Predicting effects of multiple stressors on aquatic biota.

Heugens, E.H.W.

Publication date
2003

Link to publication

Citation for published version (APA):
Modeling Life Cycle Parameters of *Daphnia magna* influenced by Multiple Stressors
ABSTRACT

Organisms living in the field are commonly exposed to combinations of stress factors. As little information is available about interactive effects between different types of stressors, this study focused on the joint effects of temperature, food, and cadmium on the water flea \textit{Daphnia magna}. Data obtained from a life history experiment were analyzed with a dynamic energy budget model (DEBtox) to disclose underlying processes accounting for temperature- and food-modified effects of cadmium on development and reproduction of \textit{D. magna}. Although more extensive modeling work needs to be done, results of the model analyses suggested that increased cadmium toxicity at high temperature and low food level was due to increased sensitivity of the daphnids to cadmium. The model parameters obtained by fitting the model to the data were used to simulate population growth of \textit{D. magna} under multiple stress conditions. The results indicate that at high temperature and limiting food conditions cadmium causes adverse effects at levels that are not toxic under laboratory conditions.

INTRODUCTION

Field populations in their natural environment are often subjected to combinations of chemical, physical and biological stressors, but it is still unclear how these stressors interact with each other. Although a number of studies considered the joint effects of chemical and physical stressors (reviewed in Cairns et al. 1975, McLusky et al. 1986, Hall and Anderson 1995, Heugens et al. 2001), an advanced understanding of the underlying processes is lacking. Insight into these processes is essential to understand and predict the dynamics of natural populations living under multiple stress conditions. For this reason, the present study analyzes the joint effects of temperature, food and cadmium on life cycle parameters of the water flea \textit{Daphnia magna} in a model setting. The data were obtained from a previous study (Chapter 4). This study revealed that temperature, food and cadmium alone and in combination had a high impact on the performance of the daphnids. In general, population growth of the daphnids was highest at high temperature and food level. Adverse effects of cadmium were enhanced at higher temperatures and lower food levels. Cadmium accumulation in the daphnids
was elevated under these conditions, but it remained unclear if other factors were important as well, such as altered sensitivity of the daphnids to the metal, as was previously observed in short-term experiments (Heugens et al. 2003).

The mechanistic DEBtox model (Kooijman and Bedaux 1996) was chosen to analyze the data of the study described in Chapter 4. DEBtox is based on the dynamic energy budget theory (Kooijman, 2000) and describes how energy is assimilated by organisms and subsequently allocated to maintenance, growth, development and reproduction. De Coen and Janssen (2003) linked energy budgets to life history characteristics of control and cadmium-exposed *D. magna* by measuring changes in available energy reserves and energy consumption. The use of the DEBtox allows the disentangling of processes responsible for the observed responses of the daphnids to the different temperature, food and cadmium treatments. Instead of fitting the data of each observation time separately which leads to several response curves, all data is fitted simultaneously. This results in a response surface, which contains information on the dynamic expression of toxicant effects. Moreover, DEBtox estimates a true no effect concentration (NEC), which is process-based and independent of exposure time.

Previous studies showed that the DEBtox model was successful in analyzing the effects of jointly acting stressors: acute effects of cadmium and temperature on daphnids (Heugens et al. 2003) and chronic effects of copper and food on chironomids (Péry et al. 2003). The results presented in the current study are preliminary, as the modeling operation is still in progress and the fitting routine is under review. At this time, observations at 10 °C have not been included in the model analyses. Nevertheless, the outcomes of the present study can be considered as an example of the application of the DEBtox model in toxicity testing, the results of which are used to evaluate the influence of multiple stressors on field populations.

**MATERIALS AND METHODS**

**Chronic experiments**

The joint effects of cadmium, temperature and food on population parameters of *Daphnia magna* cultured in the laboratory were studied in chronic
experiments. An extensive description of these experiments is given in Chapter 4. In short, an experiment consisted of 12 treatments, composed of four cadmium concentrations (including one control) and three food levels (0.50, 1.0 and 2.0 mg C L\(^{-1}\) of the algae *Selenastrum capricornutum*), which was consecutively performed at 10, 20 and 26 °C. Extra cadmium concentrations were included in an additional experiment at 20 °C, since the exposure concentration in the first experiment proved to be too low to exert effects. The experimental design is given in Table 4.1.

To maintain constant cadmium and food levels, an intermittent flow-through system was used. Per cadmium and food treatment in each temperature experiment, 10 neonates (<24 h) were exposed. As temperature accelerates physiological processes in ectotherms such as *Daphnia*, the experiment was ended when the daphnids reached a marked developmental endpoint: the production of the third brood. This led to exposure durations of 41, 15 and 11 d at 10, 20 and 26 °C, respectively. Several life history features of the daphnids were determined, from which the amount of living neonates produced over time, and body length and tissue cadmium concentration at the start and the end of the experiment were used in the present study. Data of the 10 °C experiment was not yet analyzed in the present study, because a missing control treatment at high food level complicates the model analysis. The effects of temperature, food, and cadmium on survival were small, and to reduce the amount of model parameters to be fitted, survival was not estimated.

**Model description**

DEBtox is a mechanistic model that rests on the Dynamic Energy Budget (DEB) theory (Kooijman 2000). This theory describes the transfer of energy from food to processes involved with maintenance, growth and reproduction. The model is able to describe time-dependent data, wherein information about the dynamic aspect of the occurrence of effects is enclosed. A full description of the DEBtox model is given in Kooijman and Bedaux (1996), but an overview of the most important assumptions, equations and extensions are given in the following paragraphs.
Growth and reproduction in the absence of cadmium

When no toxicants are present and food levels are constant, growth of the isomorphic growing daphnids (body shape remains unchanged during growth) can be described by a Von Bertalanffy growth curve:

\[ l(t) = l_\infty - (l_\infty - l_b) \exp(-r_B t) \quad (5.1) \]

where \( l(t) \) is body length as fraction of the maximum length \( l_\infty \) that the daphnids can reach when food is abundant (i.e. scaled length) at time \( t \) \((\text{d})\), \( l_b \) is scaled length at birth (\(l_0\) initial length in the present study was \(0.853 \pm 0.0074\) (SE) and \(0.874 \pm 0.0088\) mm for the 20 and 26 °C experiment, respectively), \( r_B \) is the Von Bertalanffy growth rate \((\text{d}^{-1})\). To include dependency on different food levels, DEBtox uses \( f \) instead of \( l_\infty \), which is the ingestion rate as a fraction of the maximum ingestion rate (i.e. scaled food density).

Both body size and food availability control reproduction. In the offspring number used to estimate the reproduction rate \( R \) in number \( \text{d}^{-1} \) in the present study, the number of living and dead neonates, and the number of aborted eggs \( \text{d}^{-1} \) were included, since energy costs are involved in all three types of reproductive output. At a constant food level, \( R \) as a function of scaled length is given by:

\[ R(l) = \frac{R_m}{1 - l_r^3} \left( \frac{g + l}{g + f} \right) l_r^2 - l_r^3 \quad (5.2) \]

with \( R_m \) the maximum reproduction rate at maximum size and abundant food (number \( \text{d}^{-1} \)), \( g \) is the energy investment ratio (\( \cdot \)) (see Kooijman and Bedaux [1996]), and \( l_r \) is the scaled length at puberty (i.e. length at which for the first time energy is invested in reproduction) (\( \cdot \)).

When food is abundant, \( f \) in Equation 5.1 and 5.2 equals 1, but this value becomes smaller than 1 under limiting food conditions. By use of this parameter, food limitation was included in the model.
Growth and reproduction in the presence of cadmium

Exposure to toxicants may either result in direct or indirect effects on growth and reproduction. Growth is directly affected by toxicants when they increase the costs for growth, but indirectly when they lower the amount of incoming energy by reducing the assimilation rate or efficiency. Similarly, reproduction is directly influenced by toxicants when they decrease the survival probability of eggs or increase the energy costs per egg. When the assimilation is lowered by exposure to chemicals, or when the energy costs for maintenance or growth are increased, reproduction is indirectly affected. Indirect effects of toxicants on reproduction are associated with a delay of the start of the reproductive cycle, whereas this effect is not seen with direct effects. The first step in this study was to select which mode of toxic action was most relevant for cadmium. This was accomplished by fitting the model to the data while assuming different modes of toxic action. The results (not shown here) suggested that cadmium is affecting growth and reproduction of the daphnids indirectly by reducing the assimilation rate. This mode of toxic action was therefore used for all subsequent model analyses.

DEBtox assumes the existence of a no-effect concentration (NEC). Below this concentration, no toxic effects occur, independent of exposure duration. In the present study, cadmium concentrations in the daphnids were determined, which allowed the estimation of the internal analog of the NEC: the internal threshold concentration (ITC) for growth and reproduction. For this purpose, the original model was extended with additional equations, which were programmed in Matlab 6.1 (The Mathworks, Inc.). The effect of cadmium on the assimilation rate was considered to be proportional to the tissue cadmium concentration exceeding the ITC.

The accumulation kinetics of cadmium by the daphnids is assumed to follow a simple linear one-compartment model, provided that the cadmium and food concentrations in the water are constant and the absorption of cadmium to the algae cells, and the partitioning of cadmium over the different body compartments are instantaneous. Instead of estimating the uptake rate constant, $k_1$, DEBtox fits the ratio of $k_1$ and the elimination rate constant, $k_2$, which is also known as the bioconcentration factor ($BCF$ in L kg$^{-1}$). For growing animals, the accumulation kinetics of cadmium can then be described by:
wherein $C_i$ is the tissue cadmium concentration (mg Cd kg\(^{-1}\) dw [dry weight]), $C_r$ is the cadmium concentration in the environment (dissolved fraction and fraction absorbed to food particles) (mg Cd L\(^{-1}\)) which is assumed to be constant in time, and the term $d/dt \ln l^3$ accounts for dilution by growth. In the present study, the likelihood of the model fits was not influenced when the BCF and $k_2$ were considered to be independent of food quantity. Therefore, to reduce the number of model parameters, one BCF and one $k_2$ were fitted for all food levels.

The effects on growth and reproduction rates resulting from cadmium-induced decrease in assimilation can be then described by:

$$\frac{d}{dt} l = r_B (f(1 - s(C_i)) - l)$$  \hspace{1cm} (5.4)

and

$$R = (1 - s(C_i))^3 \frac{R_m}{1-l_r^3} \left( \frac{g+l}{g+f} l^2 - l_r^3 \right)$$  \hspace{1cm} (5.5)

respectively, with $s(C_i)$ as the stress function that is linear in the tissue cadmium concentration above the ITC:

$$s(C_i) = \left( \frac{C_i - ITC}{C_A} \right)_+$$  \hspace{1cm} (5.6)

where $C_A$ is the tolerance concentration for assimilation (mg Cd kg\(^{-1}\) dw), which determines the magnitude of the effect caused by the tissue cadmium concentration exceeding the ITC. The '+' indicates that the stress function should be positive. Both the ITC and the tolerance concentration ($C_A$) are measures for the intrinsic sensitivity of the daphnids to cadmium. Estimation of these parameters at the different temperature and food treatments will therefore reflect changes in the sensitivity of the daphnids that may contribute to the observed temperature- and food-dependent cadmium toxicity.
Model parameters were estimated simultaneously (additional equations were programmed in MatLab 6.1 (The Mathworks, Inc.) by use of maximum likelihood methods and 95% confidence intervals were obtained by using the profile likelihood (Meeker and Escobar 1995).

**RESULTS**

Figures 5.1 and 5.2 present the data and model fits of the tissue cadmium concentration, body length and cumulative reproduction of *D. magna* exposed to different combinations of cadmium and food levels at 20 and 26 °C, respectively. In general, the model fits that were obtained matched the data well. Parameter estimates with their likelihood-based 95% confidence intervals are given in Table 5.1. Non-overlapping confidence intervals indicate a significant difference between treatments.

Parameter estimates for the scaled food density (Table 5.1) show that the performance of the daphnids at the three food levels used in the experiments differed significantly from each other. Comparison of the scaled food densities at 20 and 26 °C reveals that the effects of food limitation imposed on the daphnids was more severe at low than at high temperature.

The course of the calculated tissue cadmium concentration over time was influenced by growth dilution (Figures 5.1 and 5.2). Once growth ceased (see growth curves), the effect of growth dilution disappeared and the cadmium tissue concentration increased rapidly. Some of the fits for cadmium accumulation (especially at 26 °C, low and medium food level) underestimated the tissue cadmium concentration. This was likely due to the small amount of data as the tissue concentrations and body lengths were only measured at the beginning and the end of the experiments. When comparing the different temperature, food and cadmium treatments, the tissue cadmium concentration of the daphnids turned out to be higher at elevated cadmium concentrations in the water and lower food levels. Corresponding parameter estimates (Table 5.1) show that the bioconcentration factor of cadmium was slightly lower at 26 °C than at 20 °C, but this difference was not significant as the confidence intervals overlapped. For the elimination rate an opposite temperature effect was found, with a significantly higher rate at 26 °C than at 20 °C.
Figure 5.1. Cadmium accumulation (left panels), growth (center panels), and reproduction (right panels) of D. magna exposed to various cadmium concentrations at 20 °C and 0.50, 1.0, and 2.0 mg C L⁻¹ of S. capricornutum as a function of time. The numbers on the right side of each line correspond to the actual cadmium concentration in the water (μg Cd L⁻¹). Symbols represent data points, while lines are fitted to the data by the DEBtox model.
Figure 5.2. Cadmium accumulation (left panels), growth (center panels), and reproduction (right panels) of *D. magna* exposed to various cadmium concentrations at 26 °C and 0.50, 1.0, and 2.0 mg C L\(^{-1}\) of *S. capricornutum* as a function of time. The numbers on the right side of each line correspond to the actual cadmium concentration in the water (μg Cd L\(^{-1}\)). Symbols represent data points, while lines are fitted to the data by the DEBtox model.
With respect to body length, daphnids grew less at low food level (Figures 5.1 and 5.2). Growth reduction by cadmium was pronounced at high temperature and low food level. Parameter estimates in Table 5.1 show that the maximum body size achieved by the daphnids at the two temperatures did not differ, but the Von Bertalanffy growth rate parameter was significantly higher at 26 °C than at 20 °C. Increasing food level increased this length at both temperatures, although this increase was stronger at 20 °C than at 26 °C.

The onset of reproduction can be seen in the reproduction curves (Figures 5.1 and 5.2), which allow a day-to-day comparison of simulated values and observations. The age at first reproduction increased at low temperature and low food conditions. Cadmium prolonged the juvenile period, and this effect was more pronounced at low food levels. These findings were supported by the length at puberty (Table 5.1), which was significantly lower at 26 °C compared to 20 °C, indicating that reproduction was enhanced at the high temperature. Increasing food level increased this length significantly at both temperatures, although this increase was stronger at 20 °C than at 26 °C. The number of neonates produced during the test period was similar at both temperatures (Figures 5.1 and 5.2). However, the number was lower at limiting food levels, and exposure to cadmium and high temperature enhanced this effect. The maximum reproduction rate was significantly higher at 26 °C than at 20 °C (Table 5.1).

As measures for the intrinsic sensitivity of the daphnids to cadmium, the ITC for growth and reproduction and the tolerance concentration for assimilation (Cₐ) (see Equation 5.6) with likelihood-based 95% confidence intervals were determined at the different temperature and food treatments (Figure 5.3 and 5.4). The overlapping confidence intervals in the 20 °C experiment reveal that there is no significant effect of food level on the ITC (Figure 5.3). At 26 °C however, the ITC at low food level was a factor of three lower than those at medium and high food levels, which were not significantly different. Comparison of the estimates made at the two temperatures points out that the ITC at low and intermediate food levels was significantly lower at 26 than at 20 °C, respectively. The difference is five-fold and two-fold for the low-food and medium-food treatment, respectively. Temperature did not affect the ITC at the high food level. The results indicate that the daphnids became more sensitive to cadmium under the combined conditions of high temperature and low food dose.
## Table 5.1. Parameters estimated by the DEBtox model for the different temperature and food treatments

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Unit</th>
<th>Temperature (°C)</th>
<th>Food level (mg of C L⁻¹)</th>
<th>Parameter value</th>
<th>Likelihood-based 95% confidence intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>f</td>
<td>Scaled food density</td>
<td>-</td>
<td>20</td>
<td>0.50</td>
<td>0.821</td>
<td>0.81 - 0.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
<td>0.926</td>
<td>0.91 - 0.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.0</td>
<td>1.00³</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>26</td>
<td>0.50</td>
<td>0.852</td>
<td>0.84 - 0.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
<td>0.960</td>
<td>0.95 - 0.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.0</td>
<td>1.00³</td>
<td>-</td>
</tr>
<tr>
<td>l_m</td>
<td>Maximum length</td>
<td>mm</td>
<td>20</td>
<td>0.50 - 2.0</td>
<td>4.02</td>
<td>4.0 - 4.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>26</td>
<td>0.50 - 2.0</td>
<td>3.93</td>
<td>3.9 - 4.2</td>
</tr>
<tr>
<td>r_B</td>
<td>Von Bertalanffy growth rate</td>
<td>d⁻¹</td>
<td>20</td>
<td>0.50 - 2.0</td>
<td>0.186</td>
<td>0.18 - 0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>26</td>
<td>0.50 - 2.0</td>
<td>0.236</td>
<td>0.23 - 0.28</td>
</tr>
<tr>
<td>l_r</td>
<td>Length at first investment in</td>
<td>mm</td>
<td>20</td>
<td>0.50</td>
<td>2.63</td>
<td>2.6 - 2.6</td>
</tr>
<tr>
<td></td>
<td>reproduction</td>
<td></td>
<td></td>
<td>1.0</td>
<td>2.95</td>
<td>2.9 - 3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.0</td>
<td>3.12</td>
<td>3.1 - 3.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>26</td>
<td>0.50</td>
<td>2.59</td>
<td>2.6 - 2.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
<td>2.88</td>
<td>2.8 - 2.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.0</td>
<td>2.94</td>
<td>2.9 - 3.0</td>
</tr>
<tr>
<td>R_m</td>
<td>Maximum reproduction rate</td>
<td>number d⁻¹</td>
<td>20</td>
<td>0.50 - 2.0</td>
<td>24.4</td>
<td>24 - 27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>26</td>
<td>0.50 - 2.0</td>
<td>28.2</td>
<td>27 - 32</td>
</tr>
<tr>
<td>BCF</td>
<td>Bioconcentration factor</td>
<td>L kg⁻¹</td>
<td>20</td>
<td>0.50 - 2.0</td>
<td>2.07·10³</td>
<td>2.0·10³ - 2.4·10³</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>26</td>
<td>0.50 - 2.0</td>
<td>1.85·10³</td>
<td>1.8·10³ - 2.0·10³</td>
</tr>
<tr>
<td>k_2</td>
<td>Elimination rate</td>
<td>d⁻¹</td>
<td>20</td>
<td>0.50 - 2.0</td>
<td>0.0423</td>
<td>0.039 - 0.050</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>26</td>
<td>0.50 - 2.0</td>
<td>0.0676</td>
<td>0.061 - 0.082</td>
</tr>
</tbody>
</table>

*Estimations for ITC and C_x are given in Figure 5.3 and 5.4, respectively.

*Not fitted.
Estimations for the tolerance concentration for assimilation ($C_A$) at the different temperature and food combinations are presented in Figure 5.4. At 20 °C, the tolerance concentration was four times higher at the high food level than at the intermediate and low food level, where the tolerance concentrations did not significantly differ from each other. In agreement with the results found for the ITC, this indicates that the daphnids were more vulnerable to cadmium when less food was available. At 26 °C however, ambiguous results were obtained as a three times lower tolerance concentration was found at the medium food level compared to the low and high food levels, where the concentrations were comparable. This suggests that the sensitivity of the daphnids was increased at the intermediate food level. However, these findings may also be the result of experimental variation in this food treatment, since the daphnids at the two lowest cadmium concentrations performed better than the control animals, which may have had consequences for the model fit. When the tolerance concentrations at the two temperatures were compared, significant temperature effects were found at the high food level. At this food quantity, the tolerance concentration was two times lower at 26 °C than at 20 °C, indicating that the daphnids were more vulnerable to cadmium under these conditions.
nids were two times less resistant against cadmium at elevated temperature, which is in accordance with the findings for the ITC.

Both the ITC and the tolerance concentration reflect the intrinsic sensitivity of the daphnids. Overall, the effects of temperature and food level on the two parameters were consistent, indicating that the daphnids became more vulnerable to cadmium at high temperature and low food levels.

![Figure 5.4](image)

**Figure 5.4.** Estimations for the tolerance concentration for assimilation ($C_A$) of *D. magna* at 20 and 26°C as a function of food level. The test duration was 15 and 11 d at 20 and 26 °C, respectively. Error bars represent likelihood-based 95% confidence intervals. Non-overlapping intervals indicate significant differences between treatments.

**DISCUSSION**

The conclusions given here are preliminary and may have to be adapted when the model is run with an improved fitting routine and when the model fits for the data obtained at 10 °C become available.

In this study it was demonstrated that DEBtox was able to describe the effects of temperature, food and cadmium on life history parameters of *D. magna* adequately. The first step of the model analyses was the selection of the most relevant mode of toxic action of cadmium (direct or indirect effects...
on growth or reproduction, see materials and methods section). Based on the results (not shown here), it was assumed that cadmium indirectly influenced growth and reproduction by its effect on assimilation. Apparently, the amount of energy assimilated was reduced in cadmium-exposed daphnids, leaving less energy available for growth and reproduction. In other studies, it was indeed observed that cadmium interfered with the feeding process of cladocerans (Chandini 1988, 1989, Taylor et al. 1998, Barata et al. 2002). Bodar et al. (1988) found no effects of cadmium on assimilation efficiency of *D. magna*, but consumption and assimilation rates decreased. It was unclear if assimilation rate was directly influenced by cadmium or indirectly by reduced consumption. However, Gulati et al. (1988) reported that cadmium inhibited assimilation rates of Crustacea, including *Daphnia*, more than consumption rates, which resulted in a strong decline in assimilation efficiency. Reduction of the available energy for population growth has also been measured for *D. magna* exposed to cadmium and other toxicants (De Coen and Janssen 2003).

In Heugens et al. (submitted), the observed increase in cadmium toxicity at high temperature and low food level was partly explained by elevated cadmium accumulation under these conditions. The model analyses presented in the current study revealed that changes in the intrinsic sensitivity of the daphnids to cadmium, as defined by the ITC and the tolerance concentration for assimilation (*C_A*) also contribute to the altered cadmium toxicity. In general, the ITC and tolerance concentration decreased at elevated temperature and low food level, which points at increased vulnerability of the daphnids to cadmium at high temperature and low food levels. Increased sensitivity of *D. magna* to cadmium at elevated temperatures was also shown in short-term experiments (Heugens et al. 2003). In these acute experiments in which no food was given to the daphnids, the results suggested an interaction between starvation and increasing temperature: cadmium-exposed daphnids that survived long enough to experience starvation appeared to become more susceptible to cadmium, resulting in a lowering of the ITC for survival in time (results not published). This effect was more pronounced at high temperature.

The implications of interactive effects of temperature, food, and cadmium on field populations are difficult to predict. Positive effects of elevated tem-
perature and food level on reproduction may be canceled out by the increased sensitivity of the daphnids to cadmium at high temperature and low food quantity. Therefore, the parameters estimated with the DEBtox model were used to calculate population growth rates at different stressor combinations as a function of cadmium concentration in the water (Figure 5.5). For this purpose, survival at the different treatments was estimated, while all other parameters were fixed at the values given in Table 5.1 (for equations, and estimates of the additional parameters for survival see Annex 5.1).

As can be seen in Figure 5.5, the highest population growth rate is achieved at high temperature and high food condition. However, when cadmium is present, the performance of the daphnids declines more rapid in this treatment relative to the other treatments, even below levels attained at low temperature and low food level. In the standard *Daphnia* reproduction tests (OECD, 1998), experimental conditions equal the 20 °C and high-food treatment. Figure 5.5 shows that the daphnids in this treatment are least affected by cadmium. These conditions may not be realistic for natural

---

**Figure 5.5.** Population growth rate of *D. magna* at 20 °C and 26 °C, and 0.50 (LF), 1.0 (MF), and 2.0 mg C L⁻¹ (HF) of *S. capricornutum* as a function of cadmium concentration in the water. Solid lines represent population growth rates at tested cadmium concentrations, while dotted lines are extrapolated population growth rates at non-tested cadmium concentrations. For equations and parameter estimates see Annex 5.1.
waters, where higher water temperatures may occur during the summer season, whereas food levels generally are much lower than those used in standard toxicity tests. Regarding Figure 5.5, elevated temperature would lead to increased population growth rates relative to standard conditions, irrespective of food quantity. Simultaneous exposure to cadmium however, would decrease population growth rates especially at high temperature and low food levels, canceling out the stimulating effect of temperature. These differences in toxicity induced by changes in temperature and food level are within the ranges observed in literature (reviewed in Heugens et al. 2001). This example illustrates how natural factors may influence the outcomes of standard toxicity tests. The current risk assessment for chemicals relies heavily on such toxicity tests, which may introduce a considerable bias and may therefore fail to protect ecosystems when environmental conditions in the field differ from experimental conditions used in the laboratory.

**ACKNOWLEDGEMENTS**

The study was supported by the Institute for Inland Water Management and Waste Water Treatment. We wish to express our thanks to Jan Hendriks and Nico van Straalen for valuable comments on previous versions of the manuscript.
ANNEX 5.1

To understand the implications of the interactive effects of temperature, food, and cadmium on field populations, population growth rates were determined using the data presented in Heugen et al. (submitted). The equations given below are extensively discussed in Kooijman and Bedaux (1996). Population growth rates \( r \) at different combinations of temperature, food, and cadmium were calculated by use of the following equation:

\[
1 = \max_{t} \int_{t_0}^{t_{\text{max}}} q(t, C_e) \cdot R(t, C_e) \exp(-r(C_e) \cdot t)
\]  

(5.7)

where \( t_0 \) and \( t_{\text{max}} \) (d) represent the times at the start and end of the experiment, respectively, \( q(t, C_e) \) (-) is the probability of individuals to survive until time \( t \), and \( R(t, C_e) \) (number d\(^{-1}\)) the reproduction rate, both a function of time \( t \) and the cadmium concentration in the water \( C_e \) (mg Cd L\(^{-1}\)). \( R \) is calculated following Equation 5.2 and 5.5. The survival probability is specified via the hazard rate \( (h) \). The product \( h \Delta t \) can be interpreted as the probability to die in the small time interval \( \Delta t \), given that the animal has survived up to that moment. The survival probability can be expressed as:

\[
q(t, C_e) = \exp(-\int_{0}^{t} h(\tau, C_e) d\tau)
\]

(5.8)

where \( h(\tau, C_e) \) (d\(^{-1}\)) is the hazard rate at time \( \tau \), which is a function of the toxicant concentration in the water \( (C_e) \). When the ITC for survival is exceeded, the hazard rate is assumed to increase proportionally to the difference between \( C_i(t, C_e) \) and the ITC:

\[
h(t, C_e) = \begin{cases} 
    k_f \cdot (C_i(t, C_e) - ITC_{\text{surv}}) + h_0 & \text{if } C_i(t, C_e) > ITC_{\text{surv}} \\
    h_0 & \text{if } C_i(t, C_e) \leq ITC_{\text{surv}}
\end{cases}
\]

(5.9)

where \( k_f \) (kg dw mg\(^{-1}\) Cd d\(^{-1}\)) represents the killing rate, \( h_0 \) (d\(^{-1}\)) is the background hazard rate, and \( ITC_{\text{surv}} \) (mg Cd kg\(^{-1}\) dw) is the internal threshold concentration for survival. The killing rate is the proportionality factor that
describes the relation between the hazard rate and the tissue concentration that exceeds the ITC.

The parameters for survival and population growth rate were estimated while all other parameters were fixed at the values given in Table 5.1. For 20 °C, the following estimates were obtained: $h_0 = 8.92\times10^{-3}$ d$^{-1}$, $ITC_{surv} = 54.8$ mg Cd kg$^{-1}$ dw, $k_f = 2.47\times10^{-3}$ kg dw mg$^{-1}$ Cd d$^{-1}$. For 26 °C, $h_0 = 7.20\times10^{-3}$ d$^{-1}$. Effects of cadmium on survival were small at 26 °C, and no $ITC_{surv}$ and $k_f$ were fitted for this temperature as the best fit was obtained when mortality due to cadmium was neglected. The calculated population growth rates are presented in Figure 5.5.