; a negative one is to be expected if either the 'exclusion principle' of Hardy (Hardy & Gunther, 1935) or the 'grazing effect' hypothesis of Harvey (Harvey *et. al.*, 1935) is working (of course, time delays cannot be excluded). Our empirical data demonstrated various positive correlations between algal biomass and the population parameters 'individual density', 'growth rate' and 'egg ratio' (Table 1). Total phytoplankton and three phytoplankton groups sorted

Table 1. Correlation of various phytoplankton groups with parameters of the population dynamics of *Brachionus calyciflorus*. N = *Brachionus* per liter, t = intervals (3 days). Phytoplankton is expressed as log weight (μg dry weight per liter).

	Brachionus calyciflorus		
	$\log N$	$\Delta \log N : \Delta t$	eggs: ♀
Total phytoplankton	$r = +0.01$ $n = 219$ $p = 0.09$	$r = +0.13$ $n = 221$ $p < 0.05$*	$r = +0.29$ $n = 184$ $p < 0.0002$*
Ultra-plankton	$r = +0.10$ $n = 219$ $p = 0.13$	$r = +0.10$ $n = 235$ $p = 0.13$	$r = +0.06$ $n = 182$ $p = 0.46$
Nanno-plankton	$r = +0.05$ $n = 213$ $p = 0.44$	$r = +0.16$ $n = 224$ $p < 0.02$*	$r = +0.19$ $n = 181$ $p < 0.05$*
Micro-plankton	$r = +0.09$ $n = 142$ $p = 0.30$	$r = +0.08$ $n = 151$ $p = 0.31$	$r = +0.22$ $n = 120$ $p < 0.02$*

r = correlation coefficient, n = number of used pairs of measured data, p = probability. Significant correlation coefficients are noted with *.

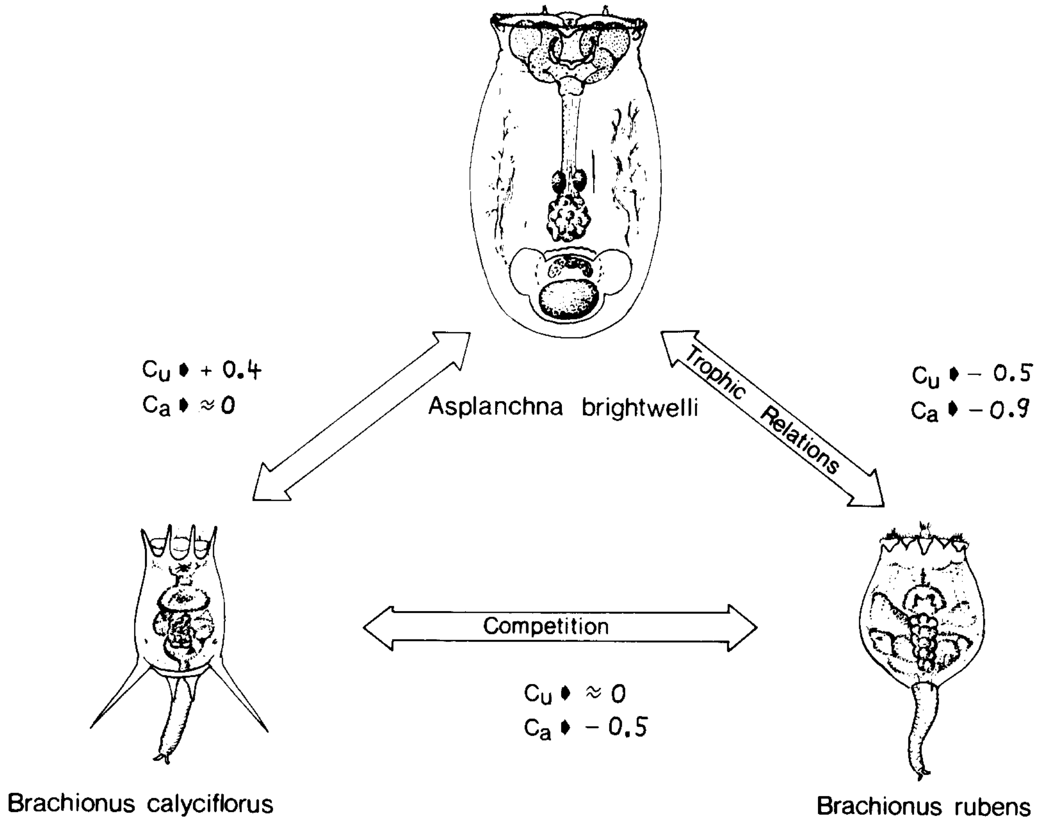

Fig. 2. Affinity coefficients of three sympatric species from several ponds. The association coefficient C_u is based on present–absent data and is density independent. It reflects primarily the influence of abiotic factors on the distribution. The density dependent association coefficient C_a is mainly influenced by biotic factors (Halbach, 1972a).

by size (ultra-, nanno- and microplankton) showed no significant correlation with population density. Cross correlations will be used to explore possible time lags (Halbach & Beuter, in prep.). There was a slight correlation ($p < 0.05$) of the growth rate with total and with nannoplankton biomass, but most significant was the correlation of egg-ratio with total phytoplankton and nanno- and microplankton. Two common algal species (*Oocystis parva* and *Scenedesmus obliquus*) were mainly responsible for this correlation (about 80%). Although other less frequent algae and bacteria were also consumed they were not frequent enough in the basins to influence the correlation significantly.

Other biotic interrelationships such as competition and predation can also be uncovered by affinity or association coefficients (Halbach, 1972b, 1973b). Presence–absence data may suggest similarities or differences in the abiotic requirements among the two species under consideration. If population densities are taken into account, the affinity coefficients may also reflect biotic interrelationships such as competition and predation.

Figure 2 shows the affinity coefficients of three rotifer species: *Asplanchna brightwelli* Gosse, *Brachionus calyciflorus* Pallas, and *Brachionus rubens* Ehrenberg. C_u refers to the density-independent association and C_a to the density-dependent affinity. Both are negative between *Asplanchna* and *B. rubens*, a consequence of heavy predation. Between the two *Brachionus*-species there exists a negative density dependent association probably reflecting competition which has also been demonstrated in the laboratory with mixed culture experiments (see Figs. 5 and 6). Unlike *B. rubens*, *B. calyciflorus* often co-occurs with *Asplanchna*, as is seen in the positive density-independent coefficient. This is due to a pair of posterolateral spines in *B. calyciflorus* induced by an *Asplanchna*-released substance (DeBeauchamp, 1952; Gilbert, 1967; Halbach,

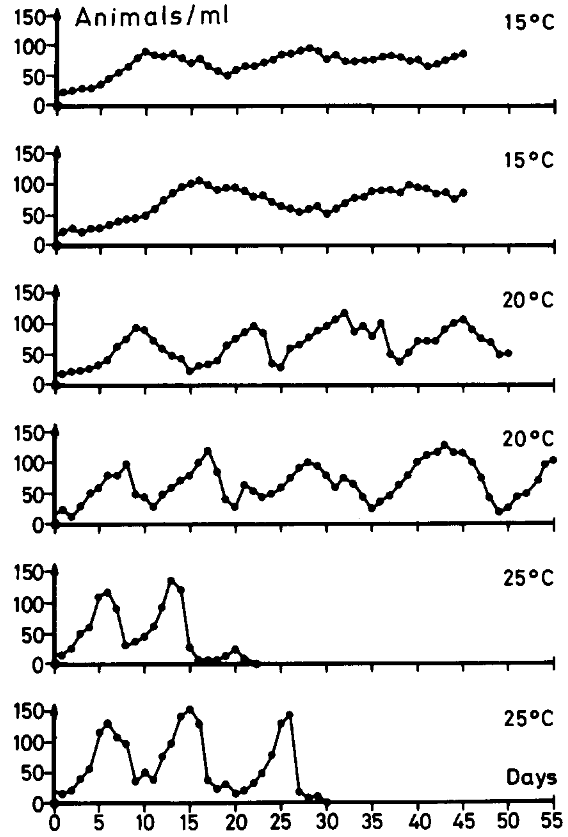

Fig. 3. Population dynamics of *Brachionus calyciflorus* in the laboratory under controlled constant conditions at three temperatures, 15, 20 and 25 °C. Note the initial sigmoid growth curves with subsequent oscillations around an equilibrium. Frequency and amplitude are increasing with temperature. At 25 °C this leads to extinction after 2 or 3 peaks.

1969a, b, 1970b). These spines prevent the potential prey from being swallowed by the predator. Therefore these two species can co-exist in a common habitat for long periods assuming there are alternative food sources available for the predator.

Another method combining abiotic and biotic parameters in an aquatic ecosystem is multivariate analysis with presentations like dendrograms (Halbach, 1979, Fig. 9). These and similar methods of multiple correlation analysis are widely used in ecology, but they are no tests for causality. Multivariate methods, however, identify key factors and can be used to develop testable working hypotheses.

Following this approach, we transferred rotifers into the laboratory and cultured them under controlled experimental conditions (i.e. constant volume, temperature, light regime and nutrition). Under these conditions we observed sigmoid growth curves with subsequent oscillations around an equilibrium (Fig. 3) which was regulated by food concentration (Halbach, 1970a, 1979). Frequency and amplitude increased with temperature. At 25 °C this led to extinction after 2 or 3 peaks.

The periodic fluctuations are cybernetic regulation cycles caused by the time delay T between food uptake and offspring production. This causes an overshoot and subsequent regulation oscillations. Thus, the instantaneous growth rate is not only dependent on the present density but also on the density at time t-T. The population curves can be described mathematically by the logistic growth function with time lag (Hutchinson, 1948, 1954):

$$\frac{dN_{(t)}}{dt} = r \cdot N_{(t)} \; \frac{K - N_{(t-T)}}{K} \qquad (1)$$

in which population growth is completely determined by the three population parameters 'intrinsic rate of natural increase' (r), 'carrying capacity' (K), and 'frequency' of the oscillations (f, defined by time-delay T, see below). If we know these parameters quantitatively for a concrete situation, we can reconstruct the complete population dynamics by computers and numerical integration (Halbach, 1979). The parameters can be calculated by data measured on single isolated specimens. The formulas are:

carrying capacity: $$K = \frac{p \cdot F \cdot L}{B} \qquad (2)$$

B = biomass of an adult animal measured with microcalorimetric combustion bomb and expressed in Joule (Störkel, 1977);

L = average lifespan expressed in days;

F = food dose expressed in Joules · ml^{-1} · d^{-1} and also measured with the microcalorimetric combustion bomb;

p = coefficient of food use efficiency. Only a fraction of the introduced algae is filtered; a fraction of the filtered algae is ingested; a fraction of ingested food is assimilated; a fraction of the assimilated food is used for growth and egg production – the rest is used for respiration. We measured the filtration rate of single specimens using Cerenkov counting of 32-P labeled algae (Haney *et al.*, 1983; Brauer, 1983), and the respiration rate of isolated specimens by Kartesian diver method (Leimeroth, 1980a, b). Both procedures assure the survival of the animals so that the measurements can be repeated during the normal lifespan. From these data we calculated p which is in the order of 0.3.

Formula (2) was developed for food-limited populations. It is based on the simple assumption that, in equilibrium, for every dying animal a newborn enters the population.

Frequency: $$f = \frac{1}{T \cdot \pi \cdot \sqrt{2}} \qquad (3)$$

T = time delay. In a small range around the stable oscillations the frequency depends only on the time lag (Cook, 1965). May (1974) gives a more detailed discussion of the dependency of f on T and confirms his results by numerical studies. Further examples are given by May (1976, 1979).

Intrinsic rate of natural increase

r is a central parameter of population dynamics. On this parameter there exists an immense amount of literature (e.g. Dublin & Lotka, 1925; Birch, 1948; Leslie & Park, 1949, Evans & Smith, 1952; Parise, 1966; Edmondson, 1968). The relevant equation is:

$$\int_{0}^{\infty} \ell_x m_x e^{-rx}\, dx = 1 \qquad (4)$$

where ℓ_x and m_x are the age-specific values of survivorship and fecundity. r can be calculated by mathematical integration methods applying iterations.

Age specific natalities and mortalities are influenced by environmental factors, for intance, temperature (Halbach, 1970b, Fig. 1). Life expectation increases with decreasing temperature. The reproductive period is prolonged, but egg development is retarded at low temperatures. Food quantity is another factor which influences life table data (Halbach & Halbach-Keup, 1974, Fig. 1). The best vitality is obtained at medium algal densities. The influence of algae is twofold. At low algal doses the animals die by starvation, at high doses by toxic metabolites. As a consequence, optimal survivorship and highest reproduction occur at a moderate algal level. This in turn causes optima in several population parameters (Halbach & Halbach-Keup, 1974, Fig. 2).

Instead of the iterative calculations, r can be determined graphically by Edmondson's model (Edmondson, 1968). In it the life history data are assumed to be identical for all animals. The timing of the life schedule is trimmed in such a way that the elementary events of birth and death are synchronized. This facilitates the calculation of r once a stable age distribution has been reached. We used this method to calculate r for different sets of environmental conditions.

The whole causal chain in rotifer population dynamics is:

Ecological factors → Physiological characters → Life table data → Population parameters → Population dynamics.

Ecological factors such as temperature, nutrition or toxic substances influence physiological characters (e.g. swimming velocity, filtration, ingestion, assimilation and respiration rate). These in turn influence life table data which determine the population pa-

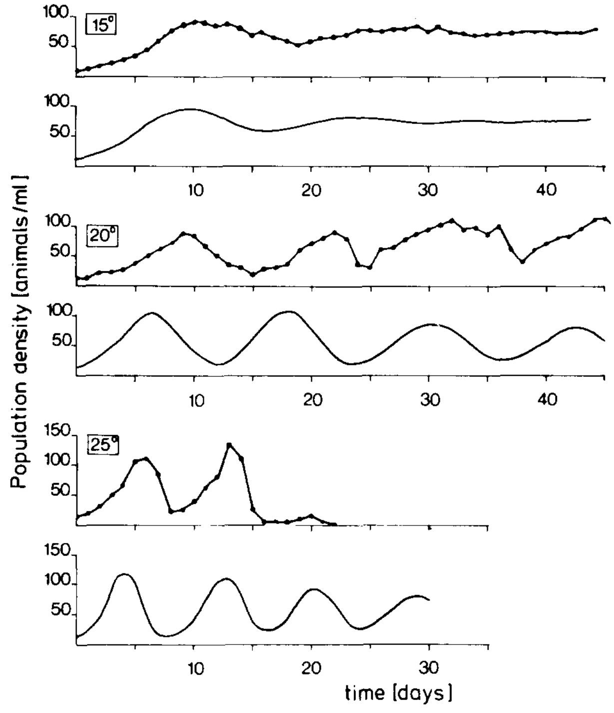

Fig. 4. Comparison of empirical population dynamics of *Brachionus calyciflorus* (upper curves with dots) and computer simulations (smooth, lower curves) at three temperatures. Simulations were carried out using the logistic growth function with simple time lag (Halbach, 1975).

rameters (r, K and f). Using these parameters, population dynamics can be simulated by deterministic models. To fill the last gap of the causal chain we have to synthesize the population dynamics by numerical integration. The similarities of empirical and simulated curves suggest that the observed oscillations are in fact caused by a time lag (Fig. 4). An important point of this heuristic approach is that we are able to predict the behaviour of the whole population cycles based on measurements of single animals without using any further information.

So far I have described simulations using just one time lag for the intrinsic rate of natural increase, r. But r depends on two parameters, birth rate, b, and death rate, m. Both are physiologically different phenomena and should have different time lags. Therefore, for some of our simulations we used equations with independent time lags for birth and death rates (Girke, 1976; Halbach, 1979, Fig. 26).

To make our simulated system still more realistic we should also incorporate changing temperatures, because in the field we rarely have constant conditions (Halbach, 1973a, Fig. 5). We ran experiments with diurnal sine-shaped temperature curves fluctuating between 15 and 25 ° C, and compared the results with 20 ° C constant conditions. With changing temperatures the general vitality (lifespan and number of offspring) was higher (Halbach, 1973a, Fig. 6). The reasons for this are not completely understood. In this case, successful simulation was complicated by the fact that changing time lags had to be incorporated into the model.

With this type of deterministic model we can also simulate multispecies systems by simultaneous integration of all differential equations describing the system. This method has been used for the world models of the Club of Rome (Forrester, 1972a, b; Meadows, 1972). As an example I examine interspecific competition as described by the well-known Lotka-Volterra equations. Figure 5 shows a computer simulation of the competition between *Brachionus calyciflorus* and *Brachionus rubens*. Independent of the initial proportions *B. calyciflorus* always dies out (in our case it goes extinct on the 35th day). This finding is confirmed by results of population experiments in the laboratory (Fig. 6). Two independent competition effects are apparent. *B. rubens* has the higher efficiency in foraging and food utilization. With the same amount of food it produces more offspring biomass than *B. calyciflo-*

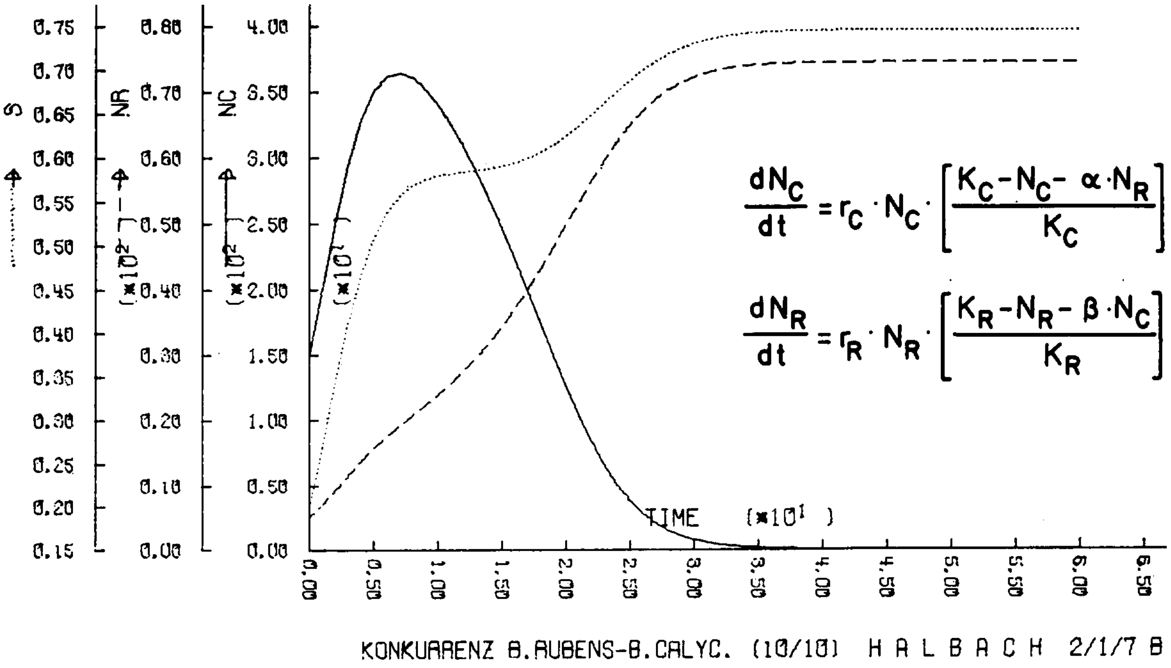

Fig. 5. Computer plot of competition between *Brachionus calyciflorus* (solid line) and *Brachionus rubens* (dashed line). The dotted line represents the sum of both. The 3 curves have different scales in order to have optimal stretching.

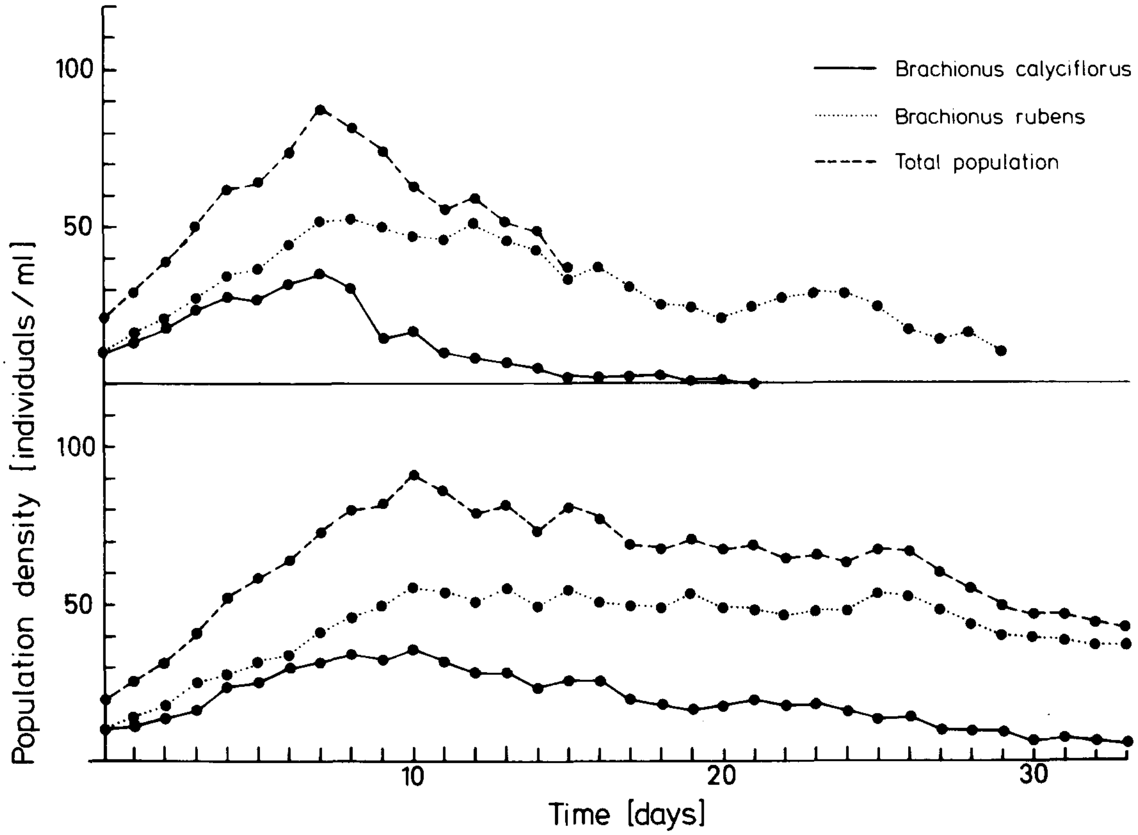

Fig. 6. Competing populations of *Brachionus calyciflorus* (solid line) and *Brachionus rubens* (dotted line) in unrenewed medium (top) and in regularly renewed medium. *B. rubens* always has a higher efficiency of food utilization for offspring production than *B. calyciflorus*. Unrenewed medium causes an additional competition effect by interference: The animals harm each other by releasing toxic substances, the interspecific effect is more harmful than the intraspecific one (Halbach, 1969b).

rus. This leads to a long-term displacement of the latter species in regularly renewed culture medium. If the culture medium is not renewed, an additional interference results in a higher depression of both species and a relatively quick extinction of *B. calyciflorus*. Supplemental analyses provide evidence that both species condition the medium, probably by metabolites which reduce the lifespan and fertility of both species. The detrimental effect is more severe between species than within species but also in this case *B. rubens* is superior to *B. calyciflorus*. In the presence of predaceous *Asplanchna* the advantage of *B. calyciflorus'* spine growing ability may possibly balance its competitive disadvantage.

Deterministic conceptual models are very clear and give good simulations of rotifer laboratory populations but they are still too simple for realistic simulations of field situations. The use of the population parameter N (population density) assumes for instance a homogeneous distribution of the individuals, which is absolutely unrealistic (Halbach, 1975). In nature we find a more or less heterogeneous distribution of organisms such as plankton patches or 'clouds'. To describe them we can use partial differential equations or diffusion equations (Halbach, 1978c). The use of N suggests also that all individuals are identical. But this of course is not the case. They differ not only in age, but also in genotype* and phenotype (e.g. morphological or behavioural modifications). In a more realistic model we should therefore first determine which individual variations are important and then incorporate the 'personal' characteristics of individuals. This can be simulated only by stochastic models

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* Our laboratory cultures are clones and thus the individuals are genetically identical. In the field populations, however, we normally have a mixture of genotypes.

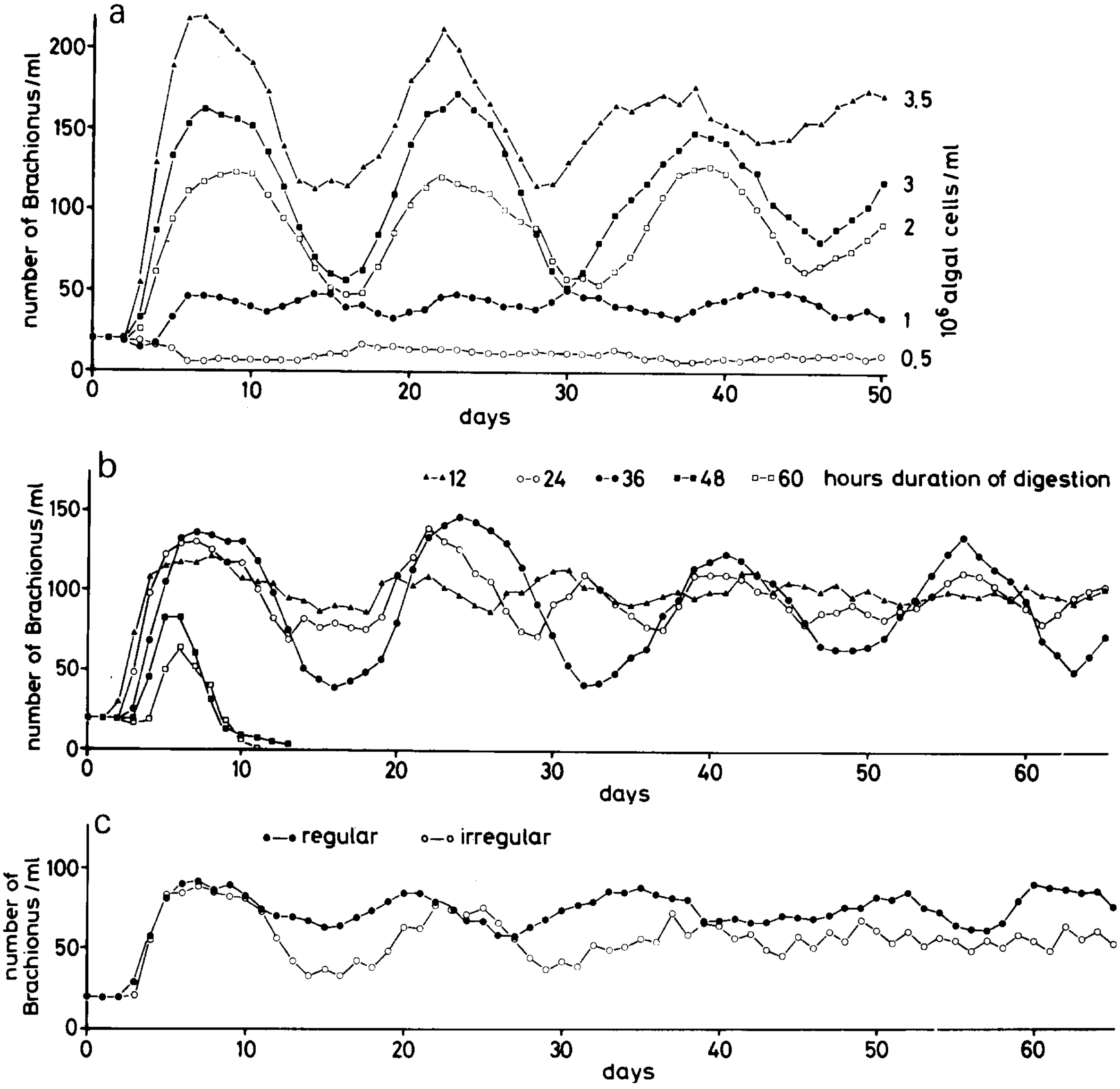

Fig. 7. Stochastic simulations of population dynamics of *Brachionus calyciflorus* testing the influence of various 'experimental' conditions. *a*. influence of food dose, *b*, influence of time lag, quantified as the time of food digestion, *c*, influence of different schedules of food supply. ●—● Regularly introduced algae (1.6 × 10^6 cells · ml^{-1} every day), O—O irregularly introduced algae (4 days with 2 × 10^6 cells · ml^{-1} followed by one food-free day etc., simulating a 'weekend'-effect).

which allow the incorporation of chance processes and the variation of the parameters studied (instead of the averages used in deterministic models). The flow chart of such a stochastic model of population dynamics is given in Halbach (1979, Fig. 27). The population density N can increase by birth or decrease by death. By Monte Carlo methods the computer synthesizes the individual life histories. The artificially composed computer animals are called 'Frankenstein' rotifers. By constructing thousands of them the computer generates population dynamics which are more realistic than those of the deterministic models and more similar to experimental populations (Halbach, 1979, Fig. 28). Such models can substitute for experiments. We can change parameters as in experiments but completely independently and over a broader range. We can thus look for important factor constellations which can then be tested experimentally. This type of deductive research may save money and time. For example, higher food doses in the simulations (Fig. 7a) led to higher carrying capacities as well as increased oscillation amplitudes. Both results are plausible. But contrary to expectation the frequency of the oscillations did not change much. We tested these results experimentally and have been able to confirm them (Halbach, 1979).

In the same way the effect of parameter variation can be tested in ranges that cannot be realized in the field or in experiments because such changes would transgress physiological possibilities. The variation of the time delay (Fig. 7b) may serve as an example. We found that by increasing time delays the amplitudes of oscillations grew higher. Time delays longer than 36 hours led to extinction. Extrapolation of the known temperature-dependence of time delays indicates that extinction by too slow regulation responses should take place below 5 °C. This is in exact accordance with the experimentally determined border of the temperature tolerance of *Brachionus calyciflorus* (Halbach, 1970b, 1979). Thus the model can test evolutionary strategies which do not occur in nature.

Also technical problems can be solved using simulation as surrogate experiments. Figure 7c shows the fluctuations of *Brachionus* density during regular and slightly irregular replenishment of the algae. In both simulations the same average amount of algae was added ($1.6 \cdot 10^6$ cells ml^{-1} d^{-1}). In one run the algae were added regularly every day; in the other one $2 \cdot 10^6$ cells ml^{-1} were introduced for four days and no algae were added every fifth day (simulating a weekend gap in feeding). The different feeding patterns led to differences in the population dynamics of which the lowered carrying capacity in populations with irregular food supply is especially remarkable.

The stochastic model is biologically very satisfying because it is realistic, precise and vivid – three important requirements for a good model (Halbach, 1974). Our type of stochastic model can be expanded to incorporate behaviour, population genetics and evolutionary theory into rotifer population ecology. A disadvantage of this approach, however, is that the construction of models for a concrete situation is extremely laborious and needs a huge amount of computer capacity because all empirical data and all characters of thousands of Frankenstein-rotifers must be stored.

If there is inadequate time to construct such a detailed model we have to look for simpler and more pragmatic solutions. In a heuristically entirely different approach we used various statistical methods to find out basic patterns in the experimental population dynamics (Halbach, 1979). Causal relations were ignored. Prognoses were made by extrapolation from these patterns. One problem here is the fact that there are always slight differences between replicates even under identical starting conditions. These 'irregularities' are due to uncontrolled features of the environmental and chance processes within the population. Most often one finds small phase differences which grow bigger with time ('historical' effects). How do we make generalizations in such a case? Averaging is one common approach (Halbach, 1979, Fig. 13), but this procedure dampens the oscillations or even removes them. In order to eliminate the irregularities which obliterate the general population patterns we made cross-correlations (Halbach, 1979, Fig. 15). After thus suppressing the 'noise' we found a periodicity with a wave length of about 10 days at 20 °C. This does not seem to be an absolutely new insight, for we had found this periodicitiy earlier in the dynamics of individual populations. The difference is that in this case the finding is based on a much larger amount of data. The result demonstrates that this type of oscillation characterizes a general pattern of *Brachionus* population dynamics and not only a single case (Wissel *et al.*, 1980, 1981).

We also used power spectra and Fourier-analyses (Halbach, 1979, Fig. 16) which indicated that there is an even more complicated underlying pattern (Beuter *et al.*, 1981). We then used this pattern for making prognoses (Halbach, 1979, Fig. 18).

I have demonstrated that rotifer population dynamics can be simulated by using the life characteristics of thousands of individuals. Even very small reductions of vitality (e.g. by low doses of chemical substances) can give rise to tiny sublethal effects such as statistical decreases in lifespan or fertility. The small effect of chronic poisoning which can be hardly detected by observing isolated animals, can be seen in the next level of integration, population dynamics. This means that population dynamics can be used like a 'magnifying glass' to detect small sublethal ecotoxicological effects. Rotifer popula-

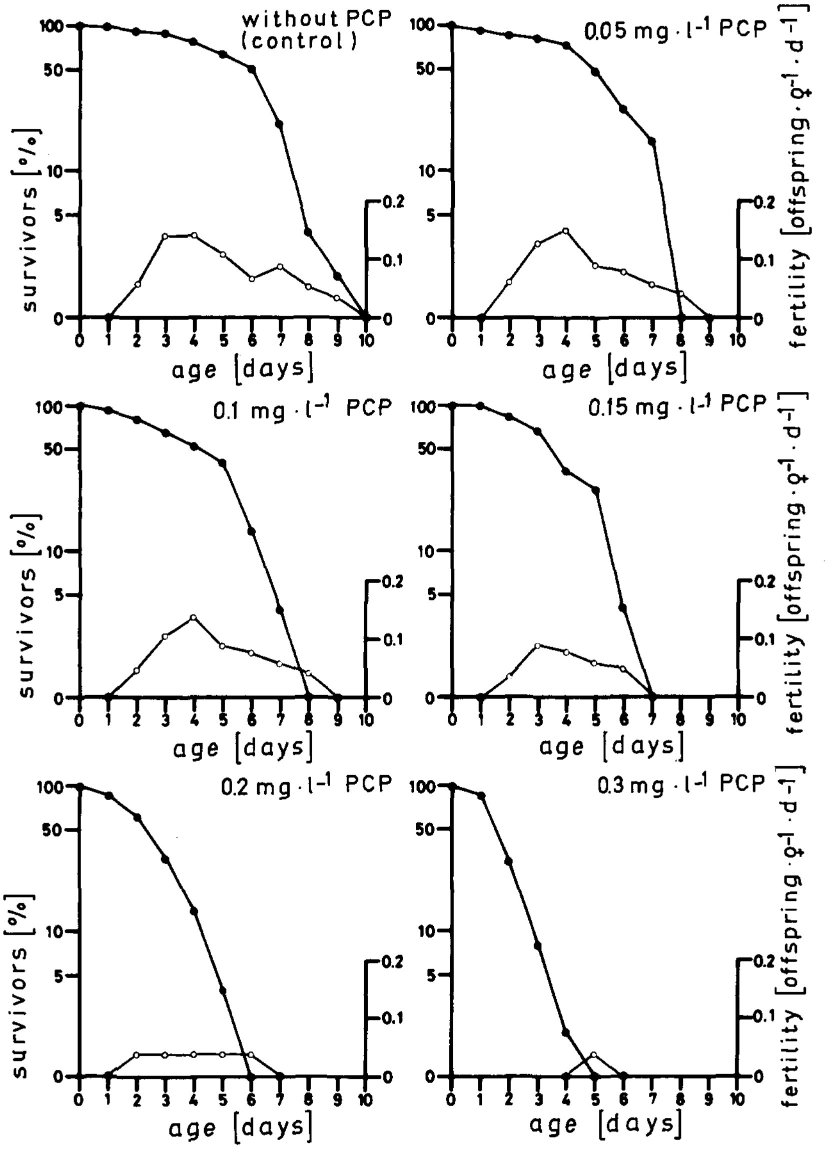

Fig. 8. Life tables of *Brachionus rubens* at 25 ° C under the influence of various concentrations of pentachlorophenol (PCP) (Halbach *et al.*, 1981).

tion dynamics may thus be a very sensitive bioassay. We have developed this method to be used as a standard test to check new chemical compounds before they are used commercially.

As an example, the life tables of *B. rubens* under the influence of the pesticide pentachlorophenol (PCP) are shown in Fig. 8. As the determination of survivorship and fertility in individuals is too complicated for routine work we used the population curves in the culture medium with different concentrations of the substance (Fig. 9). With 0.2 mg$\cdot$l^{-1} PCP the result is quite clear, the population died out after the fifth day of exposure. Equally clear are the results from 0.15 mg$\cdot$l^{-1} PCP: The intrinsic rate of natural increase as well as the carrying capacity were markedly lowered. Not so obvious are the effects of 0.10 and 0.05 mg$\cdot$l^{-1} PCP; we cannot decide by superficial consideraton whether the observed differences are significant or not. For this decision we used the statistical method of autocorrelation (Fig. 10). The frequency of density cycles can be measured much more accurately in au-

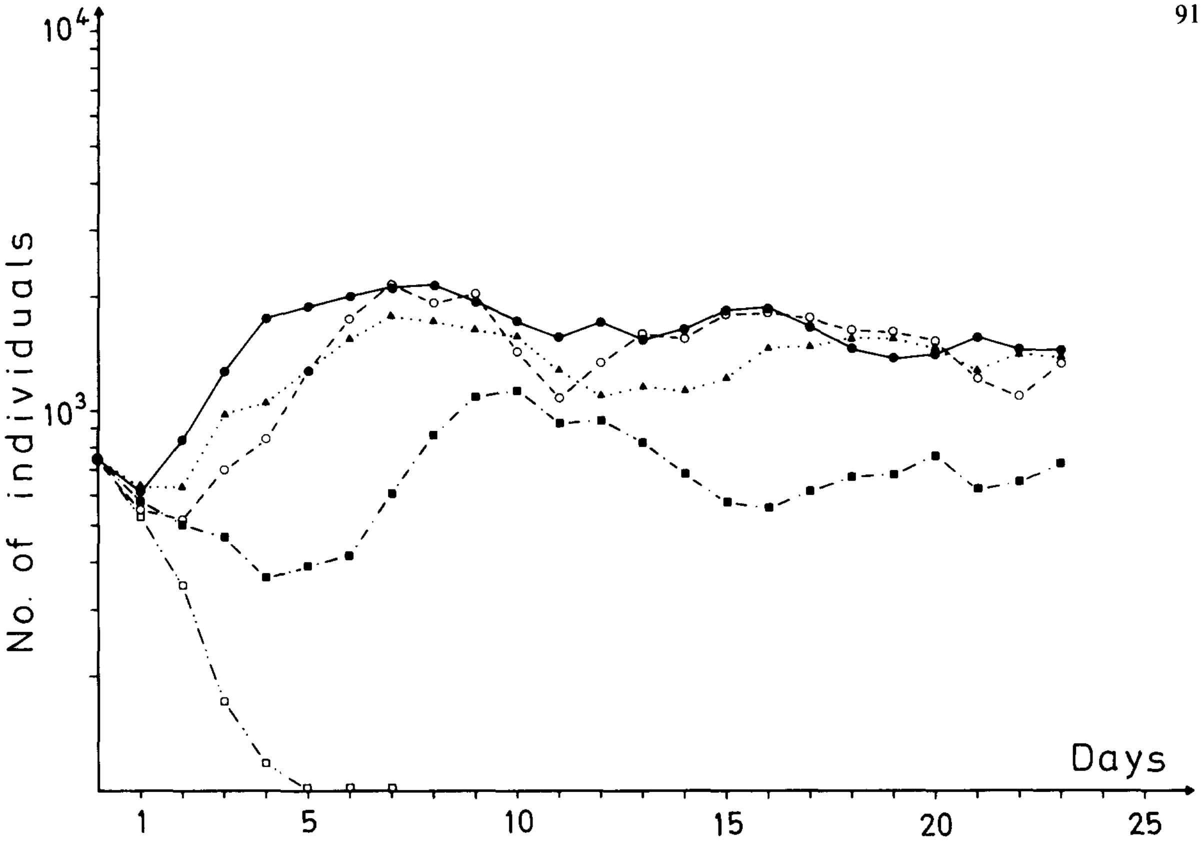

Fig. 9. Influence of pentachlorophenol (PCP) on population curves. Control (solid circles), 0.05 mg·l^{-1} (open circles), 0.10 mg·l^{-1} (triangles), 0.15 mg·l^{-1} (solid squares), 0.20 mg·l^{-1} (open squares) (Halbach *et al.*, 1981).

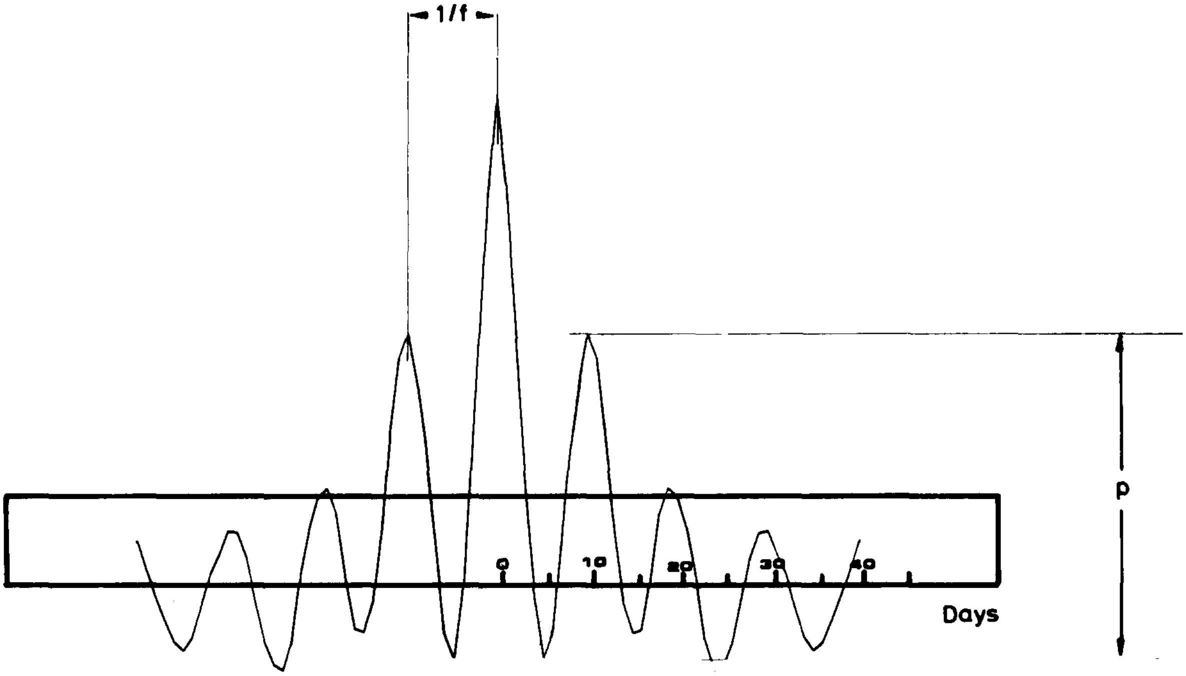

Fig. 10. Autocorrelation of a population of *Brachionus rubens* at 20 ° C. The curve is symmetrical by definition. By this procedure the periodicity is freed from stochastic noise allowing a very precise measurement of the frequency. In addition the pregnancy P of the oscillations can be measured (distance between levels of first minimum and first maximum) (Halbach, 1978c).

Fig. 11. The influence of pentachlorophenol (PCP) on the four population parameters which serve as bio-indicators: intrinsic rate of natural increase, r (No. of offspring per female and per hour), carrying capacity, K (individuals per 15 ml), frequency of the oscillations, f (oscillations per day), relative pregnancy of the oscillations, p (%). Note that the strongest changes of the four parameters occur at different concentrations of the toxic substance. Therefore the parameters can be used as specific indicators for different amounts of the poison (Halbach *et al.*, 1981).

tocorrelation curves than in the original data. I have also introduced another parameter which appears to respond to toxic substances: the 'pregnancy' (p) of the oscillations, which is defined as the distance between the levels of the first minimum and the first maximum of the autocorrelation curve. For practical purposes we use four parameters as bioindicators: the intrinsic rate of natural increase (r), the carrying capacity (K), the frequency (f), and the pregnancy (p) of the oscillations. Figure 11 shows that the strongest responses of the four parameters occur at different concentrations of the toxic substance (Halbach, 1983b). Thus we can use the different parameters as indicators for different concentrations of the substance.

There are sometimes effects which are not easy to interpret. The population curves with phenol (Fig. 12) show what seems to be a long-term adaptation: After a period of divergence, the curves converge. These effects were studied in more detail by standardizing the capacities as percentages of the control and calculating the resulting regression lines: The surprising results was that low concentrations were clearly more detrimental than high concentrations (Fig. 13a). A possible explanation could be that metabolites of phenol are more toxic than the original substance but that the metabolism is blocked at higher concentrations of phenol. We are looking now for such metabolites by careful chemical analysis. With PCP we observed a gradual convergence toward the control (Fig. 13b), with 4-chloroanilin a divergence (Fig. 13c). Since both effects are long-term phenomena, some kind of transfer over several generations seems likely. A simple explanation would be the direct transfusion of the substance or its derivatives from the yolk of the mother to the egg and thus to the next generation. This hypothesis is now being tested.

Conclusions

We have developed three philosophically different types of population models for rotifers. However, our interest is in the practical application rather than in the epistemological background. The oversimplified *deterministic models* give some insight into the basic features of the populations. They permit, for instance, the explanation of the observed population oscillations by a time lag of food utilization. Biologically more meaningful are the *stochastic models*, but we have to keep in mind that the Frankenstein-rotifers are composed of several

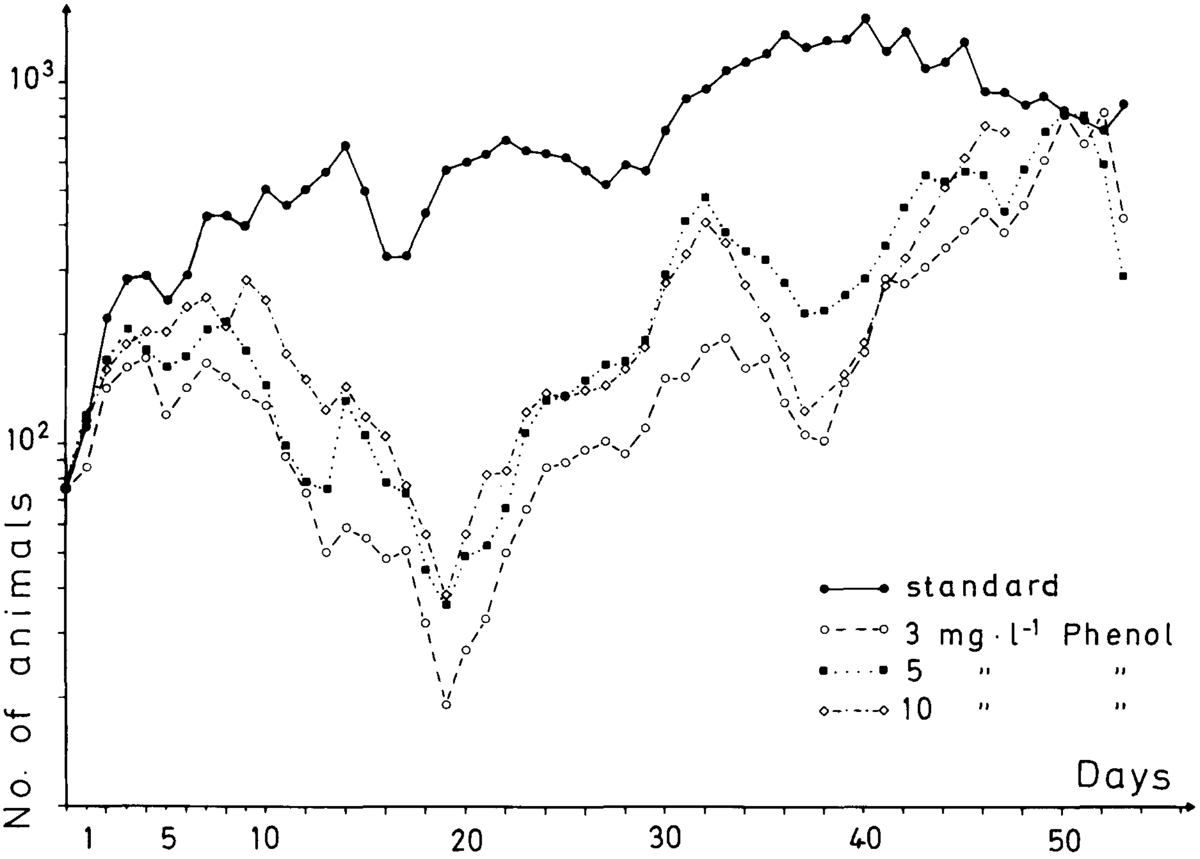

Fig. 12. Population curves of *Brachionus rubens* at different concentrations of phenol. After a phase of divergence the poisoned cultures converge again toward the control, indicating an adaptive effect.

independent characters. The assumption of independence is in fact unproved and even unlikely. Also, the high complexity of these models makes them unwieldy for routine work. Another problem is that a complex model can often be fitted to concrete situations even if the basic assumptions are meaningless or wrong. We are searching now for the actually important key factors in order to be able to gradually reduce the complexity. The resulting models would be rough but workable for routine applications without losing predictive precision. The *descriptive models* are well developed and may serve as bioassays for ecotoxicological tests. In several preliminary tests we had promising and also surprising results. Our model systems are far from natural. Under these circumstances, can we use our results to understand what occurs in nature? As our laboratory rotifers are the same as in nature we can conclude that the observed phenomena may also occur in the field. But they may of course be masked because in the field many additional environmental factors influence the population dynamics simultaneously. We are now trying to bridge the gap between laboratory and nature. By using aquaria we enlarge the volume and allow for heterogeneous distributions while keeping basic environmental factors such as light and temperature constant. The next step, which we have already started, are artificial basins with a screen roof in order to prevent the accidental introduction of frogs, salamanders, birds, insects, leaves etc. At the start of the experiment the basins are filled with fresh water, then algae and rotifers are introduced. Thus, the starting conditions are manipulated but the weather conditions like temperature, light, and rain are natural. A third approach involves the use of plastic bags which are exposed in lakes with the original plankton composition.

Preliminary results from these experiments suggest that sublethal ecotoxicological effects do in-

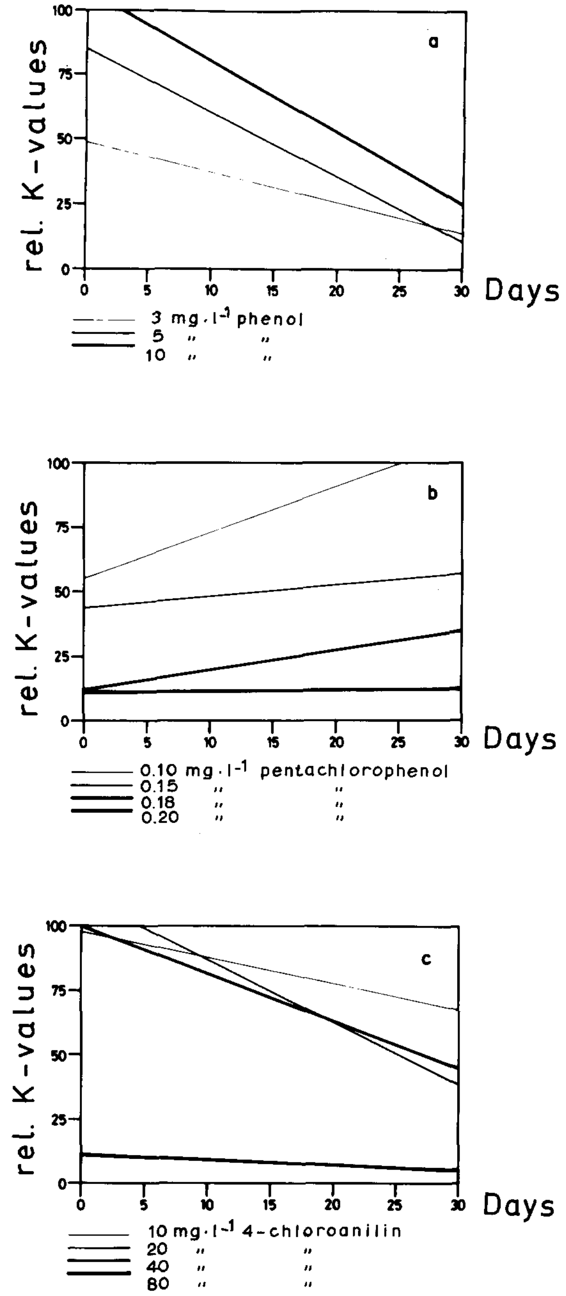

Fig. 13. Long-term influences of chemical compounds on the carrying capacity, K, of *Brachionus rubens* at 25 °C. The K-values are expressed as percent of the control. The curves are regression lines. *a. Phenol:* low concentrations are more detrimental than high concentrations. *b. Pentachlorophenol (PCP):* the curves converge toward the control suggesting a gradual adaptation over several generations. *c. 4-chloroanilin:* as with phenol, the curves gradually diverge from the control indicating that chronic detrimental effects accumulate over several generations.

deed occur under more complex field conditions. Even though the responses may not be as pronounced as in the laboratory, their detection is nevertheless possible by proper statistical analysis.

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