

Population dynamics of *Daphnia magna* as modified by chronic bromide stress

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Abstract

Two chronic toxicity experiments were conducted with *Daphnia magna*. In a semi-static experiment with cohorts, the no effect level for bromide in respect of the intrinsic rate of natural increase (derived from age-specific survival and fecundity) was 10 mg l⁻¹. A second test was started with small populations in an intermittent-flow system. These populations had a stable age distribution, were composed of cohorts of different ages, and showed an almost perfect logistic growth. Model calculations showed that bromide reduced the upper numerical limit (carrying capacity). It also increased the time-lag required to attain the maximum reproduction rate. For the first parameter, a no effect level of 14 mg l⁻¹ was calculated. For the latter a threshold could not be detected. The EC₅₀ and EC₁₀ were 27 and 18 mg l⁻¹, respectively. Additional experiments showed that individual growth of *D. magna* in time could also be described by a logistic equation. The age structure of the populations changed when food became limiting. This was paralleled by a reduction of the mean brood size. In conclusion it is stated that the results of the toxicity studies with populations support Halbach's view (1984), that population dynamics can be used like a 'magnifying glass' to detect small sublethal ecotoxicological effects of environmental pollutants.

Introduction

Most of the literature dealing with acute and chronic effects of chemicals on aquatic ecosystems is concerned with effects on a relatively few species of fish and invertebrates. The vast majority of tests on invertebrates have dealt with only one species, *Daphnia magna*. One of the reasons *Daphnia* species have been used extensively in toxicity testing programs is their demonstrated sensitivity to a broad spectrum of toxicants. Their short life-cycle and the good correlation of 21-day chronic tests with chronic fish toxicity data, made these tests an attractive alternative to long-term fish toxicity studies (Maki, 1979). As the principal objective of aquatic toxicology is to determine concentrations of pollutants which, when released into the environment, will not impact natural populations and

community integrity (Gentile *et al.*, 1982), parameters should be studied which cover these aspects. Single-species tests have a place in evaluating these effects, provided that relevant parameters are studied.

Analyses of the effects on longevity and reproductive rate can be based on the results of demographic life history data. Integrated by life-table calculations, mortality and natality data of cohorts provide information on the intrinsic rate of natural increase (r_m), an ecologically more relevant parameter than the total number of young produced in a fixed time-interval (Van Leeuwen *et al.*, 1985).

This parameter can be calculated from the formula of Lotka (1913):

$$\sum_{x=0}^{\infty} l_x m_x e^{-r_m x} = 1 \quad (1)$$

where l_x is the proportion of individuals surviving to age x , m_x is the age-specific fecundity (number of females produced per surviving female at age x), and x is time expressed in days.

Experiments with populations may provide a good alternative to life-table studies. Population toxicity studies are started with small exponentially growing populations. Slight reductions in vitality, such as a decrease in lifespan or offspring production which can hardly be detected at the level of the individual are then summed over many individuals in the next level of integration, population dynamics, thus facilitating detection (Halbach, 1983, 1984). The underlying assumption for projecting future growth with either the exponential or logistic equation, however, is that the population has a stable-age structure. At low population densities, growth will proceed exponentially and the stable-age structure can easily be calculated from:

$$c_x = (l_x e^{-r_m x}) (\sum l_x e^{-r_m x})^{-1} \quad (2)$$

where c_x is the proportion of the total population in the x^{th} age class. Derivations of these equations can be found in Roughgarden (1979) and Pielou (1977).

In routine toxicity testing, population growth is only studied in experiments with algae. The objective of this study is to extend this approach to the experiments with daphnids, and to describe several aspects of population dynamics. Bromide was chosen as the test compound because it is reported to inhibit reproduction specifically (Canton *et al.*, 1983), and can easily be detected spectrophotometrically.

Materials and methods

Experimental procedures

Experiments with *D. magna* were carried out in a constant temperature room at $20 \pm 0.5^\circ\text{C}$ with a 12 h-photoperiod. The test medium used was 50- μm filtered, UV-sterilized Lake IJssel water with a pH of 8.1 and a hardness of approximately 225 mg l^{-1} (as CaCO_3). Sodium bromide (chemical purity, 99.6%) was obtained from Baker Chemicals B.V. (Deventer, The Netherlands).

The life-table experiments were started with newborn (< 24 h) daphnids from a laboratory stock cul-

ture. The tests were conducted in 800 ml test vessels to which 500 ml of test solution was added. Daphnids were fed daily with 3×10^8 cells l^{-1} of the unicellular green alga *Chlorella pyrenoidosa*. The pooled neonates were randomly distributed into cohorts of ten animals each in 8 toxicant concentrations and a control. To enable statistical treatment of the data, the experiments were replicated five times. The number of surviving females and the number of neonates produced were recorded daily, with new neonates discarded from the test vessels after counting. After three weeks, the experiments were terminated and the carapace length of the daphnids was determined from the anterior margin of the head to the base of the caudal spine using an ocular micrometer.

Population dynamics of *D. magna* under different levels of bromide stress were studied in an intermittent-flow system, equipped with electric valves. Water was aerated before algal cells and toxicant solutions were added. The water flow through the 20 l-test vessels was 667 ml h^{-1} . The *C. pyrenoidosa* concentration was 3×10^8 cells l^{-1} . The test vessels consisted of an outer and inner container. The 16 l-inner container was divided into four compartments, each holding one population. The test was initiated with exponentially growing populations of 20 daphnids composed of cohorts of different ages. The stable-age structure was calculated from equation 2, with $r_m = 0.3$ and $l_x = 1.0$. The total number of daphnids in each test compartment (biomass) was counted at regular intervals. Concentrations of bromide were determined spectrophotometrically at 590 nm with phenol red as indicator (APHA, 1980). The results of both experiments were based on actual concentrations.

Additional intermittent-flow experiments were carried out to study several aspects of the population dynamics in further detail i.e. the development of the age structure, reproductive ratio (the proportion of adult females with broods), and mean brood size (the number of eggs per reproductive female) in time. These measurements were performed at regular intervals on the total number of daphnids in one compartment. The reproductive ratio was determined by classing as adults all females larger than the smallest reproductive female actually observed in the populations (Hebert, 1978). The determinations of the age structure were based on length measurements which were transformed to age, using

the logistic equation for growth. The development of length in time was determined in experiments with cohorts under semi-static conditions. These experiments were all performed at *Chlorella* densities of 3×10^8 cells l^{-1} .

Calculations and statistics

Results of the semi-static tests were summarized in life-table form. The intrinsic rate of natural increase was calculated for each replicate by successive approximation from equation 1. As r_m calculated after 21 days is indistinguishable from r_m estimated for the entire lifespan, due to the great importance of early reproduction (Van Leeuwen *et al.*, 1985), the calculations were based on 21-day experiments. Finally, means and standard errors were calculated.

Differences in mean survival, r_m and carapace length, between treatments and control were tested using the procedure described by Williams (1971, 1972). In case of the survival data, the arc sin square root transformation was applied. Differences were considered significant at $\alpha \leq 0.01$.

The effects of bromide on the population dynamics of *D. magna* were calculated by means of a parametric model developed by Kooymann *et al.* (1983). In this model, it is assumed that population growth is a logistic function of time and that survival probability, reproduction rate or yield (carrying capacity) decreases according to a logistic function of the concentration of the test substance. In formula

$$N(t,c) = EY\{E + (Y-E) \exp(-R_c t)\}^{-1} \quad (3)$$

where $N(t,c)$ is the mean number of daphnids per compartment at time t and bromide concentration c , E is the inoculum, i.e. mean number of daphnids per compartment at time $t=0$, Y is the yield, i.e. the mean number of daphnids per compartment obtained in the culture and R_c is the growth rate at bromide concentration c . The three types of effects can occur singly or in combination. The logistic equation was also applied to the growth of *D. magna* in time. The experimental data were fitted to

$$L(t) = L_m L_0 \{L_0 + (L_m - L_0) \exp(-\alpha t)\}^{-1} \quad (4)$$

where $L(t)$, L_m and L_0 is length at time t , ∞ and 0, respectively and α is the growth rate. The parameters

were quantified by means of non-linear regression. Calculations and curve plottings were performed with a Sperry Univac 1100/84 computer.

Total biomass estimations were based on length measurements and weight was subsequently calculated using the regression equation given by Dumont (1975, Fig. 1).

$$W = (1.5 \times 10^{-8}) L^{2.84} \quad (5)$$

where L is length in μm and W is dry weight in μg . For the weight of eggs and embryos $8 \mu g$ was substituted (Dumont, 1975, Table 3).

Results

Life-table studies

Impairment of reproduction was clearly related to exposure concentration and significantly inhibited at $32 \text{ mg } l^{-1}$ (Table 1). Bromide did not delay the onset of reproduction nor the brood frequency, but disturbed embryonic development as many aborted eggs were found on the bottom of the test vessels. At $100 \text{ mg } l^{-1}$, reproduction was completely inhibited and only aborted eggs were found. Survivorship was unaffected at this concentration, and not reduced until $10 \text{ g } l^{-1}$ (Fig. 1). It may therefore be concluded that bromide specifically inhibits the reproduction process. This inhibition, however, was reversible. Daphnids from this experiment which had previously been exposed to bromide concentrations up to $32 \text{ mg } l^{-1}$ released broods within three

Table 1. Demographic information for *Daphnia magna* from life-table experiments at various concentrations of bromide.

Conc. (mg l^{-1})	$r_m \pm SE$ (day $^{-1}$)	Survivorship (%)	Mean length $\pm SE$ (mm)
0	0.298 ± 0.006	100	4.09 ± 0.08
3.2	0.288 ± 0.008	98	4.09 ± 0.08
10	0.283 ± 0.011	100	4.03 ± 0.11
32	0.037 ± 0.042^a	92	4.05 ± 0.10
100	-	100	4.02 ± 0.07^a
320	-	100	4.01 ± 0.07
1 000	-	100	3.98 ± 0.08
3 200	-	100	3.93 ± 0.13
10 000	-	58	3.58 ± 0.16

^a Lowest concentration significantly different ($\alpha < 0.01$) from the control.

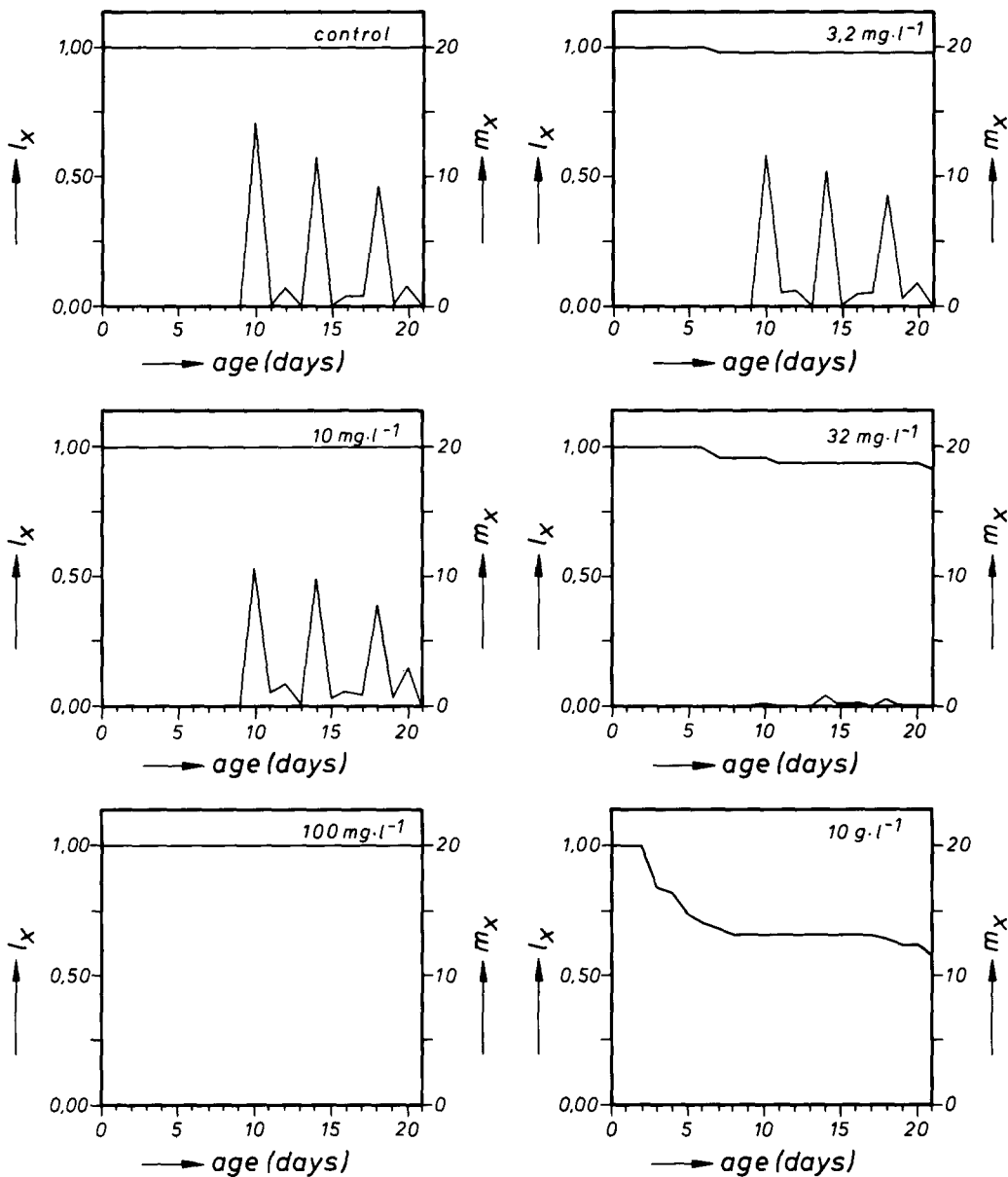


Fig. 1. Life-table of *Daphnia magna* at various concentrations of bromide: survivorship curve (thick line) and fertility curve (thin line).

days upon transferring to uncontaminated water. At higher levels of pre-exposure, this brood was skipped and a first brood appeared within 7 days. Growth was significantly inhibited at 100 mg l^{-1} (Table 1).

Population toxicity studies

The population toxicity experiment showed that

small populations of *D. magna* composed of cohorts of different ages and having a stable age structure grew in an almost ideal logistic fashion (Fig. 2). This was true provided that experimental data obtained after 15 days were excluded from the calculations. After the population had attained its maximum at day 13, the mean yield dropped. At concentrations of 0, 4.5, 8.6, 16.3, 27.9, 45.0 and 98.0 mg l^{-1} the yield at $t = 18$ and, in parentheses,

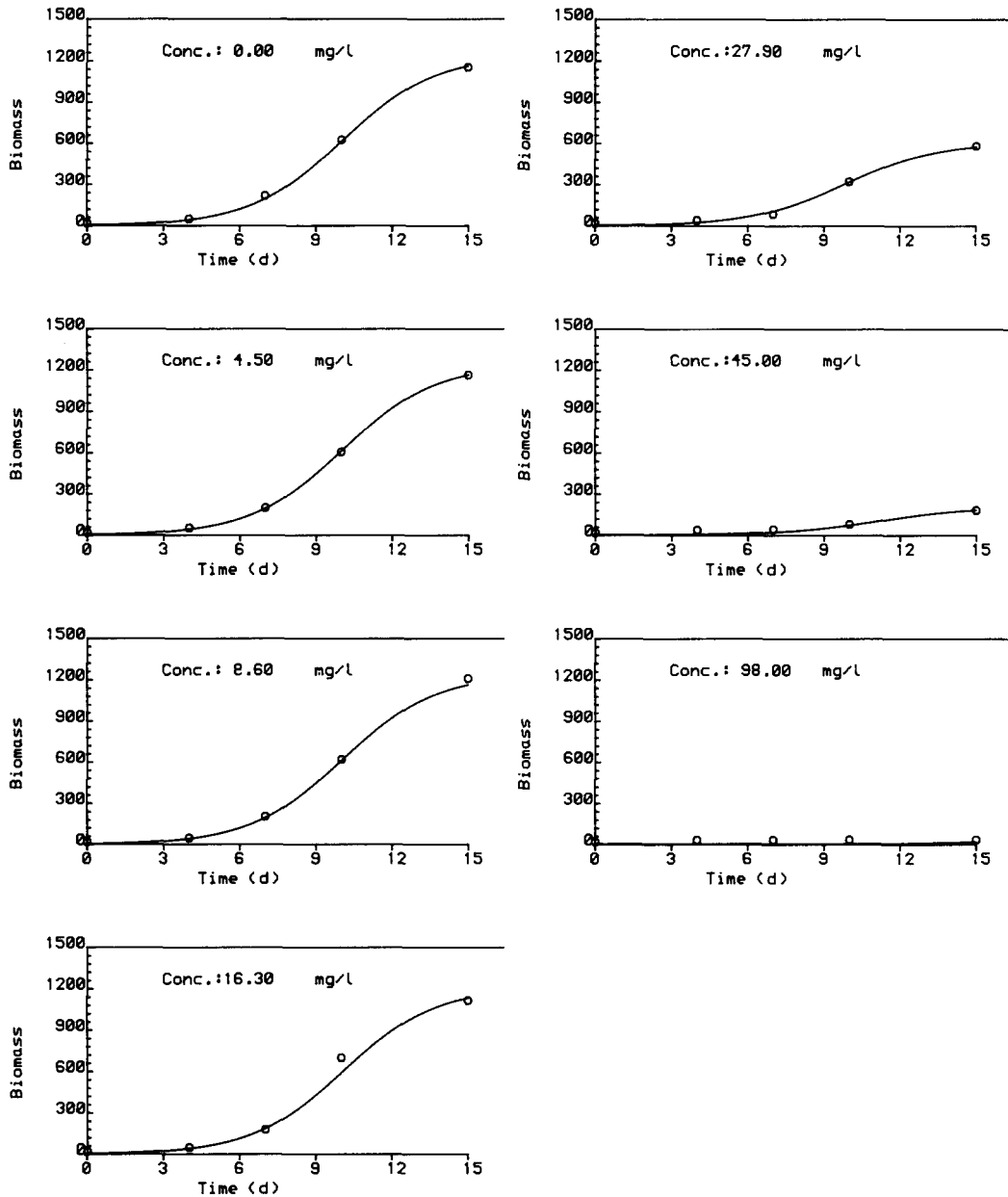


Fig. 2. The effects of bromide on the growth of *Daphnia magna* populations. Circles represent the observed and lines the expected values based on model calculations.

at $t = 21$ days was 862 (673), 1117 (821), 1236 (986), 748 (582), 463 (430), 185 (188) and 37 (36), respectively.

The ultimate factor limiting population growth and size clearly was the amount of food available for consumption. During the experiments, the chlo-

rophyll *a* concentration decreased. This was also shown by a color phase shift from 'Chlorella-green' to 'Chablis-yellow'. The depletion of food was paralleled by an increasing number of neonates which adhered to the water surface and died, likely from starvation. Resource depletion, neonatal mor-

tality, and drops in the yield were less dramatic at high levels of bromide exposure.

Bromide clearly delayed population growth. This was shown by the extension of the lag phase, i.e. the time required to attain the maximum reproduction rate. For this effect on the inoculum, a threshold could not be detected. The EC_{50} and EC_{10} were 27 and 18 $mg\ l^{-1}$, respectively. Bromide also reduced the yield, i.e. the upper numerical limit for *D. magna*. The no effect level for this parameter was 14.3 $mg\ l^{-1}$. The results of the parametric analysis are given in Table 2.

Additional experiments

The relation between growth, expressed as linear growth, and time is given in Figure 3. In *D. magna*, the growth rate seems to be exponential in the pre-adult instars. There is no appreciable growth beyond this stage in the reproductive phase, i.e. the active growth levels off, indicating that the animal reaches maximum growth at this stage.

The experimental data fit the logistic equation quite well. The standard error of estimate was 0.11. This logistic equation was used to reconstruct changes in the age structure of *D. magna* populations in time. At increasing population densities (in a numerical sense), a shift occurred towards a population with a large proportion of adults (Figs. 4 and 5). The greatest changes in age structure occurred after the population had attained its numerical maximum. As food levels declined, the proportion of poorly reproducing adults increased. This response to food limitation was shown primarily by a decrease in the mean brood size, as opposed to the

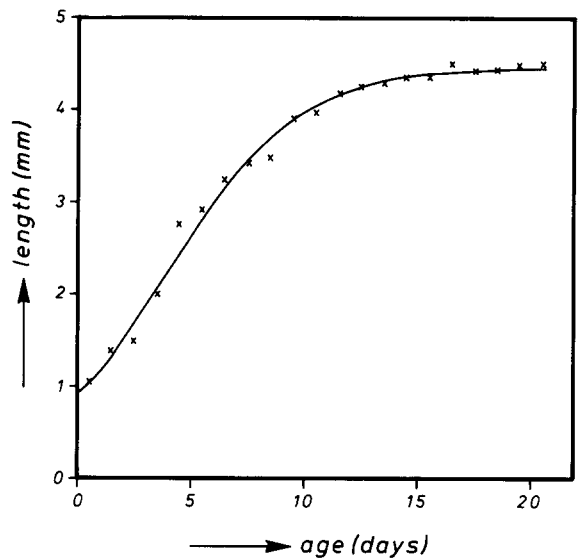


Fig. 3. Length development of *Daphnia magna* as a function of time t . The fitted curves have the form $L(t) = L_m L_0 \{L_0 + (L_m - L_0) \exp(-\alpha t)\}^{-1}$, where $L_m = 4.47$ mm, $L_0 = 0.905$ mm and $\alpha = 0.348\ d^{-1}$.

reproductive ratio, which remained approximately constant, although it dropped after 23 days (Table 3 and Fig. 5). The carapace length of the smallest reproductive female detected, necessary for the calculation of the reproductive ratio was 2.49 mm.

In the exponential phase of population growth, total biomass rose in proportion to the total number of daphnids. Subsequently, this relation became disturbed. The obvious conclusion therefore is that population counts are good estimates for total biomass, provided that no great changes in age structure occur.

Discussion

Logistic equations provide an excellent fit of the available data for both population and individual growth (Figs. 2 and 3). Population growth, however, was far from logistic when the tests were continued after the population had attained its numerical maximum (Fig. 5). This may be ascribed to the absence of an instantaneous reaction to changes in population density, i.e. time lags are likely to occur, which cause daphnid populations to 'overshoot' and then 'undershoot' the equilibrium density in a

Table 2. Results of a parametric analysis of population growth experiments with *Daphnia magna* under various levels of bromide stress ($mg\ l^{-1}$).

Model parameter	Symbol	Estimate	Variance
Inoculum in blank	E_b	5.3	3.2
Inoculum gradient	E_g	5.3	3.0
In EC_{50} for inoculum	E_c	3.3	0.008
Blank reproduction rate	R_b	0.55	0.0014
Yield in blank	Y_b	1240	1010
Yield gradient	Y_g	1.9	0.3
In EC_{50} for yield	Y_c	2.6	0.047
No effect level	γ	14.3	6.8
Model variance	σ^2		534

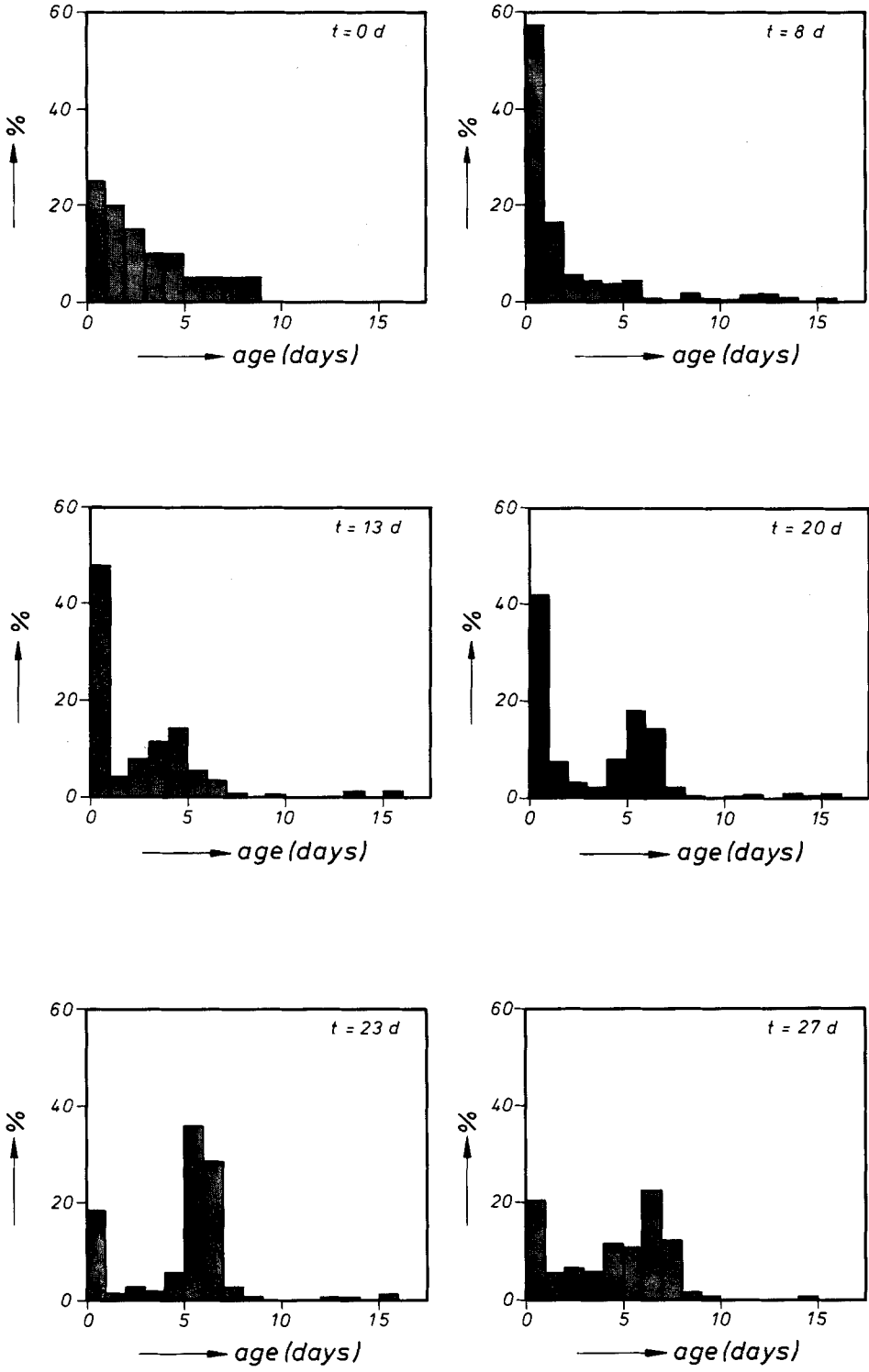


Fig. 4. Changes in the age structure of a *Daphnia magna* population in time.

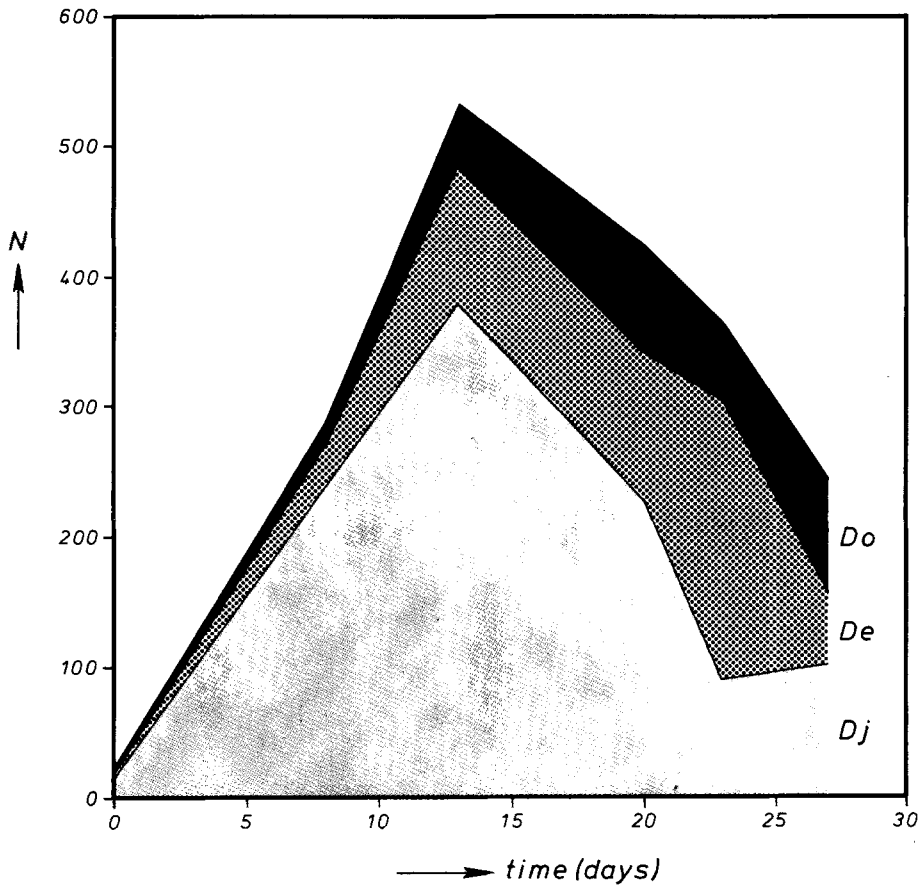


Fig. 5. Growth of a *Daphnia magna* population in time. De = egg-carrying females, Do = adults without eggs or embryos, Dj = adolescents and juveniles.

repetitive way (cf. Marshall, 1978). Changes in the age structure may also account for the absence of constant upper numerical limits (Fig. 4). It should be noted that the age structure determinations are estimations, as actual *Chlorella* densities in the

Table 3. Demographic information for *Daphnia magna* from an intermittent-flow experiment with populations.

Time (days)	Mean number per compartment	Biomass (mg)	Reproductive ratio	Mean brood size
0	20	1.98	0.59	9.9
8	286	16.03	0.65	18.8
13	533	31.24	0.67	7.5
20	424	32.36	0.57	3.5
23	365	41.74	0.78	2.5
27	246	24.05	0.37	3.2

population growth experiments diminished, whereas the equation for individual growth (Fig. 3) was derived from experiments with cohorts under an approximately constant food density.

It was striking that total biomass still increased after the daphnid population had reached its numerical maximum. This may be explained by the buildup of energy reserves in the form of lipid droplets (Tessier & Goulden, 1982) in the preceding food-unlimited phase, i.e. the production of daphnids with a high calorific value. It may also be explained by a decrease in nutritional requirements at older ages. When growth and reproduction cease, energy is spent on maintenance only. Maintenance, or respiration, i.e. oxygen consumption on a unit weight basis, is smaller in older animals (Richman, 1958).

Bromide inhibited reproduction. In the population toxicity study, this is shown by an increased time-lag prior to attainment of the maximum reproduction rate (Fig. 2). The EC_{10} for this parameter was 18 mg l^{-1} , whereas the lowest concentration which significantly reduced r_m in the life-table study was 32 mg l^{-1} . The results are consistent with those reported by Canton *et al.* (1983).

The major difference between life-table and population toxicity tests, is, that in the latter type of experiment the population will meet the inevitable situation of food limitation. In contrast life-table studies are conducted at a constant food density. The amount of food generally applied is unrealistically high (Buikema *et al.*, 1980), and clearly above the threshold food density (cf. Kersting & Van Der Leeuw-Leegwater, 1976). Thus, effects of chemicals on the threshold food density for daphnids cannot be detected in life-table studies. As pointed out by Kooyman & Metz (1983), the effects of chemicals on populations are strongly dependent on food availability. A shortage of food is likely to greatly increase chemical stress. This possibly offers an explanation for the bromide-induced reduction of the yield in the experiment with populations. By which this effect was caused, i.e., a slight reduction of the filtration rate, digestion rate or changes in basal metabolism, remains unsolved.

Yet, this result supports Halbach's view (1984), that population dynamics can be used like a 'magnifying glass' to detect small sublethal effects of environmental pollutants.

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