PHENOTYPIC PLASTICITY AND FITNESS CONSEQUENCES IN NEMATODES EXPOSED TO TOXICANTS

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PHENOTYPIC PLASTICITY AND FITNESS CONSEQUENCES IN NEMATODES EXPOSED TO TOXICANTS

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Proefschrift

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Stellingen

1. De intrinsieke kwetsbaarheid van organismen voor chemische stoffen wordt bepaald door de relatie tussen plasticiteit van levenscyclusvariabelen en fitness en is niet afhankelijk van kritische effectconcentraties voor de meest gevoelige levenscyclusvariabele (dit proefschrift).

2. Het vaststellen van concentratie- *c.q.* dosis-respons relaties voor levenscyclusvariabelen is van beperkte waarde voor de ecotoxicologische risico-analyse indien deze variatie voor toxische stress niet wordt gerelateerd aan de fitness van het desbetreffende organisme (dit proefschrift).

3. De huidige risico-analysemethoden voor het bepalen van veilige normen van chemische stoffen in bodem en water op basis van kritische effectconcentraties houden geen rekening met het vermogen van organismen zich door middel van plasticiteit aan een veranderend milieu aan te passen (dit proefschrift).

4. Fenotypische plasticiteit moet gezien worden als een intrinsieke eigenschap met een eigen genetische controle zoals alle andere onderdelen van de levenscyclusstrategie. (Via et al. 1995. Adaptive phenotypic plasticity: consensus and controversy. Trends Ecol. Evol. 10:212-217).

5. In de studie van Perrin *et al.* naar de energie-allocatie en populatiedynamiek van watervlooien wordt het begrip fitness niet eenduidig gedefinieerd. (Perrin et al. 1992. Resource allocation, population dynamics and fitness: some experiments with *Daphnia magna* Straus. Arch. Hydrobiol. 125:431-449).

6. De uitkomsten van Wright en Coleman over lokale extinctie en overleving van nematoden doet vermoeden dat nematoden in een metapopulatiestructuur voorkomen. (Wright D.H. en Coleman D.C. 1993. Patterns of survival and extinction of nematodes in isolated soil. OIKOS 67:563-572).

7. Het advies van de voormalige Centrale Raad voor de Milieuhygiëne (CRMH) dat in beginsel 100% van de soorten in een ecosysteem beschermd moet worden tegen de belasting van chemische stoffen is ten onrechte niet opgevolgd. (Anoniem 1992. Advies over de nota "Omgaan met Risico's". CRMH, VROM, 's-Gravenhage).

8. Indien de ecotoxicologie uitsluitend aangestuurd wordt door het milieubeleid is het gedoemd om als wetenschappelijke discipline ten onder te gaan.

9. Ecotoxicology is a non-issue, the scientists involved are just a bunch of people asking the same question (P.M. Kareiva).

10. Moeilijke beslissingen worden vaak niet genomen, niet omdat ze moeilijk zijn maar omdat het aan durf ontbreekt.

Stellingen behorend bij het proefschrift, getiteld '*Phenotypic plasticity and fitness consequences in nematodes exposed to toxicants*' door Jan Kammenga.

Wageningen, 12 december 1995.

Voor Renate en
mijn ouders

CONTENTS

CHAPTER 1

INTRODUCTION

The early discovery of side-effects of DDT and related compounds on non-target species (Hotchkiss and Pough 1946; Hunt and Bischoff 1960) marked the dawn of environmental concern in the scientific and public community. The publication of Rachel Carson's book *Silent Spring* (1962) on the alarming effects of pesticides was a landmark and accelerated the scientific awareness of the deterimental impact of anthropogenic pollution on the environment and sparked the birth of ecotoxicology as a scientific field. Originally defined by toxicologists, ecotoxicology is 'the branch of toxicology concerned with the study of toxic effects, caused by natural or synthetic pollutants, to the constituents of ecosystems, animal (including human), vegetable and microbial in an integral context' (Truhaut 1977). Despite the original goals, the field of ecotoxicology has been seriously criticized for its lack of ecological relevance since most scientists approached the subjects from a toxicological perspective (for a historical review see: Forbes and Forbes 1994). Already lamented in the early eighties a plea was made for ecologists to play a major role in ecotoxicology (Koeman 1983). It was even stated that 'it would be hard to find two groups with less interchange than ecologists and ecotoxicologists' (Cairns 1990).

Although advocated by Moriarty (1983), very few attempts have been made to identify important ecological tenets and to integrate this theory with toxicological concepts. At present, research has been focused on the prediction of the potential effect of chemicals on ecosystems and the evaluation of their potential ecological risk. Consequently, many theoretical and experimental explorations have been conducted to provide an adequate basis for the risk assessment of toxicants at the ecosystem level. Much attention has been paid to the development of statistical methodologies to extrapolate the results obtained from laboratory toxicity tests to the field situation (Suter et al. 1985; Stephan et al. 1985; Van Straalen and Denneman 1989; Wagner and Løkke 1991; Aldenberg and Slob 1993). Basically these methods estimate safe environmental concentrations of hazardous compounds from distribution models of critical effect levels obtained from single species

toxicity tests for different organisms. In general, toxicity tests are used to estimate critical effect levels (EC_x or LOEC) from concentration-response relationships for single life-cycle variables such as mortality, growth, reproduction or breeding success. Numerous results concerning these tests have been presented for studies using both aquatic and terrestrial organisms (Jørgensen et al. 1991), and currently a large part of ecotoxicological research is focused on the development of standardised tests for a wide range of terrestrial and aquatic species (Maltby and Calow 1989; Van Gestel and Van Straalen 1994).

A prevailing view in many of these studies is that the estimation of critical effect levels for sensitive life-cycle traits are highly relevant for the ecological effect assessment of toxicants. This premise stems from classic toxicological studies which aim for sensitive indicators in test organisms to evaluate the biological hazard of chemical compounds to humans. The rationale of applying the most sensitive life stage as a suitable parameter has often been adopted (Marchini et al. 1993; McKim 1985; Norberg-King 1989). Indeed, a large number of ecotoxicological papers have studied the effect of chemicals on sensitive life-cycle variables, such as juvenile mortality, growth and reproduction assuming them to be relevant. To mention a few, it was argued that aquatic toxicity tests should be based on the most sensitive stages of the life cycle if the results are to be ecologically meaningful (Green et al. 1986). Also Call et al. (1987) and Nagel et al. (1991) implicitly focus on susceptible life-cycle components in early life-stage experiments. In addition Coyle et al. (1993) and DeLonay et al. (1993) have studied the effect on reproductive success and early life-stages respectively. However, these papers do not consider life-history theory and assume that these sensitive factors are higly relevant from an ecological point of view. At present standardised toxicity tests, which are implicitly based on the sensitivity concept, with earthworms and daphnids have been adopted by the Organisation for Economic Cooperation and Development (OECD) and the European Union (EU) (Anonymous 1984; 1985). Although these studies and approaches may be useful for the ranking of the potential toxicity of chemicals, this thesis will examine whether the general premise of sensitive variables being ecologically relevant is supported by life-history theory.

ADAPTIVE PHENOTYPIC PLASTICITY AND THE FITNESS CONCEPT

An important tenet in life-history theory is the concept of adaptive phenotypic plasticity (Via et al. 1995). Phenotypic plasticity represents the range of phenotypes not originating from genetic differentiation that can develop if an organism is exposed to heterogeneous or changing environments. Plastic phenotypes offer an important solution in the adaptation to a wide spectrum of abiotic and biotic environmental factors (in accordance with current literature on adaptive plasticity, no distinction is made between adaptation and acclimatisation). Many experimental approaches have shown phenotypic alterations for a variety of traits in plants as well as animals to a range of ambient conditions such as temperature (Williams and Black 1993), nutrient content (Sultan and Bazzaz 1993a), light (Sultan and Bazzaz 1993b) and the presence or absence of a predator (Stibor 1992). It appeared that the relative changes in characteristics differed among traits and that the degree of change depended on the genotype and the type of environments under consideration (see Via et al. 1995).

Toxicants can be regarded as an additional ambient factor to the aforementioned ones which poses stress when organisms are exposed to relatively high concentrations. Following this equality assumption the possibility of accumulation of contaminants in tissues of the organism is excluded. Consequently the classic concentration-response relationship to a toxicant may be conceived as the plastic response of a trait to a range of discrete environments *i.e.* the concentration range of the toxicant. An important aspect of plasticity is that organisms subjected to non-homogeneous or adverse conditions may be able to maintain fitness by changing phenotypic characteristics. This presumption is the heart of the present thesis and provides the basis for the experimental investigations.

Defining fitness has been subjected to much dispute and controversy in life-history theory over the last centuries. In 1958 Fisher published his tractate on the 'Genetical Theory of Natural Selection' in which fitness was defined for the first time:

'The vital statistics (i.e. survival and reproduction, JK) of an organism in relation to its environment provide a means of determining a measure of the relative growth rate of the

population, which may be termed the Malthusian parameter of population increase, and provide also a measure of the reproductive values of individuals at all ages or stages of their life-history. The Malthusian parameter will in general be different for each genotype, and will measure the fitness to survive each.'

Based on life-table data, thus including reproduction and mortality statistics, Lotka developed the now classic equation relating these statistics to fitness (r) by:

$$1 = \sum_{t=0}^{t=\infty} e^{-rt} l_t(p,E) n_t(p,E)$$

where t is age, $l_t(p,E)$ is survivorship ($0 \leq l_t(p,E) \leq 1$) during time t of a genotype with its corresponding phenotype p in environment E and $n_t(p,E)$ is the number of female offspring per time unit ($n_t \geq 0$) at age t of phenotype p in environment E (Sibly and Calow 1983). For any age-dependent and constant-in-time offspring and survival the population will decrease or increase exponentially after reaching a stable age distribution. This holds only for density-independent populations, without competition and abundant food supply. The genotype with the highest r is the fittest (Kozłowski 1993). A different, and also commonly used, definition of fitness is life-time offspring production, or the net reproductive rate, which is a proper measure only in stationary populations (Kozłowski 1993). By using life-table data however it is widely acknowledged that the Malthusian parameter is a correct fitness measure, although Stearns (1992) argued that fitness is a problem solving tool rather than a well-defined parameter.

A limited number of papers have evaluated the effect of chemicals based on life-history theory by using the Lotka equation. Daniels and Allan (1981) and Allan and Daniels (1982) were among the first who used the rate of population increase (r) as a measure of toxicity in aquatic invertebrates. Using soil invertebrates, Van Straalen et al. (1989) and Crommentuijn (1994) stressed that the relationship between life-cycle variables and

population growth are of paramount importance for evaluating the impact on single traits. Most of these studies however did not gain insight into the actual cause of the fitness decrease and the observed changes were discussed in a qualitative way. Moreover, they concentrated on the decrease of trait values at various toxicant concentrations and did not address the variation expressed in phenotypic characteristics.

CHALLENGING TOXICOLOGICAL PERCEPTIONS

The aim of this thesis was to evaluate underlying premises on current risk assessment procedures of toxicants on organisms. By using the concept of phenotypic plasticity and fitness maximisation the intention was to challenge present toxicological perceptions from a life-history point of view. By regarding concentration-response relationships as plastic responses to ambient factors, this thesis questions current methodologies on effect assessment of toxicants based on sensitive and single life-cycle traits.

NEMATODES

Free-living bacterivorous nematodes provide an excellent organism to investigate the relationships between plasticity of life-cycle traits to toxic stress and fitness alterations since many species are parthenogenetic. Commonly found in soils and sediments nematodes may occur in densities depending on soil structure, texture and vegetation (Sohlenius 1980). On the basis of their ecology and morphological structures, nematodes can be divided into plant-associated or free-living species (Yeates et al. 1993). Identification of the free-living species has become within reach of non-specialists and they can be classified into different feeding groups such as bacterial and fungal feeders, predators and omnivores (Bongers 1988). After extraction from the soil, many free-living bacterivorous nematodes can easily be reared on bacteria in the laboratory in agar thus providing a suitable substrate for living and offering the opportunity for complete life-

cycle analysis.

A small number of papers have investigated the intricacies of life cycles in free-living nematodes, some of which will be mentioned here. Observations on life cycles have been reported mainly for Rhabditid nematodes which have fairly short generation times (2-5 days). Population growth rates from life-table data were determined for *Rhabditis marina* (Vranken and Heip 1983) and comparative studies into resource allocation among life-cycle traits have been performed with *Caenorhabditis briggsae* and *Plectus palustris* (Plectidae) (Schiemer et al. 1980; Schiemer 1983). Also detailed life-cycle studies have been undertaken for some marine nematodes (Vranken 1987).

At present, only few authors have focused on the use of bacterivorous nematodes in life-cycle toxicity studies. Some of these have used the same test species, the rhabditid nematode *Caenorhabditis elegans* aiming to develop toxicity tests. Van Kessel et al. (1989) reported on the influence of cadmium on life-cycle traits and Williams and Dusenbery (1990) focused on the impact of other heavy metals on reproductive output.

PLASTICITY-TO-FITNESS ANALYSIS

The present thesis provides a comprehensive analysis of the relationships between toxicant-induced phenotypic plasticity and fitness reductions in divergent life-history strategies which will be denoted by 'plasticity-to-fitness analysis'. Both theoretical and experimental approaches will be presented aiming to unify life-history theory and toxicological concepts on deriving critical effect levels for ecological risk assessment procedures. The objectives are threefold:

i) to determine the relationship between plasticity to toxicants in life-cycle traits to changes in fitness.

ii) to evaluate critical effect levels of toxicants by means of the plasticity-to-fitness relationships.

iii) to explore the susceptibility of divergent life-history strategies to toxic stress using the plasticity-to-fitness relationships.

To gain insight into these relationships a general rationale is presented in Chapter 2 which aims to identify the influence of plastic responses in life-cycle traits on fitness for different iteroparous strategies. To verify these theoretical explorations, bacterivorous nematodes are used in a case study to explore the plasticity-to-fitness relationships for various toxicants. Chapter 3 focuses on the selection of *Plectus acuminatus* (Bastian 1865). Consequently, Chapter 4 aims to tailor the plasticity-to-fitness relationships for *P. acuminatus* by constructing a mathematical model based on life-cycle data in control and cadmium exposed females. This model provides the basis for the evaluation of critical effect levels of cadmium and pentachlorophenol in *P. acuminatus* which is described in Chapter 5. To investigate the susceptibility of divergent life-history strategies to toxic stress, the impact of copper is studied for *P. acuminatus* and *Heterocephalobus pauciannulatus* using plasticity-to-fitness relationships in Chapter 6. Finally, the principle conclusions are summarised and the ecotoxicological implications are briefly explored by focusing on plasticity for age and size at maturity to toxicants using literature data for the springtail *Folsomia candida*.

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CHAPTER 2

GENERAL RATIONALE *

ABSTRACT - *Fisher's postulate on fitness maximisation was used to outline a concept for the quantitative evaluation of toxicant induced life-cycle alterations by relating reaction norms for different traits to fitness variation. For this purpose the reaction norm was defined as the range of effect levels of life-cycle phenotypes which can be developed by one genotype. A plasticity-to-fitness analysis illustrated that i) the impact of toxicants on fitness was not linearly related with the reaction norms for different traits to these toxicants, and ii) the relationship between reaction norms and changes in fitness depended on the life-history strategy. These findings denounce current procedures for the risk assessment of toxicants which are based on effect levels of single and sensitive life-cycle variables. Hence these procedures need to be revisited by life-history theory to ensure a proper evaluation of the potential ecological hazards of toxicants.*

* *Based on: Kammenga J.E., Korthals G.W., Bongers A.M.T. and Bakker J. Reaction norms for life-history traits as the basis for the evaluation of critical effect levels of toxicants. In: Ecological principles for risk-assessment of contaminants in soil (Van Straalen N.M. and Løkke H. eds.). Chapman and Hall, (in press).*

2.1 INTRODUCTION

A concept is presented for the quantitative evaluation of toxicant induced life-cycle alterations within the context of the life-history strategy of the exposed organism. The evaluation is based on the reaction norm for different life-cycle traits which mirrors the range of toxicant concentrations to which these traits are exposed.

The concept asserts from Fisher's postulate on the tendency of natural selection to maximize fitness by optimizing different components of the life cycle (Fisher 1958). Following this theorem Charlesworth (1980) and Kozlowski (1993) showed that fitness is defined by the intrinsic rate of natural increase which is the root 'r' of the following Euler-Lotka equation:

$$1 = \sum_{t=0}^{t=\infty} e^{-rt} l_t(p,E) n_t(p,E) \quad (1)$$

where t is age, $l_t(p,E)$ is survivorship during time t of phenotype p in environment E and $n_t(p,E)$ is the number of female offspring per time unit at age t of phenotype p in environment E (Sibly and Calow 1983; Smith 1991).

To maintain maximum fitness in a changing and less favourable or contaminated environment many species are able to adapt life-cycle phenotypes within one generation, a phenomenon which is called phenotypic plasticity (Stearns 1983). Plasticity refers to differences in life-cycle components within populations which do not originate from genetic differentiation (Stearns 1992). For example, phenotypic plasticity has been found by Dangerfield and Hassal (1992) in breeding phenology for the woodlouse *Armadillidium vulgare*. They observed a range in life-cycle phenotypes due to spatial as well as temporal variation and argued that plasticity can be appropriate in the attempt to maximize fitness in a changing environment. Another example is a study by Stibor (1992) who reported that life-history shifts in cladocerans can be induced by external factors such as chemical

stimuli released by a predator.

The range of potential phenotypes that a single genotype can develop if exposed to a specified range of ambient conditions is called the reaction norm (Woltereck 1909). At present reaction norms have been found to temperature for eye-size genotypes in *Drosophila* and to altitude for plant height (see Griffiths et al. 1993). The findings of Stearns (1983) on developmental plasticity in life-cycle traits in fish have led to the definition of the reaction norm for traits such as age and size at maturity (Stearns and Koella 1986). However, the classic toxicological concentration-response relationship can also be conceived of as a reaction norm. The concentration range of the toxicant represents the discrete ambient environment and the variation in phenotypes is represented by the range of effect levels of different life-cycle traits.

A fundamental aspect of plasticity is that life-cycle variables can be flexible over a certain range without being disadvantageous to the species in question, *i.e.* without any significant fitness reduction. These findings have serious consequences for the evaluation of effect levels of toxicants which will be exemplified by means of a life-cycle analysis for two different iteroparous strategies.

2.2 Theoretical framework

ANNUAL ITEROPAROUS LIFE CYCLE WITH EQUAL JUVENILE AND ADULT PERIODS

Consider a sexual reproducing organism with homozygous alleles and an annual iteroparous life cycle. This means that each genotype has the same life cycle and that the adults survive after reproduction and live on to next year for the following breeding season (*e.g.* some invertebrates). Following the approach of Sibly (1989) we further assume that the period between breeding seasons is equal to the length of the juvenile period (Fig. 1). Hence equation (1) becomes (Sibly 1989):

$$\tfrac{1}{2}nS_j e^{-rj} + S_a e^{-rj} = 1 \quad (2)$$

where n = the age specific reproduction rate, S_j = survival from birth to the end of the juvenile period, r = fitness, t_j = the juvenile period and S_a = survival during the reproductive period.

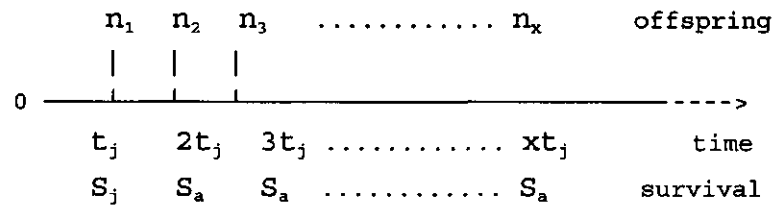

Figure 1. Annual iteroparous life cycle with equal juvenile and adult periods.

The hypothetical reaction norm ▲x for the genotype is defined as the toxicant-induced change in a life-cycle variable x from 0 (no change) to 1 (100% change). The reaction norm can be related to changes in fitness (▲r) by performing a sensitivity analysis of equation (2) using multiple iteration processes. The software package MathCad 5.0 (Mathsoft Inc. USA) was used for this purpose. Different maps can now be constructed illustrating the relative sensitivity of fitness to ▲x.

Map of ▲r versus ▲S_a

Assuming S_j=0.63 and t_j=30.6, which are realistic values obtained from life-cycle experiments with invertebrates, figure 2a shows the relationship between changes in fitness (▲r) and the reaction norm for S_a (▲S_a) for n=4, n=6 and n=30. It is illustrated that ▲S_a influences ▲r most strongly when n is low. However, when n is high the effect is negligible. This implies that species with a high reproduction rate are less vulnerable to stress induced reduction in adult survival. On the contrary species with low reproduction

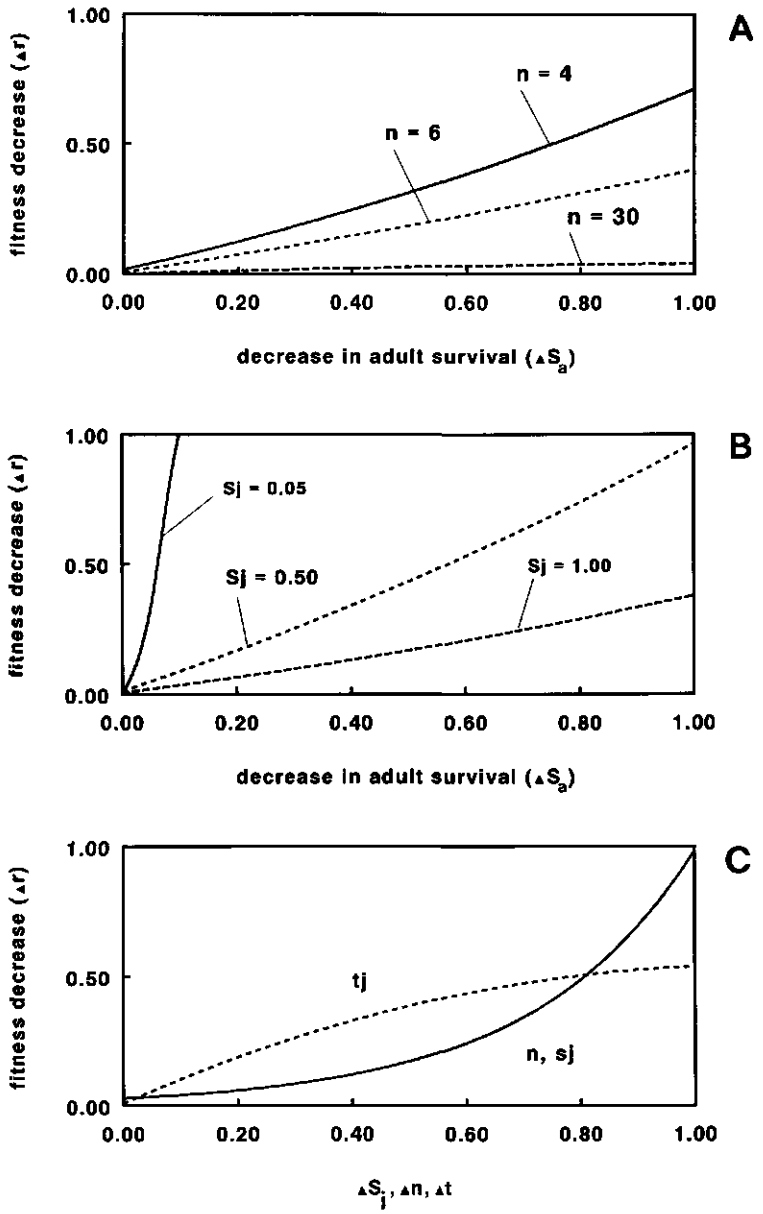

Figure 2. Maps relating ▲r and the reaction norm of S_a where n=4, n=6, n=30 (A); S_a where S_j=0.05, S_j=0.1, S_j=1.0 (B) and S_j, n, t_j (C) for an annual iteroparous life cycle with equal juvenile and adult periods.

rates are most susceptible to impairment of adult survival. The length of the juvenile period did not have any effect on the relationship between $\blacktriangle S_a$ and $\blacktriangle r$.

Figure 2b shows the same map, however in this case S_j=0.05, 0.5 and 1.0 for n=4 and t_j=30.6. It shows that $\blacktriangle S_a$ has a very strong effect on $\blacktriangle r$ when S_j is low. The influence on $\blacktriangle r$ will never be negligible because even at maximum juvenile survival (S_j=1.0) there is a significant decrease in $\blacktriangle r$. The map illustrates that organisms with low S_j are extremely vulnerable to stress induced reductions in S_a. Even when S_j is 1, a change in S_a will have detrimental consequences for fitness.

Map of $\blacktriangle r$ versus $\blacktriangle n$, $\blacktriangle S_j$ and $\blacktriangle t_j$

Figure 2c shows the reaction norm for the length of the juvenile period ($\blacktriangle t_j$), juvenile survival ($\blacktriangle S_j$) and reproduction rate ($\blacktriangle n$) in relation to changes in fitness. It is illustrated that $\blacktriangle t_j$ influences $\blacktriangle r$ differently compared to $\blacktriangle S_a$ (Fig. 2c) when n=4, S_j=0.63, t_j=30.6. However changing n, S_j or S_a does not affect this relationship, indicating that the influence of t_j on fitness is not determined by these variables. Also the relationship between $\blacktriangle n$ or $\blacktriangle S_j$, and fitness appears to be very rigid and non-sensitive to changes in the other variables.

These findings may have far reaching consequences for the evaluation of critical effect levels of toxicants. For example, a 50% effect (EC_{50}) in t_j or n has less influence on fitness (Fig. 2c) than 10% (EC_{10}) reduction in S_a if S_j=0.05 (Fig. 2b). On the other hand a 50% reduction in S_a when S_j=1 (Fig. 2b) has a smaller effect on fitness than even 20% effect on t_j (Fig. 2c). Also a 50% reduction of S_a when n=30 (Fig. 2a) has less impact on fitness than a 10% increase in t_j (Fig. 2c).

ANNUAL ITEROPAROUS LIFE CYCLE WITH UNEQUAL JUVENILE AND ADULT PERIODS AND TIME DEPENDENT MORTALITY

Different results are obtained by focusing on the same iteroparous life cycle except that t_j is unequal to t_a and mortality time dependent following an exponential decrease $S_j = e^{-\mu t}j$, $S_a = e^{-\tau t}a$ (*e.g.* some birds and small vertebrates). Assuming a difference between t_j and t_a and time dependent mortality (Fig. 3), then equation (1) can be rewritten as (Sibly 1989):

$$\tfrac{1}{2} n e^{-\mu t}j - {}^{-\pi}j + e^{-\tau t}a - {}^{-\pi}a = 1 \qquad (3)$$

where n = age specific reproduction rate, μ = juvenile mortality rate from birth to the end of the juvenile period, r = fitness, t_j = juvenile period, τ = adult mortality rate and t_a = adult period. The reaction norm can be related to $\blacktriangle r$ by performing a sensivity analysis equivalent to equation (2).

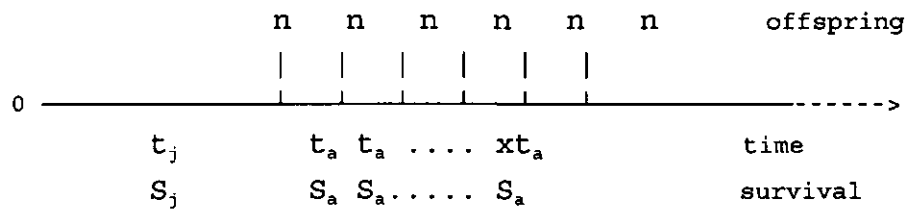

Figure 3. Iteroparous life cycle where t_j is not equal to t_a and time dependent mortality.

Map of $\blacktriangle r$ versus $\blacktriangle n$

Taking t_j=30, τ=0.02 t_a=60 and n=100..1 (where 100 is set to 0 and 1 is set to 1), values which are commonly found for some small vertebrates, figure 4a shows the relationship between $\blacktriangle r$ and the reaction norm $\blacktriangle n$ (0 to 1) for μ=0.01, μ=0.05 and μ=0.08. Although impairment of reproduction rate is detrimental in all three cases, it

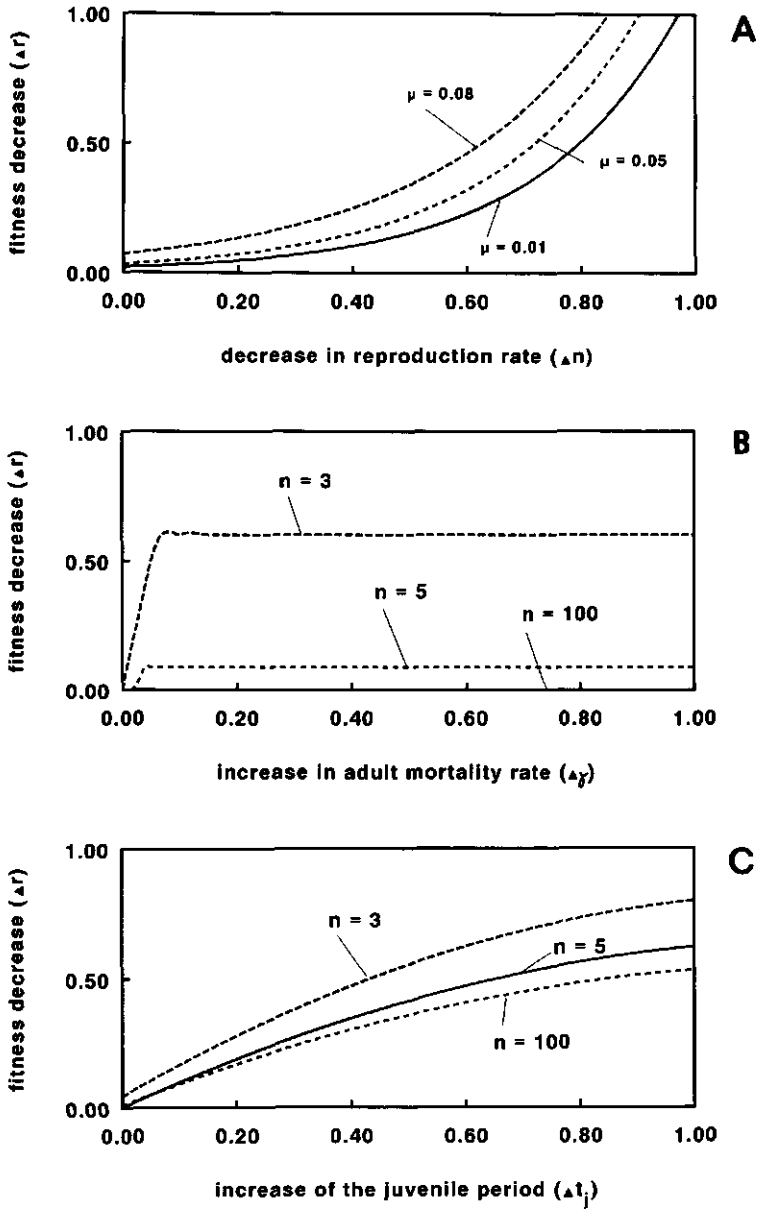

Figure 4. Maps relating ▲r and the reaction norm of n where μ=0.01, μ=0.05, μ=0.08 (A); τ where n=3, n=5 and n=100 (B) and t_j where n=3, n=5, n=100 (C) for an iteroparous life cycle with unequal juvenile and adult periods.

appears that the effect of stress induced reductions in n on fitness is most important in species with high juvenile mortality rates.

Map of ▲r versus ▲τ

Figure 4b shows the map of ▲τ and ▲r for t_j=30, τ=0.02, t_a=60, n=3, n=5 and n=100. It is illustrated that ▲τ influences fitness strongly from 0 to 0.1 when n=5 and n=3, whereas further impairment does not affect fitness further. Also it appears that at low n the effect on ▲r is very strong compared to high n. At n=100 there is no detrimental effect on fitness. This implies that increased adult mortality rate is only important in species with low reproductive output.

Map of ▲r versus ▲t_j

Figure 4c illustrates the relationship between ▲t_j (here t_j increases from 30 (set to 0) to 60 (set to 1)) and ▲r for μ=0.01, τ=0.02, n=3, n=5 and n=100. The map for ▲t_j and ▲r is similar as for the other life cycle, however in contrast it depends on n. At low n fitness is much more influenced by ▲t_j then at high n, indicating that species with low reproduction rates are more vulnerable to impairment of t_j.

It was indicated that a 50% effect (EC_{50}) in τ when n=5 (Fig. 4b) has a smaller impact on fitness than a 20% effect (EC_{20}) in t_j (Fig. 4c). Also a 50% reduction in n (Fig. 4a) has a smaller effect on fitness than 15% increase in τ (Fig. 4b) when n=3. The conclusion may be that the influence of toxic stress on fitness is not determined by the effect on sensitive traits but depends on the reaction norm for traits and the life-history strategy which comprises the relationship between all variables and fitness.

2.3 Discussion

This theoretical paper illustrates that the impact of a toxicant on fitness is determined by i) the reaction norm for various life-cycle traits to chemical stress and ii) the life-history strategy. Reaction norms define the variation of life-cycle traits to a range of environmental conditions for one genotype. In the field, populations consist of many different genotypes, each having a specific reaction norm to certain stress factors. Using different genotypes of *Daphnia magna*, relatively large differences in reaction norms were observed for stress tolerance to cadmium (Baird et al. 1990). Also an extensive altitudinal distribution of *Pennisetum setaceum* was noted which could be explained by phenotypic plasticity for leaf photosynthesis to temperature stress (Williams and Black 1993). Determination of reaction norms requires that different individuals of the same genotype have to be reared in different environments. Therefore, experimental research in this field requires that numerous eggs or juveniles with identical genotypes are produced. At present only for a few organisms, which are mainly parthenogenetic invertebrates, it is possible to obtain a large number of identical genotypes and hence to determine reaction norms for traits to a range of toxicant concentrations.

In this paper the reaction norm was hypothesized for various traits from 0 to 100% variation. Experimental data confirm this large variation in phenotypic plasticity for a range of species. A change of 40% was found in plasticity of age at first reproduction in freshwater cladocerans (Stibor 1992) and in size of reproductive females in woodlice (Dangerfield and Hassall 1992).

At present much effort is taken in risk assessment procedures to verify the results obtained from laboratory toxicity tests to the field situation. However, the verification of the test results requires a fundamental understanding of the underlying mechanisms at the individual level. Life-history theory states that in the attempt to maximize fitness, natural selection favours traits which have a strong impact on fitness. Hence impairment of these traits may give rise to enhanced vulnerability of species to environmental contamination which eventually may lead to extinction. Analysis of the relationship between reaction norms and fitness provides the key to identify these traits for different strategies.

General rationale

The relationship between the reaction norm and fitness depends on the life-history strategy and determines the ultimate effect of a toxicant on fitness. It was shown in this paper that for two different life cycles each variable contributes differently to fitness which in many cases depended on the value of the other variables. Strong effects on certain parameters did not always influence fitness to the same extent because reaction norms and fitness were not linearly related. Different maps were drawn relating changes in fitness to the reaction norm of life-cycle traits for each life-history strategy. Annual iteroparous species with equal juvenile and adult periods appeared to be vulnerable to stress induced increase of adult mortality when the juvenile survival or reproduction rate was low. Within the range investigated here, the influence of the juvenile period on fitness was not affected by the value of the other variables. However, species with an iteroparous life cycle and with unequal juvenile and adult periods appeared to be susceptible to increased adult mortality rates when reproduction rate was low, and in this case the relationship between the juvenile period and fitness depended on reproduction rate.

It was reported by other authors that, in general, the length of the juvenile period has the strongest impact on fitness (Lewontin 1965; Sibly and Calow 1986). My results imply that for annual iteroparous organisms with equal juvenile and adult periods the adult survival may have a stronger impact on fitness depending on the value of the juvenile survival. Also in case of the iteroparous life cycle with unequal juvenile and adult periods the influence of adult mortality rate can be stronger at low reproductive output compared to the length of the juvenile period.

An important presumption in the aformentioned life-cycle analysis is the independency of traits to one another, *i.e.* to investigate the relative contribution of each trait to fitness, a sensitivity analysis is performed *ceteris paribus*. Although each trait may be traded against another trait, this is the only way to gain insight into plasticity-to-fitness relationships and therefore the chapters 4, 5 and 6 will also assume independency. Moreover, based on this assumption, the present paper clearly outlines the neccessity of novel concepts in the ecological risk assessment of toxicants which unify both ecological and toxicological methodologies.

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CHAPTER 3

SELECTION OF A SUITABLE TEST SPECIES *

ABSTRACT - *To investigate plasticity-to-fitness relationships for toxicants in nematodes, selection of a suitable test species was based on the variation of acute toxicity data among nematode species belonging to different taxonomic and ecological groups. Twelve different nematode species were extracted from the soil and directly exposed to cadmium and pentachlorophenol. LC_{50}-values were estimated after 24, 48, 72 and 96 h of exposure in aqueous solutions. The species exhibited large differences in sensitivity. LC_{50}-values (72 h) for pentachlorophenol ranged from 0.5 to more than 34.5 µM and for cadmium from 29 to more than 800 µM. These toxicity data could be described by a log-logistic distribution function. LC_{50}-values for cadmium were not correlated with those for pentachlorophenol. Species of the subphylum Secernentia were less sensitive to pentachlorophenol than species of the subphylum Penetrantia, while no differences were observed for cadmium. In addition, no relationship was found between toxicity data and life-history strategies. Slow colonizers (K-strategists, sensu lato) were not more sensitive to cadmium and pentachlorophenol than opportunistic species (r-strategists, sensu lato). Nematodes appeared to be as sensitive to pentachlorophenol as other soil invertebrates but were generally tolerant to cadmium. On the basis of these findings, Plectus acuminatus Bastian 1865 (Plectidae) was selected for further life-cycle investigations.*

* *Based on: Kammenga J.E., Van Gestel C.A.M., Bakker J. (1994) Patterns of sensitivity to cadmium and pentachlorophenol among nematode species from different taxonomic and ecological groups. Arch. Environ. Contam. Toxicol. 27: 88-94.*

Chapter 3

3.1 INTRODUCTION

Nematodes, or threadworms, are ubiquitous in aquatic and terrestrial habitats and constitute one of the largest animal phyla. Population densities depend on various biotic and abiotic factors and may range to $1.10^7/m^2$ in soil (Sohlenius 1980). Soil inhabiting nematodes can be divided into different feeding groups: plant, bacterial and fungal feeders, predators and omnivores (Yeates et al. 1993). Plant feeding nematodes are of agricultural importance because of their detrimental effects to crops (Wallace 1977). Bacterivorous and fungivorous nematodes are key intermediaries in decomposition processes of organic matter in soil (Freckman 1988). These nematodes stimulate N-mineralisation and play an important role in the nutrient cycle (Anderson et al. 1981; Yeates and Coleman 1982; Woods et al. 1982). Predators and omnivores regulate the density of prey populations by feeding on nematodes, algae and other soil organisms.

Nematodes offer perspectives for ecotoxicological research because of their abundance, species diversity and differences in sensitivity to chemicals. They can be extracted from the soil efficiently and identification has become within reach of non-specialists (Bongers 1988). A large number of species can be reared in the laboratory and are relatively easy to handle.

Nematodes live in the interstitial water between soil particles, therefore toxicity studies can be conducted in water. Since the bioavailability of toxicants for soil organisms depends largely on the concentration in the interstitial water (Van Gestel and Ma 1988; Aben et al. 1992) nematodes are directly exposed to environmental contaminants.

Various papers have investigated the toxicity of chemicals, e.g. pesticides (Bunt 1980; Kämpfe and Wischgoll 1984) and heavy metals (Vranken and Heip 1986; Williams and Dusenbery 1990; Vranken et al. 1991) to nematodes. However, only a limited number of species have been tested, and routes of uptake and test conditions differed too much to allow for a proper comparison. Therefore, these studies provide no insight in the patterns of sensitivity among species in the phylum Nematoda.

This chapter proposes to select a suitable species for life-cycle studies by i) obtaining insight in the sensitivity of nematode species for two distinct toxicants and ii) determining to which extent acute toxicity data are correlated with taxonomic and ecological similarities.

Therefore 12 species, representing distinct ecological groups and belonging to different subphyla, families and genera, were exposed to cadmium and pentachlorophenol in aqueous solutions. Both compounds are ubiquitous in the environment, have different modes of action and their properties are well documented (Ros and Slooff 1988; Anonymous 1987). Cadmium occurs in zinc and phosphate ores and is mainly emitted into the environment by ore mining industries. Cadmium accumulates in the top layer of the soil where it may affect terrestrial organisms. Pentachlorophenol is widely used as a wood preservative or fungicide. Once emitted into the air, pentachlorophenol is readily deposited on the soil.

3.2 Materials and Methods

CHEMICALS

Cadmium chloride ($CdCl_2$) was purchased from Merck, Darmstadt, Federal Republic of Germany, and pentachlorophenol from Fluka Chemie A.G., Switzerland. The compounds were >99% pure. All other chemicals (Merck) used were of the highest analytical grade.

CHEMICAL ANALYSIS

Analysis of cadmium and pentachlorophenol in water was performed with Atomic Absorption Spectrophotometry (Perkin Elmer 3030 AAS, flame furnace, detection-limit: 0.1 μg/L) and HPLC (Spectro Flow 757, Lichrosorb RP18 column (7 μm x 20 cm), UV-detector, 254 nm, detection limit: 50 μg/L, flow rate 0.7 mL/min), respectively.

NEMATODES

Nematodes were extracted from the top mineral layer of arable soil, forest floor and stream sediment. Sites were selected where different species predominate. Samples were taken near Wageningen, The Netherlands, and recovered from the soil by means of the modified

Oostenbrink-method (Oostenbrink 1954; 1960) during late spring. In this way multi-species suspensions were obtained, free from debris and soil particles. Before toxicity testing, adult nematodes were identified to species level. Representatives of different trophic levels were selected: bacterial feeders (*Rhabditis sp.*, *Cephalobus persegnis*, *Plectus acuminatus*, *Acrobeloides buetschlii* and *Diplogasteritus sp.*), a fungal feeder (*Aphelenchus avenae*), plant feeders (*Tylenchus elegans*), carnivores (*Prionchulus punctatus, Tobrilus gracilis*) and omnivores (*Dorylaimus stagnalis*, *Aporcelaimellus obtusicaudatus*). The bacterial feeder *Caenorhabditis elegans* (Bristol, N2 strain), was obtained from a laboratory culture. Representatives of the genera *Rhabditis* and *Diplogasteritus* could not be identified to species level due to inconsistent taxonomical status. Figure 1 shows the taxonomical classification of the phylum Nematoda and the species which were used for this study.

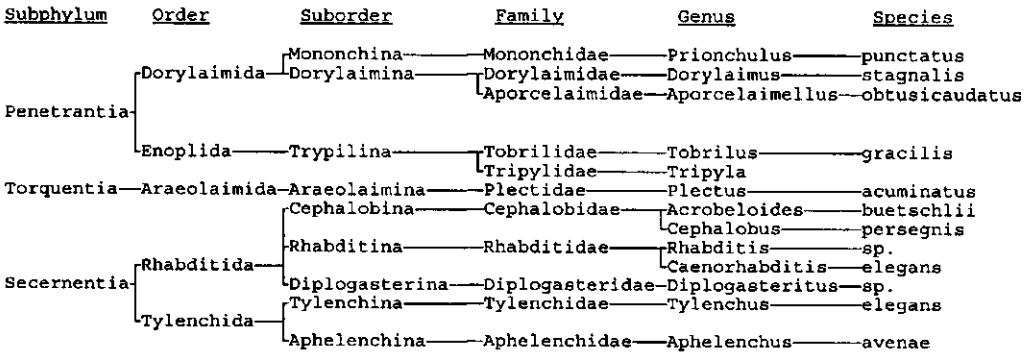

Figure 1. Taxonomic classification of the nematode species tested.

TOXICITY TESTS

LC_{50}-tests were performed in water containing a defined mixture of minerals with concentrations resembling those found in interstitial water of sandy forest soils (K^+: 0.1 mM,

Na^+: 0.2 mM, Ca^{2+}: 0.35 mM, Mg^{2+}: 0.3 mM, NH_4^+: 0.3 mM, NO_3^-: 1.7 mM, Cl^-0.3 mM) (Schouten and Van der Brugge 1989). The pH was adjusted to 6.0 ± 0.1 with NaOH and checked at the end of each experiment. In the control, all tested nematode species survived in this water for at least 3 days. Pentachlorophenol was initially dissolved in ethanol (96%) after which stock solutions in water were made. The stock solutions were sonificated for 60 min. The amount of ethanol did not exceed 0.1 mL/L of the total test volume. The control contained 0.1 mL/L ethanol.

Two replicates of the tests were carried out in multi-dishes (Greiner, 24 compartment plate, nr. 662160) with lid and sealed with parafilm to minimize volatilization. Dishes were stored at 20 ± 0.1 °C in the dark. Each compartment was filled with 0.9 ml of water containing toxicant. Samples of 0.1 mL of the multi-species suspension, containing 10 to 50 adult individuals of each species, were taken and suspended in each compartment. The toxicity tests were carried out with 6 - 8 concentration steps, the ratio between successive concentrations being 1.8. The concentrations used differed between each species due to differences in sensitivity observed in preliminary range-finding tests. Overall, the concentrations ranged from 7 μM to 2 mM for cadmium and 0.1 μM to 38 μM for pentachlorophenol. Mortality, which was recognizable by the decayed bodies, of the species was recorded after 24, 48, 72 or 96 h. Dead nematodes were not removed. Observations were done using an inverted microscope (magnification of 40 - 100).

DATA ANALYSIS

LC_{50}-values and their confidence intervals were calculated according to the trimmed Spearmann-Kärber method (Hamilton et al. 1977; 1978). Differences between LC_{50}-values were tested using a Student's t-test. It was assumed that the LC_{50}-values of the different nematode species were log-logistic distributed (Kooijman 1987), hence the cumulative frequency distribution can be written as:

$$\text{Prob}(\ln LC_{50} \leq x) = \{1 + \exp[(\alpha - x)/ß]\}^{-1}$$

where α = sample mean LC_{50} of m species, $\beta = S_m \sqrt{3}/\pi$ and S_m = sample standard deviation (Kooijman 1987).

3.3 Results

For most species LC_{50}-tests were conducted for 72 h because longer exposure times resulted in increased mortality in the control groups. Table 1 shows the LC_{50}-values of cadmium for twelve species after different exposure times. There are large differences in sensitivity, with LC_{50}-values ranging from 29 to more than 800 μM after 72 h. *Diplogasteritus sp.* was the most sensitive followed by *D. stagnalis*, *P. acuminatus*, *A. obtusicaudatus*, *T. gracilis*, *C. persegnis*, *Rhabditis sp.* and *C. elegans* as intermediate and *A. buetschlii*, *T. elegans* and *A. avenae* as insensitive. For *T. elegans* and *A. avenae* no mortality was recorded below 800 μM cadmium after 96 h. There was no significant difference in sensitivity between species from the Secernentia and the Penetrantia. The LC_{50}-value decreased with time and this decrease was most pronounced for *D. stagnalis* and *P. acuminatus*.

The LC_{50}-values for pentachlorophenol at subsequent time periods are given in Table 2. LC_{50}-values ranged from 0.5 to more than 34.5 μM after 72 h exposure. *P. punctatus* appeared to be the most sensitive followed by *D. stagnalis*, *A. obtusicaudatus*, *T. gracilis* and *T. elegans* as intermediate and *C. persegnis*, *Rhabditis sp.*, *P. acuminatus* and *Diplogasteritus sp.* as relatively tolerant. *C. elegans*, *A. avenae* and *A. buetschlii* were rather insensitive. The genus *Tripyla* was found in every sediment sample although densities were too low to calculate an LC_{50}-value. It was observed, however, that some specimens were still alive at 33 μM pentachlorophenol after 48 h. The LC_{50} declined most apparently with time for *P. punctatus*. Species of the subphylum Secernentia were less sensitive to pentachlorophenol as compared to the Penetrantia (t-test, $p < 0.05$).

The obtained toxicity data could be considered as independent trials from a log-logistic distribution. Figure 2 shows the calculated (α = 4.83, β = 0.42) and observed cumulative frequency data for cadmium, including the LC_{50}-value of 302 μM cadmium in sea water for *Monhystera disjuncta* (Vranken et al. 1991). Figure 3 shows the calculated (α = 1.75, β =

Table 1. Acute toxicity of cadmium to nematodes in aqueous solutions. LC_{50}-values (μM) at different exposure times (95% confidence intervals in brackets) (- : not determined).

Species	exposure time (h)		
	24	48	72
Prionchulus punctatus	216.0 (124.1-375.9)	-	-
Dorylaimus stagnalis	336.0 (170.2-663.2)	154.1 (130.8-181.6)	91.8 (65.9-127.9)
Aporcelaimellus obtusicaudatus	-	214.9 (171.4-269.3)	131.1 (101.0-170.2)
Tobrilus gracilis	204.0 (163.9-253.9)	177.1 (129.0-243.1)	120.2 (90.1-160.3)
Plectus acuminatus	398.0 (322.0-492.4)	132.8 (98.2-179.7)	107.0 (82.4-138.9)
Acrobeloides buetschlii	885.2 (701.7-1117.0)	838.6 (541.7-1298.5)	527.4 (395.0-704.2)
Cephalobus persegnis	174.6 (134.3-227.1)	105.9 (79.1-141.9)	82.9 (58.3-117.8)
Rhabditis sp.	270.2 (215.4-339.0)	170.4 (132.5-219.1)	125.3 (97.4-161.2)
Caenorhabditis elegans	262.0 (224.8-305.4)	134.6 (105.3-172.1)	130.9 (104.1-164.5)
Diplogasteritus sp.	37.3 (29.3- 47.7)	29.4 (23.5- 36.7)	29.4 (23.5- 36.7)
Tylenchus elegans	>800 (96h)		
Aphelenchus avenae	>800 (96h)		

Table 2. Acute toxicity of pentachlorophenol to nematodes in aqueous solutions. LC_{50}-values (μM) at different exposure times (95% confidence intervals in brackets).

Species	exposure time (h)		
	24	48	72
Prionchulus punctatus	6.4 (4.9-9.7)	1.1 (0.7-1.9)	0.5 (0.2-1.5)
Dorylaimus stagnalis	5.0 (3.8-7.0)	3.6 (2.9-4.6)	3.6 (2.9-4.6)
Aporcelaimellus obtusicaudatus	7.5 (3.3-17.2)	3.8 (3.5-4.2)	3.6 (3.2-4.1)
Tobrilus gracilis	2.8 (2.0-3.9)	2.6 (1.6-4.1)	1.9 (1.1-3.5)
Plectus acuminatus	19.5 (17.8-21.3)	19.1 (17.6-20.7)	18.7 (17.4-20.2)
Acrobeloides buetschlii	>34.5 (96h)		
Cephalobus persegnis	>34.5	>34.5	9.6 (3.5-26.1)
Rhabditis sp.	>34.5	>34.5	9.1 (5.8-14.2)
Caenorhabditis elegans	>34.5 (96h)		
Diplogasteritus sp.	26.6 (22.9-30.5)	26.4 (22.0-32.0)	25.4 (11.7-54.7)
Tylenchus elegans	13.9 (11.3-17.1)	6.5 (5.3-8.0)	4.5 (3.8-5.5)
Aphelenchus avenae	>34.5 (96h)		

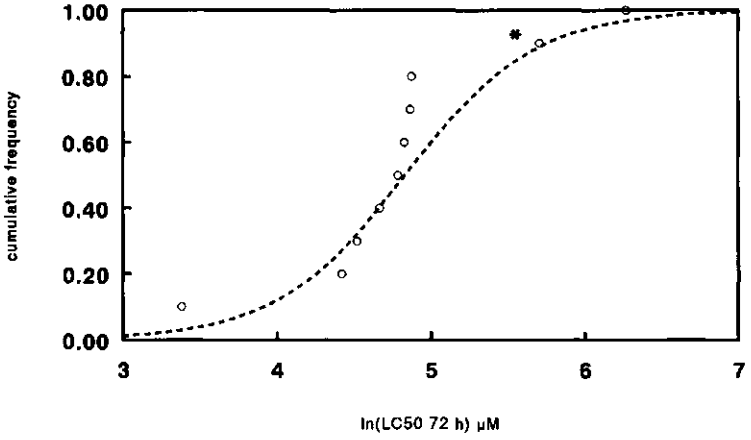

Figure 2. Cumulative frequency distribution of ln(LC_{50}-values) for 10 nematode species exposed to cadmium. Log-logistic curve (- - -) and empirical values (○), * = data obtained from Vranken et al. (1991).

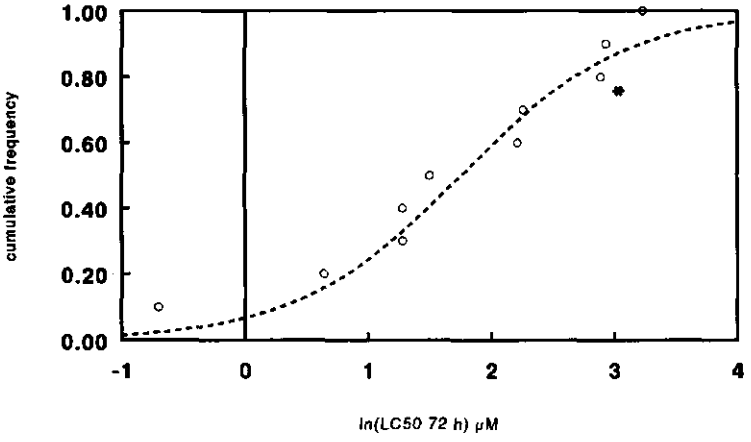

Figure 3. Cumulative frequency distribution of ln(LC_{50}-values) for 10 nematode species exposed to pentachlorophenol. Log-logistic curve (- - -) and empirical values (○), * = data obtained from Vranken et al. (1991).

0.66) and observed cumulative frequencies for pentachlorophenol, including the LC_{50}-value (18 μM) in sea water for *M. disjuncta* (Vranken et al. 1991). There appears to be no

correlation between the sensitivity to cadmium and pentachlorophenol for different species. *D. stagnalis* and *A. obtusicaudatus* were intermediate sensitive to cadmium but relatively susceptible to pentachlorophenol. *A. avenae* was tolerant to both compounds whereas *C. elegans* was insensitive to pentachlorophenol but more sensitive to cadmium.

Chemical analysis revealed that the concentrations of cadmium and pentachlorophenol in the test compartments remained constant in time, so no loss due to adsorption, volatilization or biodegradation occurred during the test. The pH measurements did not indicate a change throughout the test.

3.4 Discussion

Large variations in sensitivity to cadmium and pentachlorophenol were observed among the twelve tested nematode species. Acute toxicity levels differed with a factor > 27 (from 29 to more than 800 μM) between lowest and highest LC_{50}-values for cadmium and a factor > 69 (from 0.5 to more than 34.5 μM) for pentachlorophenol. The variation of LC_{50}-values among the tested nematode species could be described by a log-logistic distribution. These findings agree with the results obtained for aquatic organisms (Kooijman 1987).

Taxonomic similarities were only partly reflected in patterns of sensitivity. For pentachlorophenol all tested species of the subphylum Secernentia were significantly less sensitive than species from the subphylum Penetrantia. No consistent pattern was observed between the sensitivity to pentachlorophenol and cadmium. For example, the species *D. stagnalis* and *A. obtusicaudatus* were intermediate in sensitivity to cadmium but relatively susceptible to pentachlorophenol. *C. elegans* was insensitive to pentachlorophenol but more sensitive to cadmium. *A. avenae* was tolerant to both compounds. *T. elegans* was susceptible to pentachlorophenol but insensitive to cadmium. Based on these results, *Plectus acuminatus* Bastian 1865 (Nematoda, Plectidae) was selected as a suitable test species because of its moderate sensitivity to cadmium and pentachlorophenol, widespread occurence in soils and ease of culturing (Bongers pers. comm.).

The LC_{50}-values for both cadmium and pentachlorophenol decreased in time for all

nematode species tested. LC_{50}-values were still declining after 72 h exposure indicating that equilibrium between uptake and elimination had not been reached yet. The LC_{50} decline for cadmium appears to be slower as compared to pentachlorophenol suggesting that cadmium accumulates more slowly into the nematode body than pentachlorophenol. These results indicate that uptake and elimination rates of cadmium and pentachlorophenol differed between the various species.

There appears to be a correlation between feeding groups and pentachlorophenol sensitivity. Carnivorous, omnivorous and plant feeding nematodes are relatively sensitive, whereas bacterial and fungal feeders are tolerant (Table 2). These findings indicate the vulnerability of particular feeding groups to pentachlorophenol contamination. Physiological research may gain insight into these apparent discrepancies.

Compared to other soil invertebrates, nematodes are relatively insensitive to cadmium (Table 3). Table 4 shows that the sensitivity of nematodes to pentachlorophenol is comparable to that of other invertebrate species.

The observed variations in sensitivity can not be explained by differences in life-history strategies. It is often assumed, either explicitly or implicitly, that slow colonising species (K-strategists, *sensu lato*) are more sensitive to toxicants than opportunistic species (r-strategists, *sensu lato*) (Warwick 1986; Zullini and Peretti 1986; Wodarz et al. 1992). However, colonising abilities are regulated by factors such as reproductive output, population growth rate and density. As Boyce (1984) pointed out in a comprehensive review on r and K selection, the r/K model does not provide a causal relationship between demographic processes and environmental pertubations. Therefore, it is not surprising that no correlation was found between colonizing abilities and sensitivity to toxicants. It was shown that a rapid colonizer, *Diplogasteritus sp.*, was relatively more sensitive to cadmium than *A. obtusicaudatus*, a slow colonizing species. For pentachlorophenol, *T. elegans*, a fast coloniser, was equally sensitive to *D. stagnalis*, a K-strategist. These results corroborate with the findings for terrestrial arthropods (Van Straalen et al. 1989).

The heterogeneity of the ectodermal tissue among nematode species is assumed to play an important role in explaining the variation in acute toxicity data. Chemical compounds enter the nematode body mainly through the cuticle and ingestion does not play an important role

Table 3. LC_{50}-values (μM) for cadmium for different soil invertebrates including the highest compared to the lowest value in this study.

Organism	Exposure time (h)	pH	LC_{50} (μM)	Author
T. elegans/A. avenae	96	6.0	>800	This study
Diplogasteritus sp.	72	6.0	29	This study
Panagrellus silusiae	24	?	480	Haight et al. (1982)
Monhystera disjuncta	24	7.5	302	Vranken et al. (1991)
Parastenocaris germanica	96	6.8	20	Notenboom et al. (1992)
Lumbricus rubellus	96	7.2	3	Ma (1982)
Tubifex tubifex	48	?	0.3	Brkovic-Popovic (1977)
Hydra oligactis	48	7.0	14	Slooff et al. (1983)

Table 4. LC_{50}-values (μM) for pentachlorophenol for different soil invertebrates including the highest compared to the lowest value in this study.

Organism	Exposure time (h)	pH	LC_{50} (μM)	Author
Caenorhabditis elegans *Acrobeloides buetschlii* *Aphelenchus avenae*	96	6.0	>34.5	This study
Prionchulus punctatus	72	6.0	0.5	This study
Monhystera disjuncta	24	7.5	18	Vranken et al. (1991)
Parastenocaris germanica	96	6.8	0.14	Notenboom et al. (1992)
Tubificids	24	7.5	1.2	Whitley (1968)
Eisenia fetida	14 days	5.6	5.67[a]	Van Gestel and Ma (1990)
Lumbricus rubellus	14 days	5.6	28.6[a]	Van Gestel and Ma (1990)

[a] values calculated from total soil concentrations using adsorption data.

(Castro and Thomason 1971). Low molecular weight compounds, like ethylene-dibromide and water, reside less than one second in *A. avenae* and *C. elegans*. The permeation of these compounds through the cuticle and hypodermata is a dynamic process which is actively controlled. Each substance enters the body at its own rate independent of the other chemical compounds and permeation rates differ strongly among different nematode species (Castro and Thomason 1971). In addition, it was observed that the internal concentrations were generally higher than the external concentrations (Marks et al. 1968).

Large differences in heavy metal uptake can be observed in closely related species. Accumulation varies between species and they exhibit distinct uptake and elimination rates. Howell (1983) showed that two taxonomic closely related species, *Enoplus brevis* and *Enoplus communis*, exhibited distinct differences in heavy metal accumulation through the cuticle. A marked uptake of copper by *E. communis* was found (accumulation factor of 10.6), whereas in *E. brevis* the copper uptake was relatively smaller (accumulation factor of 5.0).

The observation that the subphylum Secernentia was less susceptible to pentachlorophenol than the subphylum Penetrantia might also be the result of differences of cuticle characteristics as such. Subphyla of nematodes can be distinguished on basis of their ectodermal characteristics (Maggenti 1981). The endo-cuticle, for instance, differs largely in thickness and collagen density between the subphyla. Although there appears to be a structural pattern in cuticle and hypodermal morphology within the phylum Nematoda, further investigation is needed to ascertain these assumptions.

This chapter illustrates that a large diversity exists within the phylum Nematode with regard to sensitivity to toxicants. The response to xenobiotics seems difficult to predict and depends on the species tested and the nature of the chemical compound. This should be taken into account when assessing the hazard of chemical substances, based on toxicity tests with only few organisms (e.g. Van Straalen and Denneman 1989). Also environmental quality criteria should be based on the diversity of the physiological spectrum which exists in living organisms.

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CHAPTER 4

TAILORING THE CONCEPT TO FREE-LIVING NEMATODES *

ABSTRACT - *In ecotoxicology it is widely assumed that toxicants affect organisms by impairment of those life-cycle variables that are most sensitive to these toxicants. This premise was tested by contrasting a fitness assessment, presented in Chapter 2, with the most sensitive life-cycle variable approach using cadmium and the free-living nematode Plectus acuminatus as a case study. Based on complete life-cycle experiments, a deterministic model was constructed relating phenotypic plasticity of juvenile and adult variables to fitness, which was defined as the intrinsic rate of population increase. A sensitivity analysis of the model indicated that the impact of cadmium on fitness was not correlated with the most sensitive life-cycle component. These findings suggest that cadmium induced life-cycle changes of P. acuminatus require a robust evaluation within the framework of the life-history strategy in order to predict effects on fitness.*

* *Based on: Kammenga J.E., Busschers M., Van Straalen N.M., Jepson P.C. and Bakker J. Stress induced fitness reduction is not determined by the most sensitive life-cycle trait. Funct. Ecol. (in press).*

Chapter 4

4.1 INTRODUCTION

Declining biodiversity and sustainability of ecosystems due to environmental contamination have led to an increased scientific as well as public and political scrutiny of anthropogenic pollution. A prevailing view is that toxicants affect organisms by impairment of those life-cycle variables that are most sensitive to these toxicants (Langston et al. 1990; Calow 1992; DeLonay et al. 1993; Coyle et al. 1993), a concept also adopted by international legislative authorities for deriving safe standards for contaminants in soil and water (OECD 1984; EEC 1985). For example, to assess the impact of contaminants on organisms, a general toxicological approach is to quantify juvenile survival, since this is often known to be the most sensitive life-cycle variable with respect to chemical stress (Nagel et al. 1991; DeLonay et al. 1993). The theoretical problem with this approach is that a chemical could dramatically reduce juvenile survival, yet has negligible effect on fitness, or it could cause minor reductions in juvenile survival yet drastically reduce fitness. To assess whether these theoretical concerns amount to much in the real world we contrasted the fitness assessment with the most sensitive life-cycle variable approach using cadmium and nematodes in a comprehensive life-cycle analysis.

The present chapter investigates the effect of cadmium stress on fitness of *Plectus acuminatus* (Nematoda, Plectidae) Bastian 1865 a bacterivorous nematode which is ubiquitous in terrestrial habitats. *P. acuminatus* is parthenogenetic, has a relatively short life cycle and can be reared easily in the laboratory. It represents the type of organism used in international regulatory testing for the determination of chemical hazard and critical loads for toxicants (Van Gestel and Van Straalen 1994).

The fitness concept has received much attention in life-history theory (Fisher 1958; Charlesworth 1980; Sibly and Calow 1983; Murray 1985; Nur 1987) and it is widely acknowledged that fitness can be defined by the intrinsic rate of population increase (r) in the Lotka equation.

4.2 LIFE-CYCLE ANALYSIS

To obtain insight into the relationship between the phenotypic plasticity to cadmium of single life-cycle variables and changes in fitness for *P. acuminatus* we defined three different stages: i) a juvenile stage (t_j) which includes the egg stage, ii) a reproductive stage (t_r) and iii) a non-reproductive, senescent stage which continues to death. The Lotka equation can then be rewritten as:

$$1 = \sum_{t=0}^{t=t_j} e^{-r.t}.L_t.n_t + \sum_{t=t_j+1}^{t=t_j+t_r-1} e^{-r.t}.L_t.n_t + \sum_{t=t_j+t_r}^{t=t_m} e^{-r.t}.L_t.n_t$$

Since $n_t = 0$ for $0<t<t_j$ and for $t>(t_j+t_r)$, only the second term is important, it follows that:

$$1 = \sum_{t=t_j+1}^{t=t_j+t_r-1} e^{-r.t}.L_t.n_t \qquad (1)$$

Adult mortality is time dependent for many nematode species (Vranken 1987; Schiemer 1982) and the survival curve can be described by a Weibull distribution function:

$$L_t = a.e^{-b(t)^c} \qquad (2)$$

where a, b and c are constants.
In addition it is assumed that:

$L(t_j) = s_j$ and

$$L(t_j+t_r) = s_j.s_r$$

so $L_t = s_j.e^{-b(t-t_j)^c}$ and

$$s_j.s_r = s_j.e^{-b.(t_j+t_r-t_j)^c} \rightarrow s_r = e^{-b.(t_r)^c} \rightarrow b = -(\ln s_r)/(t_r)^c \quad (3)$$

substituting (3) in (2) gives

$L_t = s_j.e^{(\ln s_r/t_r^c).(t-t_r)^c}$ which can be rewritten as:

$$L_t = s_j.s_r^{[(t-t_j)/t_r]^c} \quad (4)$$

Now the Lotka equation becomes:

$$1 = \sum_{t=t_j}^{t=t_j+t_r} e^{-r.t}.s_j.s_r^{[(t-t_j)/t_r]^c}.n_t \quad (5)$$

Fitness (r) can be estimated by means of iterative procedures from equation 5 when s_j, t_j, t_r, n_t are obtained from detailed observations of individual organisms over their life cycles. The parameters s_r and c can be derived from the Weibull distribution function. To determine the relationship between plasticity in each variable to r, a sensitivity analysis of equation 5 was performed.

4.3 Materials and methods

CHEMICALS USED

Cadmium chloride ($CdCl_2$, > 99% pure) was obtained from Merck, Darmstadt, Federal Republic of Germany. Yeast extract was purchased from Difco Laboratories, Detroit, Michigan, USA, and technical agar was supplied by Oxoid Ltd, Basingstoke, Hampshire, England. All other chemicals (Merck) were of the highest analytical grade. The water used for culturing and experiments contained a defined mixture of minerals resembling those found in interstitial water of sandy forest soils (K^+: 0.1 mM, Na^+: 0.2 mM, Ca^{2+}: 0.35 mM, Mg^{2+}: 0.3 mM, NH_4^+: 0.3 mM, NO_3^-: 1.7 mM, Cl^-: 0.3 mM) (Schouten and Van der Brugge 1989). The pH was adjusted to 6.0 ± 0.1 with NaOH.

NEMATODE CULTURING

P. acuminatus was reared in agar with bacteria as food source. The soil bacterium, *Acinetobacter johnsonion*, was obtained from the Department of Microbiology of Wageningen Agricultural University and stock cultures were stored in a cryo-protectant in glass beads at - 80°C (Jones et al. 1984). Bacteria were cultured in an autoclaved batch of aerated yeast extract (4.0 g/L, 28°C, 16 hrs.) after which they were centrifuged (13,170 g, 2 min.) and washed with water to obtain a fresh supply for culturing and experiments. Bacterial densities were measured with a spectrophotometer (Shimadzu, UV-160), at a wavelenght of 560 nm.

Nematodes were cultured in agar in multi compartment plates (Greiner, nr. 662160) with 24 wells. Small agar droplets (80 µL, 0.5%) were applied in each well on the inside of the lid. Bacteria were mixed through the agar in a water bath of 37°C to obtain a density of 2.10^9 cells/mL. Each compartment of the bottom plate contained 1 mL water to prevent evaporation.

Nematodes were initially extracted from arable land in Wageningen, The Netherlands by means of the modified Oostenbrink method (Oostenbrink 1954;1960). The worms were identified to genus level and *Plectus spp.* females were transferred to sterile water for two days before culturing to reduce bacteria and debris. One adult female was transferred to each agar droplet and allowed to reproduce at 20°C for 2 days after which they were removed and identified to species level. After identification all *P. acuminatus* juveniles

were mixed randomly through the plates. The plates were sealed with Parafilm and placed on metal grids in plastic boxes with a small amount of water to prevent evaporation. The boxes were stored at 15°C in the dark. Nematodes were transferred to fresh plates every week, although populations could be maintained for 3 weeks without increased mortality.

TOXICITY EXPERIMENTS

One clone of *P. acuminatus* was reared in agar with the soil bacterium *Acinetobacter johnsonion* as food source. Life-cycle experiments were conducted in 4 plates (Greiner, nr. 662160) each containing 24 agar droplets (80 μL, 0.5% agar) with a bacterial density of 2.10^9 cells/mL. Bacterial densities were measured with a spectrophotometer (Shimadzu, UV-160), at a wavelenght of 560 nm. Each compartment of the bottom plate contained 1 ml water to prevent evaporation. Two plates contained 7.6 μM in agar. Fourth stage juveniles were randomly selected from the stock culture of the clone which had been kept in the laboratory for more than one year, and individually transferred to the agar plates. Each droplet contained one female which was transferred to fresh plates every 5 days. The plates were sealed with Parafilm and stored at 15°C in the dark. The number of eggs produced was recorded for each female during the complete reproductive period. Also longevity of adults was registered. The eggs as well as the juveniles were observed for mortality during the complete juvenile period. Observations were done with a stereo-microscope (magnification 40 - 64).

STATISTICAL ANALYSIS

The obtained values for the life-cycle variables were used to estimate fitness values for the different treatments by means of equation 5. A specific algorithm was constructed in SAS-software (SAS 1990) using non-linear regression procedures. Application of the jack-knife procedure (Meyer et al. 1986) for estimates of fitness resulted in pseudo-values. Longevity data were used to construct a Weibull distribution function where survival L_t is given by: $L_t = e^{(-\ln 2.(t/LT50)^C)}$ where t is age in days, LT_{50} is the median survival time in days

and c determines the shape of the curve. Data were analysed using SAS software. The least square method was used in an iterative non-linear regression procedure to estimate LT_{50} and c. A likelihood ratio test was applied to analyse differences in survival curves.

4.4 RESULTS

The influence of cadmium on fitness was studied by analysing the effects on single life-cycle variables (Table 1). Reproduction, the reproductive period and the survival over this

Table 1. Effect of cadmium on different life-cycle variables and parameters (means ± S.E., number of replicates in brackets) of *P. acuminatus* at 15°C. (n_t: daily reproduction; t_r: reproductive period in days; S_r: survival over t_r; t_j: juvenile period in days, including the egg stage; S_j: survival over t_j; LT_{50}: median survival time in days; c: shape parameter of the Weibull survival curve; r: jack-knife estimate for fitness; * = significantly different from control, *t-test* or likelihood ratio test in the case of LT_{50}-values, $p<0.05$; ** = $p<0.01$).

life-cycle variable	control	cadmium
n_t	5.5 ± 0.4 (32)	4.3 ± 0.3 * (31)
t_r	16.2 ± 1.1 (32)	8.9 ± 0.7 * (31)
S_r	0.96	0.87
t_j	30.6 ± 0.3 (19)	32.9 ± 0.4 ** (16)
S_j	0.63 ± 0.07 (38)	0.57 ± 0.07 (39)
LT_{50}	33.4 ± 0.2	24.6 ± 0.6 *
c	3.7 ± 0.1	1.8 ± 0.1
r	0.10 ± 0.01 (32)	0.06 ± 0.02 * (31)

period decreased at this level of contamination. The juvenile period increased as a result of cadmium exposure. The LT_{50} was significantly reduced and also the slope of the mortality curve (c) showed a decrease in cadmium exposed populations (Fig. 1). The survival over t_r, as estimated from the Weibull curve decreased (Table 1). However, the

duration of the egg stage (6.5 days) and the juvenile survival (s_j) were not affected. It was observed that juvenile mortality mainly occurred during the egg stage, afterwards no significant mortality was recorded. Overall, fitness was significantly impaired.

A sensitivity analysis of equation 5 was conducted by using life-cycle values of the control group. The relationship between plasticity of s_j, t_j, t_r, n_t, s_r and fitness decrease was determined using SAS software. The analysis showed that the most sensitive trait to cadmium, the reproductive period (t_r) which was reduced by 45%, did not have any effect on fitness (Fig. 2a). Hence, an effect assessment for cadmium based on nematode reproductive period overestimates the effect on fitness dramatically. Also the 9.4% effect on the survival over the reproductive period (s_r) did not influence fitness (Fig. 2b). A prolongation by cadmium of the juvenile period (t_j) by 7.5%, the least sensitive trait, resulted in a fitness decrease of 5% (Fig. 2c). Furthermore, inhibition of reproduction (n_t) by 22% decreased fitness with 5% (Fig. 2d).

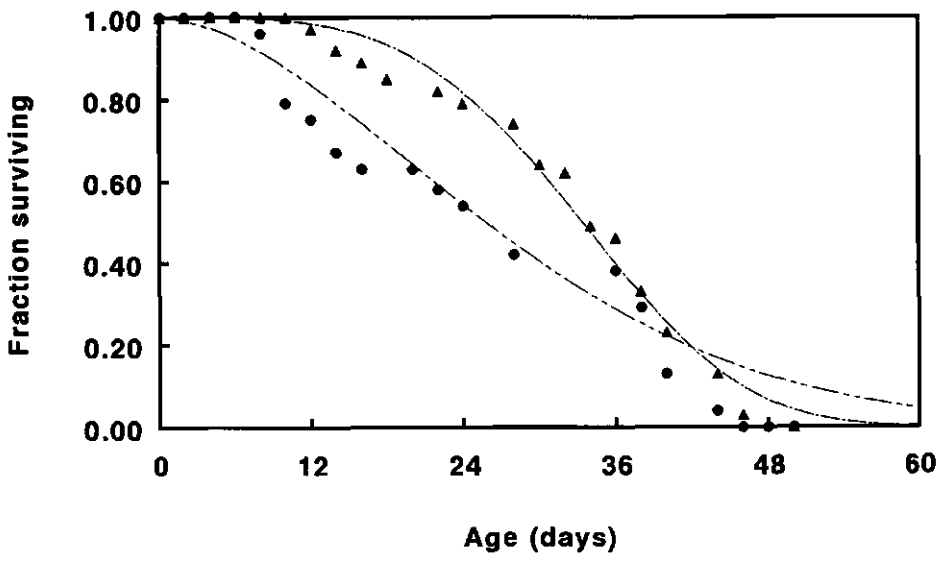

Figure 1. Effect of cadmium on survival of adults. (▲ : data from control group, —·— Weibull curve of control group; ● : data from cadmium treated group, —··— Weibull curve of cadmium treated group).

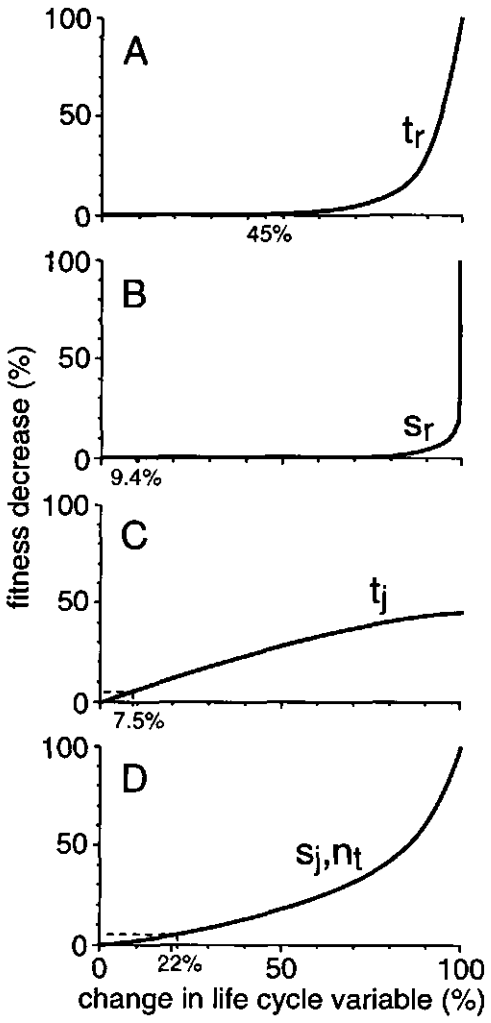

Figure 2. Effect of cadmium on different life-cycle variables and the sensitivity analysis of equation 5 on fitness by A): decreasing reproductive period (t_r), B): decreasing survival (S_r) over t_r, C): increasing juvenile period (t_j) and D): decreasing reproduction (n_t) and juvenile survival (S_j). The effect of cadmium (|) on : the reproductive period was 45%, reproduction was 22%, survival over the reproductive period 9.4% and on t_j 7.5%.

4.5 Discussion

It was demonstrated that impairment of the most sensitive trait to cadmium in *P. acuminatus*, the reproductive period which was reduced with 45%, did not have any effect on fitness. A prolongation by cadmium however of the juvenile period by 7.5%, the least sensitive trait, resulted in a fitness decrease of 5%. Overall, fitness was reduced by cadmium with 40% indicating the non-linear relationships among different life-cycle traits which do not allow for straightforward adding of the reduction in fitness calculated for single trait values.

The life-cycle analysis did not support the key premise that was under test, *i.e.* that effects on the most toxicant-sensitive life-cycle variables should have the greatest implications for fitness. Importantly, fitness was more impaired by small changes in less sensitive traits. From these results it can be concluded that the impact on single traits needs to be evaluated from a life-cycle perspective to ensure a proper assessment of the potential ecological risk of toxicants. Moreover, ecotoxicologists need to rethink current risk assessment procedures based on single life-cycle traits by implementing life-history theory in a quantitative way.

A limited number of papers have evaluated the effect of chemicals based on life-history theory by using the Lotka equation. Daniels and Allan (1981) were among the first who used the rate of population increase (r) as a measure of toxicity, estimated from the age-specific survival and fecundity schedules for a cladoceran and a copepod. They speculated that population growth is an ecologically realistic parameter for sublethal stress in these invertebrates. Also Allan and Daniels (1982) assessed the chronic toxicity to a copepod and estimated r from complete life-cycle experiments and discussed the relative contribution of different life-cycle variables to r. Meyer et al. (1987) focused on the sensitivity of r in daphnids compared to life-table data and argued that, although r was not the most susceptible, it could be used for comparison with the effect on survival and reproduction. Using soil inverebrates, Van Straalen et al. (1989) reported that as a result of differing physiological responses, differences in population growth rates were observed. In addition Crommentuijn (1994) stressed that the relationship between life-

cycle variables and population growth are of paramount importance for evaluating the impact on single traits.

It was noted by several authors that delayed reproduction caused by toxicants is a significant factor in the estimation of r, but is often underestimated (Van Leeuwen et al. 1985; Meyer et al. 1987; Bengtsson et al. 1985). Also Baird et al. (1990) mentioned the importance of development time on the fitness of a population. However, most of these studies did not gain insight into the actual causes of the fitness decrease and the observed changes were discussed in a qualitative way. This chapter presents a mathematical framework which can be used for the quantitative evaluation of toxicant stress on fitness by describing the relationship between fitness and plastic responses in life-cycle traits. Application of the approach to other species may provide a versatile tool for the effect assessment of toxicants.

The relationship between different traits and population growth rate or extinction rates has quantitatively been analysed by ecologists applying different mathematical techniques. Caswell (1978) presented a formulation which was applicable to any linear population growth model. The model showed that the population growth rate was more sensitive to changes in somatic growth and survival than to changes in total fecundity. Lande (1988) used classic demographic methods and reported that the adult annual survivorship had a strong influence on population growth in the Northern spotted owl compared to annual fecundity. Using stochastic models it was estimated that population viability in the badger was very much dependent on changes in adult mortality (Lankester et al., 1991). Although these papers provide valuable insight into life-cycle changes and population reduction, they do not however contribute towards a better understanding of the underlying mechanisms of toxicant-induced fitness impairment.

By analysing juvenile and adult variables in a deterministic model it was demonstrated that for *P. acuminatus* the length of the juvenile period had the strongest influence on fitness when impairment was below 80%, and that reproduction, *e.g.* the number of eggs per female, was less important. Hence, the mathematical approach presented in this chapter allows for a more refined insight in the contribution of each variable to fitness and shows that critical threshold concentrations for the most sensitive variables may not

necessarily be correlated with the vulnerability of a species to toxicants. Since organisms have developed a variety of life-history strategies, detailed analysis of life-cycle variables in relation to toxicant induced fitness reduction is essential to gain insight into the population dynamics of stressed organisms and may provide a more rational basis for assessing the ecological impact of toxicants and, hence the conservation of biodiversity in contaminated environments. If potential hazards and safe environmental concentrations are to be estimated from selected life-cycle traits, it is advocated that the biological impact must be investigated from a life-history perspective. Using inappropriate criteria for selecting test variables introduces the potential for incorrect estimations of the biological hazards that toxicants cause. This is especially important with an international regulatory system that relies heavily on the use of single species laboratory test methods.

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CHAPTER 5

EVALUATION OF CRITICAL EFFECT LEVELS *

ABSTRACT - *Using the nematode Plectus acuminatus and the toxicants cadmium and pentachlorophenol as a case study, life-cycle experiments were conducted to evaluate critical effect levels for various traits by means of a plasticity-to-fitness analysis. For cadmium the most sensitive trait was reproduction (EC_{20}=2.0 μM) followed by the juvenile period (EC_{20}=8.9 μM). Using the demographic model presented in Chapter 4, plastic responses in life-cycle traits were related to fitness. Plasticity-to-fitness analysis indicated that a 20% decrease in daily reproduction resulted in a fitness impairment of less than 5% whereas a 20% increase in the juvenile period resulted in a fitness decrease of nearly 15%. Fitness however remained constant over all concentrations (0.14 d^{-1}), indicating that impairment of reproduction was compensated for by various changes in other traits. Hence, plasticity allows P. acuminatus to maintain fitness in cadmium contaminated environments despite the observed effects in life-cycle traits. For pentachlorophenol the juvenile survival was less sensitive compared to the reproductive period (EC_{20}=4.3 μM and 1.3 μM respectively). Analysis of plasticity revealed that 20% decrease in the reproductive period did not influence fitness whereas, a 20% reduction in juvenile survival resulted in a 5% fitness decrease. Fitness was reduced from 0.12 d^{-1} in the control to 0.02 d^{-1} at the highest concentration. In contrast to cadmium exposure, P. acuminatus was not able to maintain fitness by means of phenotypic plasticity. This may be caused by the specific mode of action of pentachlorophenol i.e. uncoupling of the oxidative phosphorylation. These results imply that i) critical effect levels for sensitive life-cycle traits are not sufficient for assessing the potential impact of toxicants on fitness and ii) insight into the relationship between plasticity of life-cycle traits and fitness provides a proper basis for the ecological risk assessment of toxicants on populations.*

* *Based on: Kammenga J.E., Van Koert P.H.G., Koeman J.H. and Bakker J. Fitness consequences of toxic stress evaluated within the context of phenotypic plasticity. Ecol. Appl. (submitted).*

5.1 Introduction

Phenotypic plasticity represents the range of phenotypes that can be developed by an organism in heterogeneous or changing environments (Via et al. 1995). It may provide a mechanism for adaptation to alterations in ambient conditions such as temperature (Williams and Black 1993), water stress (Newman 1988) or the presence of a predator (Stibor 1992). Toxicant-induced changes in life-cycle traits can be expressed by the concentration-response relationship which represents the plasticity to toxic stress. Analysis of life-cycle plasticity provides insight into the vulnerability of organisms to chemicals and may contribute to evaluating critical effect levels with a view to risk assessment (Chapter 2).

At present, many theoretical and practical explorations have been made to provide adequate tools for the ecological risk assessment of toxicants (Wagner and Løkke 1991, Van Straalen and Denneman 1989, Stephan et al. 1985, Suter et al. 1985, Blanck 1984, Slooff et al. 1986) in soil and water. In general, these methodologies are based on the results of toxicological experiments that provide estimates for critical concentrations such as the NOEC, LOEC, EC_{50} or EC_{20}. Over the last decades, effect levels for toxicants have been obtained from concentration-response relationships using sensitive life-cycle variables such as juvenile mortality, growth, reproduction or breeding success for a wide range of species (Jørgensen et al. 1991). This traditional concept, which assumes that sensitive variables are ecologically relevant, is however not supported by life-history theory (Chapter 4).

Life-history theory emphasizes that the impact of stress on organisms is determined by the effect on fitness (Sibly and Calow 1989). More specifically, the concept of maximizing fitness is the key to gain insight into the demographic consequences of toxic insults on the individual level (Sibly and Calow 1989). This fundamental principle of population dynamics is of paramount importance for the evaluation of critical effect levels on the dynamics of populations and provides guidance to the understanding of the effect that toxicants may have on the viability of species in contaminated environments.

This chapter presents a unification of life-history theory and toxicology by describing a

model which relates toxicant-induced alterations in life-cycle components to changes in fitness. The life cycle of the bacterivorous soil inhabiting nematode *P. acuminatus* was used as a case study. Cadmium and pentachlorophenol were used as test compounds since their toxicological properties are well known (Anonymous 1987, Ros and Slooff 1988). The model consists of two parts and is based on the classical Lotka equation. The first part describes the relationship between plasticity of life-cycle variables and fitness by dividing the life cycle into a juvenile and adult stage (see Chapter 4). The second part of the model is the estimation of effect levels from the concentration-response relationship for life-cycle variables to cadmium and pentachlorophenol. As an example this chapter focuses on the EC_{20}, but the concept holds for other effect levels *e.g.* LOEC or EC_{50} as well. Concentration-response relationships were constructed for various traits and survival curves for different chemicals to estimate EC_{20}-values. The obtained effect concentrations were related to changes in fitness using the first part of the model.

5.2 Materials and methods

CHEMICALS USED

Cadmiumchloride ($CdCl_2$) was obtained from Merck, Darmstadt, Federal Republic of Germany and pentachlorophenol was purchased from Fluka Chemie A.G., Switzerland. The compounds were >99% pure. All other chemicals used (Merck) were of the highest analytical grade available. Pentachlorophenol was initially dissolved in ethanol after which stock solutions were made in water. The stock solutions were sonificated for 60 min. The water used for culturing and experiments contained a defined mixture of minerals resembling those found in interstitial water of sandy forest soils (K^+: 0.1 mM, Na^+: 0.2 mM, Ca^{2+}: 0.35 mM, Mg^{2+}: 0.3 mM, NH_4^+: 0.3 mM, NO_3^-: 1.7 mM, Cl^-: 0.3 mM) (Schouten and Van der Brugge 1989). The pH was adjusted to 6.0 $\pm$ 0.1 with NaOH.

NEMATODE CULTURING

Nematodes were reared in agar on the soil bacterium *Acinetobacter johnsonion* which was obtained from the department of Microbiology at Wageningen Agricultural University in The Netherlands. Bacterial stock cultures were stored at -80°C in a cryo-protectant on glass beads (Jones et al. 1984). Bacteria were cultured in an autoclaved batch of aerated yeast extract (4.0 g/L, 28°C, 16 h) after which they were centrifuged (13,170 g, 2 min) and washed with water to obtain a fresh supply for culturing and experiments. Bacterial densities were measured with a spectrophotometer (Shimadzu, UV-160, 560 nm).

LIFE-CYCLE EXPERIMENTS

Life-cycle studies were conducted in agar in multi compartment plates (Greiner, nr. 662160). Twenty-four agar droplets (70 μl, 0.5%) were applied on the inside of the lid. Bacteria were mixed through the agar at 37°C to obtain a density of 2.10^8 cells/ml. Each compartment of the bottom plate contained 1 ml water and plates were sealed with Parafilm to prevent evaporation.

A concentration range of 0, 0.5, 0.9, 1.6, 2.8 and 5.0 μM cadmium and a range of 0, 2.6, 4.6, 8.3, 14.9 and 26.8 μM pentachlorophenol in agar was used. Both compounds were added to fluid agar at a temperature of 37°C. The ranges were based on LC_{50}-values in water for 72 h (Chapter 3). Two compartment plates were used for each treatment.

Nematodes were used from a clone which has been maintained in the laboratory for more than two years. Gravid females were individually transferred to the agar droplets in the multi-compartment plates. After 24 h the adults were removed and the eggs were each placed in fresh agar droplets. The hatching period of the eggs was recorded as well as the length of the juvenile period. The duration of the reproductive period was studied and the daily reproduction was recorded. Eggs were counted each day and the females were placed on fresh plates every 5 days. The observations covered the total life time of the nematode and included adult and juvenile mortality. Juvenile mortality was recorded by studying the eggs which were produced during the life cycle. Observations were done

with a stereo-microscope (magnification 40 - 64). Experiments were conducted at 20°C in the dark.

CHEMICAL ANALYSIS

To gain insight into adsorption of cadmium and adsorption or breakdown of pentachlorophenol by agar and bacteria, chemical analysis was performed on the supernatant of centrifuged agar with and without bacteria added. Sealed plastic centrifuge tubes with 10 ml agar suspension (0.5% agar) were made with and without added bacteria (2.10^8 cells/mL) and stored at 20°C. Nominal concentrations of 5.0 and 8.3 μM were used for cadmium and pentachlorophenol respectively. After 0 and 120 h, the agar was centrifuged (13,170 g, 20 min.) and 1.5 mL of supernatant was sampled for analysis. The cadmium samples were acidified with 1 N HNO_3. Cadmium concentrations were measured with Atomic Absorption Spectrophotometry (Perkin Elmer 3030 AAS, furnace, detection-limit: 0.1 μg/L). Pentachlorophenol concentrations were determined with HPLC (Spectro Flow 757, Lichrosorb RP18 column (7 μm x 20 cm), UV-detector, 254 nm, detection limit: 50 μg/L, flow rate 0.7 mL/min). Experiments were conducted with two replicates in the dark.

STATISTICAL ANALYSIS

Results were analysed with the statistical package of SAS (Anonymous 1990). Differences between control and treatments of t_j, s_j, n_t, t_r were tested using Student's t-test. Longevity data were used to construct a Weibull distribution function. The least square method was used to estimate LT_{50}, c and s_r (Chapter 4). A likelihood ratio test was applied to analyse differences in survival curves between treatments.

The obtained values for the life-cycle variables were used to estimate fitness values for the different treatments by means of equation 5 (Chapter 4). A specific algorithm was constructed in SAS-software using non-linear regression procedures (PROC NLIN). Application of the jack-knife procedure (Meyer et al. 1986) for estimates of fitness

resulted in pseudo-values which were analysed by analysis of variance. EC_{20}-values were estimated using non-linear regression procedures in SAS (Bruce and Versteeg 1992). Results of chemical analyses were compared with Student's t-test for comparison between means.

5.3 RESULTS

LIEFE-CYCLE EXPERIMENTS

The results are presented for nominal exposure concentrations of cadmium and pentachlorophenol.

Cadmium - Table 1 shows the effect of cadmium on different life-cycle variables. A concentration-response relationship was found for the daily reproduction showing a decrease from 6.8 eggs/day in the control group to 5.4 eggs/day in the group with the highest concentration; EC_{20} was estimated as 2.0 $\pm$ 1.3 μM.

A less pronounced relationship was observed for the length of the juvenile period. Cadmium increased t_j from 24.4 days in the control group to 25.3 days at the highest concentration. At 0.5 μM however t_j was significantly reduced from 24.4 to 23.6 days, which may indicate a stimulation of maturation at low cadmium concentrations. An EC_{20} of 8.9 $\pm$ 2.1 μM was estimated, although a less accurate estimation could be made than for the daily reproduction. The duration of the egg stage, which is included in t_j, was 4.7 days and was not changed by cadmium.

The juvenile survival was only significantly affected by cadmium at 1.6 μM but remained constant at nearly 0.8 at other concentrations (Table 1). It was noted that mortality mainly occurred during the egg stage, whereas during the rest of the juvenile period, mortality was negligible.

Figure 1a shows the adult survival curves at different cadmium concentrations. There was a good fit of the Weibull curve ($p < 0.001$) but there appeared to be no concentration-response relationship, consequently the median survival time, LT_{50}, was not related with

Table 1. Effect of cadmium on different life-cycle variables of P. acuminatus in agar. Means ± SE, number of replicates in brackets. t_j = juvenile period in days, S_j = juvenile survival over t_j, n_t = daily reproduction, t_r = reproductive period in days, S_r = survival over t_r (* = significantly different from control at $p<0.05$, t-test).

µM	t_j (days)	s_j	n_t (day^{-1})	t_r (days)	s_r
0.0	24.4 ± 0.1 (44)	0.84 ± 0.02 (300)	6.8 ± 0.3 (43)	61.7 ± 0.8 (43)	0.61
0.5	23.6 ± 0.1 (42) *	0.83 ± 0.02 (252)	6.6 ± 0.3 (27)	83.2 ± 7.3 (27)	0.78
0.9	24.1 ± 0.3 (38)	0.84 ± 0.02 (179)	5.8 ± 0.4 (25)	74.5 ± 9.4 (25)	0.51
1.6	24.5 ± 0.5 (20)	0.78 ± 0.02 (149) *	5.6 ± 0.7 (23) *	66.2 ± 7.7 (23)	0.72
2.8	25.1 ± 0.4 (35)	0.82 ± 0.02 (219)	4.9 ± 0.3 (30) *	79.4 ± 7.3 (30)	0.67
5.0	25.3 ± 0.3 (31) *	0.82 ± 0.02 (145)	5.4 ± 0.5 (20) *	71.6 ± 9.5 (20)	0.61

Table 2. Influence of cadmium and pentachlorophenol (PCP) on median survival time (LT_{50} ± SD) and the shape of the Weibull curve (c ± SD) obtained from survival curves of P.acuminatus in agar (*: $\alpha = 0.05$, **: $\alpha = 0.01$, ***: $\alpha = 0.001$, likelihood ratio test).

cadmium (µM)	LT_{50} (days)	c
0.0	71.1 ± 0.9	2.6 ± 0.1
0.5	108.2 ± 3.4 **	4.0 ± 0.8
0.9	77.1 ± 5.5 *	1.1 ± 0.2
1.6	84.4 ± 1.7	3.1 ± 0.3
2.8	97.7 ± 3.5 *	2.6 ± 0.4
5.0	84.9 ± 3.3	1.9 ± 0.3
PCP (µM)		
0.0	89.7 ± 0.8	4.1 ± 0.2
2.6	67.7 ± 1.0 **	2.8 ± 0.2
4.6	54.1 ± 0.6 ***	3.2 ± 0.2
8.3	52.9 ± 0.8 ***	3.2 ± 0.2
14.9	-	-
26.8	-	-

Table 3. Effect of cadmium and pentachlorophenol (PCP) on estimated fitness pseudo-values (r ± SE) of P. acuminatus in agar (* = significantly different from control at $p<0.05$, t-test).

cadmium (µM)	r (d^{-1})
0.0	0.13 ± 0.01
0.5	0.14 ± 0.01
0.9	0.15 ± 0.03
1.6	0.13 ± 0.01
2.8	0.14 ± 0.02
5.0	0.14 ± 0.01
PCP (µM)	
0.0	0.12 ± 0.02
2.6	0.12 ± 0.01
4.6	0.11 ± 0.02
8.3	0.02 ± 0.03 *
14.9	-
26.8	-

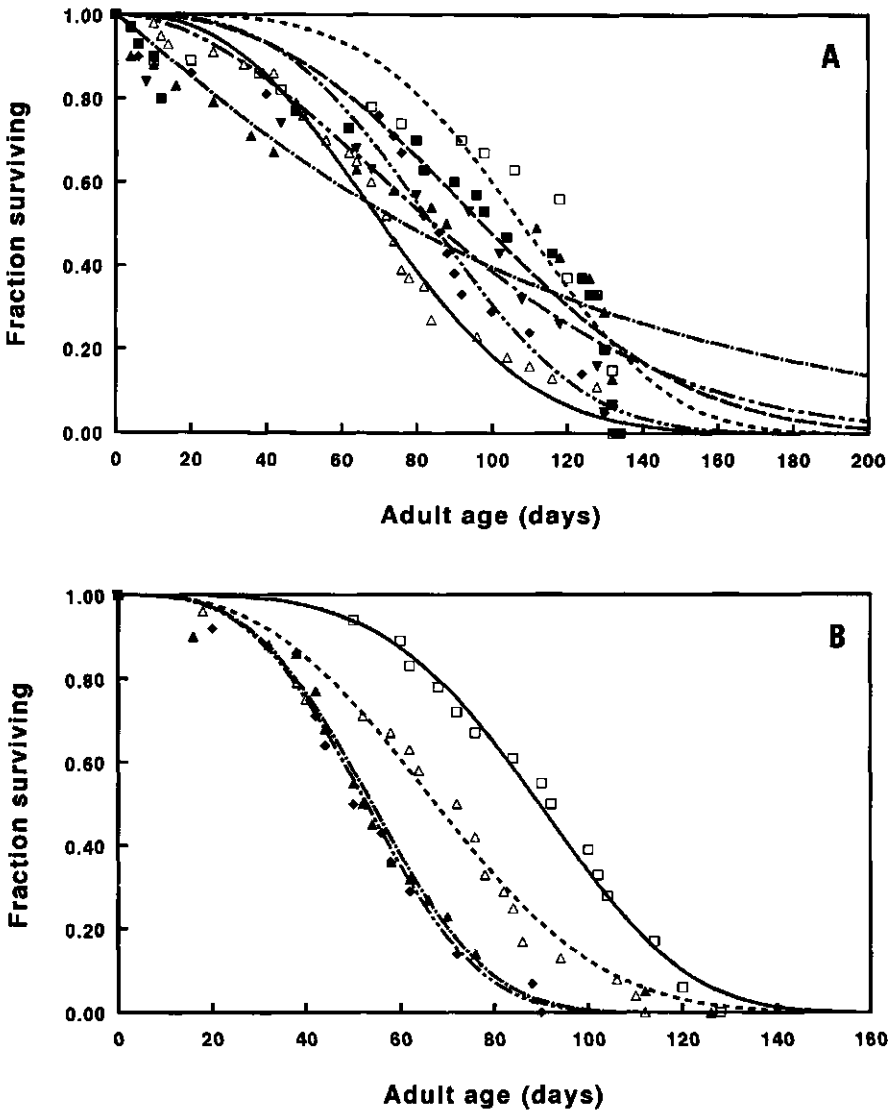

Figure 1. A) Effect of cadmium on survival of adults. △: data from control group, ——— Weibull curve of control group; □: 0.5 μM Cd, - - - - Weibull curve; ▲: 0.9 μM, — · — · — Weibull curve; ◆ 1.6 μM, — · · — Weibull curve; ■:2.8 μM, — — Weibull curve; ▼:5.0 μM, — · · · Weibull curve. B) Effect of PCP on survival of adults. □: data from control group, ——— Weibull curve of control group; △: 2.6 μM Cd, - - - - Weibull curve; ▲: 4.6 μM, — - — - — Weibull curve; ◆: 8.3 μM, — · · — Weibull curve.

the concentration. Also the slopes of the curves were not clearly influenced (Table 2). Comparison between different curves indicated a significant increase compared to the control group for 0.5, 0.9, and 2.8 μM. The survival over the reproductive period, s_r, was also not related to the concentration and ranged from 0.51 to 0.78 (Table 1).

The reproductive period showed large variations between different treatments, but was not dose related. A minimum was observed in the control group of 61.7 days whereas a maximum of 83.2 days was recorded at 0.5 μM.

Table 3 shows the jack-knife estimates for fitness at different cadmium concentrations. There appeared to be no concentration-response relationship for fitness, nearly all values were 0.14 d^{-1} or close to this value.

The results presented here for control situations differed from Chapter 4, where the ambient temperature was 15°C. Also food levels were very high in Chapter 4 indicating that these levels may not be favorable for *P. acuminatus* since total life-time and reproduction was much lower.

Pentachlorophenol - Table 4 shows the effect of pentachlorophenol on different life-cycle variables. The juvenile survival showed a steep concentration-response relationship with a survival of 0.64 for the control and 0.04 at 8.3 μM with EC_{20}=4.3 $\pm$ 0.03 μM. At higher concentrations the juvenile survival was 0.0.

It was observed that the length of the reproductive period was also related to the concentration (EC_{20}=1.3 $\pm$ 0.3 μM). In the control t_r was 69 days decreasing to 29.4 days at 8.3 μM.

The daily reproduction and the length of the juvenile period were not affected by pentachlorophenol. Nearly 5 eggs/day were produced at all concentrations. The juvenile period remained constant at an average of 22.5 days for all treatments.

Figure 1b shows the adult survival curves of the different groups (Weibull fit: $p<0.0$-01). A concentration-response relationship was found for LT_{50} ranging from 89.7 days in the control to 52.9 days at 8.3 μM. However s_r and the slope of the curve c were not affected (Table 2 and 4). Clearly, concentration-related mortality occurred only after the reproductive period. Table 3 shows the estimated pseudo-values for fitness at different pentachlorophenol

Table 4. Effect of pentachlorophenol on different life-cycle variables of P. acuminatus in agar. Means ± SE, number of replicates in brackets. t_j = juvenile period in days, S_j = juvenile survival over t_j, n_t = daily reproduction, t_r = reproductive period in days, S_r = survival over t_r (* = significantly different from control at $p<0.05$, t-test).

µM	t_j (days)	s_j	n_t (day^{-1})	t_r (days)	s_t
0.0	22.9 ± 1.0 (27)	0.67 ± 0.04 (93)	4.1 ± 0.4 (18)	69.0 ± 9.1 (18)	0.78
2.6	22.1 ± 0.9 (27)	0.58 ± 0.06 (49)	4.9 ± 0.5 (25)	43.4 ± 4.6 (25) *	0.82
4.6	22.6 ± 1.1 (23)	0.46 ± 0.06 (38) *	4.6 ± 0.4 (23)	39.9 ± 6.3 (23) *	0.75
8.3	22.2 ± 1.1 (17)	0.02 ± 0.02 (18) *	5.4 ± 0.5 (15)	29.4 ± 4.8 (15) *	0.89
14.9	-	0.0	-	-	-
26.8	-	0.0	-	-	-

Table 5. Nominal and actual concentrations of cadmium and pentachlorophenol (PCP) in the supernatant of centrifuged agar, with or without added bacteria, after 24 and 120 h incubation at 20°C (means ± SD, n=2) (* = significantly different from treatment with added bacteria at $p<0.05$, t-test).

nominal (µM)		intrinsic (t=24 h)		intrinsic (t=120 h)	
		+ bact.	- bact.	+ bact.	- bact.
Cadmium:	5.0	3.6 ± 0.03	4.5 ± 0.1 *	3.9 ± 0.5	4.3 ± 0.1
PCP:	8.3	6.5 ± 1.1	7.9 ± 0.6	4.1 ± 0.1	7.2 ± 0.3 *

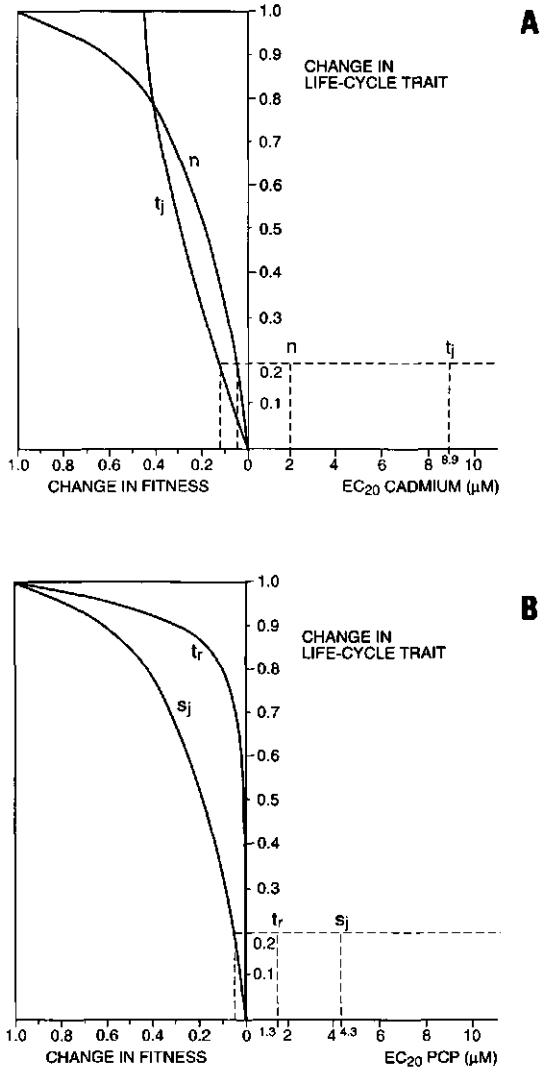

Figure 2. Relationship between critical effect levels (EC_{20}) for cadmium (A) and PCP (B) and changes in fitness. The left-hand side of the figure represents the sensitivity analysis of equation 5, Chapter 4, which relates plasticity in life-cycle traits to changes in fitness. The right-hand side of the figure shows the EC_{20} (μM) and the change in life-cycle traits. n = daily reproduction, t_j = length of the juvenile period (days), S_j = juvenile survival, t_r = length of the reproductive period (days).

concentrations. In the control group, fitness was 0.12 d^{-1} which decreased with increasing concentrations to 0.02 d^{-1} at 8.3 μM. At higher concentrations fitness could not be estimated because juvenile survival was zero.

RELATIONSHIP BETWEEN PLASTICITY OF LIFE-CYCLE TRAITS TO FITNESS

The values of the life-cycle variables from the two control groups were averaged and used to estimate fitness values. A sensitivity analysis was conducted for each life-cycle variable using these average data. Simulations were run using equation 5 in Chapter 4 to obtain a fitness value for each 5% step of reduction in s_j, t_r, n_t and s_r and each 5% step increase in t_j. Grahps were constructed consisting of three axes relating plasticity in life-cycle variables to i) a decrease in fitness and ii) EC_{20}-values of cadmium (Fig. 2a) and pentachlorophenol (Fig. 2b).

From a toxicological point of view the daily reproduction appeared to be nearly four times more sensitive (EC_{20}=2.0 μM) than the juvenile period (EC_{20}=8.9 μM). However, figure 2a illustrates that a 20% decrease of the daily reproduction, has less impact on fitness than a 20% effect on the juvenile period.

Figure 2b shows that although the reproductive period was more sensitive to pentachlorophenol than the juvenile survival, their impact on fitness differs strongly. A decrease of 20% in the reproductive period has no influence on fitness whereas a 20% decrease in the juvenile survival almost decreases fitness with 5%. The toxicant induced changes in fitness may seem negligible, however fitness represents the rate of population increase. Hence small changes may ultimately lead to large differences in population size in the long term.

CHEMICAL ANALYSIS

Table 5 shows the nominal and actual concentrations of cadmium and pentachlorophenol in the supernatant of centrifuged agar after 24 and 120 h. It appeared that actual concentrations of pentachlorophenol were 20% lower than nominal. Furthermore, adding

of bacteria significantly decreased pentachlorophenol concentrations after 120 h. Also actual concentrations of cadmium were lower than nominal. After 24 h, bacteria significantly decreased the concentration in the supernatant, whereas after 120 h this difference was not significant anymore.

5.4 Discussion

This chapter demonstrates the need to evaluate critical effect levels of toxicants for life-cycle traits within the context of phenotypic plasticity in order to predict the impact on fitness. Daily reproduction was the most sensitive trait to cadmium (EC_{20}=2.0 μM) followed by the length of the juvenile period (EC_{20}=8.9 μM). However, the analysis of plasticity in life-cycle traits indicated that a 20% decrease in daily reproduction resulted in a fitness impairment of less than 5% whereas a 20% increase in the juvenile period resulted in a fitness decrease of nearly 15%. Also a 20% reduction in juvenile survival and the reproductive period by pentachlorophenol (EC_{20}=4.3 and 1.3 μM respectively) leads to nearly 5% fitness decrease for the juvenile survival compared to zero impairment in the case of a 20% reduction in the reproductive period. These results imply that critical effect levels for sensitive life-cycle traits are not sufficient for assessing the potential impact of toxicants on population growth rates. Moreover, derivation of environmental quality criteria of toxicants based on these effect levels may lead to false estimates of the ecological hazards. For instance, a risk assessment for cadmium based on daily reproduction will lead to the underestimation of fitness effects. Insight into the relationship between plasticity of life-cycle traits and fitness will provide guidance for a proper ecological risk assessment.

Phenotypic plasticity comprises environmentally based changes in the phenotype and is an important feature of adaptation to heterogeneous or unfavorable environments (Via et al. 1995). For instance, discrete phenotypes were encountered in amphibians where one of the morphs was able to accelerate its metamorphosis in submerging waters (Newman 1988) and in some insects with seasonal polyphenism (Moran 1992). Continuous

phenotypes for example were present in the grass *Pennisetum setaceum* which occurs widely among various altitudes resulting from a strong plasticity for leaf photosynthesis in response to differences in temperature (Willams and Black 1993). Toxicants are an additional stress factor to natural occurring (un)favorable conditions thus adding new physiological constraints which, apart from accumulation of compounds in tissues, are comparable to ambient stressors. At present little is known about phenotypic plasticity to toxicants *per se*. Steep concentration-response relationships reflect a strong plasticity over a short concentration range whereas relatively insensitive traits are less plastic to that particular toxicant. The present chapter shows that daily reproduction in *P. acuminatus* was plastic to cadmium compared to other traits. Fitness however was not affected implying that either changes in reproduction are compensated for by various changes in other traits or that the combined alterations of traits eventually do not lead to expected fitness changes due to the intricacy of the model used. It can be concluded that plasticity allows *P. acuminatus* to maintain fitness in cadmium contaminated environments in which reproduction is signifinantly affected, although from a toxicological point of view impairment of reproduction is often appreciated as an important impact of toxicants.

Exposure to pentachlorophenol resulted in fitness impairment from 0.12 d^{-1} in the control to 0.02 d^{-1} at the highest concentration. In contrast to cadmium exposure, *P. acuminatus* was not able to maintain fitness by means of phenotypic plasticity which might be caused by the specific mode of action of pentachlorophenol compared to cadmium *i.e.* uncoupling of the oxidative phosphorylation vs. aspecific binding of cadmium to proteins and blocking of calcium channels (Neathery 1981, Verbost 1989). These findings are supported by toxicity results obtained for the springtail *Folsomia candida* (Crommentuijn et al. in press). Here, life span increase was compensated by slower somatic growth for springtails exposed to cadmium or triphenyltin hydroxide. No such trade-off was found for springtails exposed to chlorpyrifos. The mode of action of triphenyltin hydroxide is unsure but appears to be non-selective (Hassall 1982) whereas chlorpyrifos specifically inhibits functioning of the nervous system.

Long-term environmental contamination by toxicants with a non-specific mode of action, may thus lead to adaptation by changing life-cycle phenotypes. It was shown that these

changes influence fitness in various degrees depending on their mutual relationship (Chapter 2). Based on Fisher's postulate of fitness maximisation (Fisher 1958), it can be argued that risk assessment cannot be based on critical effect levels of single traits as such, since each trait has its own plasticity and a different relationship with fitness depending on the life-history strategy of the organism.

Recent literature suggests that plasticity is genetically controlled by either allelic sensitivity, where the expression of single genes is changed by external conditions, or regulatory plasticity, where gene receptors detect changes in external conditions and alter other gene expressions (Schlichting and Pigliucci 1995). Gene regulation by toxicants, such as the induction of heat-shock proteins, metal-binding proteins and esterases, produces discrete phenotypes, offers the potential to anticipate to adverse environmental conditions and allow organisms to change allometric relationships (Falconer 1990). On the other hand toxicant induced plasticity may be controlled by allelic sensitivity thus leading to phenotypic modulation as observed in many sublethal toxicity experiments. Both types of genetic control are extremely important for the evolutionary trajectories of plasticity (Via et al. 1995) and hence for the adaptibility of organisms in contaminated environments.

Because of the attribution of plasticity to the genotype, it is subjected to natural selection which appears to be similar to the responses of trait means. For instance it was reported that the plasticity of body weight in *Drosophila melanogaster* across two food environments responded relatively rapidly within approx. 10 generations (Hillesheim and Stearns 1991). Also it was clearly demonstrated that thermal tolerance in the same fly species was subjected to swift natural selection in changes in the thermal environment (Huey et al. 1991). From an ecotoxicological perspective this may imply that plasticity to toxicants is also subjected to selective forces thus modifying relationships between life-cycle traits and fitness. Although it is already known that toxicant-induced genetic adaptation changes life-history patterns in soil organisms (Donker 1992 ; Posthuma 1992) more research is required on the evolution of the shape of reaction norms in contaminated environments and its relation to optimal fitness.

Phenotypic adaptation to toxic stress indicates that the genotype is able to survive in

different environments by having reduced fitness plasticity. This was shown for *P. acuminatus* where fitness was not influenced over a range of cadmium concentrations. Hence, *P. acuminatus* can be regarded as a generalist to these cadmium environments since plasticity in fitness characters assures rigid fitness reaction norms, assuming maintenance of fitness in widely different habitats. Contrasting, genotypes which are not able to perform in different environments by means of plasticity indicate a plastic fitness reaction norm, with plasticity generally leading to fitness impairment.

The results have been discussed on the basis of nominal toxicant concentrations. Table 5 however shows that agar and bacteria adsorb cadmium to a level of approx. 25%. PCP concentrations also decreased due to breakdown or adsorbtion by bacteria and agar. Hence, care must be taken when deriving critical effect levels on a nominal or actual base.

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CHAPTER 6

COMPARISON OF LIFE-HISTORY STRATEGIES: DEFINING TOXIC ELASTICITY CONCENTRATIONS *

ABSTRACT - *Using theoretical life-cycle models in Chapter 2 it was illustrated that the impact of toxicants on fitness was determined by i) the plasticity of traits to toxic stress, ii) the relationship between plasticity and fitness and iii) the life-history strategy. To compare these theoretically obtained results with experimental observations, the present chapter explores the susceptibility of fitness in the nematodes Plectus acuminatus and Heterocephalobus pauciannulatus, each having distinct life-history strategies. to copper-induced life-cycle alterations. For both species, a strong response was found for the length of the reproductive period t_r. From a toxicological point of view t_r in P. acuminatus was more sensitive to copper than in H. pauciannulatus. However, a plasticity-to-fitness analysis revealed that although copper affected t_r stronger in P. acuminatus (60% reduction at 35 μM) than in H. pauciannulatus (10% reduction at 35 μM) both reductions resulted in approx. 2% fitness reduction. Hence small effects on t_r in H. pauciannulatus had the same impact on fitness as a large effect in P. acuminatus.*

To quantify these relationships, a '0% toxic elasticity concentration' (TEC) was defined denoting the maximum concentration of a toxicant leading to a significant effect on a particular trait but which does not influence fitness i.e. 0% fitness impairment. $TECt_r$ = 25 μM was found for the reproductive period for P. acuminatus and $TECt_r$ = 0 for H. pauciannulatus. These findings imply that fitness in P. acuminatus was less susceptible to toxicant-induced changes in t_r than H. pauciannulatus. The use of toxic elasticity concentrations facilitates the identification of ecotoxicologically important traits from a life-history perspective, since it quantifies the relationship of the classic concentration-response curve for various traits to fitness.

* *Based on: Kammenga J.E., Riksen, J.A.G., Bakker J. Challenging classic toxicological perceptions: phenotypic plasticity vs. concentration-response relationships (Submitted).*

Chapter 6

6.1 INTRODUCTION

Adaptation of organisms to heterogeneous or unfavourable environments requires the ability to respond to ambient stimuli by either genetic alterations or phenotypic plasticity (Sibly and Calow 1989; Via et al. 1995). Genetic adaptation may involve the re-allocation of resources among various phenotypic characteristics following environmentally induced changes in the genotype (Sibly and Calow 1986). Trophic conditions in freshwater sediments, for instance, may have substantial impact on the intraspecific variation in trade-off between survival of the parents and reproductive investment in the leech *Erpobdella octoculata* (Maltby and Calow 1986). In addition to these environmental factors, toxicants can also act as selective agents thus altering life-history strategies. In contaminated habitats, organisms are subjected to selection for toxicant resistance which may result in life-history shifts aiming to maximise fitness, *i.e.* intrinsic rate of population increase of a particular phenotype (Sibly and Calow 1989). Metal-adapted populations of the terrestrial isopod *Porcellio scaber* showed a strong allocation for early maturation and increased reproduction (Donker et al. 1993). Also for the springtail *Orchesella cincta* life-history patterns appeared to differ genetically among populations which have experienced various toxicant exposure levels (Posthuma et al. 1993).

Phenotypic plasticity comprises the ambient induced change in phenotypic traits without genotype alterations and is a specific type of genotype-environment interaction that reduces the need to match genotypes to the environment (Sultan and Bazzaz 1993a). For instance the extent of the light regime was found to induce changes in the allocation of metabolic energy in perch thus enabling the fish to respond sensitive to changes in external conditions (Wieser and Medgyesy 1991). Also variation in nutrient concentrations gave rise to plastic responses in either the size or the tissue nitrogen concentration of propagules in the plant *Polygonum persicaria* reflecting the adaptive capacities to a heterogeneous environment (Sultan and Bazzaz 1993b). In addition to these more general ambient factors, toxicants have been known to alter phenotypic characteristics thus leading to physiological trade-offs without genetic alterations in invertebrates (Chapter 5; Crommentuijn et al. in press). Toxicants can therefore be regarded as just an additional

environmental factor such as temperature, light regime or nutrient concentration modulating various phenotypic characteristics by re-allocation of recources.

In Chapter 2 it was illustrated that the impact of toxicants on fitness was determined by i) the plasticity of traits to toxic stress, ii) the relationship between plasticity and fitness and iii) the life-history strategy. This provided valuable information on the vulnerability of distinct strategies to toxic stress, indicating that the final impact is unique for each strategy and depends on a range of different relationships between fitness and plasticity of traits.

This chapter aims to explore the value of the results presented in Chapter 2 by investigating the impact of copper on the plastic responses and fitness in the nematodes *P. acuminatus* and *Heterocephalobus pauciannulatus* each having different life-history strategies. Complete life-cycle experiments were conducted at various copper concentrations to estimate fitness and to relate phenotypic variation in life-cycle traits to changes in fitness. Fitness in the two nematode species was estimated using a model as described in Chapter 4.

6.2 Materials and methods

CHEMICALS USED

Copperchloride ($CuCl_2.2H_2O$, 100% pure) was obtained from Sigma. All other chemicals used (Merck) were of the highest analytical grade available. The water used for culturing and experiments contained a defined mixture of minerals resembling those found in interstitial water of sandy forest soils (K^+: 0.1 mM, Na^+: 0.2 mM, Ca^{2+}: 0.35 mM, Mg^{2+}: 0.3 mM, NH_4^+: 0.3 mM, NO_3^-: 1.7 mM, Cl^-: 0.3 mM) (Schouten and Van der Brugge 1989). The pH was adjusted to 6.0 $\pm$ 0.1 with NaOH.

NEMATODE CULTURING

Laboratory stock cultures of the bacterivorous, parthenogenetic soil inhabiting nematodes *P. acuminatus* and *H. pauciannulatus* were used. Both nematode species were reared in agar on the soil bacterium *Acinetobacter johnsonion* which was obtained from the department of Microbiology at Wageningen Agricultural University in The Netherlands. Bacterial stock cultures were stored at -80°C in a cryo-protectant on glass beads (Jones et al. 1984). Bacteria and nematodes were cultured as described in Chapter 4.

LIFE-CYCLE EXPERIMENTS

Life-cycle studies were conducted in agar in multi compartment plates (Greiner, nr. 662160). Agar droplets (70 μL, 0.5%) with a bacterial density of 2.10^8 cells/mL were applied on the inside of the lid. Each compartment of the bottom plate contained 1 mL water and plates were sealed with Parafilm to prevent evaporation.

Following the outcomes of pilot experiments, the following concentration ranges were used: 0, 9.7, 17.5, 31.4, 56.6, 101 μM and 0, 12.4, 22.4, 40.4, 72.8 and 131 μM copper in agar for *P. acuminatus* and *H. pauciannulatus* respectively. Copper was added to fluid agar at a temperature of 37°C. Fourty-eight replicates were used for each treatment. Experiments were conducted at the same temperature in the dark as described in Chapter 4.

DATA ANALYSIS

Results were analysed using SAS software (Anonymous 1990). Differences between control and treatments of t_j, s_j, n_t, t_r were tested using Student's t-test. Longevity data were used to construct a Weibull distribution function. The least square method was used in an iterative non-linear regression procedure to estimate LT_{50} and c which were used to estimate s_r. A likelihood ratio test was applied to analyze differences in survival curves between treatments (see Chapter 4). The obtained values for the life-cycle variables were used to estimate fitness values for the different treatments by means of equation 5 in Chapter 4. A specific algorithm was constructed in SAS-software using non-linear

regression procedures (PROC NLIN). Application of the jack-knife procedure (Meyer et al. 1986) for estimates of fitness resulted in pseudo-values which were analysed by analysis of variance.

6.3 Results

Figure 1 and 2 show the phenotypic variation in four different life-cycle traits of *P. acuminatus* and *H. pauciannulatus* to various copper levels. For *P. acuminatus* the highest copper concentration resulted in 100% mortality among juveniles and was

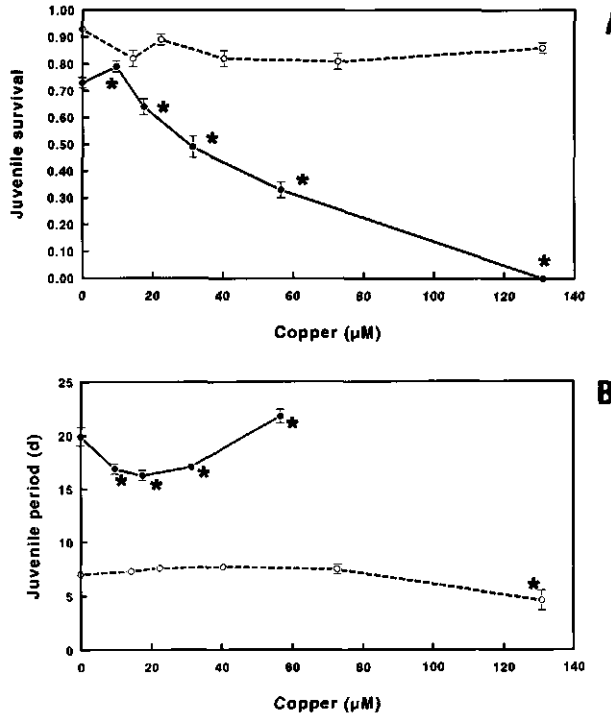

Figure 1. A) The effect of copper on the juvenile survival for *P. acuminatus* (●) and *H. pauciannulatus* in agar (○), B) the effect of copper on the juvenile period for *P. acuminatus* (●) and *H. pauciannulatus* (○) in agar (*: significantly different from control, Student's t-test, $p<0.05$, bars are standard errors).

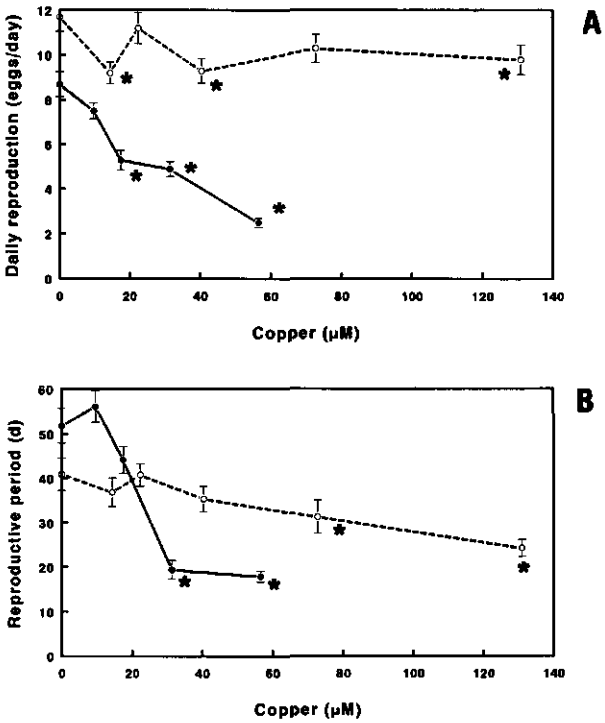

Figure 2. A) The effect of copper on the daily reproduction for *P. acuminatus* (●) and *H. pauciannulatus* (○) in agar, B) the effect of copper on the reproductive period for *P. acuminatus* (●) and *H. pauciannulatus* (○) in agar (*: significantly different from control, Student's t-test, $p<0.05$, bars are standard errors).

therefore discarded from further analysis. Variation in *P. acuminatus* was observed for the survival over the juvenile period s_j, ranging from 0.33 to 0.79 (Fig. 1a). A significant stimulation was found by copper at 9.7 µM. The length of the juvenile period t_j decreased at the three lowest concentrations and ranged from 16.3 to 21.8 days (Fig. 1b) whereas daily reproduction n_t decreased from 8.7 to 2.5 eggs/day (Fig. 2a). The reproductive period varied between 17.8 and 51.8 days with the highest value at 9.7 µM (Fig. 2b). In general, high copper concentrations resulted in impairment of most life-cycle traits except for the survival over the reproductive period s_r which increased during increased copper levels from 0.57 in the control to 0.99 at 56.6 µM copper. The shape of the survival curve varied between 2.1 and 5.9 (Table 1). Figure 3 shows that the median lethal time

LT_{50} in *P. acuminatus* ranged from 46.2 to 78.8 days at 9.7 μM. Figure 4 shows a variation in fitness in *P. acuminatus*, fluctuating between 0.09 d^{-1} and 0.18 day^{-1} with the highest value at 9.7 μM.

Life-cycle traits in *H. pauciannulatus* appeared to be less plastic to copper compared to *P. acuminatus*. Also contrasting to *P. acuminatus*, no significant juvenile mortality due to copper exposure was recorded (Fig. 1a). The juvenile period t_j significantly varied from the control only at the highest concentration (Fig. 1b). Reproduction ranged from 9.2 to 11.7 eggs/day (Fig. 2a). Only the reproductive period t_r appeared to be related to increased copper concentrations, decreasing from 40.9 days in the control to 24.2 days at the highest concentration (Fig. 2b). Figure 3 shows that the LT_{50} fluctuated between 47.3 and 28 days. Fitness ranged from 0.41 d^{-1} in the control to 0.29 d^{-1} at the highest concentration (Fig. 4). The survival over the reproductive period s_r varied from 0.5 to 0.73 and c fluctuated between 1.7 and 5.3 (Table 1).

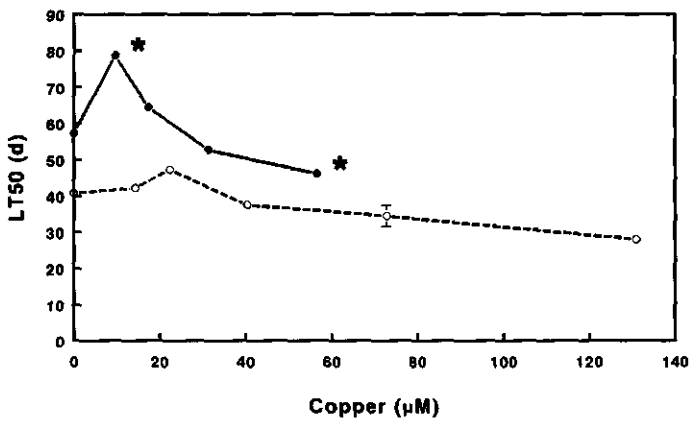

Figure 3. Impact of copper on the LT_{50} in P. acuminatus (●) and H. pauciannulatus (○) in agar. (*: significantly different from control, bars fall within the markers).

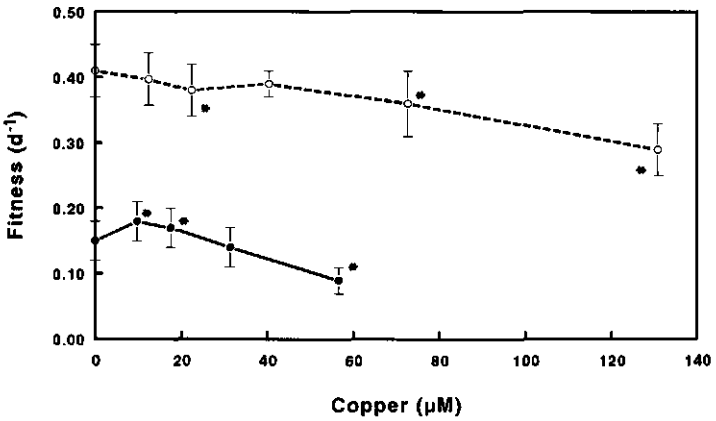

Figure 4. Impact of copper on the fitness in P. acuminatus (●) and H. pauciannulatus (○) in agar. (*: significantly different from control, Student's t-test, $p<0.05$, bars are SD).

Table 1. Effect of copper on estimated survival over the reproductive period (S_r) and the shape of the Weibull survival curve (c ± SD) for *P. acuminatus* and *H. pauciannulatus* in agar.

P. acuminatus			*H. pauciannulatus*		
µM	s_r	c	µM	s_r	c
0.0	0.57	2.1 ± 0.1	0.0	0.50	2.2 ± 0.2
9.7	0.80	3.3 ± 0.2	14.4	0.65	3.5 ± 0.3
17.5	0.78	2.7 ± 0.2	22.4	0.73	5.3 ± 0.3
31.4	0.89	3.7 ± 0.3	40.4	0.55	2.6 ± 0.1
56.6	0.99	5.9 ± 0.3	72.8	0.56	1.7 ± 0.4
101.0	-	-	131.0	0.65	3.3 ± 0.5

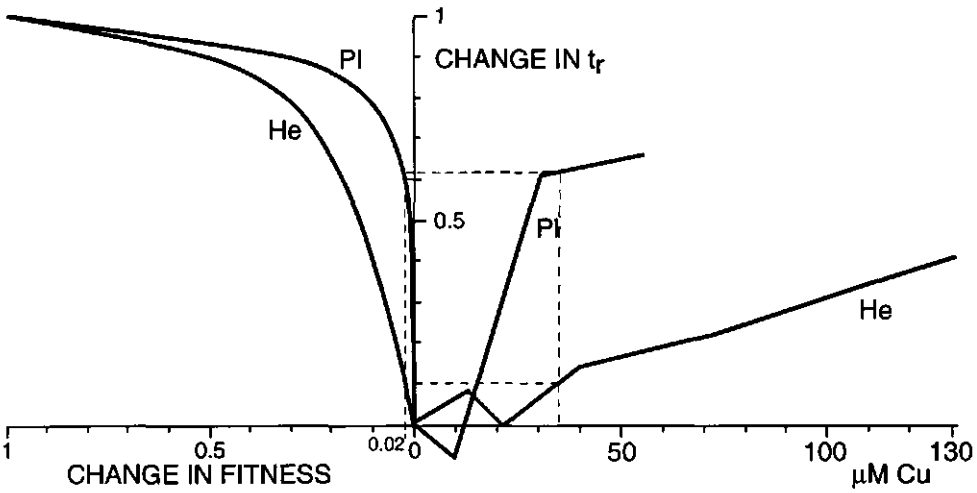

Figure 5. Plasticity-to-fitness relationship for copper and the length of the reproductive period (t_r) for *P. acuminatus* (Pl) and *H. pauciannulatus* (He). The left-hand side of the graph shows the relationship between a proportional decrease in t_r and impairment of fitness. The right-hand side of the graph shows the plasticity of t_r to copper for *P. acuminatus* (Pl) and *H. pauciannulatus* (He).

RELATING PLASTICITY OF t_r TO FITNESS

To compare the distinct plastic responses between the different nematode species in the most sensitive trait for both species, figure 5 shows the plasticity-to-fitness relationships for the length of the reproductive period t_r. Fitness in *H. pauciannulatus* was stronger related to changes in t_r than in *P. acuminatus*. A 45% reduction in t_r did not influence fitness in *P. acuminatus* whereas fitness was impaired with 13% in *H. pauciannulatus*.

From a toxicological point of view *P. acuminatus* was more sensitive to copper-induced changes in t_r than *H. pauciannulatus*, since a steep concentration-response relationship was found at relatively low concentration levels (right-hand side of Fig. 5). However, although copper affected t_r stronger in *P. acuminatus* (60% reduction at 35 µM) than in

H. pauciannulatus (10% reduction at 35 μM) both reductions resulted in approx. 2% fitness reduction. Hence small effects on t_r in *H. pauciannulatus* had the same impact on fitness than a large effect in *P. acuminatus*.

6.4 Discussion

This chapter underlines the importance of a plasticity-to-fitness analysis for assessing the impact of toxicants on fitness in species with divergent life-histories. These findings are in agreement with the theoretical explorations in Chapter 2, where it was illustrated that the impact of toxicants on fitness was not linearly related with the reaction norms for different traits and that the relationship between reaction norms and changes in fitness depended on the life-history strategy. By conceiving the concentration-respons relationship as a plastic response to stress it was shown in this chapter that for *P. acuminatus* the most sensitive trait t_r did not influence fitness below 45% reduction. The most sensitive trait in *H. pauciannulatus* was also t_r but, in contrast to *P. acuminatus*, fitness was influenced stronger. To quantify these discrepancies in the relationships between plasticity to toxicants and fitness impairment, the term '0% toxic elasticity concentration (TEC)' is introduced, denoting the maximum concentration of a toxicant leading to a significant effect on a particular trait but which does not influence fitness *i.e.* 0% fitness impairment. Thus, a TEC_{t_r} = 25 μM was found for t_r for *P. acuminatus* and TEC_{t_r} = 0 for *H. pauciannulatus*. TEC facilitates the identification of ecotoxicologically important traits from a life-history perspective, since it quantifies the relationship of the classic concentration-response curve for various traits to fitness. The proposed toxic elasticity concept follows the rationale of the projection matrix approach for conducting perturbation analysis of population growth rates (Van Groenendael et al. 1989). The analysis involved the estimation of proportional elasticities directly from the projection matrix entries which represent coefficients of life-cycle traits. The proportional elasticities have often been estimated using mathematical perturbation models, however, to my knowledge the application to assess 0% toxic elasticity concentrations is unique.

It appeared that life-cycle traits were more plastic to copper in *P. acuminatus* than *H. pauciannulatus*. In addition, the juvenile survival and period, daily reproduction and the reproductive period were all more sensitive in *P. acuminatus* than in *H. pauciannulatus*. However, at low copper levels *P. acuminatus* was able to increase fitness due to hormesis *i.e.* increase in juvenile survival, reproductive period and LT_{50}, and a decrease in juvenile period. Although the survival over the reproductive period s_r in *P. acuminatus* increased from 0.57 in the control to almost 1 at the highest concentration, fitness was still impaired at this level. Hence, *P. acuminatus* was not able to maintain fitness by having a plasticity in various traits as was found for cadmium (Chapter 5).

The decrease of 28% fitness reduction in *H. pauciannulatus* (0.41 day^{-1} to 0.29 day^{-1} at the highest concentration of 131 μM) can be largely explained by the 41% reduction in t_r (from 40.9 in the control to 24.2 at 131 μM, see Fig. 2b) which resulted in a fitness impairment of 10% (Fig. 5). The other 18% fitness reduction can be attributed to impairment in the other life-cycle traits. Clearly there appeared to be no adaptive plasticity in traits at these concentration levels as was found for cadmium in *P. acuminatus* (Chapter 5), where plasticity resulted in constant fitness.

The interspecific discrepancies in life-cycle responses may be explained by means of new insights into the theory on the evolution of generalists and specialists (Van Tienderen 1991). This theory states that in a defined environment with two distinct habitat qualities, rigid reaction norms found for traits in generalists will favour selection for stasis, *i.e.* homeostatic reactions. This may occur in organisms which regulate their body temperature at a constant level in response to fluctuating ambient temperatures or, for instance, in organisms which regulate copper metabolism during exposure to a range of copper environments. *P. acuminatus* showed a large phenotypic variation in response to copper in contrast to *H. pauciannulatus*, where traits were relatively slightly impaired. These results may be explained by assuming *H. pauciannulatus* to be less susceptible to copper stress due to physiological mechanisms involved in copper regulation. In this view, *H. pauciannulatus* is a generalist to copper environments with aplastic traits whereas *P. acuminatus* is a specialist with a plastic response to copper. The assumption that heavy metals may not have entered the body of *H. pauciannulatus* and consequently

may not exert any effects can be refuted on the basis of previous work on the accumulation of metals in various nematode species (Howell 1983; Popham and Webster 1979).

Large differences were demonstrated in the response of traits to copper in two nematode species with divergent life-history strategies. Quantitative evaluation of the life-cycles provided insight into the subtle interchanges among the reproductive period and fitness and it was illustrated by figure 5 that outcomes can be non-intuitive thus underlining the theoretical considerations presented in Chapter 2. In contrast to the results on toxicity of copper to single traits it was found that fitness in *H. pauciannulatus* was more susceptible to changes in t_r than in *P. acuminatus*. These findings indicate that the impact of copper on fitness depends on the interchange and connections between various life-cycle traits.

Only few papers have compared differences in susceptibility of fitness to toxic stress from a life-history perspective. In a comparative study of sensitivity of soil organisms to toxicants it was argued that the ratio between LC_{50} and the No-Observed-Effect-Concentration for reproduction was important for population response to toxic stress (Crommentuijn et al. in press). These results point out that the distance between reaction norms for survival and reproduction may offer potential for the evaluation of toxic insults on organisms. Survival over the reproductive period increased when most other traits, including reproduction, decreased in *P. acuminatus* leading to low fitness values. Hence there may be a cost of reproduction favoring survival over the reproductive period. Both these traits and fitness were less sensitive in *H. pauciannulatus*. These outcomes agree with Van Straalen et al. (1989) who reported that as a result of differing physiological responses in soil arthropods, differences in population growth rates were observed.

The present chapter exemplifies that the effect of toxicants on fitness in species with divergent life-history strategies depends on the plasticity-to-fitness relationships. By regarding the concentration-response relationship as a plastic response to stress, we obtained insight into the underlying mechanisms of fitness impairment.

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SUMMARY AND ECOTOXICOLOGICAL IMPLICATIONS

SUMMARY

A prevailing view in ecotoxicology is that toxicants affect organisms by impairing life-cycle traits that are most sensitive to these toxicants, a concept also adopted by national and international legislative authorities for deriving safe standards for contaminants in soil and water. For example, a classic approach in toxicity testing is to quantify juvenile survival or reproduction, since these are often known to be the most sensitive traits with respect to toxicants. However, organisms can tolerate stressors such as toxicants by having plastic life-cycle traits thus enabling them to maintain population growth rate or fitness.

The main objective was to test the classic premise on the most sensitive life-cycle trait by investigating toxicant-induced plasticity in life-cycle traits and fitness alterations.

Using theoretical life-cycle models, Chapter 2 showed that the impact of toxicants on fitness was determined by its relationship with plastic responses in life-cycle traits and the life-history strategy. These results were compared with experimental observations using nematodes as a case study. The nematode *Plectus acuminatus* was selected for chronic life-cycle investigations in Chapter 3 on the basis of its moderate sensitivity to cadmium and pentachlorophenol. Chapter 4 presented a mathematical life-cycle model for *P. acuminatus* to estimate fitness reductions from plastic responses in life-cycle traits to cadmium. This model was used in Chapter 5 to evaluate the impact of critical effect concentrations (EC_{20}) for cadmium and pentachlorophenol on fitness. It was demonstrated that fitness in *P. acuminatus* was not determined by the most sensitive life-cycle trait. Moreover, less sensitive traits appeared to have a stronger impact on fitness. In addition it was found that *P. acuminatus* was able to maintain fitness due to plasticity in life-cyle traits when exposed to cadmium. Finally Chapter 6 explored the effect of copper on fitness in two nematode species with divergent life-history strategies (*P. acuminatus* and *Heterocephalobus pauciannulatus)*. From a toxicological point of view the reproductive

period in *P. acuminatus* was 6 times more sensitive to copper than in *H. pauciannulatus*. However, the relationship between plasticity in the reproductive period and fitness showed that fitness was equally reduced in both species.

The present findings agree with the theoretical results obtained in Chapter 2 and it can be concluded that the impact of toxicants on fitness depends on: 1) the plasticity of life-cycle traits to toxicants, 2) the relationship between the plastic responses in life-cycle traits to fitness and 3) the life-history strategy of the organism. These conclusions do not support the general premise in ecotoxicology that the impact of toxicants on organisms is determined by the most sensitive life-cycle trait. It is therefore advocated that future ecotoxicological research with other organisms should put more emphasis on the ability of species to adapt to toxic stress by having plastic life-cycle traits in order to enhance the predictive value of toxicity tests which provide the basis for ecological risk assessment procedures.

ECOTOXICOLOGICAL IMPLICATIONS

The present thesis outlines the importance of investigating the plasticity in life-cycle traits to toxic stress for the effect assessment of toxicants on organisms. It appeared that fitness reductions were determined by the relationship with plastic responses in life-cycle traits rather than by critical effect concentrations of toxicants. This section briefly describes the implications for ecotoxicological research by using plastic responses as a tool for predictive ecological effect assessment of toxicants.

The impact of toxicants on organisms can be predicted from the relationship between two plastic responses which is defined as the *plastic trajectory*. Plastic trajectories mirror the i) plasticity in life-cycle traits and ii) a strategy traject under toxic stress conditions (after Stearns 1983). To illustrate this, life-cycle options under toxic stress will be explored by constructing plastic trajectories for age and size at maturity in the springtail *Folsomia candida* exposed to triphenyltin-hydroxide (TPT) and chloropyrifos (CPF) based on complete life-cycle data from Crommentuijn et al. (in press) (Fig. 1).

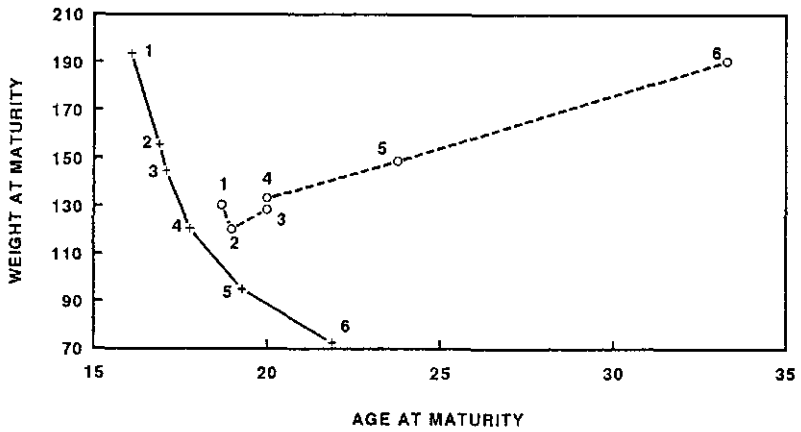

Figure 1. Plastic trajectories for age and weight at maturity to TPT (+) and CPF (O) in *F. candida* (The increasing concentrations are denoted by nr. 1-6).

The trajectory to TPT is L-shaped and shows that in *F. candida* age at maturity was only slightly changed when TPT concentrations were low, (concentrations 1-4). Contrasting however, at high TPT concentrations, (concentrations 5-6), maturation tended to start at a fixed size, changing only age at maturity. In the case of CPF, the trajectory for age and size at maturity is keel-shaped. On the left-hand side of the trajectory, (concentration 1 and 2), weight at maturity decreased and age at maturity increased. On the right-hand side of the trajectory, (concentrations 3-6), *F. candida* delayed maturity to such an extent that size at maturity was increased.

These findings agree with the general models of Stearns and Koella (1986) on the prediction of optimal reaction norms to food quality and temperature (results not shown). It may be concluded that trajectories to toxicants follow the same rules as dictated by life-history theory indicating that organisms respond to these stressors in the same way as they respond to other ambient factors. If we are able to construct graphs of trajectories relating plasticity in life-cycle traits for different organisms under different toxic stress regimes we are in a position to predict the impact of toxicants on life-cycle attributes and patterns of susceptibility of strategies to these stressors.

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SAMENVATTING EN ECOTOXICOLOGISCHE IMPLICATIES

SAMENVATTING

Binnen de ecotoxicologie wordt algemeen aangenomen dat de invloed van toxische stoffen op organismen bepaald wordt door de meest gevoelige levenscyclusonderdelen. Deze aanname vormt ook de basis voor de ecotoxicologische risico-analysemethoden van stoffen in bodem en water zoals die nationaal en internationaal geaccepteerd zijn. Een klassieke benadering in toxiciteitstoetsen is bijvoorbeeld het vaststellen van effecten op juveniele overleving en reproductie omdat die vaak het meest gevoelig zijn. Organismen zijn evenwel in staat om stressfactoren, zoals toxische stoffen, te weerstaan d.m.v. plasticiteit in levenscyclusonderdelen teneinde de populatiegroei of fitness constant te houden.

Het doel van dit onderzoek was om de klassieke aanname van de gevoeligste variabele te toetsen door het bestuderen van plasticiteit in levenscyclusonderdelen en fitnessreductie o.i.v. toxische stress.

Analyse van theoretische levenscycli toonde aan dat het effect op fitness niet bepaald wordt door de gevoeligheid van levenscyclusonderdelen, maar door de relatie met de plasticiteit van de verschillende levenscyclusonderdelen voor toxische stress en de levenscyclusstrategie. Om deze theoretische bevindingen te kunnen staven met empirische gegevens, werd de relatie tussen plasticiteit voor toxische stoffen en fitness in vrijlevende nematoden onderzocht. Hiertoe is een demografisch model ontwikkeld waarmee de invloed van veranderingen door cadmium in de levenscyclusonderdelen op fitness vastgesteld kan worden. Het model werd geparametriseerd door data in te voeren verkregen uit complete levenscyclus-experimenten en gebruikt om kritische effect concentraties voor cadmium en pentachloorfenol op fitness te evalueren. Hieruit bleek dat het effect op fitness niet wordt bepaald door het meest gevoelige levenscyclusonderdeel maar door minder gevoelige onderdelen. Dit was sterk afhankelijk van de relatie tussen plasticiteit en fitness. Vervolgens werd het belang van plasticiteit voor het effect van

koper op fitness onderzocht in twee nematodensoorten (*Plectus acuminatus* en *Heterocephalobus pauciannulatus*) met een verschillende levenscyclusstrategie. De reproductieve periode bleek 6 keer gevoeliger voor koper in *P. acuminatus* dan in *H. pauciannulatus*. Fitness werd evenwel voor beide soorten in gelijke mate gereduceerd.

Geconcludeerd kan worden dat de invloed van toxische stoffen op fitness afhankelijk is van: 1) de plasticiteit van levenscyclusonderdelen voor toxische stress, 2) de relatie tussen de plastische respons en fitness en 3) de levenscyclusstrategie.

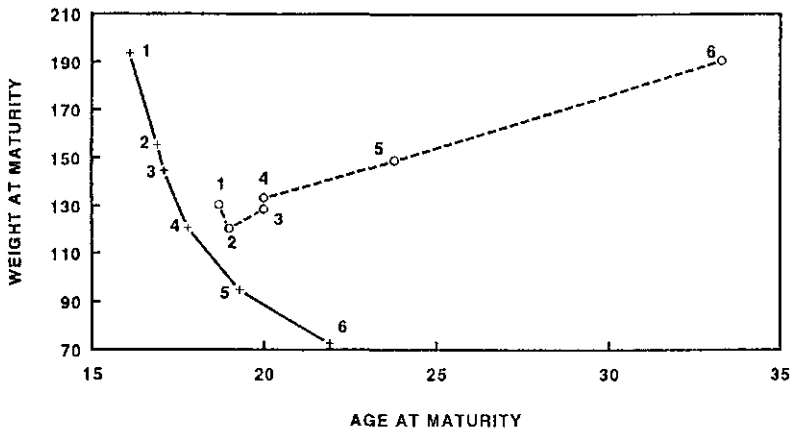
Deze conclusies geven aan dat de huidige manier van denken binnen de ecotoxicologie, namelijk dat gevoelige levenscyclusonderdelen ecologisch relevant zijn, niet gesteund wordt door empirisch onderzoek en de levensgeschiedenistheorie. Tevens bleek dat organismen in staat kunnen zijn fitness te handhaven d.m.v. plasticiteit ongeacht de afname van bepaalde levenscyclusonderdelen. Inzicht in deze plasticiteit draagt bij tot een realistische evaluatie van de effectbeoordeling vanuit de levensgeschiedenistheorie. Aanbevolen wordt om in toekomstig onderzoek met andere organismen de nadruk te leggen op de plasticiteit van levenscyclusonderdelen voor chemische stress. Dit verhoogt de voorspellende waarde van toxiciteitstoetsen die de basis vormen voor de huidige ecologische risico-analyse modellen.

ECOTOXICOLOGISCHE IMPLICATIES

Dit proefschrift toont het belang aan van plasticiteit voor de effectbeoordeling van stoffen op organismen. De afname van fitness bleek meer af te hangen van de relatie met plasticiteit in de verschillende levenscyclusonderdelen dan van kritische effect niveau's. Deze laatste sectie is gebaseerd op vervolgonderzoek en beschrijft in het kort de implicaties voor ecotoxicologisch onderzoek door gebruik te maken van de plastische respons op toxische stress als instrument voor de ecologische effectbeoordeling van stoffen.

De invloed van stoffen op organismen kan voorspeld worden m.b.v. de relatie tussen de plastische respons van twee verschillende levenscyclusonderdelen, ook wel *plastic traject*

genoemd. Plastische trajecten weerspiegelen i) de plasticiteit en ii) een strategisch traject onder invloed van toxische stress (naar Stearns 1983). Dit wordt geïllustreerd aan de hand van plastische trajecten voor leeftijd en gewicht bij volwassenheid in de springstaart *Folsomia candida* blootgesteld aan tri-fenyltinhydroxide (TPT) en chloorpyrifos (CPF) (Fig. 1). De data hiervoor zijn verkregen uit de literatuur van Crommentuijn et al. (in press). Het L-vormig traject voor TPT laat zien dat de leeftijd bij volwassenheid enigszins hoger wordt in *F. candida* bij een lager gewicht, dus bij lage TPT concentraties (1-4). Bij hoge TPT concentraties (5-6) wordt de volwassenheid later bereikt bij een vast gewicht.



Figuur 1. Plastische trajecten voor leeftijd en gewicht bij volwassenheid voor *F. candida* blootgesteld aan TPT (+) en CPF (○). De nrs. 1-6 geven toenemende concentraties weer.

Bij blootstelling aan CPF blijkt het traject een kiel-vorm te hebben. Aan de linkerkant van het traject, (concentratie 1 en 2), neemt het gewicht bij volwassenheid af terwijl de leeftijd bij volwassenheid toeneemt. Aan de rechterkant van het traject, (concentratie 3-6), vertraagt *F. candida* het volwassen worden zodanig dat het gewicht bij volwassenheid toeneemt.

Deze resultaten komen goed overeen met de theoretische voorspellingen van Stearns en Koella (1986) omtrent trajecten voor voedselkwaliteit en temperatuur. Het blijkt dus dat

plastische trajecten voor toxische stoffen sterk overeenkomen met trajecten die voorspelt worden door de levensgeschiedenistheorie. Dit impliceert dat organismen op gelijke wijze op toxische stress reageren als op algemene stressfactoren. Het opstellen van plastische trajecten voor verschillende organismen en stoffen biedt de mogelijkheid om voorspellingen te doen omtrent de gevoeligheid van strategieën voor toxische stress. Dit kan leiden tot het formuleren van algemeen geldende regels voor gevoeligheid waarbij rekening wordt gehouden met de plasticiteit.

LITERATUUR

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Jan Kammenga

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Curriculum vitae

Jan Edward Kammenga werd geboren op 3 november 1961 te Deventer. In 1982 behaalde hij het VWO diploma te Deventer. Na het voldoen van de militaire dienst bij de Koninklijke Marine begon hij in 1984 aan de studie Milieuhygiëne die hij in 1989 voltooide met de specialisaties Toxicologie en Biochemie. Eveneens in 1989 begon hij als assistent-in-opleiding bij de vakgroep Nematologie aan de Landbouwuniversiteit in Wageningen. In 1991 werd hij toegevoegd onderzoeker om in 1992 als universitair docent in vaste dienst te treden bij dezelfde vakgroep. De resultaten verkregen tijdens deze periode zijn deels vastgelegd in dit proefschrift.

Momenteel geeft hij leiding aan het ecotoxicologisch onderzoek en onderwijs van de vakgroep Nematologie en coördineert hij een aantal internationale EG-projecten in samenwerking met verschillende onderzoeksgroepen.