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Ecotoxicology and population dynamics: Using DEBtox models in a Leslie modeling approach

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Abstract

Although the ecological risks of toxic chemicals are usually assessed on the basis of individual responses, such as survival, reproduction or growth, ecotoxicologists are now attempting to assess the impact of environmental pollution on the dynamics of naturally exposed populations. The main issue is how to infer the likely impact on the population of the toxic effects observed at the individual level. Dynamic energy budget in toxicology (DEBtox) is the most user-friendly software currently available to analyze the experimental data obtained in toxicity tests performed on individuals. Because toxic effects are diverse and because the sensitivity of individuals varies considerably depending on life-cycle stage, Leslie models offer a convenient way of predicting toxicant effects on population dynamics.

In the present study, we first show how parameter inputs, estimated from individual data using DEBtox, can be coupled using a Leslie matrix population model. Then, using experimental data obtained with *Chironomus riparius*, we show how the effects of a pesticide (methiocarb) on the population growth rate of a laboratory population can be estimated. Lastly, we perform a complex sensitivity analysis to pinpoint critical age classes within the population for the purposes of the field management of populations.

Keywords: Ecotoxicology; Leslie matrix population model; DEBtox; *Chironomus riparius*; Methiocarb

1. Introduction

For practical reasons, ecotoxicology initially focused on the effect of pollutants on organisms by means of bioassays performed in the laboratory. Nowadays, ecotoxicologists are attempting to assess the impact of environmental pollution on the dynamics of exposed natural populations (Baird et al., 1996; Spromberg et al., 1998; Caswell, 1996), in order to reach conclusions with greater relevance for the ecosystem. The main problem is that experiments involving an entire population can be very onerous in terms of time and expense.

Modeling offers a possible intermediate in this transition from individual to population level. One

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major advantage of mathematical modeling, compared to descriptive and/or purely statistical methods, is that it can be used to test various hypotheses and scenarios, in order to predict the outcome of some effects, of an ecological state, or simply to identify the most relevant biological variables. In this paper, we have chosen to use matrix population models, in which individual-level data can be input and used to calculate characteristic endpoints for the population (Caswell, 2001). Among the possible endpoints, Forbes and Calow (2002) recommend using the population growth rate; "although the most sensitive individual-level variables are likely to be equally or more sensitive to increasing concentrations of toxic chemicals than population growth rate, they are difficult to identify a priori and, even if they could be identified, integrating impacts on key life-cycle variables via population growth rate analysis is nevertheless a more robust approach for assessing the ecological risks of chemicals".

Kooijman and Bedaux (1996) have suggested a way to analyze aquatic toxicity data using a biology-based model known as dynamic energy budgets in toxicology (DEBtox). One of the aims of DEBtox is to estimate a no-effect concentration (NEC), defined as the highest concentration having no effect on the test organism. DEBtox models have many advantages over the descriptive methods usually used to analyze toxicity data. The assumptions on which the predictions concerning survival are based, are realist with the kinetics of the chemical compound. Furthermore, DEBtox models involve only three effect parameters: the elimination rate, the NEC and the killing rate, this latter being the effect observed as soon as the toxicant concentration exceeds the NEC. Another parameter can be deduced from these three; the hazard rate in the control. All these parameters are toxicologically meaningful, and so, the model is of real relevance and provides information of biological interest. DEBtox models also have the advantage of being able to allow for changes in toxicant concentration over time. The final advantage of these models is that they estimate time-independent parameters, unlike no observed effect concentration (NOEC), which is estimated after a statistical testing procedure, or the lethal concentration leading to 50% mortality (LC_{50}), which has been widely criticized in many publications (Chapman et al., 1996). Time-independent parameter estimates make it possible to compare different bioassays, making it easier to forecast the effects in situ, where these parameters are not standardized (Kooijman and Bedaux, 1996).

The aim of our paper is to introduce DEBtox models into a matrix population model, in order to determine the effect of a pesticide, methiocarb on a Chironomidae population. *Chironomus riparius* was chosen, as it is a commonly used species in toxicity laboratory tests and because it is widespread in river sediments. *C. riparius* organisms are considered to be good bioindicators of water quality, and they have characteristics that are advantageous for bioassays: they are easy to culture in the laboratory (short generation time) and are able to tolerate a wide range of physicochemical sediment characteristics (Ingersoll et al., 1995).

Our paper is organized as follows; we first describe the biological data we used to estimate demographic and toxicological parameters; second, we present effect models relating these parameters to the toxicant concentration; and third, we construct a population dynamics model according to the Leslie theory (Leslie, 1945, 1948). In the last part of the paper, we report the effect of the toxicant on the population growth rate and provide a sensitivity analysis that we have found to be very helpful.

2. Biological data

2.1. C. riparius

C. riparius (Diptera: Chironomidae) is a non-biting midge widely distributed in the northern hemisphere (Armitage et al., 1995). Its life-cycle comprises aquatic stages (eggs, larva, pupae) and aerial ones (adults). These four stages are shown in Fig. 1 (Ali and Morris, 1992).

(1) *Stage one*: Females deposit egg masses on the water surface, each of which may contain up to 600 eggs and which hatch after a few days.
(2) *Stage two*: The larval stage involves four instars; the first (L_1) is predominantly planktonic, whereas the second (L_2), third (L_3) and fourth (L_4) instars live in the sediment, where they construct tubes from detritus, algae and sediment particles (Armitage et al., 1995).
(3) *Stage three*: Pupae actively swim to the surface, the pupa stage being a characteristic stage of Diptera.

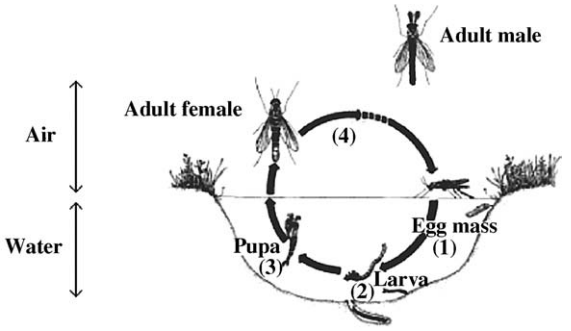

Fig. 1. The life-cycle graph of *Chironomus riparius*.

(4) *Stage four*: The adults emerge a few hours later into the aerial compartment, where they copulate.

In the *C. riparius* species, individuals are synchronous, with a diapause period in the winter—during the fourth larval stage (Goddeeris et al., 2001). Under laboratory conditions, the life-cycle lasts about 17 days, with the stages occurring in rapid succession if food is not a limiting factor.

C. riparius populations are commonly used in laboratory toxicity tests (bioassays) because they are good indicators of water pollution. There are three main reasons for this; first, they play an important ecological role in freshwater ecosystems due to their abundance and the fact that they are a food source for fish and predatory aquatic insects (Burton et al., 1992). Second, they have a short life-cycle under laboratory conditions and the different stages can easily be identified, which makes experiments easier to perform. Finally, the larvae are relatively sensitive to pollution (Ingersoll et al., 1995).

2.2. Methiocarb

2.2.1. Choice of toxicant

Methiocarb is a carbamate pesticide used in agriculture, mainly to protect against insects and molluscs. We chose this chemical compound for three main reasons. First, it has been shown that it is more toxic than other similar chemicals (Marking and Chandler, 1981). Second, it has been found in field sediments at concentrations between 10 and 268 μg/kg (data from the Water Agency of Rhône–Méditerranée–Corse). Third, few studies are available concerning the effects of this compound on benthic organisms and those existing, report an effect on individual survival rates (Péry et al., 2003b, 2004).

2.2.2. Choice of test concentrations

For L_2–L_4, we used the survival test data from a previously published study (Péry et al., 2003b). Six toxicant concentrations were tested: 0 (control), 25, 50, 280, 310 and 360 $\mu g\,L^{-1}$. Seven concentrations were tested in this study for the egg, L_1 and pupa stages, which are the most sensitive stages: 0 (control), 10, 20, 30, 40, 60, 80 $\mu g\,L^{-1}$. These concentrations were determined during preliminary experiments. Péry et al. (2003a,b) showed that the concentration of methiocarb can be considered to be constant throughout an exposure period lasting 3 days.

2.3. Survival experiments in the laboratory (bioassays)

Survival data for the L_2–L_4 stages, are fully reported elsewhere (Péry et al., 2003b). Here we will just recall the main points. At the beginning of each survival test, 20 organisms were randomly placed in beakers. The instar was identified on the basis of head capsule width measurements. Each instar was exposed to the toxicant for 3 days, and the survivors were counted after the first, second and third days of exposure. The experimental conditions were as follows: temperature maintained at 21 °C, a 16-h light:8-h dark photoperiod, a pH between 8.1 and 8.4 and conductivity between 300 and 400 μS/cm.

Survival tests with eggs and first instar larvae were performed by placing individual egg masses in beakers containing 300 mL water (pH 8.1, conductivity 400 μS/cm) and 100 mL of methiocarb dissolved in the same water to yield the exposure concentrations we had chosen. A small amount of silicate was added. The egg masses came from our laboratory culture, which is the same as the one used for survival tests with L_2–L_4. We used five replicates per concentration. The egg masses we used all contained approximately the same number of eggs. We roughly estimated this number by counting the number of rings and the mean number of eggs on three different rings, using a binocular microscope. A given egg mass was only selected if the number of eggs it contained was estimated to be between 250 and 350. During the experiment, the beakers were placed in water maintained at 21 °C to

avoid temperature variations. We used a 16-h light:8-h dark photoperiod. Because the amount of food present could dramatically affect water quality, we used pipettes, pipes and an aeration system to add air to the medium. Larvae were counted after 4 days of exposure. The survivors were too small to be counted daily.

Survival tests with pupae were performed using pupae from our laboratory culture. The experimental conditions were the same as those used previously, with the same exposure concentrations and three replicates per concentration. We used the pupae inside their tubes, because preliminary experiments had shown that the mortality was considerably increased in the control when pupae were removed from their tubes before being introduced into the beakers. The beakers were covered with a net trap to prevent the adults from escaping. Emergence was monitored after 2 days of exposure.

3. Models

3.1. Survival modeling of the egg, first larval and pupa stages versus methiocarb concentrations: logistical models

As we saw in Section 2, the survival data for the egg, L_1 and pupa stages depended solely on the toxicant concentration, and the survivors were counted only once; after exposure for 4 days for the egg and L_1 stages and for 2 days for the pupae. The data obtained were, therefore, the number of survivors in the five replicates of each concentration tested, but we did not know how many chironomids were present initially. This made it impossible to use DEBtox models or generalized linear models.

3.1.1. Construction of the model

Given the data available, the only way to fit a survival model was to take the mean of the five replicates at the null concentration as the reference (when the survival rate equals natural survival rate) and then, to calculate the survival rates of each replicate compared to this reference. The resultant data are shown in Fig. 2a for the egg and L_1 stages and in Fig. 2b for the pupae. We can see that daily survival rates decreased as a function of methiocarb concentration. We, therefore, used a decreasing logistical model as expressed in the following Eq. (1):

$$q(C) = s\,\alpha(C) \text{ with } \alpha(C) = \frac{1 + \exp(a)}{\exp(a) + \exp(bC)} \qquad (1)$$

where C is the toxicant concentration ($\mu g\,L^{-1}$), s the natural survival rate (day^{-1}), $\alpha(C)$ the survival reduction function for a given toxicant concentration C and b is a curvature parameter.

The LC_{50}, i.e. the concentration lethal for 50% of the individuals, is equal to $(\ln(2 + \exp(a)))/b$.

The parameter s had been estimated in a previous study (Charles et al., 2004) and found to be 0.836 for eggs and L_1 and 1 for pupae.

3.1.2. Data analysis

The logistical model (1) was fitted to the experimental data using a non-linear minimization

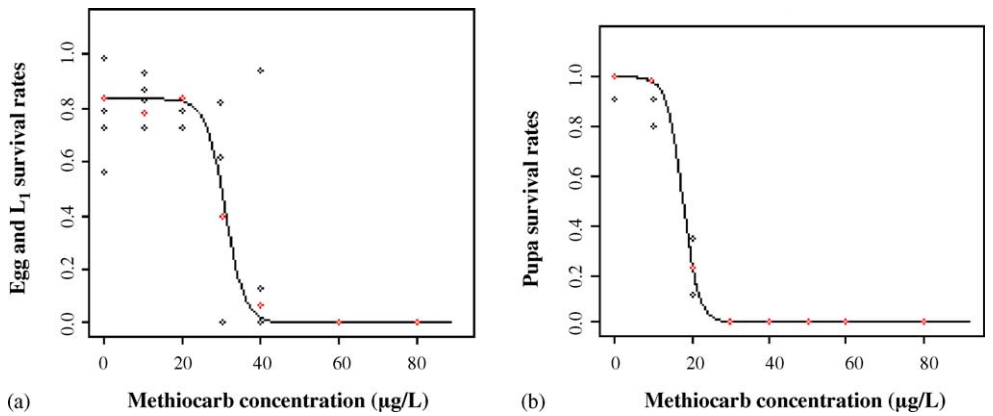

Fig. 2. Logistical effects models eq. (1) fitted to survival experimental data for the: (a) egg and first larval stages and (b) pupa stage.

Table 1
Parameter estimation using the model (1) for the egg and L_1 stages (in bold) and for pupa (ordinary type)

Parameters	Estimation	Standard error[a]	Correlation
a	**8.478**, 8.749	**1.603**, 0.082	**0.996**,
b	**0.282**, 0.499	**0.053**, 0.004	0.998

[a] The total number of experimental points is $n = 35$.

function (nlm) of the software package R (Ihaka and Gentleman, 1996). This iterative fitting procedure is based on a modified Levensberg–Marquardt algorithm (Meyer and Roth, 1972). It minimizes the sum of the squares (RSS) of the differences between the calculated (C, q) and observed values (C_i, q_i) and the initial values of the parameters (Bates and Watts, 1988):

$$\mathrm{RSS} = \sum_{i=1}^{n} [q_i - q(C_i)]^2 = \sum_{i=1}^{n} \left(q_i - s\frac{1+\exp(a)}{\exp(a)+\exp(bC_i)} \right)^2 \quad (2)$$

where n is the number of experimental data.

This algorithm makes it possible to estimate parameters a and b, corresponding to the lowest residual sum of squares. Parameter estimates, the corresponding standard errors and the correlation between the two estimated parameters are shown in Table 1. First, the closeness of the fit can be estimated visually from Fig. 2a by examining the theoretical curve superimposed over the experimental points. The residual standard error was estimated to be 0.008 for the eggs and L_1 and 4×10^{-7} for pupae, with 33 degrees of freedom ($n-2$). The elliptic form of the contour lines (not shown) indicates the good identification of the parameters, even though the elongated shape suggests a strong correlation between the two parameters, as confirmed by the correlation given in Table 1. This correlation is due to the data set itself and not to the mathematical expression of the model. It cannot be avoided, as both parameters are required to describe the decrease in survival.

3.2. Survival modeling of the second, third and fourth larval stages versus methiocarb concentration: DEBtox models

It should be recalled that the data available for these stages are functions of both the toxicant concentration and the exposure time, as the survivors were counted every day for 4 days after exposure. This type of data can easily be analyzed using DEBtox models, as fully described in Kooijman and Bedaux (1996). We just recall here the main equations used in the survival model.

3.2.1. Kinetics module: from exposure to the concentration in the body

A simple, linear, one-compartment model describes the kinetics of the chemical compound. The uptake of the compound is assumed to be proportional to its concentration in the solution, whereas its elimination is assumed to be proportional to its concentration in the body. This leads to the following equation:

$$\frac{\mathrm{d}c_i}{\mathrm{d}t}(t) = \varepsilon(C - c_i(t)) \quad (3)$$

where ε is the elimination rate, C the concentration in the solution (external concentration) and $c_i(t)$ is the scaled internal concentration, related to the original one by the equation $c_i = C_i/\mathrm{BCF}$. C_i is the internal concentration, i.e. the ratio of the amount of compound in the body to the body volume and BCF is the bioconcentration factor, defined as the ultimate ratio between the concentration in the body of an organism and concentration in the solution when the latter is kept constant. We assume that the initial amount of compound can be neglected, that is, $c_i(0)$ equals 0, because the organisms exposed came from a laboratory culture and, since methiocarb is not an essential composite, the organisms were healthy initially. This assumption makes it possible to avoid having to estimate this parameter $c_i(0)$.

3.2.2. Effect module: from the concentration in the body to the produced effects

The survival probability at time t, $q(t)$, is defined as the probability of surviving until time t, and can be expressed as the exponential of minus the cumulated hazard function, as shown in (4):

$$q(t) = \exp\left[-\int_0^t h(\tau)\mathrm{d}\tau \right] \quad (4)$$

where $h(\tau)$ is the hazard rate at time τ. For a small interval $\mathrm{d}\tau$, $h(\tau)\mathrm{d}\tau$ represents the probability of dying between τ and $\tau + \mathrm{d}\tau$ for an organism who has survived until time τ. In the DEBtox approach, we assume

that there is an NEC, i.e. the concentration that has no effect on the survival of the organisms during the bioassay, regardless of how long this lasts. As soon as the concentration in the organism, $c_i(t)$, exceeds this NEC, the hazard rate is assumed to increase proportionally to the difference between $c_i(t)$ and the NEC:

$$h(t) = \begin{cases} k\left(c_i(t) - NEC\right) + m & \text{if} \quad c_i(t) > NEC \quad (5) \\ m & \text{if} \quad c_i(t) < NEC \quad (6) \end{cases}$$

where k is the killing rate and m is the natural death rate, which is assumed to be constant. The model being a hazard model, the description of the data is based on the percentages of organisms dying between two measurements. These percentages are assumed to be statistically independent.

In our case, DEBtox models were used to express survival rates of L_2, L_3 and L_4 according to the toxicant concentration, C, and the time, t. The survival probability between 0 and t for a given stage, denoted by $q(t, C)$, is expressed as shown in (7) and (8):

- If $C < \text{NEC}$, the toxicant has no effect, and so only the natural mortality is taken into account:

$$q(t) = \exp[-mt] \quad (7)$$

- If $C > \text{NEC}$, we have:

$$q(t, C) = \exp[f(t, C)] \quad (8)$$

with

$$f(t, C) = -mt + \frac{k}{\varepsilon} C \left(1 - \frac{\text{NEC}}{C} - \exp[-\varepsilon t]\right) - k(C - \text{NEC}) \left(t + \frac{1}{\varepsilon} \ln\left(1 - \frac{\text{NEC}}{C}\right)\right)$$

The biological rhythm of *C. riparius* being circadian, the survival probability must be accounted for a day t to the next one $(t + 1)$. Therefore, these equations become:

- If $C < \text{NEC}$

$$q = \exp[-m] \quad (9)$$

- If $C > \text{NEC}$

$$q(t, C) = \exp[f(t + 1, C) - f(t, C)]$$

or

$$q(t, C) = \exp\left[-m + \frac{k}{\varepsilon} C \exp[-\varepsilon t](1 - \exp[-\varepsilon]) - k(C - NEC)\right] \quad (10)$$

3.2.3. Fitting procedure

The DEBtox software package (Kooijman and Bedaux, 1996) can be used in survival models to estimate the four parameters, namely m, k, ε and the NEC ((9) and (10)). DEBtox is a user-friendly package intended for use in the analysis of standard aquatic toxicity test data: acute and chronic tests of survival, growth and reproduction. The biological variable measured is known as the "response", and the main goal of DEBtox models is to characterize the effect of the chemical compound on this response. Parameter estimation is performed by maximizing the likelihood function (ln), which is described by (11) for the survival experiment:

$$\ell[\theta, (x_{ij})] = \sum_{i=1}^{r+1} \sum_{j=1}^{z} n_{ij} \ln(p_{ij}) \quad (11)$$

where θ is the parameter set to estimate: $\theta = (m, k, \varepsilon, \text{NEC})$, p_{ij} the probability that an organism exposed to concentration c_j will die between t_{i-1} and t_i, the index $i = 0, \ldots, r$ corresponds to the duration of the test ($r = 3$ days in the case of *C. riparius*), the sub-index $j = 1, \ldots, z$ corresponds to concentrations used in the bioassays ($z = 6$ for L_2, L_3, L_4), n_{ij} the number of organisms dying during that period and x_{ij} is the number of surviving organisms at t_i that have been exposed to a toxicant concentration of c_j: $n_{ij} = x_{i-1,j} - x_{ij}$.

Maximum likelihood estimates of θ can be found by solving the vector (12):

$$\frac{\partial \ell}{\partial \theta} = 0 \Rightarrow \sum_{i=1}^{r+1} \sum_{j=1}^{z} \frac{n_{ij}}{p_{ij}} \frac{\partial p_{ij}}{\partial \theta} = 0 \quad (12)$$

The DEBtox software also provides a 95% confidence interval for all estimates.

3.2.4. Data analysis

Parameter estimates and correlations are given in Tables 2a and b, respectively. Note that the correlations

Table 2a
Parameter estimation using the DEBtox model (10) for each stage (in bracket the standard error)

	m	NEC	k	ε
L_2	0.051 (0.070)	3.73×10^{-7} (5.667)	0.023 (0.006)	3.474 (2.167)
L_3	0.038 (0.009)	236 (176)	0.014 (0.003)	3.171 (0.451)
L_4	0.033 (0.008)	255 (6.155)	0.022 (0.004)	4.234 (0.582)

The total number of experimental points is $n = 72$.

Table 2b
Correlations between parameter estimates for the L_4 stage from the DEBtox model (10)

	m	NEC	k
NEC	0.091		
k	0.038	0.727	
ε	−0.054	0.329	−0.202

are not strong, which confirms that the parameters have been clearly identified, and that the choice of a model with four parameters was appropriate.

Parameter values are similar to those found by Péry et al. (2003b). Nevertheless, we show here the time profile (survival curve for each concentration, superimposed over the $n = 12$ experimental points) and the concentration profile (survival curve for each day, superposed over the $n = 18$ experimental points), as given by the DEBtox software (Fig. 3a and b, respectively, for the L_4 stage). In the concentration profile, we can see that the toxicant mainly impacts on survival from a threshold concentration corresponding to the NEC (255 $\mu g\,L^{-1}$ for L_4, see Fig. 3a), and above which the survival falls sharply. On the time profile, we can see that the decline in survival accelerates as the concentration increases; after 3 days, survival reaches zero.

3.3. Population dynamics modeling: a Leslie-type matrix model

All data used from here on the population dynamics modeling process refer exclusively to females.

We used a linear standard Leslie matrix model (Leslie, 1945, 1948; Caswell, 2001) with a pre-breeding census. This type of model was chosen because it takes into account the internal structure of the population in development stages, and the instars do not all have the same toxicant sensitivity (Caswell, 2001). Given the circadian rhythm of the life-cycle, we used a daily time step.

The dimension of the Leslie matrix is equal to the total duration of the life-cycle. In a previous study (Charles et al., 2004), the duration of each stage under laboratory conditions and with a non-limiting food supply had been determined: 2 days for the egg, L_1 and L_2 stages, 3 days for L_3, 7 days for L_4 and 1 day for adults, since all adult females reproduce only once during the first day of their adult life. Hence, the total duration of the life-cycle is 17 days, leading to Leslie matrix of dimension 17.

The matrix population model we used for the Chironomidae population dynamics can be written

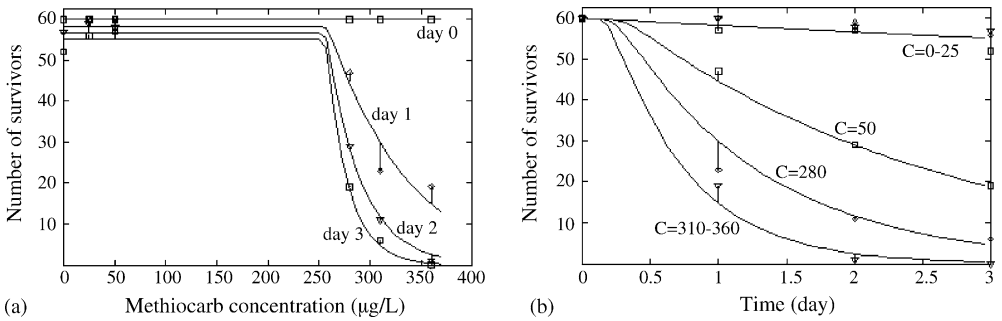

Fig. 3. DEBtox model ((9) and (10)) fitted to survival data for the L_4 stage: (a) concentration profile and (b) time profile.

as follows:

$$\vec{N}_{t+1} = \boldsymbol{L}_{(C)}\ \vec{N}_t \tag{13}$$

where the population vector at day t is $\vec{N}_t = (n_{1,t}, n_{2,t}, \ldots, n_{w,t})^{\mathrm{T}}$, where T denotes the transposition, w the total duration of the life-cycle (17 days) and $n_{i,t}$ the individual number aged i at day t. $\boldsymbol{L}_{(C)}$ refers to the Leslie matrix, which can be written as follows:

$$\mathbf{L}_{(C)} = \begin{pmatrix}
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & F \\
S_{2,1}(C) & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & S_{3,2}(C) & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & S_{4,3}(C) & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & S_{5,4}(C) & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & S_{6,5}(C) & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & S_{7,6}(C) & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & S_{8,7}(C) & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & S_{9,8}(C) & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & S_{10,9}(C) & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & S_{11,10}(C) & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \ddots & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & S_{z,z-1}(C) & 0
\end{pmatrix}$$

Egg L_1 L_2 L_3 L_4 Adult

where

- $S_{i+1,i}(C)$ is the survival probability of larval stages from day i to day $i+1$ at a given the toxicant concentration C.

  Note that the z subscript in $S_{z,z-1}$ refers to the last day of the fourth larval stage ($z = 16$ for *C. riparius* under non-limiting food conditions). As the pupa stage lasts less than one day, it has been combined with the fourth larval stage. Thus, $S_{Z,Z-1} = pq$, with p (the pupa survival probability) is estimated by the logistical model (1), and q (the L_4 survival probability), is estimated using DEBtox models ((9) and (10)).
- F is the fecundity of adult females, estimated to be 208.1 in a previous study (Charles et al., 2004).

The effects of methiocarb on the population dynamics will subsequently be quantified from the population growth rate λ, corresponding to the first eigenvalue of $\boldsymbol{L}_{(C)}$, according to the Perron–Froebenius theorem (Caswell, 2001).

4. Results

In the absence of the toxicant, we found the same data as reported Charles et al. (2004). Hence, the population growth rate was equal to $\lambda = 1.28$, corresponding to a hypothetical daily increase of 28%. This huge value of λ is consistent with the opportunistic characteristics of *C. riparius*, which is able to colonize organically enriched aquatic habitats very quickly (Armitage et al., 1995). As in the previous study (Charles et al., 2004), the Leslie matrix $\boldsymbol{L}_{(C)}$ was imprimitive (due to the fact that reproduction only lasts 1 day) with one real eigenvalue corresponding to λ, and 16 conjugates of complex eigenvalues. Consequently, the age distribution does not converge to a stable distribution, but oscillates with a period of 17 days, as does the total population size. Cull and Vogt (1973) showed that a running average of $\vec{N}_t$, taken over the period of oscillation, does converge to the right eigenvector associated with λ and gives a growth rate of $\lambda = 1.28$.

4.1. The effect of pollution on population dynamics

For C varying from 0 to 120 $\mu g\,L^{-1}$, the decrease in λ versus methiocarb concentration was simulated from the population model (13). Simulations were performed using Maple® software. As shown in Fig. 4, methiocarb had a major impact on population growth rate, λ, which rapidly decreased when the methiocarb concentration rose above a threshold value of around 21 $\mu g\,L^{-1}$. Above this threshold, the population became extinct, whatever the value of λ, as indicated in Fig. 4 by the dotted horizontal line corresponding to $\lambda = 1$.

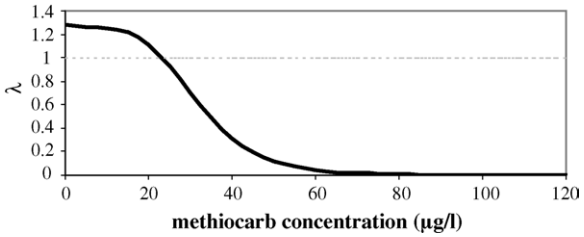

Fig. 4. Effect of the methiocarb concentration on population growth rate, λ, of *C. riparius*.

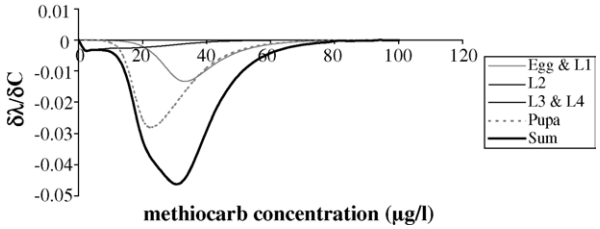

Fig. 5. Results of the sensitivity analysis by decomposition of the population response (14).

4.2. Sensitivity analysis: decomposition of the population response

4.2.1. Principle

The influence of each of the parameters in a Leslie matrix on λ is usually assessed separately using eigenvectors associated with λ. However, in our case, we have a second-order variable, namely the concentration of methiocarb, *C*. As shown by Caswell (1996), the sensitivity of λ to the methiocarb concentration can be decomposed according to the following linear expression:

$$\frac{\partial\lambda}{\partial C} = \sum_{x,y} \frac{\partial\lambda}{\partial l_{xy}} \frac{\partial l_{xy}}{\partial C} \tag{14}$$

where

- $\partial\lambda/\partial C$ can be calculated numerically from the curve $\lambda = f(C)$ (Fig. 3);
- l_{xy} is the coefficient located in row x and column y of the Leslie matrix;
- $\partial\lambda/\partial l_{xy}$ is the sensitivity of λ to the change in an l_{xy} coefficient. This term can be calculated analytically for a given concentration *C*, with right and left eigenvectors associated with λ:

$$\frac{\partial\lambda}{\partial l_{xy}} = \frac{v_x w_y}{\langle w, v\rangle} \tag{15}$$

  where $\langle\,\rangle$ symbolize the scalar product, v_x the xth coordinates of the right eigenvector $\vec{v}$ of $\boldsymbol{L}_{(C)}$ and w_y the yth coordinates of the left eigenvector $\vec{w}$ of $\boldsymbol{L}_{(C)}$ (Caswell, 2001).
- $\partial l_{xy}/\partial C$ is the sensitivity of an l_{xy} coefficient to the toxicant concentration. This term can be calculated analytically by deriving effect models (1), (9) and (10).

From an ecotoxicological point of view, this decomposition (14) is very interesting, because it allows to see how and which, demographic parameters contribute the most to the change of λ versus the methiocarb concentration.

4.2.2. Results

As shown by (Péry, 2003) methiocarb only affects survival rates. Consequently, only sub-diagonal terms of the Leslie matrix contribute to the sensitivity of λ to the toxicant concentration. Analytical calculations and numerical simulations were performed with the software Maple®. The results are shown in Fig. 5. The egg and L_1 stages had a moderate impact on λ in the middle-range concentrations, whereas the L_2 stage weakly contributed at low concentrations. No effect could be detected for L_3, L_4 or the adult stages; only individual effects were detected at very high concentrations. Finally, we observed that the pupa stage made a major contribution at mid-range concentrations, which can be accounted for by the emergence of strong individual effects during the brief period corresponding to this stage. From this sensitivity analysis, we concluded that the egg, L_1 and pupa stages strongly influenced population growth rate as a result of the impact of the concentration of the toxicant on survival rates.

5. Discussion

The matrix population model presented in this paper (13) could be used to describe the dynamics of a laboratory population of Chironomidae exposed to a toxicant such as methiocarb. This work showed how nested modeling methods, which are used in both ecotoxicology and in ecology, can help us to understand

responses at the population level by extrapolating from the effects observed at the individual level. Indeed, logistic and DEBtox models nested in a matrix population model can be used to estimate the population response to a toxicant in terms of the change in population growth rate λ. In this way, we have demonstrated that methiocarb has a rapid effect on Chironomidae population dynamics; above a threshold toxicant concentration of 21 $\mu g\,L^{-1}$. The population became extinct when λ fell below a value of 1.

Our analytical approach to mathematical modeling also allowed us to use a complex sensitivity analysis method, making a connection between an output population variable (λ) and individual input parameters of the Leslie matrix. This decomposition of the population response, described by Caswell (1996, 2001), highlights the critical age classes for population dynamics; the egg, L_1 and pupa stages, the survival rates of which strongly affect λ in the case of *C. riparius*. These results are consistent with the fact that younger stages are those most sensitive to pollution (Williams et al., 1986).

Furthermore, our approach, based on a simple and realistic Leslie model, which is particularly easy to construct, analyze and interpret (Caswell, 2001), is suitable for use in animal species displaying discrete life-cycle stages, and it is general enough to make it easy to extend it to other toxic compounds, or/and other species. Indeed, in natural aquatic ecosystems, several toxicants are present in the same time, and a large number of species are exposed to them simultaneously. Moreover, the pesticide we studied only affected the survival of the exposed individuals, whereas other toxicants can also affect their fecundity. Copper, for example, is a major toxicant that is widely employed in laboratory toxicological experiments (Janssens De Bisthoven et al., 1998; Groenendijk et al., 2002; Girling et al., 2000; Fargosova, 1994). It has been shown that copper can affect not only survival, but also the fecundity and growth of *C. riparius* (Péry et al., 2003a, 2004; Ducrot et al., 2004). Our group is currently engaged in attempting to transfer our extended Leslie model for used in in-situ situations. Hence, various scenarios in which toxicant concentrations vary over time are tested, in order to help decision-makers change the rules governing acceptable pollution levels, with a view to preserve *Chironomidae* species in field.

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