Predicting effects of multiple stressors on aquatic biota.

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Chapter 6

Predicting Effects of Multiple Stressors

This thesis addresses the joint impact of natural factors and toxicants on aquatic species and studied the nature of the interaction between the two types of stressors. The findings showed that environmental conditions may influence toxicity of chemicals to a great extent. The experimental chapters gave more insight into the mechanisms responsible for the interactive effects of temperature, food level, and cadmium (i.e. changes in accumulation kinetics and sensitivity of the test organisms).

Knowledge of multiple stressor effects is vital for the protection of species living under variable field conditions. Risk assessment for chemicals is used for the regulation of chemical emissions, which aims at protecting ecosystems against adverse effects of man-made substances. One of the elements of the risk assessment process, effects assessment, relies heavily on laboratory toxicity tests in which the toxicity of single chemical is determined for a few species (algae, invertebrates, and fish). When the amount and quality of toxicity data is sufficient, species sensitivity distributions can be used to derive a concentration of the chemical below which adverse effects in the specific environmental compartment are not expected to occur (i.e. predicted no-effect concentration [PNEC]) (Van Straalen 2002). However, when the amount of toxicity data is limited, a PNEC is estimated by applying a constant assessment or uncertainty factor on the data for the most sensitive species to adjust for intra- and interspecies variation. The magnitude of these factors depends on the type (acute, chronic, or field) and quantity of toxicity data available. In the European Union, a constant factor of 10 is applied for each extrapolation step: the assessment factor ranges from 10 when sufficient chronic toxicity data is available to 1000 for acute toxicity data for a few species (Van Leeuwen and Hermens 1996). Similar approaches are used by the U.S. EPA (see Chapman 1998).

Current risk assessment for chemicals does not take the potential effects of multiple stress conditions into account. Therefore, the question remains how to predict the effects of multiple stress conditions in the field. This question is addressed here by discussing the findings of this thesis in the context of the predictability of multiple stress responses. The mechanistic approach that was followed in the previous chapters is extrapolated to the current practice in risk assessment.

PREDICTING MULTIPLE STRESS RESPONSES

Two approaches will be evaluated to predict the joint effects of temperature, food level, and cadmium on population growth rates of *D. magna* that were presented in Chapter 4. The first approach has previously been used in Chapter 2 in which data obtained from literature studies were analyzed quantitatively. This resulted in coefficients for temperature, nutritional state, salinity, and toxicant concentration (when parameters other than LC50 or EC50 were considered), which specify the change in toxicity in relation to the change in the natural factor. With the second approach, multiple stress responses are calculated using the responses to single stress factors following Folt et al. (1999). The DEBtox model is not discussed as a predictive risk assessment tool here, as the model was fitted to the data of Chapter 4 and no other data set is available to validate the estimated parameters.

Coefficients for temperature- and food-modified cadmium toxicity

In Chapter 2, coefficients for temperature and food level were derived from literature data using linear regression. With these coefficients, changes in cadmium toxicity due to variation in temperature and food level can be calculated. Here, this predicted change in toxicity is compared with the actual change in chronic cadmium toxicity observed in Chapter 4 for the population growth rate of *D. magna*.

The coefficients obtained in Chapter 2 were used to assess the change in toxicity due to shifts in temperature and food level by applying equation 1, which is based on Equation 2.2:

$$\frac{\log y_1}{\log y_2} = \frac{\beta_T \cdot \log T_1 + \beta_F \cdot \log F_1}{\beta_T \cdot \log T_2 + \beta_F \cdot \log F_2} \qquad (6.1)$$

in which y_1 and y_2 are the responses under the two treatments to be compared, β_T and β_F are the coefficients for temperature and food level, -14.5 and 0.357 (see Table 2.2), respectively, T_1 and T_2, and F_1 and F_2 are the temperatures (Kelvin) and food levels (mg C L^{-1}) of treatment 1 and 2, respectively. The coefficient for temperature (-14.5) was the average of temperature coefficients obtained from studies focusing on combined effects of tem-

perature and salinity (Annex 2.6). This coefficient was based on seven sets of LC50 data for marine and brackish water species, as all other available data were fitted to another model (Equation 2.1). This resulted in disparate coefficients that cannot be compared. For food, a coefficient of 0.357 was used (Table 2.2). This coefficient was based on 13 sets of reproduction data, mainly for crustaceans. As mortality was low in the experiments described in Chapter 4, the use of this coefficient seems appropriate for population growth rate as well. Population growth rates in cadmium treatments presented in Chapter 4 were normalized by dividing the population growth rates by the rates obtained in the corresponding control treatments. By this scaling, the cadmium toxicity-modifying effects of temperature and food were separated from the direct effects of the natural factors on population growth rates. Temperature- and food-induced toxicity changes were determined by calculating the ratios of population growth rates obtained in treatments with different temperatures and food levels, but similar cadmium exposure concentrations. These experimentally derived ratios were compared with the ratios calculated with Equation 6.1.

The ratios of the observed and the predicted change in toxicity due to shifts in temperature and/or food are given in Table 6.1. Values smaller than one indicate that the observed change in toxicity was lower than predicted, while values higher than one denote the opposite. The values were skewed to the right, with a geometric mean of 1.57 (1.0 - 3.5 (5^{th} - 95^{th} percentile)), and a maximum of 4.3. This deviation is considered to be acceptable. The results indicate that the coefficients calculated from literature data of multiple stressor experiments were appropriate to predict the temperature- and food-modified effects of cadmium on population growth rates of *D. magna* measured in Chapter 4 (i.e. similar patterns as in literature were observed in Chapter 4).

A major drawback of this method is that scaling of experimental data to the corresponding control treatment eliminates the direct effects of temperature and food. Although this is an advantage for the separation of the influence of natural factors on toxicity and their direct effects on population growth rate, and for the comparability of studies (see Chapter 2), the influence of ambient temperature and food levels may be of greater importance to field populations than the toxicity-modifying nature of the factors. To distinguish between these two aspects, coefficients for temperature, food level, and

cadmium were determined using population growth data of *D. magna* (Chapter 4), but now by using both normalized and non-normalized data. The coefficients were estimated with multiple regression (SPSS version 10.0.5, SPSS Inc.) by use of Equation 6.2 (see also Equation 2.2):

$$\log y = \alpha + \beta_T \cdot \log T + \beta_F \cdot \log F + \beta_{Cd} \cdot \log Cd \qquad (6.2)$$

in which y is either population growth rate normalized to the rate in the corresponding control treatment, or non-normalized population growth rate (d^{-1}), α is the intercept, β_T, β_F and β_{Cd} are coefficients for temperature, food and cadmium, respectively, T is temperature (Kelvin), F is food level normalized to the highest food level of 2 mg C L^{-1}, and Cd is the actual cadmium concentration in the water (µg Cd L^{-1}).

The coefficients for temperature, food and cadmium calculated using normalized and non-normalized population growth rates are summarized in Table 6.2. The coefficients for normalized data specify the effects of temperature and food level on cadmium toxicity. The negative temperature and cadmium coefficients imply that a lower population growth rate, i.e. increased cadmium toxicity, is achieved at elevated temperatures and cadmium concentrations. The opposite occurs for food level, where the positive coefficient indicates that population growth is increased, i.e. cadmium toxicity is reduced, at elevated food levels. The overall effects of temperature and food level on population growth rate (i.e. cadmium toxicity-modifying effect *and* enhancing effect on population growth rate) are given by the coefficients for non-normalized data. In contrast to negative temperature coefficient for normalized data, the coefficient for non-normalized data is positive. Apparently, the influence of temperature on cadmium toxicity is of lesser importance than the stimulating effect of temperature on population growth. No differences were observed for the food coefficients for normalized and non-normalized data as the confidence intervals overlap. Apparently, the beneficial effect of food was not large enough to become apparent in the food coefficient for non-normalized data. However, the cadmium coefficient for non-normalized data is smaller than that for normalized data, suggesting that the favorable effect of temperature on population growth rate partly canceled out the adverse effects of cadmium.

Table 6.1. Comparing observed and predicted change in toxicity of cadmium to *D. magna* due to shifts in temperature and food density. The comparison is based on the ratio of observed (derived from data presented in Chapter 4) and predicted effects (ratio obs/pred). The predicted change in cadmium toxicity induced by temperature and food density is described in Equation 6.1. If the ratio is greater than unity, cadmium is more toxic than expected, in other words, temperature and food density in combination with cadmium reduces population growth rate more than was expected from literature data. Treatments with similar cadmium concentrations in the water were compared[a]

Treatments compared		[Cd]	Ratio obs/pred	Treatments compared		[Cd]	Ratio obs/pred
26 HF	26 MF	31	0.749	26 LF	20 LF	31	1.43
		52	0.907			52	0.733
	26 LF	31	0.653		10 MF	52	1.74
		52	1.09		10 LF	52	1.30
	20 HF	85	1.25	20 HF	20 MF	85	2.33
	20 MF	31	1.06		20 LF	85	0.815
		52	1.22		10 HF	145	1.17
		85	2.91			246	0.450
	20 LF	31	0.763		10 MF	85	3.48
		31	0.933			145	0.988
		52	0.802	20 HF	10 LF	85	0.992
		85	1.02			145	2.58
	10 MF	52	1.91	20 MF	20 LF	31	0.723
		85	4.35			31	0.884
	10 LF	52	1.43			52	0.780
		85	1.24			85	0.351
26 MF	26 LF	19	1.89		10 MF	52	1.85
		31	0.872			85	1.50
		52	1.21		10 LF	52	1.39
	20 MF	31	1.41			85	0.427
		52	1.35	20 LF	10 MF	52	1.85
26 MF	20 LF	31	1.02			85	4.27
		31	1.25		10 LF	52	1.78
		52	0.885			85	1.22
	10 MF	52	2.10	10 HF	10 MF	145	0.845
	10 LF	52	1.57		10 LF	145	2.20
26 LF	20 MF	31	1.62	10 MF	10 LF	52	0.748
		52	1.11			85	0.285
	20 LF	19	3.95			145	2.61
		31	1.17				

[a]Symbols indicate the following: [Cd], cadmium concentration (µg Cd L^{-1}), here given as nominal concentrations; 10, 20, 26: 10, 20, and 26 °C; LF, MF, HF: 0.50, 1.0, and 2.0 mg C L^{-1} of *Selenastrum capricornutum*.

Summarizing, the coefficients determined from literature data were successful in predicting the temperature- and food-modified effects of cadmium on population growth rates presented in Chapter 4. Estimation of coefficients for temperature, food and cadmium using normalized and non-normalized population growth data gave insight into the importance of temperature

and food level as factors that modified cadmium toxicity and population growth. The results indicate that the performance of field populations can only be accurately predicted when the effects of all prevalent environmental conditions, consisting of both natural and anthropogenic stressors, are considered.

Table 6.2. Coefficients with 95% confidence intervals (in brackets) for temperature (β_T), food (β_F), and cadmium (β_{Cd}), as well as the intercept α, and R-square derived from population growth rates of *D. magna* presented in Chapter 4. The coefficients were estimated using Equation 6.2. Both normalized and non-normalized population growth rates were used as effect parameter[a]. The coefficients for normalized data represent temperature- and food-induced changes in cadmium toxicity whereas the coefficients for non-normalized data also denote the change in toxicity, as well as the direct effects of temperature and food density

	Effect parameter			
Regression parameter	Normalized population growth rate		Non-normalized population growth rate	
β_T	-4.48	(-13.1 – 4.17)	24.3	(18.3 – 30.3)
β_F	0.268	(-0.0528 – 0.589)	0.0971	(-0.129 – 0.323)
β_{Cd}	-0.513	(-0.787 – -0.239)	-0.0187	$(-0.0377 - 3.63 \cdot 10^{-4})$
α	11.9	(-9.78 – 33.6)	-60.4	(-75.2 — 45.7)
R-square	0.443		0.704	

[a]The negative population growth rate at 10 °C, high food and 370 µg Cd L^{-1} could not be used in Equation 6.2 and was discarded.

Calculating effects of multiple stressors from effects of individual stress factors

The second approach to predict the joint effects of temperature, food, and cadmium on population growth rates obtained in Chapter 4 explores to which extent combined effects of multiple stressors can be predicted from effects of the individual factors. Following Folt et al. (1999), the interaction between the single factors temperature, food, and cadmium is assumed to be comparative (joint effect equals effect of dominant or single worst stressor (Bruland et al. 1991), additive (joint effect equals sum of individual effects (Hay et al. 1994, Hay 1996), or multiplicative (joint effect equals multiplication of individual effects (Begon 1996, Pennings 1996)).

Population growth rates in all treatments were expressed as ratios of the performance under optimal conditions, where the highest population growth rate was achieved (see also Folt et al. 1999). This was in the treat-

ment with 26 °C, high food, and 30 µg Cd L^{-1} (Figure 4.6). Since the performance in this treatment did not differ significantly from that in the corresponding control treatment, and to facilitate comparisons between different exposures, the 26 °C, high-food, and no-cadmium treatment was considered to be optimal. Effects of individual stressors effects were calculated by comparing population growth rate under optimal conditions to rates obtained in treatments where only a single factor was altered. For instance, the low temperature effect is the population growth rate in the 10 °C, high-food and no-cadmium treatment divided by the population growth rate in the 26 °C, high-food, no-cadmium treatment. As cadmium concentrations could differ between temperature and food treatments, not all combinations of stressors could be analyzed. Responses to the individual stressors were used to calculate multiple stress responses by assuming a comparative (dominant stressor determines multiple stress response), additive (responses to individual stressors are added), and multiplicative interaction (single stress responses are multiplied) between the separate factors. For example, when the population growth rates at low temperature, and at low food level were 0.9 and 0.8 times the population growth rate at optimal conditions, the population growth rate at the combination of low temperature and low food level is 0.8 (comparative), 1 - (1 - 0.9 + 1 - 0.8) = 0.7 (additive), and 0.9 x 0.8 = 0.72 (multiplicative) times the population growth rate at optimal conditions.

Table 6.3 shows the ratios of the observed and predicted multiple stress responses at various treatments for the three methods. While the predictions made with the comparative and multiplicative interaction were generally underestimating the actual responses (20 and 15 times out of 21, respectively), the additive interaction mostly overestimated (13 times out of 21) the effects induced by multiple stressors. The deviations of the predictions from the actual observed values were skewed to the right. When responses were overestimated, the geometric means of the differences between observed and predicted responses were 1.25 (1.0 – 2.5 (5^{th} – 95^{th} percentile)) and 1.08 (1.0 – 1.2) and a maximum of 2.6 and 1.2 for the additive and multiplicative interactions, respectively. No geometric mean was determined for the comparative interaction as only one of the predictions overestimated the response (by a factor of 1.06). The geometric means of the differences between observed and predicted multiple stress responses when underestimation occurred were 1.35 (1.1 – 3.1), 1.34 (1.0 – 2.3), and 1.28 (1.0 – 2.6) for the comparative, additive, and multiplicative interaction, respec-

tively. The predicted response differed from the observed response up to a maximum of 3.6, 2.5, and 2.8 for the three interactions. As was argued above, these deviations are regarded as acceptable.

Table 6.3. Comparing observed effects of temperature, food, and cadmium on population growth rate of *D. magna* (data presented in Chapter 4) and those predicted by assuming a comparative, additive, and multiplicative interaction between the stressors (ratio obs/pred)[a]

		Comparative		Additive		Multiplicative	
Treatment	[Cd]	Ratio obs/pred	Under- or overestimation	Ratio obs./pred.	Under- or overest	Ratio obs./pred.	Under- or overest.
10 LF	0	1.09	-	2.43	+	1.13	+
10 MF	0	1.08	-	1.38	+	1.03	+
20 LF	0	1.34	-	1.01	-	1.09	-
20 LF	0	1.17	-	1.13	+	1.05	+
20 MF	0	1.13	-	1.02	+	1.02	-
10 LF	52	1.16	-	2.58	+	1.08	+
10 LF	85	1.15	-	>10	+	1.24	+
10 MF	52	1.20	-	1.33	+	1.06	-
10 MF	85	3.09	-	1.34	+	2.42	-
20 LF	31	1.23	-	1.05	+	1.02	-
20 LF	31	1.32	-	1.02	-	1.09	-
20 LF	52	1.33	-	1.02	+	1.07	-
20 LF	85	1.68	-	1.02	+	1.19	-
20 MF	31	1.12	-	1.01	+	1.02	-
20 MF	52	1.12	-	1.04	+	1.00	+
20 MF	85	3.59	-	2.52	-	2.82	-
20 HF	85	1.07	-	1.13	+	1.08	+
26 LF	31	1.06	-	1.07	-	1.07	-
26 LF	52	1.82	-	1.79	-	1.80	-
26 MF	31	1.06	+	1.04	+	1.04	+

[a]Symbols indicate the following: 10, 20, 26: 10, 20, and 26°C; LF, MF, HF: 0.5, 1.0, and 2.0 mg C L^{-1} of *S. capricornutum*; [Cd]: cadmium concentration in the water (µg Cd L^{-1}), here given as nominal concentrations; -, +: under- or overestimation of actual response.

Considering these results, the actual multiple stress responses are worst predicted by the comparative interaction, since this interaction nearly always underestimated responses and the deviations were largest. The additive interaction mostly overestimated the effects of multiple stressors, whereas the multiplicative interaction generally underestimated effects. Although it seems safer to use a method that is overprotective than to have a chance of underestimating risk, the predictions made with the multiplicative interactions differed less from the actual responses than those made with the additive interaction. It is therefore concluded that of the three

methods (comparative, additive, and multiplicative), the multiplicative method predicted the joint effects of temperature, food, and cadmium on population growth rates of *D. magna* presented in Chapter 4 best.

Comparison of the two approaches used to predict multiple stress responses

Joint effects of temperature, food, and cadmium on population growth of *D. magna* were predicted by use of coefficients that were derived from literature data, and by calculating multiple stress responses from responses induced by individual stress factors relative to optimum conditions. Since the results of the second approach revealed that multiplication of fractional responses to single stressors gave the best predictions, only this method is compared with the first approach using coefficients.

Both methods were able to predict the results of Chapter 4 adequately. Still, the predictions made by multiplying the responses to single stressors differed less from the actual observed effects than the use of literature-derived coefficients. However, to use the methods for a range of values for the natural factors, both methods require many data to obtain accurate coefficients for natural factors or to quantify the responses to single stress factors correctly. However, once appropriate coefficients for the natural stress factors have been determined, they can be used for a range of values for the natural factors to predict multiple stress effects, except values exceeding the tolerance range of species. In contrast, when predicting effects by multiplying responses to separately acting stressors, the response to every new value of the factor that deviates from the optimum conditions needs to be established first, for example by performing new experiments.

Both methods were able to predict the joint effects of temperature, food, and cadmium on population growth of *D. magna* presented in Chapter 4 within acceptable ranges. However, the use of literature-derived coefficients is less laborious because effects of non-tested values of natural factors can also be determined once coefficients are available. Therefore, it is concluded that this approach has the best potential for extrapolating toxicity estimates from one condition to another (for example, from the laboratory to the field).

Although environmental factors may affect both the exposure of organisms to substances and toxicity of these substances to organisms, current risk assessment for chemicals does not take the potential effects of multiple stressors into account. While much progress is made in predicting the influence of natural factors on the bioavailability of chemicals with biotic ligand models (Paquin et al. 2002, Janssen et al. 2003), the assessment of multiple stressor responses is still lacking. A straightforward implementation of multiple stressors in legislation (for example, by multiplying existing quality criteria for temperature, eutrophication, and chemicals) seems problematic because the units of multiple stressors are unlike and risks induced by multiple stressors can only be expressed in units of responses rather than in concentrations. However, the use of responses is not practical for legislation of emissions and translation of altered responses to safe concentrations is therefore crucial.

As was argued above, the uncertainties related to differences between laboratory test conditions and natural conditions in ecosystems can be estimated by the use of literature-derived coefficients. These uncertainties can be accounted for by applying an assessment factor for multiple stressor effects on toxicity data obtained under standard laboratory conditions. In Chapter 2, the magnitude of these factors with respect to effects of temperature, food level, and salinity on toxicity was discussed. It was argued that the factor should be smaller than one when extrapolating to ecosystems with lower temperatures or higher salinities than in the laboratory. On the other hand, the value should be greater than one when field temperatures are higher and food levels are lower than those in the laboratory. Translating the changes in toxicity due to the three natural factors found in literature to relevant field conditions suggests that the magnitude of the factor should fall with the range of 0.01 to 15 for temperature, and 1.2 to 10 for nutritional state. The range for salinity could not be determined. These values only give an indication of the change in toxicity due to environmental factors and should not be overvalued. The use of assessment factors seems valuable in the short term. However, on the long term it would be more effective to replace the use of constant assessment factors with statistical and mechanistic models that also specify the margins of uncertainty for the range of factors that modify the toxicity of chemicals.