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"Sublethal Narcosis and Population Persistence: A Modeling Study on Growth Effects"

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SUBLETHAL NARCOSIS AND POPULATION PERSISTENCE: A MODELING STUDY ON GROWTH EFFECTS

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Abstract—This study of a *Daphnia* population model suggests that sublethal effects of nonpolar narcotics on growth of individual organisms can result in ultimate extinction of the population at chronic chemical concentrations near the effect concentration that leads to a 50% reduction in individual growth (the 16-d EC50 for growth). A quantitative dose-response relationship (QDRR)—a population extinction threshold, relating the minimal chronic chemical concentration that yields population persistence and the octanol/water partition coefficient (K_{ow})—is generated from numerical simulation studies of a *Daphnia* population model. The transition from population persistence to extinction is examined in terms of density functions in the lipid, structure, and age variables associated with models of individual daphnids. The densities of populations undergoing transition from persistence to extinction due to increased toxicity suggest that critically stressed populations are age-bimodal with a component of old, large individuals and a component of small, nonreproductive individuals. The populations that persist near the threshold for extinction are unexpectedly diverse in age density and consist of relatively small individuals.

Keywords—Sublethal effects Model *Daphnia* Dose response Narcosis

INTRODUCTION

Ecological risk assessment approaches are maturing in that determinations of probable effects of toxicant stress sometimes are based on factors other than mortality of organisms. Although it is generally recognized that sublethal effects on individuals are important, it is not known how they are manifested at the population level. To better understand implications of sublethal chemical stresses, we employ a modeling approach to study the effects of a class of chemicals—those with the nonpolar narcosis mechanism of action. In spite of narcotics being widely studied and constituting about 70% of all industrial chemicals, their effects at the population and higher ecological organizational levels are relatively unknown [1].

We investigate the ramifications of a particular sublethal effect—growth of individuals—on a model *Daphnia* population. Clearly, growth governs ultimate size of an organism. For many organisms, including daphnids, an individual's size explicitly relates to many life history attributes such as filtering, digestion, maintenance, and reproduction [2].

We focus on a *Daphnia* population as our study organism for several reasons. Quantitative structure-activity relationships (QSARs) for nonpolar narcotics are available for daphnids [3–5]. *Daphnia* is the designated test organism for many toxicity tests. A physiological, energetics-based model of an individual daphnid exists [2]. It has been coupled with an exposure model, FGETS [6], and the resulting composite model has been used to investigate the mortality effects of chemicals on populations [7]. An analogous methodology is employed here, but in addition to mortality, sublethal effects of growth are incorporated into the individual model and the effects on the population are investigated.

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Individual daphnid model

To be useful in risk assessment, factors relevant to the growth of a daphnid, the dynamics of the population, and the chemical stress should be depicted in the model of the individual organism. A brief overview of the individual daphnid model follows. The references indicate our approach and should be consulted for additional detail [2,7].

The individual model has five components. Two components represent labile and nonlabile lipid phases, and two represent labile and nonlabile structure phases (primarily protein) as a function of age of the individual [2]. The fifth phase is the aqueous phase of the organism. Structured population models traditionally have represented individuals in terms of age and size [8]; however, the basal lipid or storage components have been included only relatively recently in mathematical models [9], even though the representation of lipid appears necessary from both the biological and the toxicological perspectives. Lipid is fundamental to reproduction because most organisms cannot reproduce if labile lipid is unavailable for reproductive allocation. It is necessary for the determination of time to effect of certain chemical stresses [10]. Structure is regarded here as a variable from which organism size can be computed. The aqueous portion of the individual is needed for transport and determination of the net affinity of the chemical to an organism's body phases.

The life history of our model daphnid is now summarized. Each daphnid is assumed to feed at a constant resource level. However, because we allow the resource level to vary from individual to individual, a diversity of types occurs in the population. The organism grows until it reaches a prescribed size at which it may reproduce. After attaining reproductive size, the organism is assumed to reproduce periodically. The number of eggs produced by an individual at a reproductive time is a function of labile lipid and labile structure in the daphnid model. Progeny are assumed to be both genetic and environmental clones of their parthenogenetically reproducing parent. The "production of genetic clone" assumption generally is adequate for parthenogenetically reproducing organisms such as *Daphnia*.

THE MODEL

Model of an individual daphnid

The model [2] used to study the sublethal effects on an individual is

$$\begin{aligned} dm_l/da &= g_L(a, m_l, m_s) \\ &= XA_{OL}x_L m_s/(A_1 m_s^{1/3} + A_2 x_T) \\ &\quad - f_L(m_l, m_s), \\ dm_s/da &= g_S(a, m_l, m_s) \\ &= XA_{OS}x_S m_s/(A_1 m_s^{1/3} + A_2 x_T) \\ &\quad - f_S(m_l, m_s) \end{aligned} \tag{1}$$

In Equation 1, the parameters are the assimilation efficiencies for lipid, A_{OL} (assimilation rate/ingestion rate; nondimensional), and structure, A_{OS} (assimilation rate/ingestion rate; nondimensional). A_1 is the reciprocal of the constant allometrically relating the maximal filtering rate to organism length squared ($mg^{2/3}\ d^{-1}\ mm^{-3}$). A_2 is the reciprocal of the constant allometrically relating the maximal ingestion rate to organism length squared (d). x_L is the density of the lipid in the resource (mg/mm^3), x_S is the density of the structure in the resource (mg/mm^3), and x_T is the total resource density (mg/mm^3). The variables m_l, m_s, and a are the mass (mg) of the lipid, mass (mg) of the structure and age of the individual, respectively. The loss rates of the components, f_L and f_S, are described in detail elsewhere [2]; because they are complex and are assumed not to be affected by the chemical, the precise forms are omitted. The expression X represents the reduction in component growth rate caused by chemical inhibition and is explained in the next section.

Sublethal effects representation

Exposure of a daphnid to a nonpolar narcotic chemical is assumed to cause effects ranging from no observed effect to reduction of growth to lethality [3–5,9]. Lassiter [11] assumes that narcosis occurs through the mechanism of competitive inhibition of a cellular enzyme system and that the chain of mechanisms between the site of action and the observable effect alters the magnitude of the parameters but does not change the form of the relationship of toxicant concentration to enzyme-system response. He derives the response relationship

$$\log C_A = \log[rK_i/(1 - r + rK_i)] - \log K_{op} - \log K_{ow} \tag{2}$$

where C_A is the internal aqueous concentration of chemical in the individual organism; r is the frac-

tional reduction of enzyme sites that occurs through competitive inhibition; K_i is a factor that is set by the ith effector system that modulates the toxicant level at which the response occurs [12]; and K_{op} is the product of the concentration of site-bound toxicant per quantity of total sites and the octanol/water partition coefficient, K_{ow}, divided by the internal aqueous concentration of toxicant.

This representation demonstrates conditions under which the widely observed (−1) slope found in QSARs for nonpolar narcotics is valid. Such QSARs [3–5] relate the logarithm of the *ambient* concentration of chemical that causes the effect to the logarithm of the octanol/water partition coefficient. Equation 2 utilizes *internal* aqueous concentration of chemical. Toxicant concentration is maintained at the sites of action by exposure to the ambient concentration of chemical, by the organism's exchange mechanisms, and by the organism's body composition and circulation. For prolonged exposures in the aqueous medium to constant concentration, c, the internal aqueous concentration will stabilize at the level c, provided there is no significant metabolic loss of chemical. For fluctuating ambient concentrations, the internal concentration also will fluctuate in a manner reflecting both the external fluctuations and the time constants of the organism's exchange mechanisms. We assume that the characteristic time for internal distribution is short compared with the characteristic time for exchange with the environment. This internal equilibrium assumption is important for this approach in that it allows computation of internal chemical distribution as a steady-state process that changes in all model phases of the organism as the organism responds kinetically to the external concentration fluctuations. Furthermore, this assumption, together with the tendency for internal aqueous chemical concentration to approach external concentration, allows interpretation of the ambient chemical concentration used in QSARs to be applied as the ultimate internal aqueous chemical concentration. Solving Equation 2 for r yields a hyperbolic function in C_A:

$$r(C_A) = K_{op}K_{ow}C_A/[K_i + (1 - K_i)K_{op}K_{ow}C_A] \quad (3)$$

The quantitative dose-response relationship (QDRR; Eqn. 2) is a continuous function of the reduction fraction r of inhibited sites. Equation 3 implies that effects occur continuously as a function of site inhibition. Because a small number of inhibited sites is generally unobservable, we modify the form of Equation 3 to impose a no-observed-effect-level threshold (NOEC) to be used for the onset of effects. The resulting hyperbolic form, $x = x(C_A)$, differs from Equation 3 only in the numerator, where evaluation of x at the NOEC results in $x(\text{NOEC}) = 0$. It is difficult to measure several quantities in Equation 3, so we write $x(C_A)$ in the form

$$x(C_A) = \alpha(C_A - \beta K_{ow}^{-1})/(\gamma K_{ow}^{-1} + C_A) \quad (4)$$

and determine the parameters from available data. In Equation 4, the parameters α, β, and γ are determined such that the percentage growth reductions in the individual model given in Equation 1 agree with average fitted aspects of the QSARs presented by Hermens and co-workers [3–5], assuming that the slope of their QSARs are −1 as indicated in Lassiter's derivation [11]. The value, $10^{-1.83}K_{ow}^{-1}$, is the computed average of the 16-d NOEC(growth) obtained in De Wolf et al. [5], stated here in molar units rather than micromoles given in each of the QSAR papers. This threshold level for onset of effects determines the parameter β in Equation 4.

The determination of the remaining two parameters, α and γ, in Equation 4 is accomplished by utilizing the computed average effect concentration that reduces the growth by 50% in a 16-d test, $10^{-1.34}K_{ow}^{-1}$ (the EC50 for growth [4]). This translates numerically into $x(10^{-1.34}K_{ow}^{-1}) = 1/2$. The 16-d mortality assay, LC50, in which 50% of the test organisms are killed, $10^{-0.8}K_{ow}^{-1}$ yields $x(10^{-0.8}K_{ow}^{-1}) = 1$; consequently, $X(10^{-0.8}K_{ow}^{-1}) = 0$. A more appropriate value for this computation would be a QSAR that gives complete growth inhibition. Unfortunately, none was available. The reduction in growth due to chemical stress is applied to the growth rates of the lipid and structure components. However, the growth data in Hermens et al. [3–5] are presented in terms of changes in the length of the daphnid. We have measured length in our individual model through an allometric relationship for length that is a function of the structure component m_s [2]. It is this measured length that is used to determine appropriate reduction in m_s so that the parameters in Equation 4 can be related to the reductions in component growth due to chemical stress. The use of the above numerical information in Equation 4 leads to two equations in

the two unknowns, α and γ, whose unique solution is found as $\alpha = 1.378$ and $\gamma = 0.0395$.

The resulting form of x is incorporated into the growth rate functions of the individual daphnid model through the function X; specifically, the growth rates of the lipid and structure components of the individual daphnid model are reduced by the factor $X = 1 - x$, where

$$x = 1.378(C_A - 0.01479K_{ow}^{-1})/(0.0395K_{ow}^{-1} + C_A) \quad (5)$$

provided C_A satisfies $10^{-1.83}K_{ow}^{-1} \leq C_A \leq 10^{-0.8}K_{ow}^{-1}$. When the chemical concentration in the aqueous phase of the individual is less than or equal to $10^{-1.83}K_{ow}^{-1}$, the 16-d NOEC, it is assumed that there is no reduction in growth, so that X is taken to be 1 in the growth model, Equation 1. When the aqueous chemical concentration is between the NOEC and the 16-d LC50 (mortality) concentration, $10^{-0.8}K_{ow}^{-1}$, then the growth rate is reduced by the factor X. Mortality occurs when the QSAR intercept is -0.8 or, equivalently, at a concentration of $10^{-0.8}K_{ow}^{-1}$. If $C_A \geq 10^{-0.8}K_{ow}^{-1}$ then X is taken to be 0. In this situation, the net growth rate is negative because the compartments of the organism have no positive growth component, but metabolism continues.

The representation X presumes instantaneous effect occurrence, that a reduced growth rate occurs exactly at the time chemical activity reaches the effect level. Our representation of sublethal effects results in the growth reduction occurring continuously as a response to continuous internal toxicant concentration. Death of the organism is assumed to befall exactly at the time when the concentration in the aqueous phase of the daphnid reaches the value LC50 and, different from the growth, is quantally rather than continuously assessed.

Population model

The individual model is incorporated into an extended McKendrick-von Foerster model to study population dynamics [7,8]:

$$\partial\rho/\partial t + \partial\rho/\partial a + \partial(\rho g_L)/\partial m_l + \partial(\rho g_S)/\partial m_s = -\mu\rho \quad (6)$$

In Equation 6, g_L and g_S are prescribed by Equation 1. This hyperbolic partial differential equation describes the population in terms of the density function $\rho = \rho(t, a, m_l, m_s)$, which is measured in terms of numbers per age, per mass lipid, per mass structure. We assume that reproduction is clonal, and consequently, Equation 6 tracks cohorts of individuals, the type of individual described by Equation 1, in the population [7]. Equation 6 contains nonlinear mortality representations due to density dependence in the function μ (number of deaths per time) [7]. In addition to the density-dependent mortality in Equation 6, we include formulations for age-dependent mortality and size-dependent mortality. Size-dependent mortality is representative of predation, which we do not model explicitly. It is also assumed that an individual will ultimately die of old age. In the case of *Daphnia magna*, this maximal attainable age is assumed to be 50 d. For the mathematical problem to be well posed, an initial distribution, $\rho(0, a, m_l, m_s)$, and a boundary condition, $\rho(t, 0, m_l, m_s)$, indicating the birth process must be specified. The boundary condition is computed by accumulating all births as determined by the individual model [2].

The metapopulation, the combination of numerous ecotypes of individuals, can be of arbitrary dimension. We use a model that consists of 27 extended McKendrick-von Foerster partial differential equations like Equation 6—one equation for each of 27 different ecotypes of individuals in the initial distribution. Hallam et al. [7] delineate characteristics of the ecotypes employed here. The diversity of ecotypes in the population is determined by three distinct choices of the three parameters: resource level (x_T), quality of resource (x_L), and filtering rate (A_1). The metapopulation composition reflects age, lipid, and structure variation. We use the same initial population distributions of age, lipid, and structure in all of our simulations. This initial distribution contains a spectrum of individuals whose distributions of age, size, and lipid are realizable in known laboratory populations of *D. magna*.

SIMULATION RESULTS

QDRR

The methodology agendum, numerical simulation of a *Daphnia* population, focuses on individual survival in toxicant-stressed populations. We say a population is *persistent* if the population density function ρ is nonzero throughout the simulation run time. A population goes to *extinction* if $\rho = 0$ at some time during the simulation run. This definition of survival is clearly a function of the length of the simulation; however, for sublethal exposures that produce growth effects, we find that extinction is independent of simulation run time if the run time is sufficiently long, generally >100 d.

4.

In our simulations, the *exposure scenario* consists of chronically exposing all individuals in a population to a toxic chemical at a constant concentration. The *population extinction threshold* (PET) is the ambient concentration of a chemical such that if the ambient chemical exposure concentration, C, for the stressed population is less than PET then the population is persistent, and if C is greater than or equal to PET then the population goes to extinction. A main objective of this article is to present a QDRR (Fig. 1) that represents the PET as a function of the chemical property log K_{ow}. A point on the PET for the *Daphnia* population model is generated by selecting K_{ow} within the interval from 1 to 10^7, a range appropriate for narcotic chemicals, and performing numerous chemical stress simulation runs to calculate the minimum concentration at which extinction of the population results. The process, repeated for numerous values of K_{ow}, generates the PET QDRR. The summary of these computations is the essence of Figure 1. The three most important features of the PET QDRR are

1. It is linear in log K_{ow}
2. It has a slope of -1 (as we have assumed the QSARs for nonpolar narcotics do)
3. It lies below the known QSARs for the EC50 for growth and reproduction.

In Figure 1, the upper line (with intercept -0.8) is the 16-d LC50 and the lower line (with intercept -1.83) is the NOEC for *Daphnia* growth [3,4]. The lower boundary line (with intercept -1.40) of the filled region is the computed PET for the nominal simulation of the model *Daphnia* population. The nominal population model includes all types of mortality representations with parameters as in [7]. The upper boundary line (with intercept -1.38) of the filled region is the computed population extinction threshold found when density-dependent mortality is completely removed. The line with intercept -1.34 is the 16-d EC50 for growth, and the line with intercept -1.28 is the computed PET obtained when there is only maximum age mortality, that is, no density-, age-, or weight-dependent mortality in the model. For exposure concentrations above the 16-d EC50, the chemically stressed model population always goes to extinction.

Population structure near the PET

The structure of the population as it evolves from a state of persistence to one of extinction is revealing because there is a distinct change in the population structure. This extinction scenario is a possible indicator of strongly affected, stressed populations, and it might prove useful as an indicator of stressed populations that are in danger of going to extinction. In the computational studies near the

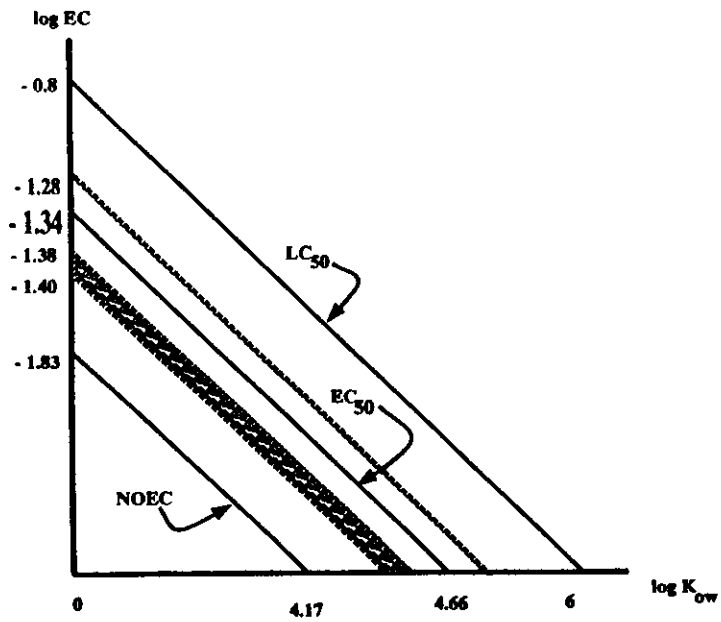

Fig. 1. A comparison between quantitative structure-activity relationships (QSARs) and quantitative dose-response relationships (QDRRs) for population extinction thresholds (PETs) obtained from the simulations under different assumptions about mortality. The axes are the logarithm of the octanol/water partition coefficient and the logarithm of the effect concentration. The solid lines are QSARs and the squiggled lines are computed. See text for description of linear functions and shaded area.

PET, a robust pattern emerged that was associated with extinction and independent of the toxicity of the chemical. Shortly before the population went to extinction, the age distribution in the population was bimodal. One population mode consisted of older individuals who had survived the chemical stress, the other consisted of younger, subsisting organisms that had not grown sufficiently large to reproduce during their lifetime (Fig. 2). This behavior has been observed in laboratory populations of *D. magna* stressed by the herbicide diuron [13].

The structure of populations surviving a sublethal chemical stress at concentrations near the PET also has an interesting, demonstrable behavior. Chemical concentrations in which the population is persistent show a wide spectrum of ages within that population; in our simulations, age distribution in these populations is more diverse than in less stressed populations or even unstressed populations (Fig. 3). The individuals in stressed but persistent populations near the PET are much smaller and less fat than those in healthy populations. The diversity of age apparent in the persistent, stressed populations in concentrations near the PET concentration is unexpected but, perhaps, reasonable. This phenomenon, attributed to a combination of population mortality representations and a lower growth rate in individuals whenever a longer time is required to reach a particular size, results in a continuing supply of individuals of reproductive size that cover a spectrum of ages.

DISCUSSION

Fundamental to our conclusion about the structure of the population near the PET concentration is the assumption that an individual reproduces after it attains a prescribed size. This reproductive trait is documented for some laboratory and field populations of *D. magna* [14], although it may not hold in all environments. The bimodal age distribution that results as the chemically stressed population evolves to extinction is obtained as a consequence of this assumption. Before extinction, the mode containing the older individuals was composed of organisms that were of reproductive size before the exposure began; the other mode consisted of individuals born to the older individuals after the exposure started but were not able to grow to reproductive size. The older individuals eventually attained maximum age and died. The smaller individuals, which never attained reproductive size, also died. Consequently, the population ultimately went to extinction.

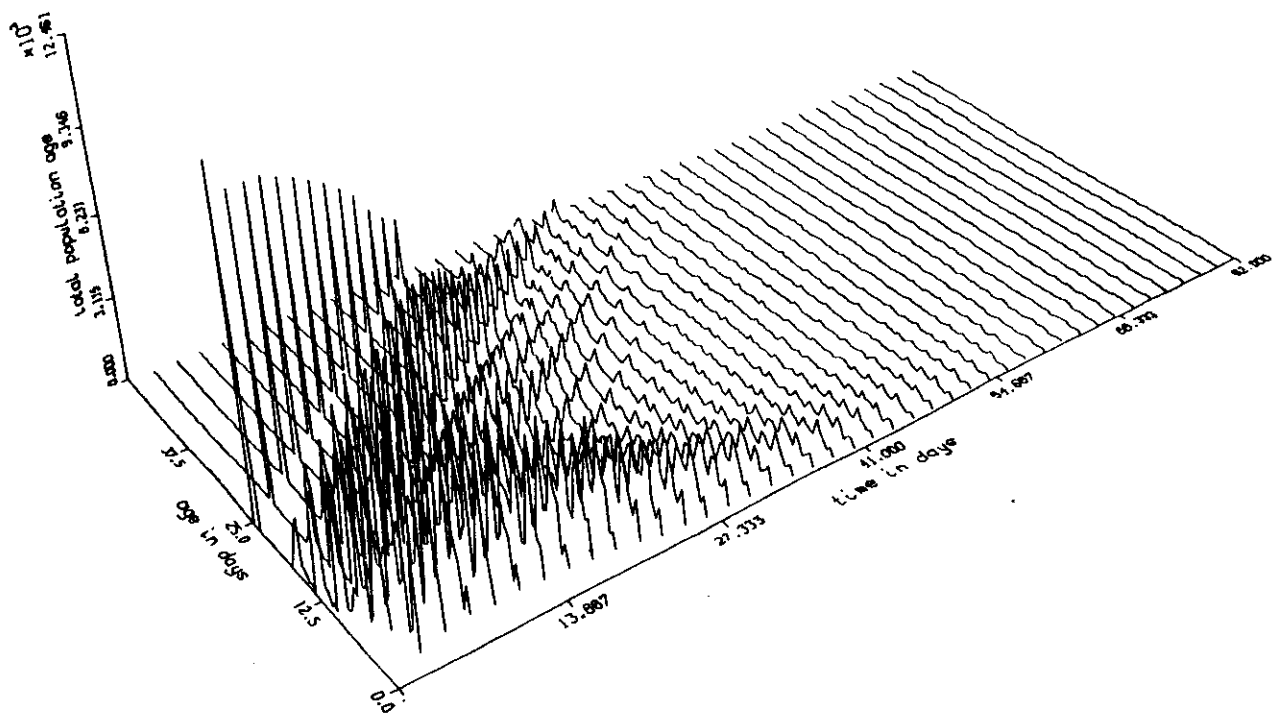

Fig. 2. The bimodal age density of a chemically stressed model population as it nears extinction close to the extinction threshold. The axes are age, time, and number of individuals times the age of the individuals. $K_{ow} = 100$, and the concentration of chemical in the water is 0.362×10^{-3} (mg/L).

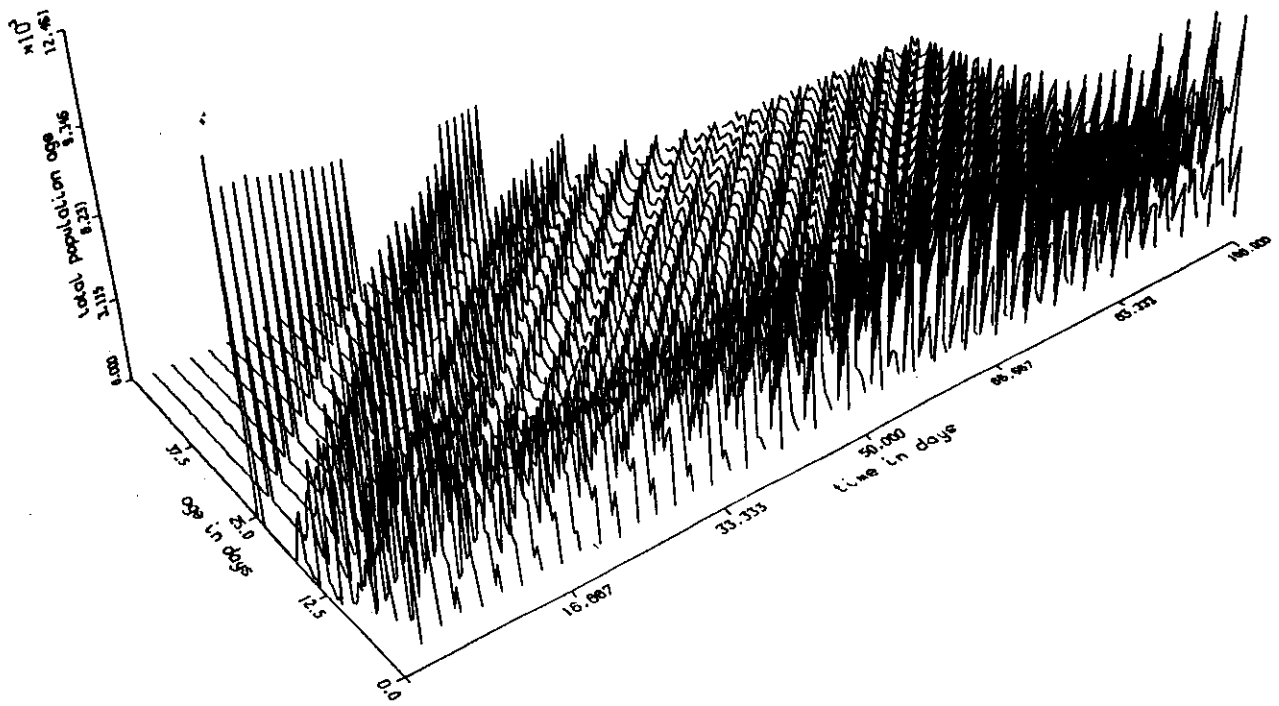

Fig. 3. The age density of a chemically stressed population that is persistent but near the threshold concentration. The axes are age, time, and number of individuals times the age of the individuals. $K_{ow} = 100$, and the concentration in the water is 0.36×10^{-3}; this is only 0.000002 lower than the concentration that led to extinction (Fig. 2).

Sensitivity

Any modeling approach is limited by assumptions. We believe that the physiology represented in our individual model is reasonable and probably adequate for the population time scales considered here; however, the conclusions must be examined in reference to the model assumptions. The part of the model for which there are the fewest data is the density-dependent component of the mortality function. We performed a sensitivity analysis to investigate the change in the QDRR by changing the parameter values associated with the density-dependent mortality representation. Whereas the specific threshold concentrations were affected by the mortality representation, the resulting thresholds of persistence were always below the EC50 for growth and the maximal threshold concentration level (the line with intercept −1.38 in Fig. 1) was never >10% of the nominal threshold values obtained when dependent mortality was included. This maximal threshold level corresponded to a decrease in growth of about 40% in the daphnid model.

We also completely removed all age-, size-, and density-dependent mortality from our model to find the maximum possible PET. In this instance, mortality occurred only when individuals died from attaining a maximum age. Even in this unrealistic setting, the threshold (the line in Fig. 1 with intercept −1.28) was well below the LC50 and only slightly above the experimental EC50. This threshold corresponded to a decrease in growth of approximately 60%.

We have considered only a single sublethal effect of chemicals on growth of individuals. There are other QSARs, such as direct reproductive effects, that might be incorporated. That we have not incorporated other effects in our model indicates that our results present a more positive perspective than a more complete toxicity syndrome, one that includes other sublethal effects such as reduced clutch size, would reflect.

This modeling investigation, focusing on the effects of sublethal concentrations of a chemical on growth of individuals in a model *Daphnia* population, reinforces knowledge that sublethal effects are important for population risk assessment. Furthermore, there may be a transitional mode between extinction and persistence of certain stressed populations that can be identified in natural populations. The QDRR found for threshold concentrations of chemicals that result in extinction of the stressed population needs experimental verification; however, that this computed QDRR clearly lies near the derived EC50 for growth and reproduction in *Daphnia* could have important ramifications for

risk assessment. The extrapolation of information from laboratory bioassays to natural populations has been fraught with difficulty and uncertainty. The transfer of information from the individual to the population level through mathematical models provides a theoretical basis for extrapolation from laboratory to natural populations.

We conclude that chemical stress, evaluated at the individual level and perceived to be sublethal, could ultimately be fatal to a population. The relationship between chemical stress and population survival, as reflected here through continuous spectra of effects, suggests that an assessment summary of a small number of isolated discrete bioassay end points may not reveal crucial information about population dynamics.

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