Benthic macroinvertebrates and multiple stressors

Quantification of the effects of multiple stressors in field, laboratory and model settings

Promotor: prof dr M. Scheffer
Hoogleraar Aquatische Ecologie en Waterkwaliteitsbeheer,
Wageningen Universiteit

Copromotor: dr A.A. Koelmans
Universitair Hoofddocent,
Leerstoelgroep Aquatische Ecologie en Waterkwaliteitsbeheer,
Wageningen Universiteit

Samenstelling promotiecommissie:
Prof dr W. Admiraal, Universiteit van Amsterdam
Prof dr H.H..T Prins, Wageningen Universiteit
Dr Ir H.H. Tolkamp, Zuiveringschap Limburg
Dr C.J.F. Ter Braak, Alterra
Dr Ir A.J. Hendriks, RIZA, Lelystad

Benthic macroinvertebrates and multiple stressors

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Edwin Theodoor Herman Maria Peeters

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STELLINGEN

1 De effecten van sediment-gebonden contaminanten op *in situ* benthische levensgemeenschappen zijn met behulp van statistische technieken te kwantificeren.
Dit proefschrift.

2 Verandering van de energiehuishouding van een organisme is een goede maat voor de stress ondervonden door het organisme, ongeacht de aard van de stressor.
Dit proefschrift.

3 In het ecotoxicologisch onderzoek zijn de ecologische factoren vaak het ondergeschoven kindje.
Dit proefschrift.

4 Een stressfactor komt nooit alleen.
Dit proefschrift.

5 Wetmatigheden vormen de basis voor een goed ecosysteembeheer hoewel uitzonderingen de regel lijken.

6 Door internet en de globalisering vervagen de grenzen tussen oost en west, maar het blijft thuis best.

7 Een variabele is geen parameter.

8 Ook binnen de waterkwaliteitsbeoordeling bestaan succes en falen alleen bij de gratie van gedefinieerde doelen.

9 Modellen zijn als Plato's ideeën: goed voor het begrip, gevaarlijk om een wereld op te bouwen.

10 Het studiehuis in het voortgezet onderwijs noopt universiteiten meer dan voorheen studenten een kritische, wetenschappelijke houding aan te leren.

11 Natuur in Nederland is wat mensen ervan maken.

12 Een leven zonder stress is geen leven.

Stellingen behorende bij het proefschrift:
"Benthic macroinvertebrates and multiple stressors
Quantification of the effects of multiple stressors
in field, laboratory and model settings"
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CHAPTER 1

GENERAL INTRODUCTION

There are three kinds of intelligence:
one kind understands things for itself,
the other appreciates what others can understand,
the third understands neither for itself nor through others.
The first kind is excellent, the second good,
and the third kind useless.

Machiavelli
The Prince, 1513

Aim of thesis

The structure and the functioning of ecosystems depend on responses to natural and anthropogenic stressors that influence the physiology and behavior of organisms, ecological interactions within assemblages, and ecosystem processes (BREITBURG ET AL. 1998). Natural fluctuations in the environmental conditions have altered and regulated ecosystems throughout their evolutionary history. However, nowadays, the impact of human activities overrules natural fluctuations and has caused major environmental changes (BIJLSMA AND LOESCHCKE 1997), such as climatic shifts, chemical pollution, destruction and loss of habitats, and eutrophication. One of the major negative effects of these impacts is the rapid decline in the world's biodiversity, impairing ecosystem functions such as primary production, carbon and nutrient conservation and cycling, decomposition, and food webs and resilience of ecosystems (e.g. PERRINGS ET AL. 1995, SCHWARTZ ET AL. 2000). Also, as a result of increased stress an ecosystem may shift in a difficult to reverse way from one alternative stable state to another (SCHEFFER ET AL. 1993).

The impact on ecosystems can be caused by a variety of mechanisms and the list of potentially dangerous chemical, physical, and biological stressors is still growing (FOLT ET AL. 1999). Usually, the effects and risks of stressors are studied individually (VOUK ET AL. 1987) by means of field and laboratory studies and models. In nature, however, organisms are always exposed to several simultaneously operating stressors (SCHINDLER ET AL. 1996, YAN ET AL. 1996) that may be natural or anthropogenic. Several studies addressing the assessment of risks associated with contaminated aquatic sediments indicate that the structure of the biotic community depends on the impact of both contaminants and other environmental variables (e.g. DEN BESTEN ET AL. 1995, CHAPMAN ET AL. 1997). However, the magnitude of the effects of contaminants in relation to the contribution of other stressors such as habitat variables remains usually unclear. Laboratory studies also demonstrated that the impact of a certain stressor depends on the intensity of other stressors (e.g. FOLT ET AL. 1999). Therefore, the combined effects of multiple stressors cannot be understood as a simple product of the individual effects. To understand how multiple stressors affect the composition and functioning of ecosystems it is necessary to know their quantitative contributions but also to explore their interactions.

The central theme of this thesis is the quantification of the combined effects of multiple stressors on benthic aquatic macroinvertebrates and their communities. Although stress can come to expression at different levels of biological organization, this thesis focuses on the community and organismal level. The following specific scientific questions are addressed: 1) Is it possible to quantify the impact of multiple stressors on *in situ* macroinvertebrate communities? 2) How do multiple stressors affect the growth of a single species under laboratory conditions? 3) Is it possible to explain the effect of multiple stressors on individual animals from their effects on the energy budget?

Definition of stress

There is no consensus about the definition of stress, as shown by reviews of LUGO (1978), SELYE (1973), IVANOVICI AND WIEBE (1981), and GRIME (1989). According to HOFFMANN AND PARSONS (1991) one of the difficulties in defining stress is that two components are involved: on the one hand, the factors that affect organisms, and on the other hand the responses in biota that occur as a consequence of these factors. The term "stress" is often used to designate either the environmental factor(s) or the biological response(s). Furthermore, "stress" is often associated with the intensity of stress. In this view, a factor is considered to be stressful only if the response it causes exceeds an arbitrary threshold, e.g. when more than a certain fraction of the population is affected. Others, however, consider the intensity of stress to be continuous, including zero (BIJLSMA AND LOESCHCKE 1997).

Due to these problems, it is not surprising that many different definitions of stress have been formulated. According to BIJLSMA AND LOESCHCKE (1997) biological definitions of stress fall into two classes. The first class of definitions considers stress in a physiological context. These definitions only focus on the physiological effects in the organism. SELYE (1973) for example defines stress as a series of physiological responses that affect the well being of individuals. In this view, a similar physiological response can be caused by various non-specific, stress causing factors. The second class of definitions focuses on the relationship between the environmental factors and their specific biological responses. For example, SIBLY AND CALOW (1989) define stress as "an environmental condition that impairs Darwinian fitness". KOEHN AND BAYNE (1989) define it as "any environmental change that acts to reduce the fitness of an organism" and GRIME (1989) as "external constraints limiting the rates of resource acquisition, growth or reproduction of organisms". These definitions have in common that they emphasize that the reduction in fitness is caused by an environmental factor and that there is no implication with respect to the intensity of the biological response. The effect may vary from zero to complete inhibition and depends on the characteristics of the subject and the nature, severity and periodicity of the stressor (GRIME 1989). In this thesis the definition of GRIME (1989) for stress is followed and stressors are all kinds of factors (natural and anthropogenic, abiotic and biotic) inducing a response.

Type of stressors

Stressors can be classified in several ways. For the purpose of this thesis, stressors will be classified into three main groups: 1) regular environmental variables; 2) food quantity and quality; and 3) contaminants. The first type includes all regular environmental factors such as habitat factors (e.g. current flow, dimensions), and water and sediment variables (e.g. oxygen concentration). The second type covers factors that determine the food resources of the macroinvertebrates (e.g. biochemical composition of organic matter). The third type covers all agents that are in general foreign to the ecosystem (e.g. pesticides, organic micropollutants) although some of them may also be natural to the system in low concentrations (e.g. trace metals).

Multiple stressors

Analyses of the response of organisms to stressors typically address a single species in relation to a single stressor (VOUK ET AL. 1989). Much literature is available that deals with the effect of a single contaminant on a single species (e.g. CAIRNS ET AL. 1994). Recently, attempts have been made in laboratory studies to relate whole communities changes to the impact of a contaminant (BROCK AND BUDDE 1994, BROCK ET AL. 1992a, 1992b, 1993, GIDDINGS ET AL. 1996, MAXON ET AL. 1997). A single stressor can induce a wide range of responses (FROST ET AL. 1999) and stressors can vary in the consistency and magnitude of their effects (BREITBURG ET AL. 1999). Susceptibility to stressors can vary among species (DIAZ AND ROSENBERG 1995, WILLIAMSON ET AL. 1999) and may be influenced by the presence or intensity of other stressors in the environment (e.g. FOLT ET AL. 1999, LENIHAN ET AL. 1999). Also, as ODUM (1981) noted, the response of biotic communities to perturbations varies with the stage in their development, i.e. the stage in their ecological succession.

The combined effect of two simultaneously operating stressors can be quite different from what would be expected on the basis of effects of the individual stressors. Various studies, not focussing on macroinvertebrates but on other biological groups, show that the combined effect of two contaminants may exceed the summed effects of the individual contaminants (e.g. HANAZATO AND DOBSON 1995) and that the impact of a contaminant depends on other non-contaminant factors (e.g. LEMLY 1993, ADAMS ET AL. 1998, PRESTON ET AL. 1999). Comparable results have been obtained in studies that focus on the effect of combined environmental stressors (e.g. GUZMAN-URIOSTEGUI AND ROBLEDO 1999, PORTER ET AL. 1999, RALPH 1999), on the effect of food in combination with environmental stressors (PILDICHT AND GRANT 1999), on the effect of parasites and environmental stressors (LAFFERTY AND KURIS 1999), and on the effect of biological and environmental stressors (DUDGEON 1993). The interaction between stressors can be classified in distinct categories. The effect of multiple stressors is called "comparative" when the effect in combination is equal to the effect of the single worst or dominant stressor (BRULAND ET AL. 1991). Additive effects are called "synergistic" or "antagonistic" when the combined effect of multiple stressors is greater or less than the sum of effects elicited by individual stressors (HAY ET AL. 1994).

To date, the combined effects of multiple stressors on freshwater macroinvertebrates have been explored only in a limited number of studies. Table 1 provides an overview of studies addressing this research area.

Table 1: Studies addressing combined effects of multiple stressors on macroinvertebrates.

Stressors	Organisms	Reference
Flow, substratum	*Paragnetina media*	FELTMATE ET AL. 1986
Copper, anoxia, temperature, salinity	*Mytilus edulis*	WEBER ET AL. 1992
Leaf litter, flow	Assemblages	LANCASTER AND HILDREW 1993
Predation, substratum, flow	Assemblages	DUDGEON 1993
PAH, UV	*Lumbriculus variegates*	ANKLEY ET AL. 1995
Density, predation, metals	Assemblages	KIFFNEY 1996
Substrate, competition	Isopods	DEFEO ET AL. 1997
Diet, temperature	*Pseudochironomus richardsoni*	GRESENS 1997
Discharge, predation	Assemblages	RAKOCINSKI 1997
Salinity, temperature	*Hydroides elegans*	QIU AND QIAN 1998
Two predators	Assemblages	STELZER AND LAMBERTI 1999
Oxygen, parasites	*Cerastoderma edule*	WEGEBERG ET AL. 1999
Flow, substratum	*Gammarus pulex* *Oreodytes sanmarkii* *Baetis rhodani*	LANCASTER AND MOLE 1999
Hypo-osmotic stress, Cd	*Arenicola marina*	RASMUSSEN 2000
Organic matter, toxicants	*Chironomus riparius*	STUIJFZAND ET AL. 2000

Stress and Scope for Growth

Stress can come to expression at different biological levels, e.g. at the molecular, physiological, organismal, population and community level (RYKIEL 1985). Cells in organisms exposed to stressing agents, usually have a rapidly expressed set of metabolic changes, referred to as the "stress response". These changes include the activation and elevated expression of a small set of genes, resulting in the increased synthesis and accumulation of stress proteins, and a concomitant reduction in the translation of most species of pre-existing m-RNAs, and thus a reduction of the normal protein synthesis (SCHLESINGER 1986, SANDERS 1990). This "stress response" has been reported in a variety of organisms under different stressors such as temperature (e.g. ASHBURNER AND BONNER 1979), chlorinated effluent (LAWRENCE AND NICHOLSON 1998), petroleum (NASCIMENTO ET AL. 1998), trace metals (SANDERS ET AL. 1991, BAUMAN ET AL. 1993, WILLIAMS ET AL. 1996), organic pollutants (SANDERS 1990), and exposure to ultraviolet radiation (NEPPLE AND BACHHOFEN 1997).

The abundance of most organisms shows a unimodal response in relation to environmental gradients. SHELFORD (1913) already recognized this in his Law of Tolerance. He stated that the occurrence of an organism is bounded by a minimum and a maximum value for any environmental variable, representing the limits of its tolerance. Normal metabolic processes occur within these environmental limits. Scope for growth (SfG), the difference between energy intake and energy metabolized by an organism, gives an indication of its metabolic condition, i.e. how much energy is available for growth and reproduction (WARREN AND DAVIES 1967). A positive SfG indicates that energy is available for production, while a negative SfG indicates that reserves have to be used to maintain the individual (MALTBY 1999). Beyond the limits of an organism's tolerance, metabolism fails although conditions are not immediately lethal, but they will limit distribution (CALOW AND SIBLY 1990).

Several studies show that stressors affect elements of the energy budget of organisms (e.g. KOOIJMAN AND BEDAUX 1996, KOOIJMAN 2000). Ecotoxicological studies show that organisms make direct energy costs to resist contaminants due to the costs of defense and repair processes. Thus, exposure to chemicals may result in a decrease in feeding and hence in energy acquisition (MALTBY 1999). If the concentration of a contaminant exceeds a certain value, organisms need energy for repair mechanisms and consequently pathological effects and exhaustion can occur (CALOW 1989). Energy spent to resist effects of contaminants may thus reduce the energy left for regular processes like growth and reproduction. Although it may be difficult to construct complete energy budgets (DAVIES AND HATCHER 1998), SfG has been used as an indicator of stress in a number of marine invertebrates and appears to be sensitive to a wide range of factors (MALTBY ET AL. 1990).

The reproductive capacity of a species depends on the available assimilation energy (KOOIJMAN AND METZ, 1983). A shift in the energy allocation as a result of exposure to a stressor, may thus affect a species' population fitness, eventually leading to the extinction of a species. HALL ET AL. (1992) argue that observed distribution and abundance patterns of organisms within space and time are related directly to species-specific energy costs and gains in response to the multiple environmental gradients. Thus, a direct relationship is assumed between stressors and the scope for growth and the distribution and abundance of a species (Figure 1). Models are proposed to relate physiological responses at the organismal level to population dynamics responses through functional relationships between those physiological responses and survivorship, fecundity, and developmental rates (CALOW AND SIBLY 1990). In the dynamic energy budget (DEB) approach (KOOIJMAN 2000) quantitative rules are used which describe how organisms acquire and utilize energy and nutrients together with constraints on metabolic organization and rules for interaction between individuals.

The field studies in this thesis deal with the biological reponse of macroinvertebrates and their communities to multiple environmental gradients. Therefore, the distribution and abundance of macroinvertebrates are related to the occurrence threshold and range (Figure 1) but in multiple gradients. The laboratory experiments mainly focus on sublethal effects of stressors and thus can be related to both the occurrence and survival threshold and ranges. The model in this thesis will address mainly the reproductive threshold and range.

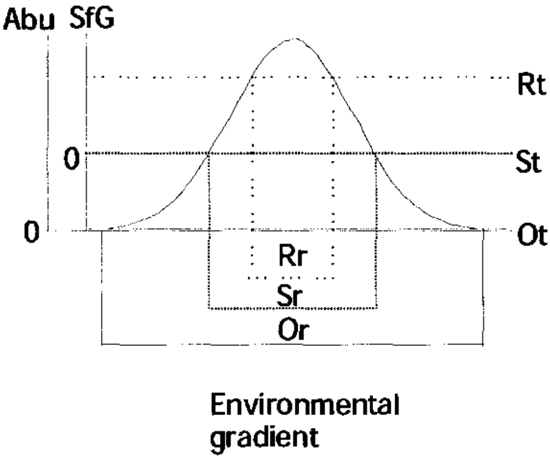

Figure 1: The relationship between abundance (Abu) and scope for growth (SfG) of a species along an environmental gradient. The horizontal line at zero abundance is the occurrence threshold (Ot) and the corresponding range is the occurrence range (Or). The dotted horizontal line represents zero scope for growth (St = survival threshold) and the corresponding range is the survival range (Sr). In this situation energy gain is sufficient to compensate for maintenance plus energy to overcome stress. Below the survival threshold, organisms can exist only by consuming energy reserves. The broken dotted line indicates the energy intake necessary to reproduce (Rt = reproductive threshold) and the corresponding range is reproductive range (Rr). The area above the reproductive threshold is the only part of the range in which the species can exist in the long term. Modified after HALL ET AL. (1992).

Outline of thesis

The effects of multiple stressors are explored in this thesis with field data, laboratory experiments, and an energy budget model (Table 2).

Table 2: Overview of the chapters of the thesis showing the research method and biological organization level.

Organization level	Research method		
	Field observations	Laboratory experiments	Modeling
Community level	2, 3, 4		
Organismal level	5	6, 7	8

The thesis can roughly be divided into two parts. The fist part focuses on the community level and aims at quantifying the effect of multiple stressors in structuring *in situ* benthic macroinvertebrate communities. To achieve this, multivariate statistical techniques are applied to data sets containing information on macroinvertebrates and different types of stressors. The second part focuses on the organismal level and deals with the effects of multiple stressors on the performance of single species. Laboratory experiments and a model focus on the effects of multiple stressors on the energy budget and scope for growth of the waterlouse *Asellus aquaticus,* a well studied species, which is widely distributed throughout Western Europe.

In chapters 2-4, the effect of different types of stressors on the macroinvertebrate community composition is quantified, using the method of variance partitioning which was introduced by BORCARD ET AL. (1992) to distinguish between environmental and geographical variation. In chapter 2 field observations on macroinvertebrates mainly from shallow lakes are related to environmental variables and food. In chapter 3, the benthic macroinvertebrate community structure patterns in the North Sea Canal are related to ecological factors and trace metal concentrations. Chapter 4 deals with the distribution patterns of macroinvertebrates in the Rhine-Meuse Delta in relation to environmental factors and different types of contaminants (trace metals, PCBs, PAHs). This chapter also addresses the question whether the results of laboratory bioassays better correlate with the *in situ* macroinvertebrates than the measurements of concentrations of contaminants. In chapter 5, the distribution of two common gammarids in the Netherlands is related to environmental conditions by means of univariate and multiple logistic regressions. Chapters 6 and 7 describe laboratory experiments with the waterlouse *Asellus aquaticus.* In chapter 6 the response of *Asellus aquaticus* to size of organic matter and the PAH benzo(a)pyrene concentration is analyzed, whereas in chapter 7 the response to two physical factors (current velocity and size of mineral substratum) is treated. Chapter 8 gives a simple energy budget model for *A. aquaticus,* which is used for further analysis of the results of the experiments described in chapters 6 and 7.

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CHAPTER 2

THE RELATIVE CONTRIBUTION OF FOOD AND ENVIRONMENTAL VARIABLES IN STRUCTURING *IN SITU* BENTHIC MACROINVERTEBRATE COMMUNITIES

Edwin THM Peeters, Ronald Gylstra and José H Vos

submitted

> *What is food to one, is to others bitter poison.*
>
> *Lucretius*
> *De Rerum Natura, 98-55 BC*

Abstract

The relative contribution of sediment food (e.g. organic matter, carbohydrates, proteins, C, N, polyunsaturated fatty acids) and environmental variables (e.g. oxygen, pH, depth, sediment grain size, conductivity) in explaining the observed variation in benthic macroinvertebrate species composition is investigated. Soft bottom sediments, water and benthic macroinvertebrates were sampled in several water systems in The Netherlands. The method of variance partitioning is used to quantify the relative contribution of food and environmental variables in structuring the benthic macroinvertebrate community structure.

Approximately 60% of the total variation in the macroinvertebrate community structure could be explained by the variables included in the analyses. The variation in the macroinvertebrate species composition between different water types is primarily related to differences in main environmental variables (e.g. current velocity, dimensions, pH). However, the variance partitioning method shows that food variables also contributed significantly and that the effect of food depends on the intensity of other factors.

The results of the study indicate that the method of variance partitioning is an appropriate tool for analyzing the impact of different groups of variables and thus, contributes to the understanding of the functioning of complex aquatic ecosystems.

The impact of food variables differed between the macroinvertebrate functional feeding groups. Detritivores showed significant correlations with food quantity (organic matter content) and quality (polyunsaturated fatty acids, P, and C/N ratio). Higher contents of organic matter usually go along with lower oxygen concentrations. Therefore, the observed lower species diversity and not changing macroinvertebrate densities with higher organic matter contents may be due to changes in either food quantity or oxygen concentration. Higher amounts of polyunsaturated fatty acids have a positive effect on the total macroinvertebrate density but not on the total number of taxa. It seems, therefore, that the productivity of benthic macroinvertebrates depends more on food quality than on food quantity.

Introduction

The distribution of aquatic macroinvertebrate species and communities is controlled by a variety of environmental factors such as habitat characteristics (e.g. HYNES 1970, TOLKAMP 1980, PEETERS AND GARDENIERS 1998), water quality (e.g. HELLAWELL 1986), sediment quality (e.g. REYNOLDSON ET AL. 1995, Chapman et al. 1997), contaminants (e.g. CLEMENTS AND KIFFNEY 1993, PHIPPS ET AL. 1995), and biological factors such as competition and predation (e.g. KOHLER 1992, MACKAY 1992, MACNEIL ET AL. 1999). Food quantity (organic matter content) and quality (biochemical composition) are also considered to be important factors determining aquatic invertebrate population dynamics (SWEENEY 1984). In field studies, however, it is difficult to separate the effects of food quality from food quantity, because they are interrelated.

Recent studies on the pelagic ecosystems of standing waters showed that the biochemical food composition is responsible for the impact of food on zooplankton (GULATI AND DEMOTT 1997) and invertebrates (AHLGREN ET AL. 1997). Among these biochemical compounds polyunsaturated fatty acids (PUFAs) have been found to be critical for maintaining high growth, survival, and reproductive rates of a wide variety of marine and freshwater organisms and thus play a key role in aquatic food webs (BRETT AND MÜLLER-NAVARRA 1997). Specific benthic macroinvertebrate species also showed strong correlations with biochemical food variables under laboratory conditions (e.g. MARSH ET AL. 1989, D'ABRAMO AND SHEEN 1993, VOS ET AL. 2000). Although some studies on the impact of biochemical food compounds on *in situ* benthic macroinvertebrate communities have been performed (e.g. GOEDKOOP ET AL. 1998) the magnitude of the impact is not quantified and thus remains largely unknown. Data on the impact of biochemical food compounds on freshwater benthic invertebrates are mainly available from lakes. Comparisons of the impact of biochemical food compounds across and among different water types have not been made yet.

The objective of the present study is to quantify the contribution of food variables in structuring the *in situ* benthic macroinvertebrate community. Soft bottom sediments and the inhabiting macroinvertebrates were collected from clean sediments in a number of lakes and some streams across The Netherlands. Simultaneously, environmental variables such as oxygen, conductivity, and depth were measured. In the laboratory, physical and biochemical sediment characteristics of the sediment were analyzed and macroinvertebrates were identified and enumerated. The relative importance of food and environmental variables on the total macroinvertebrate community as well as on different functional feeding groups was assessed using the variance partitioning method presented by BORCARD ET AL. (1992). This method attributes the biological variation in multivariate field data into different sources using canonical correspondence analysis (CCA). The method has been successfully applied in partitioning the variation in distribution patterns of subarctic plant species in a spatial and an environmental component (HEIKKINEN AND BIRKS 1996) and to partition macroinvertebrate variation into an ecological and an ecotoxicological component (PINEL-ALLOUL ET AL. 1996, PEETERS ET AL. 2000). Therefore, this method was considered to be a potentially effective method to compare the impact of food variables on benthic macroinvertebrate communities with that of environmental variables.

Materials and methods

Data collection

Between April 1998 and October 1998 a set of sediments was sampled across The

Netherlands. From this data set a number of 28 clean sediments (25 lakes, 3 small streams) were selected for further analysis because these sediments were classified as clean according to the Dutch standards. More information on the locations can be found in VOS (2001).

At each site water and sediment characteristics were determined as well as some general characteristics (Table 1). Temperature, oxygen, pH, and conductivity were measured in the field. Water samples (0.5 L) were taken and brought into the laboratory for further analyses on other water quality variables. The upper 4 cm of the sediments were sampled with an adjusted Ekman Bridge grab. Five sediment samples were taken, mixed, and stored at -20°C within 6 hours after sampling for physical and chemical analyses. Environmental variables were distinguished from food variables to calculate the relative contribution of both groups of variables in explaining the observed variation in the macroinvertebrate community data (Table 1). VOS (2001) showed that normalizing biochemical food variables on organic matter content correlated well with laboratory growth tests with the detritivores species *Chironomus riparius* and with the distribution and abundance of *in situ* benthic detritivores. Therefore, food variables were normalized on organic matter content.

Three replicate bottom samples of macroinvertebrates were also collected with the Ekman grab. The samples were rinsed using a sieve of 500 μm mesh size (ISO testing sieve, brass, NEN 2560 standard, ∅ 35 cm). The material retained in the sieve was preserved in 80% ethanol. In the laboratory macroinvertebrates were picked by using a stereomicroscope, sorted out, counted, and identified. The three replicates were combined and abundance was expressed as numbers/m^2.

Statistical analyses

The relative contribution of environmental and food variables in explaining the observed biological variation was determined by using the method of partial CCA as proposed by BORCARD ET AL. (1992). Detrended correspondence analysis assesses the length of the gradient and hence whether a linear or unimodal method should be used. A preliminary detrended correspondence analysis (HILL 1979) with logarithmic transformed abundance data and invoking the option 'downweighting of rare species', showed moderate to long gradient lengths. Therefore, the unimodal response model (TER BRAAK AND SMILAUER 1998) was considered appropriate.

The maximum number of variables that can be analyzed with CANOCO should be one less than the number of sites included in the analyses. Because the number of variables in the present study exceeds the number of samples the following selection procedure was followed to obtain a reduction in the number of variables. Prior to the analyses the importance of each variable was assessed using the forward selection procedure together with the Monte Carlo Permutation test. Only those variables were included in further analyses that had a significant ($P \leq 0.05$) contribution. In a next step the Variance Inflation Factors (VIFs) were inspected. High VIF values (>20) indicate multicollinearity between

Table 1: *Mean, standard deviation, minimum and maximum values for the continuous* variables.

Variable	Unit	Variable type[3]	Mean	Stdev	Min	Max
General variables						
Season[1]		E				
Watertype[1] standing/running		E				
Dimension[2]		E				
Depth	cm	E	46	25.5	10	100
Water						
Temperature	°C	E	16.3	2.9	9.7	21.4
Oxygen	mg/L	E	9.77	1.91	6.46	16.70
pH	-	E	7.9	1.1	5.2	9.6
Conductivity	µS/cm	E	789	396	44	1491
Total inorganic carbon	mg/L	E	11.9	4.9	1.2	23.0
Total organic carbon	mg/L	E	14.9	10.5	5.4	48.6
NH_4	mg/L	E	0.18	0.23	0.01	1.18
NO_{23}	mg/L	E	0.92	1.54	0.02	5.93
Ortho P	mg/L	E	0.034	0.037	0.000	0.146
Total P	mg/L	E	0.072	0.072	0.005	0.301
Total N	mg/L	E	2.16	1.58	0.65	6.23
Sediment						
Organic matter	mg/g	F	2.9	4.8	0.3	23.1
Carbohydrates	mg/g	F	0.35	1.51	0.01	8.02
P	mg/g	F	0.03	0.04	0.00	0.14
Pigments	µg/g	F	12.31	14.79	0.32	62.54
Proteins	mg/g	F	0.21	0.36	0.00	1.81
C	mg/g	F	2.51	5.11	0.15	27.06
Kjeldahl-N	mg/g	F	0.19	0.49	0.01	2.58
C/N ratio	-	F	21.9	15.0	5.9	69.3
CO_2 production	mmol/g	F	10.1	12.5	0.0	66.8
PUFAs[5]	µg/g	F	2.16	2.40	0.00	7.39
Bacterial FAs	µg/g	F	4.59	5.08	0.00	20.26
Total FA	µg/g	F	289.7	287.9	85.5	1615.5
GS[4] <63µm	%	E	23.1	28.0	0.7	79.0
GS <210µm	%	E	68.3	26.9	12.0	96.0
Dry weight	%	E	63.9	20.0	3.9	79.4

[1] *nominal variable*

[2] ordinal variable with 5 classes ranging from small (1) to large (5)

[3] E=environmental variable; F=food variable

[4] GS=grain size fraction

[5] PUFA=polyunsaturated fatty acid

[6] FA=fatty acid

variables and this should be avoided (TER BRAAK 1986). Hence, the variable with the highest

VIF was removed in the next analysis and this procedure was repeated until all VIFs had a value lower than 20.

Five different CCA runs were performed to calculate the contribution of the two groups of variables in explaining the variation in the species data (Figure 1): run 1) CCA of species data and both environmental and food variables as explanatory variables, run 2) CCA of species data constrained by environmental variables, run 3) CCA of species data constrained by food variables, run 4) the same as run 2 after removing the effect of food variables, and run 5) the same as run 3 after removing the effect of environmental variables. The effect of a set of variables can be removed by defining them as covariables within the program (TER BRAAK AND SMILAUER 1998).

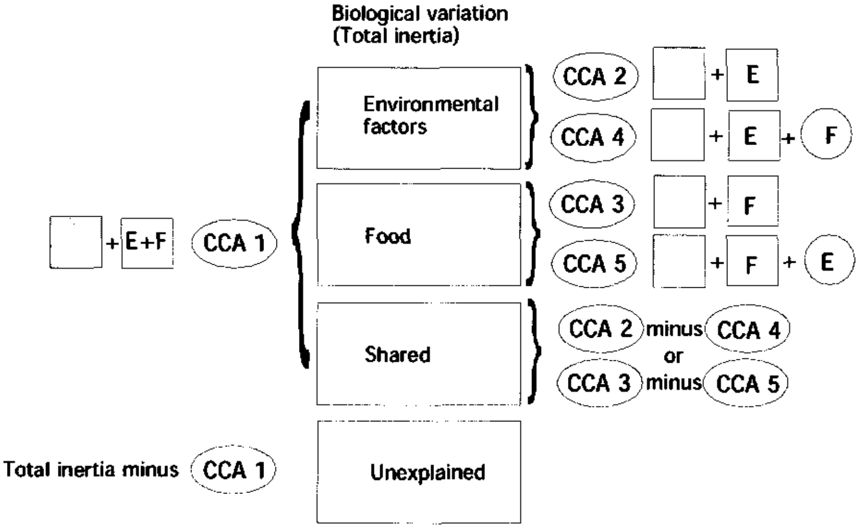


Figure 1: Diagram showing all partial canonical correspondence analyses (CCA) with the data set included in the analyses and the calculation of the partitioning of the variance. ☐ : macroinvertebrates, ☐ : explanatory variables, O: covariables, E : environmental variables, F : food variables. The number behind the abbreviation CCA refers to the numbers explained in the text.

The total variation present in the species data is called total inertia and is given by the sum of all unconstrained eigenvalues (TER BRAAK AND SMILAUER 1998). The proportion of variance explained by a set of variables is calculated as the sum of all canonical eigenvalues of a CCA divided by the total inertia. Hence, run 1 provides the overall amount of explained variation. The variation in the species matrix can further be partitioned as follows: a) variation explained solely by environmental variables (run 4); b) variation explained solely by food variables (run 5); c) variation shared by food and environmental variables (run 2 minus run 4 and run 3 minus run 5 respectively); and d) unexplained variation: total variation minus run 1 (see Figure 1). This approach was applied to different combinations of selected sites and to data sets containing different functional feeding groups (detritivores, herbivores, and carnivores). Table 2 gives an overview of the analyses performed together with the data sets included in relation to the purpose of the analyses.

Table 2: Overview of the data sets included in the canonical correspondence analyses in relation to the research question.

Purpose	Data set
Impact of food and environmental factors on benthic communities across water types	Total data set: macroinvertebrates and abiotic data
Impact of food and environmental factors on benthic communities within in lakes	Lake data set: macroinvertebrates and abiotic data
Impact of food and environmental factors on different functional feeding groups in lakes	Lake data set: macroinvertebrates and abiotic data

The statistical significance of the effect of each set of variables was tested by a Monte Carlo Permutation test with 199 permutations (TER BRAAK AND SMILAUER 1998). All CCAs were performed using the CANOCO program (TER BRAAK AND SMILAUER 1998).

Table 3: Number of taxa per taxonomic group, the number of samples and abundance data.

			Abundance		
Main group	No of taxa	Occurrence	Min	Mean	Max
Oligochaeta	1	27	0	3435	15292
Hirudinea	3	4	0	5	75
Mollusca	15	22	0	88	700
Hydracarina	1	14	0	35	450
Mysidacea	1	2	0	1	8
Amphipoda	3	15	0	246	2883
Isopoda	3	4	0	6	92
Ephemeroptera	2	10	0	124	1517
Odonata	3	4	0	1	17
Heteroptera	3	6	0	3	50
Megaloptera	2	2	0	2	44
Coleoptera	7	10	0	10	58
Trichoptera	14	12	0	37	342
Diptera					
Chironomini	22	26	0	1540	8908
Orthocladiinae	11	17	0	53	692
Tanypodinae	7	21	0	89	983
Tanytarsini	7	19	0	2107	25725
other	10	21	0	76	700
Total	115	28	42	7857	36117

Results

Macroinvertebrates

A total of 115 taxa were collected in 28 samples with an average of 14.9 ± 7.4 taxa per sample. Total number of individuals varied between 42 and 36117 per m^2. Table 3 shows the number of taxa per taxonomic order. The diversity was highest in diptera (especially chironomini and orthocladiinae) followed by mollusca and trichoptera. In general, oligochaeta, tanytarsini and chironomini were the most dominant taxonomic groups.

Table 4 shows the contribution of different taxonomic groups to the biological variation in the correspondence analysis. Approximately 80% of the variation is due to insects among which chironomidae contributed most (48%). Amphipoda and mollusca had the highest contributions among non-insects.

Table 4: Proportion of the variation (%) in the total data set (n=28) that is due to a certain taxonomic group.

Taxonomic group	Proportion of variation
Oligochaeta	2.2
Mollusca	8.1
Amphipoda	8.6
Isopoda	2.2
Ephemeroptera	3.9
Heteroptera	2.4
Coleoptera	5.7
Trichoptera	6.1
Chironomini	22.8
Orthocladiinae	8.0
Tanypodinae	7.1
Tanytarsini	11.0
Other Diptera	7.4
Other Taxa	4.4

CCA with the total data set

The forward selection procedure resulted in 15 variables that had a significant contribution. Inspection of the VIF showed that the value was high for bacterial FAs and this variable was also left out. Finally, 14 variables were included of which 6 were food variables. Figure 2 shows the distribution patterns for the samples and the variables for the first two axes of the ordination with the macroinvertebrates and all variables as explanatory. The constructed axes are linear combinations of the included variables; hence, the sequences of the samples on the axes are directly related to the variables. Three samples were separated from the others and positioned in the right hand side of the diagram (Figure 2). These samples

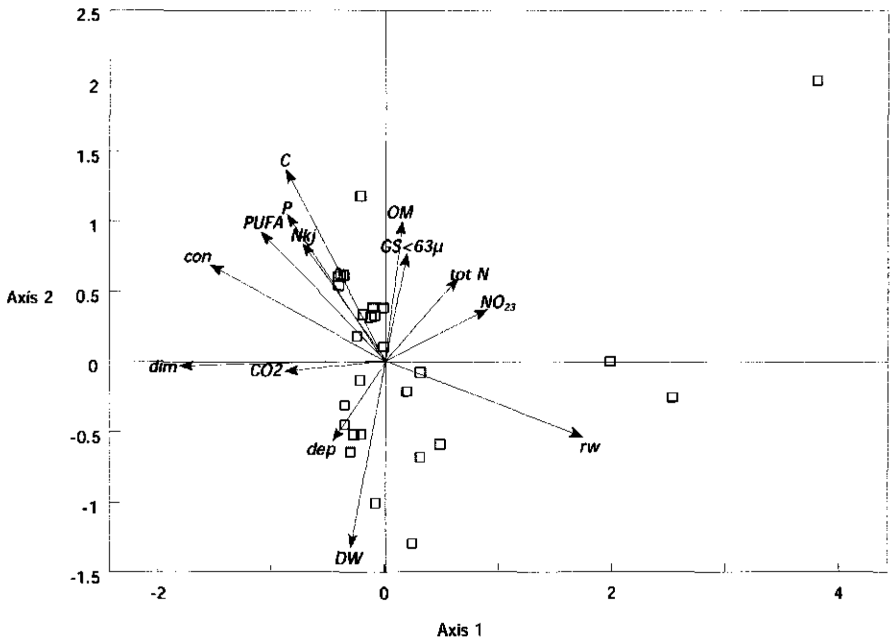


Figure 2: The position of samples and variables in the ordination diagram performed on the total data set. The diagram shows the positions for the first two axes of a partial CCA in which the species data were constrained by food and environmental variables. Samples are represented by blocks, variables by an arrow and their abbreviation: *rw*= water type: running water, *dim*=dimensions, *dep*=depth, *con*=conductivity water, NO_{23}=NO_{23} water, *tot N*=total nitrogen water, *OM*=organic matter, *GS<63μ*=grain size fraction <63μm, *C*=sediment carbon, *P*=sediment phosphorus, *NKj*=sediment Kjeldahl nitrogen, *PUFA*=sediment polyunsaturated fatty acid, CO_2= sediment CO_2 production, *DW*=sediment dry weight. The length of the arrow is a measure of the importance of the variable and the arrowhead points at the direction of increasing influence.

originate from small streams as is reflected by the position of the variables 'stream' and 'dimension'. These two variables had the highest absolute value for the first axis. Dimension is pointing to the left, indicating that dimension is increasing from right to the left. Sediment dry weight had the highest absolute value for the second axis. The food variables were placed close to each other and do not have an important impact on the sequence of the samples along the first axis although their contribution in the explanation of the total variation was significant. Approximately 65% of the variation in the species data was significantly ($P<0.05$ Monte Carlo Permutation test) explained by all variables (both environmental and food variables) included in the CCA (Table 5). Both environmental and food variables, each as a group, contributed significantly to the explanation of the observed variation in the species data. The contribution of environmental variables in explaining the biological variation was a factor 1.5 - 2 higher than the contribution of the food variables.

Table 5: Partitioning of the macroinvertebrate variance. All analyses were significant ($P< 0.05$, Monte Carlo permutation test).

Source of variation	Total data set[a] n=28 total inertia = 2.900	Lake data set[b] n=24[c] total inertia = 1.929
All factors	65.7	58.8
Food variables	21.5	20.9
Environmental variables	33.8	18.2
Shared	10.4	19.7

a: included variables: *General variables* water type, dimension, depth; *Sediment variables* organic matter, P, C, Kjeldahl N, PUFAs, CO_2 production; grain size fraction<63µm, dry weight; *Water variables* conductivity, NO_{23}, total N.

b: included variables: *General variables* dimension, depth; *Sediment variables* organic matter, P, C/N ratio, PUFA, CO_2 production, dry weight; *Water variables* conductivity, total N.

c: Lake data set is total set minus samples from 3 streams and 1 fen.

CCA with the lake data set

An initial analysis of the lake data set showed that one sample from a small acid fen dominated the results of the ordination (not shown) and this sample was left out in further analyses. The forward selection procedure resulted in 13 variables that had a significant contribution. Due to high VIFs the following variables were additionally excluded: sediment grain size fraction < 63 µm, bacterial FAs, and sediment carbon content and thus 10 variables remained in the analyses. Figure 3 shows the position of the samples and variables for the first two axes of a partial CCA in which the species data were constrained by food variables after removing the effect of the environmental variables. The sequence of the samples along the first axis is mainly related to PUFAs, P, and C/N-ratio. C/N-ratio also contributed to the second axis. The impact of organic matter content is rather low, as can be derived from the close position of this variable to the center of the diagram.

The partitioning of the variance for the lake data set is given in Table 5. The environmental and food variables contributed significantly (Monte Carlo Permutation test, $P<0.05$) and had a similar contribution in explaining the observed variation. In comparison to the results of the total data set, the explained variation by all variables dropped 7%. The contribution of the environmental variables dropped with 15% whereas the contribution of food variables remained the same and thus the importance of food variables increased.

CCA with functional feeding groups in the lake data set

The variance partitioning for the three main functional feeding groups (carnivores, detritivores, herbivores) is presented in Table 6. A large proportion of the variation in the carnivores could be explained by all variables included in the analyses, whereas for herbivores the explained fraction was rather low. The contribution of the environmental variables for both carnivores and herbivores was significant, but the proportion of the

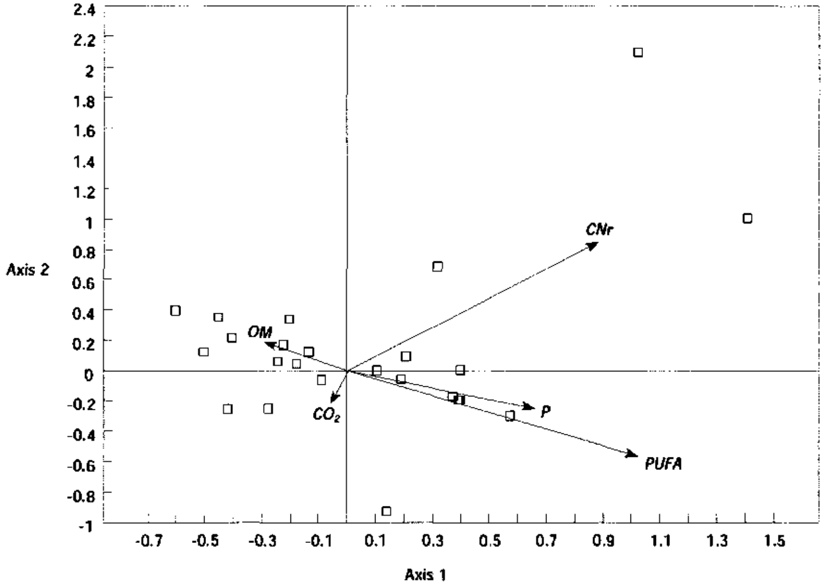


Figure 3: The position of samples and variables in the ordination diagram of the lake data set. The diagram shows the positions for the first two axes of a CCA in which the species data were constrained by food variables after removing the effect of the environmental variables (dimension, depth, conductivity, and total-N). Samples are represented by blocks, variables by arrows and their abbreviation: *OM*=organic matter, *CNr*=sediment C/N ratio, *P*=sediment phosphorus, *PUFA*=polyunsaturated fatty acid, *CO_2*=sediment CO_2 production. The length of the arrow is a measure of the importance of the variable contaminant and the arrowhead points at the direction of increasing influence.

Table 6: Partitioning of the macroinvertebrate variance for the functional feeding groups.

Source of variation	Trophic group Carnivores[A] (n=18[D])	 Detritivores[B] (n=40)	 Herbivores[C] (n=19)
All factors	71.8	51.3	27.9
Food variables	15.1[ns]	29.3	2.8[ns]
Environmental variables	34.0	14.9	18.4
Shared	22.9	7.1	6.7
Unexplained	28.2	48.7	72.1

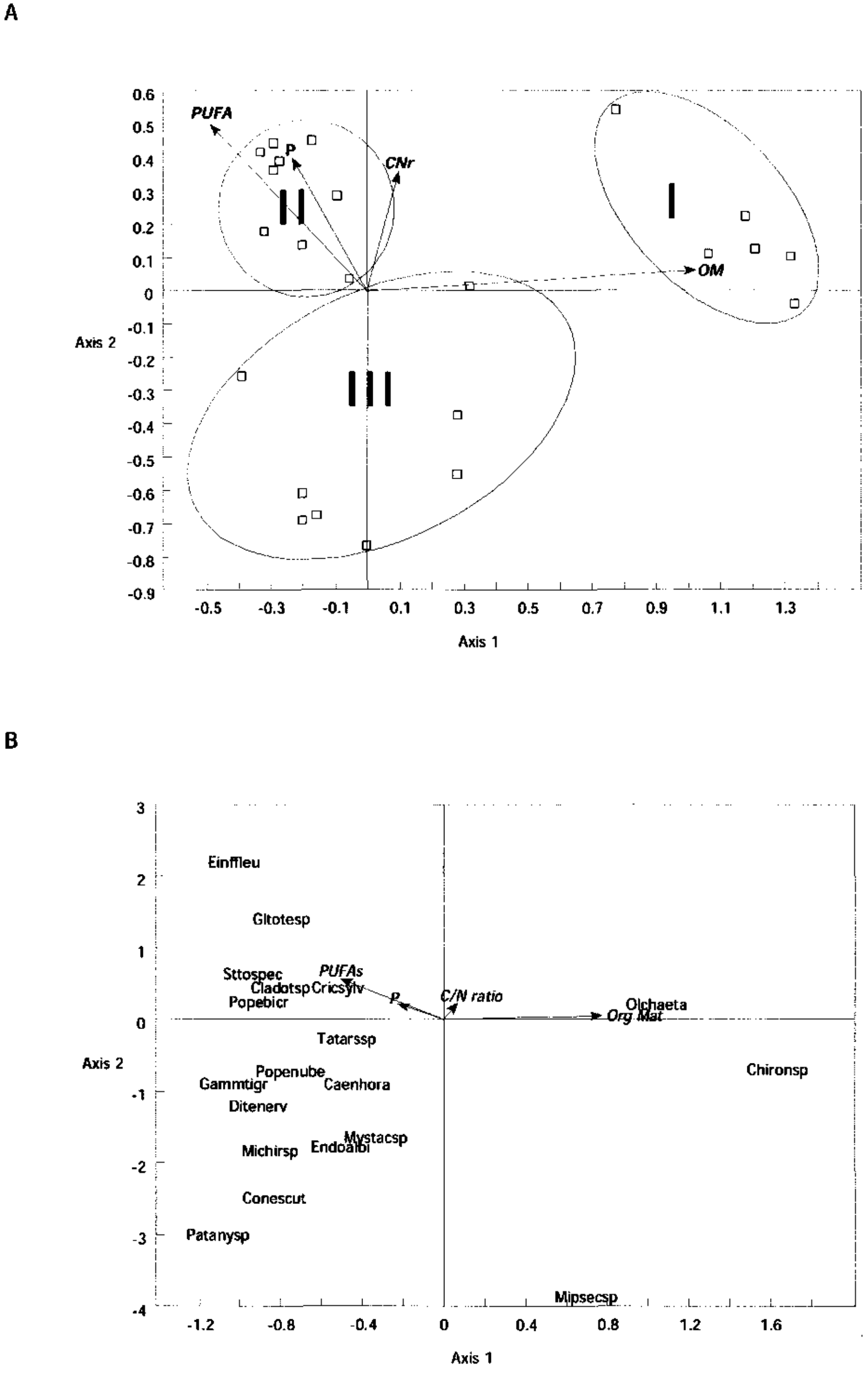
[ns]= not significant (Monte Carlo test p>0.05)

[A]: included variables: *General variables* dimension; *Sediment variables* pigments, C, Kjeldahl N, CO_2 production, PUFA, grain size fraction <63µm and <210µm, dry weight; *Water variables* pH, conductivity.

[B]: included variables: *General variables* dimension; *Sediment variables* organic matter, P, C/N ratio, PUFA; *Water variables* conductivity

[C]: included variables: *General variables* season, dimension; *Sediment variable* P; *Water variables* conductivity.

[D]: number of taxa


A
PUFA
P
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OM
I
II
III
Axis 2
Axis 1
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
-0.5
-0.3
-0.1
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Einffleu
Gltotesp
Sttospec
Cladotsp
Cricsylv
PUFAs
P
C/N ratio
Popebicr
Olchaeta
Org Mat
Tatarssp
Chironsp
Popenube
Gammtigr
Caenhora
Ditenerv
Mystacsp
Endoalbi
Michirsp
Conescut
Patanysp
Mipsecsp
Axis 2
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3
2
1
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-1
-2
-3
-4
-1.2
-0.8
-0.4
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1.2
1.6

Figure 4: Position of samples and variables (A) and taxa and variables (B) in the ordination diagram of the lake data set with detritivore taxa only. The diagram shows the positions for the first two axes of a CCA in which the species data were constrained by food variables after removing the effect of the environmental variables (dimensions and conductivity). Samples are represented by blocks; variables by arrows and their abbreviation: *OM* = organic matter, *PUFA* = polyunsaturated fatty acid, *CNr* = sediment C/N ratio; taxa by their abbreviations: Olchaeta=Oligochaeta, Gammtigr=*Gammarus tigrinus*, Caenhora=*Caenis horaria*, Mystacsp=*Mystacides* sp., Chironsp=*Chironomus* sp., Ditenerv=*Dicrotendipes gr. nervosus*, Einffleu=*Einfeldia/Fleuria*, Endoalbi=*Endochironomus albipennis*, Gltotesp=*Glyptotendipes* sp., Michirsp=*Microtendipes* sp., Popebicr=*Polypedilum gr. bicrenatum*, Popenube=*Polypedilum gr. nubeculosum*, Sttospec=*Stictochironomus* sp., Conescut=*Corynoneura scutellata agg.*, Cricsylv=*Cricotopus sylvestris agg.*, Cladotsp=*Cladotanytarsus* sp., Mipsecsp=*Micropsectra* sp., Patanysp=*Paratanytarus* sp., Tatarssp=*Tanytarsus* sp. The length of the arrow is a measure of the importance of the variable contaminant and the arrowhead points at the direction of increasing influence.

variance explained by food variables was not. Both food and environmental variables had a significant contribution in explaining the observed variation in detritivores. The contribution of food variables was two times higher than environmental variables. Figure 4A and B shows the first two axes of a partial CCA in which the species data were constrained by food variables after removing the effect of the environmental variables.

Because CA is susceptible to taxa that occurred in low numbers in only one sample, these were omitted from the analysis. The sequence of the samples and species along the first axis is mainly related to organic matter content. The ordering along the second axis, however, is due to differences in food quality. PUFA had a higher score on the second axis than P and C/N ratio, indicating the greater importance of PUFAs. Oligochaeta spp. and *Chironomus* sp. are mainly associated with high organic matter content. *Einfeldia/Fleuria* sp., *Glyptotendipes* sp., *Stictochironomus* sp., *Cladotanytarsus* sp., and *Polypedilum bicrenatum* show a relationship with elevated food quality.

In Figure 4A, three different groups of samples are distinguished. Group I consists of samples with higher organic matter content, and group II and III differed in food quality. Non-parametric ANOVA (Mann Whitney U test) showed that there were significant differences in total number of taxa between group I and group II (P=0.007), and between group I and group III (P=0.001). No significant differences were observed between group II and group III. With respect to the total number of individuals, only group II and group III were significantly different (P=0.043). Elevated organic matter content is accompanied with lower number of taxa but did not affect the total number of individuals whereas increase in food quality seems to affect the total macroinvertebrate density but not the total number of taxa.

Discussion

The communities in the present study were dominated by oligochaeta, chironomini, and tanytarsini although taxonomical diversity was highest in mollusca, trichoptera, and chironomidae. The total variation in the species data set was mainly due to chironomidae larvae. These results are in line with other observations on benthic communities in lakes (e.g. DOUGHERTY AND MORGAN 1991, PRUS ET AL. 1999). Approximately 60% of the variation in the macroinvertebrate species composition could be explained by the variables included in the analyses. This percentage is rather high in comparison to other studies (e.g. VERDONSCHOT AND TER BRAAK 1994, RODRIGUEZ AND MAGNAN 1995, PINEL-ALLOUL ET AL. 1996, PEETERS ET AL. 2000). Although a large proportion of the variation was explained in the present study, another part remains unexplained due to e.g. natural fluctuations and certain factors or aspects not considered such as predation and competition.

The variance partition method has been applied successfully to partition the variation of subarctic plant species in a spatial and an environmental component (HEIKINNEN AND BIRKS 1996) and to allocate variation in macroinvertebrates into an ecological and an ecotoxicological component (PINEL-ALLOUL ET AL. 1996, PEETERS ET AL. 2000). The method as applied in the present study showed that the impact of food variables differed between the functional feeding groups (carnivores, detritivores, herbivores). The results of the analyses with the functional feeding groups showed that significant correlations with food variables were only obtained for detritivores and not for herbivores and carnivores. This may be expected because in the present study food quality is determined from the sediment and it is well known that detritivores use sediments as a food source whereas carnivores and herbivores use other resources. Therefore, the observed significant correlation between food variables and detritivores indicates that this method may be useful tool for analyzing the impact of different groups of variables in natural ecosystems.

This study clearly showed that main environmental variables (e.g. current velocity, dimension, pH) contributed more to the explanation of the macroinvertebrate variation between different water types then food variables. This is in line with many publications concerning the distribution patterns of benthic macroinvertebrates over a wide range of water types (e.g. VERDONSCHOT 1990). The results also showed that a substantial proportion of the variation in the macroinvertebrate species composition could not be explained without including food variables. Analyses of the macroinvertebrate community variation in a more homogeneous subset of shallow lakes, as in the present study, showed that food and environmental variables had a similar contribution in explaining the observed variation and thus food variables became relatively more important. The relative effect of food seems, therefore, to depend on the variation of other factors. This is in line with the concept that abiotic factors are determining patterns in the distribution and abundance of species over broad scales whereas the relative importance of biotic factors is greater over local scales. For example, according to JACKSON ET AL. (2001) abiotic factors control fish communities at the large scale, whereas competition is of greater importance at small scale. Furthermore, the model study of PINEL-ALLOUL ET AL. (1995) on zooplankton showed that abiotic variables explained most of the variability in zooplankton community on the large scale, whereas biological bottom-up and top-down control occur at small scales.

Analyses of the lake dataset with only detrivores showed that food quantity (organic matter content) was of greater importance than food quality (e.g. PUFAs). Especially oligochaeta and *Chironomus* sp. were positively related to organic matter content. Both taxa were collected in all locations, sometimes in high numbers. Organic matter may influence oxygen levels in sediments through oxygen consumption and by increasing the packing of the sediment, usually resulting in lower oxygen concentrations in sediments with high organic matter contents (WATLING 1991). The present study showed that increased organic matter content resulted in a decrease in biodiversity, but hardly affected total abundance of macroinvertebrates. It is well known that many oligochaetes and chironomid species are resistant to low oxygen levels. Therefore, it is unclear whether the observed response is due to food quantity or oxygen concentrations.

Taxa like *Einfeldia/Fleuria*, *Glyptotendipes* sp., *Stictochironomus* sp., *Cladotanytarsus* sp., and *Polypedilum bicrenatum* were positively related with PUFAs and thus with food quality. Increase in food quality hardly affected the total number of taxa and had a positive effect on the total macroinvertebrate densities. Therefore, food quality can be considered as an important factor determining the production capacity of macroinvertebrates in shallow lakes. This finding is in line with studies in the marine environment, which showed that biotic communities were constrained more by changes in the food quality rather than food quantity (e.g. ALBERTELLI ET AL. 1999, DANOVARO ET AL. 2000).

The present study showed that sediment PUFA was the second most important food variable. Studies on pelagic invertebrates in relation to food quality showed that PUFAs can be considered as the main factor determining food quality (BRETT AND MÜLLER-NAVARRA 1997, GULATI AND DEMOTT 1997, GOULDEN ET AL. 1999). PUFAs are essential components for animals but animals cannot generate PUFAs or at most to a limited extent (NAPOLITANO 1999). Hence, they should assimilate PUFAs from their diet. In deep lakes, PUFAs are mainly produced in the pelagic part of the ecosystem and therefore the composition and functioning of the benthic macroinvertebrate communities is often linked with the production in the pelagic part (MOORE 1987, MARSH AND TENORE 1990, GOEDKOOP AND JOHNSON 1996; GOEDKOOP ET AL. 1998). Such a pelagic-benthic linkage might also be expected in shallow lakes although macrophytes, periphyton, and benthic diatoms may also contribute to the production of PUFAs.

In conclusion, the variance partitioning method enables to quantify the contribution of food and environmental variables in explaining the observed variation in the benthic macroinvertebrate community structure. The relative effect of food variables depends on the intensity of other factors. Differences in macroinvertebrate composition between communities of different water types are mainly related to environmental variables although the impact of food variables is certainly not neglectable. Detritivores showed a significant relationship with food variables: higher organic matter contents hardly affected their abundance but gave a decrease in biodiversity, whereas increase in food quality (e.g. higher content of PUFAs) did not affect biodiversity but resulted in higher densities.

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CHAPTER 3

THE CONTRIBUTION OF TRACE METALS IN STRUCTURING *IN SITU* MACROINVERTEBRATE COMMUNITY COMPOSITION ALONG A SALINITY GRADIENT

Edwin THM Peeters, Jean JP Gardeniers and Albert A Koelmans

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> *.. all our reasonings concerning causes and effects*
> *are derived from nothing but custom;*
> *and that belief is more properly an act of the sensitive,*
> *than of the cogitative part of our natures...*
>
> *David Hume*
> *An Enquiry Concerning Human Understanding, 1748*

Abstract

Macroinvertebrates were studied along a salinity gradient in the Northsea Canal, The Netherlands, to quantify the effect of trace metals (cadmium, copper, lead, zinc) on community composition. In addition, two methods for assessing metal bioavailability (normalizing metal concentrations on organic carbon and on the smallest sediment fraction) were compared. Factor analyses showed that normalizing trace metals resulted in an improved separation of trace metals from ecological factors (depth, organic carbon, granulometry, and chloride). The variation in the macroinvertebrate data was partitioned into four sources using partial canonical correspondence analysis, with the partitions being purely ecological factors, purely trace metals, mutual ecological factors and trace metals, and unexplained. Partial canonical correspondence analysis applied to total and normalized trace metal concentrations gave similar results in terms of unexplained variances. However, normalization on organic carbon resulted in the highest percentage of variation explained by purely ecological factors and purely trace metals. Accounting for bioavailability thus improves the identification of factors affecting the *in situ* community structure. Ecological factors explained 45.4% and trace metals 8.6% of the variation in the macroinvertebrate community composition in the ecosystem of the Northsea Canal. These contributions were significant, and it is concluded that trace metals significantly affected the community composition in environment with multiple stressors. Variance partitioning is recommended for incorporation in further risk assessment studies.

Keywords: Bioavailability, Ecotoxicology, Macroinvertebrates, Sediment, Trace metals, Variance partitioning

Introduction

Macroinvertebrate field surveys are frequently used in studies assessing water and/or sediment quality, e.g., as part of the sediment quality triad approach (e.g. CHAPMAN ET AL. 1997). Macroinvertebrates are directly associated with contaminants in sediments through their feeding and behavioral activities (REYNOLDSON ET AL. 1995). They take up trace metals from the ambient water and from their food (TIMMERMANS 1993) and therefore play a key role in bioaccumulation and transfer of contaminants to higher trophic levels in aquatic and terrestrial food webs. Natural macroinvertebrate communities are strongly influenced by a variety of environmental factors other than contaminants, such as salinity, bottom substratum, and pH (e.g. HYNES 1970). To date, the knowledge of the relative importance of trace metals in structuring aquatic communities in comparison to the impact of other variables is very limited.

Multivariate statistical tools such as principal component analysis, multiple regression, discriminant analysis, and multidimensional scaling, can contribute to the assessment of the relative importance of different environmental stressors. In many studies relating abiotic and biotic data, abiotic data are analyzed with multivariate techniques followed by an interpretation of the results with biotic community measures. A disadvantage of such an indirect multivariate approach is that biological information is afterwards related to the results of an analysis of the abiotic data. This means that the final results are constrained by abiotic rather than by biota data. Furthermore, overall community measures such as diversity or species richness are frequently used in the indirect approach and not data on community composition. Studies of CLEMENTS AND KIFFNEY (1994) and JOHNSON ET AL. (1992) showed that, indeed, benthic community composition was a better indicator of metal impact than species richness. Canonical Correspondence Analysis (CCA) is a direct multivariate ordination technique. This technique has the advantage that species composition is directly and immediately related to measured environmental variables (TER BRAAK 1986, 1990). Techniques like CCA are becoming more important in data handling in ecotoxicological field and laboratory studies (VERDONSCHOT AND TER BRAAK 1994, GOWERS ET AL. 1994, VAN WIJNGAARDEN ET AL. 1995).

BORCARD ET AL. (1992) presented a method for partitioning the variation of species abundance data into a spatial and an environmental component using CCA. The total variation in the species data was successfully partitioned into four independent components, with the components being purely spatial, purely environmental, a spatial component of environmental influence, and an undetermined component. The usefulness of this method was demonstrated in a study concerning the distribution patterns of subarctic plant species (HEIKKINEN AND BIRKS 1996). The method also seemed promising in partitioning the biological variation into ecological and ecotoxicological factors (PINEL-ALLOUL ET AL. 1996), but its potential has not been explored in assessing the relative contribution of trace metals in an environment with a dominant salinity gradient.

Total metal concentrations are used in most sediment risk assessment procedures to evaluate the effects of trace metals on, e.g., macroinvertebrates (VAN DER GUCHTE ET AL. 1991, CHAPMAN ET AL. 1997, MAXON ET AL. 1997). However, it is well accepted that it is not total but rather bioavailable concentrations that cause the effects on the biota (LANDRUM AND ROBBINS 1990, ALLEN 1993, WRIGHT 1995, BERRY ET AL. 1996) and thus on the community. Therefore, it may be assumed that total metal content is an inappropriate variable for characterizing metal exposure or habitat quality. Normalizing metal concentrations may approach bioavailable concentrations. Different methods, such as normalizing on organic carbon, small sediment fractions, conservative elements such as aluminum, and acid volatile sulfide, have been proposed that better approach bioavailability than does total concentrations. Two common methods in sediment risk assessment procedures in The Netherlands are normalizing on organic carbon or on clay. Both methods are simple and convenient for large sample numbers. These two methods are selected in the present study for investigating their effects on the variance partitioning because only from these normalization phases data are available. Normalizing trace metals might also reduce the covariation between metals and the variable used for normalization, resulting in a lower correlation between the variables. The more independent variables are, the more precise they can explain observed variation in biological data sets using CCA. A translation of total metal concentration to an approximate available or normalized concentration thus might improve the identification of sources of toxic stress among common ecological sources of stress in the field.

The objective of this paper is to quantify the effect of trace metals (Cd, Cu, Pb, and Zn) in structuring macroinvertebrate communities in the Northsea Canal in The Netherlands, an environment with multiple stressors. The impact of trace metals is compared with the contribution of the ecological factors chloride, depth, granulometry, and organic carbon. A secondary objective is to demonstrate that approaching bioavailability of trace metals by normalizing metal concentrations gives a better quantification of the effects of trace metals then total sediment concentrations. Two normalization procedures, normalizing trace metal concentrations on organic carbon and on sediment fraction < 63 µm, are tested in the present study.

Materials and methods

Study sites and sampling design

The Northsea Canal in The Netherlands (Figure 1) is 27 km long, 170 m wide, and 15 m deep and is the main shipping route from the North Sea to the harbors of Amsterdam.

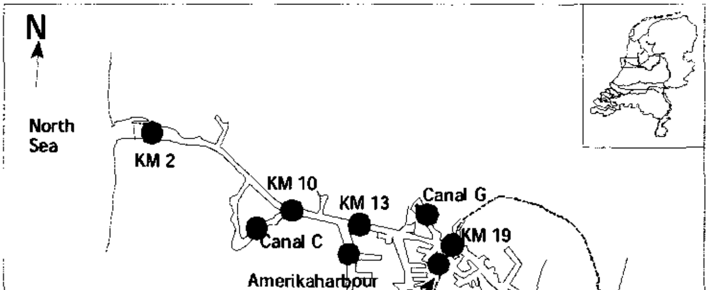

Figure 1: Map of the Northsea Canal showing the position of the sampling stations.

The eastern part of the canal is connected with Lake IJsselmeer, and from there, the canal receives freshwater from the River Vecht and some other canals. A sluice in the west end prevents the free flow of seawater entering the canal. However, opening the sluice gate for ship passage results in salt water entering the canal. Salinity decreases from west to east due to both water flows. Differences in density between salt and freshwater also create a vertical salinity gradient where salinity increases with depth. Both gradients are stable within and between years. Sediments in different parts of the Northsea Canal are polluted with a variety of substances, like oil, polyaromatic hydrocarbons, and trace metals (VAN KLAVEREN 1989).

Across the Northsea Canal ecosystem, 11 stations were selected that differed in salinity. At each station, three to four sampling sites were chosen across the canal. These sampled sites differed in depth and thus in salinity. In total, 39 sites were sampled.

Five replicate Ekman grab (Hydro-Bios, Kiel, Germany) samples were taken per sampling site and mixed for sediment analyses of the upper 10 cm bottom sediment. Sediment samples were stored in plastic bags for granulometric analyses and in acid rinsed glass jars for analyses of trace metals. Three replicate bottom samples of macroinvertebrates were collected with an Ekman grab. The samples were rinsed using a sieve of 210 µm mesh size. All material retained in the sieve was preserved in 5% (v/v) buffered formalin solution (Merck, Amsterdam, The Netherlands). All macroinvertebrates were picked in the laboratory, sorted, counted, and identified as far as possible with available keys. The three replicates were combined and abundance was expressed as numbers/m^2.

Analyses

The variables measured are given in Table 1. Ecological variables (organic carbon, granulometry, depth, chloride) are distinguished from trace metals (Cd, Cu, Pb, Zn) to facilitate the interpretation of the results and to be able to calculate the relative contribution of trace metals. Depth was measured in the field. Chloride data were obtained from published data (ANONYMOUS 1987). All sediment samples were dried and weighted for granulometric analyses. Each sample was sieved thoroughly through successive sieves of 0, 63, 125, 250, 500, 1000, and 2000 µm mesh size. Each fraction was weighted and expressed as percentage of the total weight.

Organic carbon content was determined by wet oxidation according to Kurmies as described by HOUBA ET AL. (1995). Sediment samples were digested on a hot plate (170 °C) by successive additions of small quantities of concentrated nitric acid (Merck) followed by additions of concentrated hydrofluoric acid (Merck). Trace metals were then dissolved in an hydrochloric acid solution and, from the filtrates, concentrations of Cd, Cu, Pb, and Zn were measured by flame atomic absorption spectrometer (HOUBA ET AL. 1995).

Table 1: Frequency, minimum, maximum, average and standard deviation of measured environmental variables.

Variable	Unit	Frequency	Minimum	Maximum	Average	Standard Deviation
Ecological variables						
Depth	m	39	1.63	24.14	8.42	4.85
Organic carbon	%	39	0.26	12.62	4.26	3.75
Granulometry						
Fraction <63µm	%	39	0.1	100.0	50.4	48.7
Fraction >63µm	%	39	0.0	47.5	7.1	11.6
Fraction >125µm	%	39	0.0	71.4	19.4	23.3
Fraction >250µm	%	39	0.0	44.7	9.8	11.7
Fraction >500µm	%	39	0.0	16.1	3.3	4.6
Fraction >1000µm	%	39	0.0	9.9	2.1	2.7
Fraction >2000µm	%	39	0.0	50.0	7.9	14.2
Chloride						
< 500 mg/l		2				
500 - 1500 mg/l		7				
1500 - 2000 mg/l		6				
2000 - 3000 mg/l		9				
3000 - 5000 mg/l		6				
5000 - 10000 mg/l		5				
>10000 mg/l		4				
Trace metals						
Cd	mg/kg	39	0.05	6.70	1.46	1.84
Cu	mg/kg	39	3.30	167.70	66.75	57.76
Pb	mg/kg	39	10.00	632.10	135.47	125.86
Zn	mg/kg	39	13.20	1142.70	325.74	294.32

Statistical analysis

Pearson correlation coefficients were calculated between the environmental variables. Factor analysis with Varimax rotation (HAIR ET AL. 1995) was used in order to verify the association of metals with the bulk sediment composition. Factor analyses were carried out with three different data sets. Data set 1 included the ecological variables (organic carbon, granulometry, depth, and chloride) and the total trace metal concentrations; data set 2 included the ecological variables and metal concentrations normalized on organic carbon; and data set 3 included the ecological variables and metal concentrations normalized on the sediment fraction <63 µm.

The relative contribution of trace metals in explaining the total variation in the macroinvertebrate taxonomic composition was determined by using the method of partial CCA as proposed by BORCARD ET AL. (1992). Detrended correspondence analysis assesses the length of the gradient and hence whether a linear or unimodal method should be used. A preliminary detrended correspondence analysis with logarithmic transformed abundance data and downweighting of rare species showed moderate to long gradient lengths. Therefore, the unimodal model (TER BRAAK 1986) was considered appropriate for these data sets. A preliminary CCA with ecological variables and trace metals (Table 1) as independent variables was performed in order to select an appropriate subset of independent variables. An analysis with all these variables resulted in a few high variance inflation factors (>20), indicating multicollinearity between some variables (TER BRAAK 1990). High inflation factors were obtained for the two coarsest sediment fractions. A subsequent analysis without these two fractions resulted in inflation factors lower than the maximum acceptable value. Both sediment fractions were left out in all subsequent analyses.

Five different CCAs were performed to calculate the contribution of the ecological variables and of the trace metals in explaining the variation in the species data: (1) CCA of species data and ecological variables (granulometry, organic carbon, depth, chloride) and trace metals (Cd, Cu, Pb, Zn) as independent variables, (2) CCA of species data constrained by the ecological variables, (3) CCA of species data constrained by trace metals, (4) the same as (2) after removing the effect of trace metals, and (5) the same as (3) after removing the effect of ecological variables

The total variation present in the species data is given by the sum of all unconstrained eigenvalues and is called total inertia (TER BRAAK 1990). Total inertia is equal for all five analyses. The proportion of variance explained by a set of variables is calculated as the sum of all canonical eigenvalues of a CCA divided by the total inertia. Step 1 provides the overall amount of explained variation. The total variation of the species matrix can be partitioned as follows: variation explained solely by common ecological variables (step 4), variation explained solely by trace metals (step 5), variation shared by ecological variables and trace metals (step 2 minus step 4 or step 3 minus step 5), and unexplained variation: total variation minus step 1.

The above procedure was applied to all three data sets. The contributions of the four sources were then compared for the three data sets. The data set that had the lowest percentage for shared plus unexplained variation was regarded as the best. The contribution of each single variable was assessed by a CCA of species data constrained by each single variable after removing the effect of all other variables. All (partial) CCAs were performed using the CANOCO program (TER BRAAK 1990). The statistical significance of the effect of each set of environmental variables was tested by a Monte Carlo permutation test (TER BRAAK 1990).

Results

Macroinvertebrate composition

A total of 42 different macroinvertebrate taxa were sampled in the Northsea Canal and its adjacent harbors and side canals (Table 2). The number of taxa per site ranged from 0 to

19, the number of individuals from 0 to 164133 individuals/m^2, and the shannon-wiener diversity (WASHINGTON 1984) ranged from 0 to 1.73. Tubificidae, *Corophium multisetosum*, Gammaridae, and *Streblospio shrubsolii* occurred in more than 50% of the samples. Taxa with high abundance values (more than 15% of total individuals) were *Potamopyrgus antipodarum*, *Streblospio shrubsolii*, and Tubificidae. Among the taxa found, typical freshwater taxa like *Glossiphonia heteroclita*, *Helobdella stagnalis*, and *Ophidonais serpentina* were present, as well as taxa typical for brackish waters, like Polychaeta, *Cyathura carinata*, *Congeria cochleata*, and *Corophium*.

Table 2: Frequency and abundance of taxa collected in the Northsea Canal.

Species name	Abbreviation	Frequency		Total abundance	
		Abs	Rel	Abs	Rel
Tricladidae					
Tricladida sp	TRCLADSP	3	7.7	12	0.0
Polychaeta sedentaria					
Ampharete sp.	AMPHARET	9	23.1	252	0.7
Manayunkia aestuarina	MANAAEST	2	5.1	444	1.2
Polydora sp.	POLYDOSP	18	46.2	527	1.4
Pygospio elegans	PYGOELEG	2	5.1	5	0.0
Spionidae sp.	SPIONISP	2	5.1	18	0.0
Streblospio shrubsolii	STRESHRU	20	51.3	10149	27.9
Tharyx marioni	THARMARI	5	12.8	891	2.5
Polychaeta errantia					
Nereis sp.	NEREISSP	17	43.6	197	0.5
Oligochaeta					
Enchytraeidae	ENEIDAE	3	7.7	13	0.0
Lumbriculidae	LUCULIAE	6	15.4	32	0.1
Nais sp.	NAISSPEC	10	25.6	530	1.5
Ophidonais serpentina	OPHISERP	2	5.1	24	0.1
Paranais litoralis	PARNLITO	6	15.4	76	0.2
Stylaria lacustris	STLALACU	3	7.7	20	0.1
Tubificidae	TUFICIAE	32	82.1	6818	18.7
Hirudinea					
Glossiphonia heteroclita	GLSIHETE	1	2.6	1	0.0
Helobdella stagnalis	HEBDSTAG	1	2.6	1	0.0
Bivalvia					
Cerastoderma edule	CERAELUD	1	2.6	1	0.0
Congeria cochleata	CONGCOCH	14	35.9	301	0.8
Dreissena polymorpha	DREIPOLY	4	10.3	55	0.2
Pisidiidae	PISIDIAE	1	2.6	4	0.0
Bivalvia sp. 1	BIVALSP1	3	7.7	4	0.0

Table 2: continued

Species name	Abbreviation	Frequency		Total abundance	
		Abs	Rel	Abs	Rel
Gastropoda					
Bithynia tentaculata	BITHTENT	1	2.6	1	0.0
Peringia ulvae	PERIULVA	2	5.1	4	0.0
Potamopyrgus antipodarum	POTAANTI	18	46.2	13402	36.9
Valvata piscinalis	VALVPISC	4	10.3	46	0.1
Crustacea					
Cirripedia sp.	CIRRPDSP	2	5.1	4	0.0
Rhitropanopeus harrissi ssp. *tridentatus*	RHITTRID	5	12.8	12	0.0
Neomysis integer	NEOMINTE	11	28.2	46	1.9
Cyathura carinata	CYATCARI	2	5.1	83	0.2
Asellidae	ASELLIAE	2	5.1	3	0.0
Gammaridae	GAMMARAE	20	51.3	694	1.9
Corophium lacustre	COROLACU	10	25.6	295	0.8
Corophium multisetosum	COROMULT	23	59.0	1269	3.5
Ecnomus tenellus	ECNOTENE	1	2.6	1	0.0
Chironomidae					
Chironomus plumosus i.w.s.	CHIRPLUM	4	10.3	15	0.0
Dicrotendipes gr nervosus	DITEGNER	5	12.8	24	0.1
Glyptotendipes sp.	GLTOTESP	4	10.3	81	0.2
Polypedilum sp.	POPESPEC	2	5.1	2	0.0
Procladius s.l.	PRDIUSSP	3	7.7	5	0.0
Stempellina sp.	STEMPELL	1	2.6	1	0.0

Correlation coefficients between variables

Table 3 shows the Pearsons correlation coefficients for all variables included in the analysis. It appeared that 68 out of 91 correlation coefficients were significant. High correlations were found among trace metals, among certain sediment fractions, between trace metals and organic carbon, and between depth and chloride. Trace metals had stronger correlations with organic carbon content than with sediment fraction <63 µm.

Factor analyses

Factor analysis with data set 1 (total trace metal concentrations) produced two components explaining 74.8% of the total variation; with data set 2 (normalized concentrations on organic carbon), three components explaining 72.0% of the total variation; and with data set 3 (normalized on sediment fraction <63 µm), three components explaining 78.9% of the total variation (Table 4). To which factor a variable contributed most per data set can be derived from the italicized loadings in Table 4. For example, the first factor of data set 1 was comprised primarily by sediment trace metal concentrations, organic

Table 5: Partitioning of the variance in the species data; note that the sum of variances explained by the individual ecological factors (or individual trace metals) does not necessarily equals the variance explained by the ecological factors (or trace metals) as a group.

Source of variance	Metal concentration					
	Total (data set 1)		Normalized on organic matter (data set 2)		Normalized on fraction < 63µm (data set 3)	
Ecological factors	37.5		45.4		38.4	
Depth		1.5		3.7		1.6
Organic carbon		2.2		3.6		2.7
Granulometry		13.0		17.3		14.6
Chloride		1.8		4.3		2.2
Trace metals	10.3		8.6		11.4	
Cd		2.2		2.8		2.7
Cu		2.9		2.7		3.2
Pb		2.5		1.0		2.8
Zn		1.9		2.0		2.3
Shared by ecological factors and trace metals	10.2		2.5		9.4	
Unexplained	42.0		43.5		40.8	
Total	100.0		100.0		100.0	

Although the overall contribution of trace metals is 8.6 % only, the partial CCA in which the taxa data were constrained by trace metals normalized on organic carbon after removing the effect of the other variables was significant ($P \leq 0.01$; Monte Carlo test). Figure 2 shows the positions of taxa from selected taxonomic groups in the ordination diagram of this analysis. The constructed axes are a linear combination of the variables included in the analysis (TER BRAAK 1986, 1990); hence, the metals determine the sequence of the taxa. Both Cd and Zn are pointing to the left-hand side and are closely related to the first canonical axis. The sequence of the taxa along the first axis was therefore mainly determined by these two metals. Cu is closely related to the second canonical axis, and therefore the sequence of the taxa along this second axis was mainly determined by this metal. The length of the arrow of Pb is short, indicating that the impact of Pb on the community was low.

Projection of the Chironomidae taxa (Figure 2a) shows that they were all associated with lower lead concentrations. *Procladius* sp., *Chironomus plumosus*, and *Polypedilum* sp. were associated with higher concentrations of Cu, Zn, and Cd, whereas *Dicrotendipes gr nervosus*, *Glyptotendipes* sp., and *Stempellina* sp. were not found at elevated metal concentrations. Bivalve taxa (Figure 2b) had different positions on all four metal axes. *Cerastoderma edule* and Pisidiidae were associated with higher Cd and Zn concentrations, whereas *Dreissena*

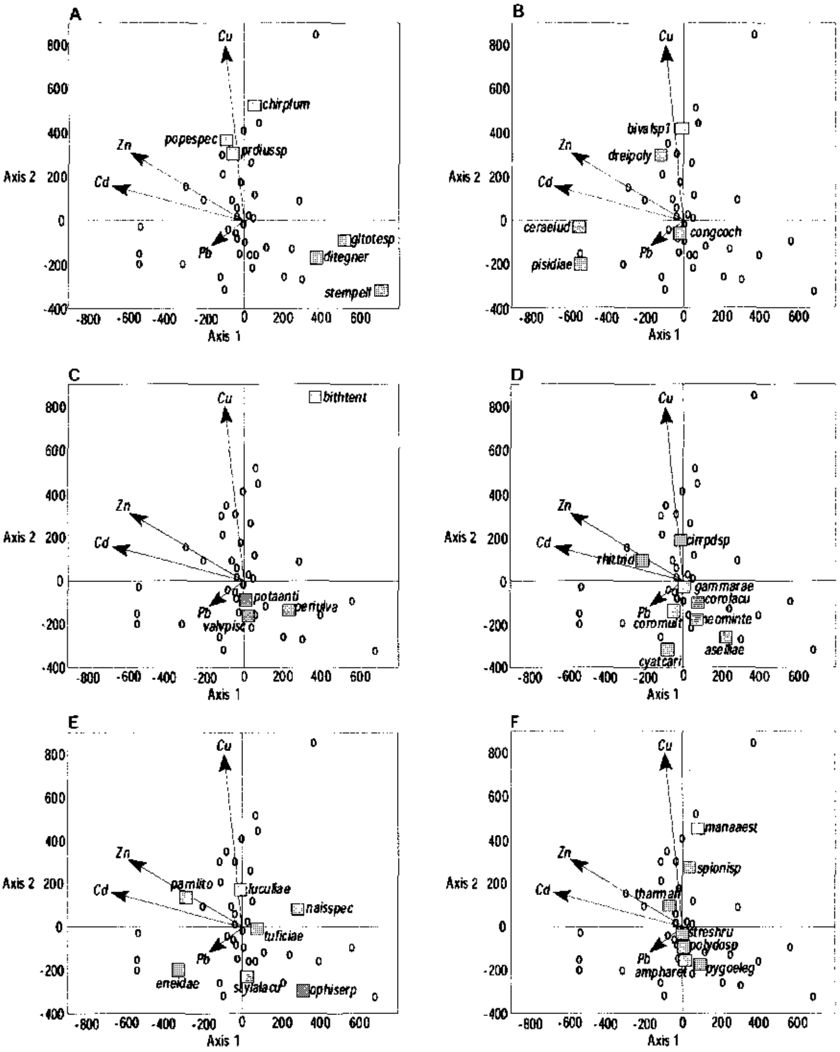

Figure 2: The positions of taxa from selected taxonomic groups in the ordination diagram of a partial canonical correspondence analysis in which the species data were constrained by trace metals normalized on organic carbon after removing the effects of other variables. (a) Chironomidae, (b) Bivalvia, (c) Gastropoda, (d) Crustacea, (e) Oligochaeta, and (f) Polychaeta sedentaria. Trace metals are represented by arrows, taxa from the selected taxonomic groups by squares and their abbreviations (see Table 2), and other species by open circles. The length of the arrow is a measure for the importance of the trace metal, and the arrowhead points at the direction of increasing influence. Projection of the taxa on the metal axes indicates the impact of the metal on the taxa such that taxa near the arrowhead are more affected by the trace metal than taxa present at the arrow tail.

polymorpha and Bivalvia species 1 were associated with higher Cu concentrations. From the gastropods (Figure 2c), only *Bithynia tentaculata* showed association with elevated Cu concentrations. *Peringia ulvae* seemed to avoid elevated metal concentrations. Crustacea (Figure 2d) were mainly positioned in the center of the diagram. Especially Asellidae and *Cyathura carinata* were not found at elevated trace metal concentrations. Projection of Oligochaeta (Figure 2e) showed that the different taxa had different tolerances for the trace metals. Especially *Stylaria lacustris* and *Ophidonais serpentina* were not found at elevated metal concentrations. Tubificidae are placed in the center of the ordination diagram, and this might be due to the level of identification (family and not species level). It is well known that some members of this family are very pollutant tolerant whereas others pollutant intolerant. The taxa of the Polychaeta sedentaria (Figure 2f) showed differences in tolerance especially for Cu and hardly for Zn, Cd, and Pb.

Discussion

The macroinvertebrate communities from the Northsea Canal and its adjacent harbors and side canals are relatively poor in species richness. REMANE AND SCHLIEPER (1958) already demonstrated that brackish water communities have a low diversity in comparison to salt or freshwater communities, and most (brackish water) communities consist of a subset of tolerant species. Although salinity was thought to be the most important factor in structuring macroinvertebrate communities, this is not supported by the results of the present study. Sediment granulometry rather than salinity was the most important factor explaining the biological variance. In part, this might be due to the use of chloride classes instead of measured chloride concentrations. Small differences in concentrations are disregarded when using classes. However, the results are consistent with other studies in which sediment granulometry is an important factor in structuring macroinvertebrate communities (TOLKAMP 1980, WATLING 1991).

Total trace metal sediment concentrations found in this study in the Northsea Canal are of the same order of magnitude as those in the deltas of other Western European rivers (BRYAN AND LANGSTON 1992, ZWOLSMAN ET AL. 1996). Trace metal concentrations exceeded Dutch standards (MINISTRY OF TRANSPORT AND WATER MANAGEMENT 1998) only at a few sites. Normalization of sediment trace metal concentrations on organic carbon or on <63 µm particle size fraction are well-established procedures to correct for differences in sorption affinity among sediments. In comparison to total metal concentrations, the normalized concentrations are supposed to better resemble the concentrations in the food of the benthic organisms (assuming that organic carbon is the dominant food) and have a closer relationship with pore-water metal concentrations to which benthic organisms are exposed. In the current study, the metal concentrations were correlated significantly with both organic carbon and with the small grain size fraction, which agrees with earlier reports (TAYLOR 1986,

FÖRSTNER 1990, SANTSCHI ET AL. 1997).

Factor analyses with normalized trace metal concentrations on either organic carbon or on smallest sediment fraction resulted in this study in a better separation of the trace metals from the other variables than with total concentrations. This agrees with the results provided by ZWOLSMAN ET AL. (1996), who found for the Scheldt estuary that metal contents normalized on organic carbon were comparable to the contents normalized on the fraction <16 μm. This may be explained from the fact that organic carbon and the clay fraction are often correlated with each other and may have similar magnitude. However, this study showed much better correlation of metals with organic carbon than with clay/silt. This suggests a dominant role of organic carbon in metal binding in the Northsea Canal as compared with the role of metal-binding phases, associated with the clay/silt particles. The method of variance partitioning applied in this study also showed that normalization on organic carbon gave a much better discrimination of the sources of variance in the biological data. Hence, it is suggested that organic carbon normalized concentrations better approach the bioavailable concentrations than total metal concentrations or clay/silt normalized concentrations.

In this study, the partial canonical correspondence analyses showed that about 56% of the total variation in the taxa could be explained by the variables used in the analyses. Compared with other studies (CATTANEO ET AL. 1995, PINEL-ALLOUL ET AL. 1996), this percentage is quite high. A considerable amount of the biological variance was explained in this study by common ecological variables (45%) such as granulometry and chloride, a smaller part by trace metals (8.6%), and a very small part by variance shared by ecological variables and trace metals (2.5%). The relatively small contribution of trace metals is, however, significant. The metal contribution in this study is comparable to that found in other studies (CATTANEO ET AL. 1995, PINEL-ALLOUL ET AL. 1996).

This study also suggested specific sensitivity of taxa to different metals. For instance, judging from their distribution in the Northsea Canal, Chironomidae, Gastropoda, and Polychaeta sedentaria seem to have low tolerances to Cd and Zn whereas Oligochaeta and Crustacea seem to have a low tolerance to Cu. These patterns are not all in accordance with various results reported in the literature such as the supposed higher tolerance to Zn of chironomids due to their capacity to regulate Zn compared with other arthropods (PHIPPS ET AL. 1995). Also, the present results are not in line with those of HALL ET AL. (1998), who found that gastropods followed by amphipods were the most sensitive species to acute Cu exposure. This study showed that some, but not all, gastropods were less abundant at sites with elevated Cu concentration, but so were some taxa among Chironomidae, Polychaeta sedentaria, Oligochaeta, and Crustacea. Although many authors have reported that some Oligochaeta and Chironomidae are tolerant to high metal contamination (ROSENBERG AND RESH 1993), this study showed that, in these taxonomic groups, several species were confined to the cleaner sites. Note that, in case of Tubificidae, a course level of identification was used and that it is well known that there are pollution-tolerant and pollution-intolerant tubificids.

Although a large part of the total biological variance was explained, another part remains unexplained due to, e.g., stochastic events, natural fluctuations, random error, and certain factors or aspects not considered in this study. For example, concentrations of mineral oil and PAH in some of the Northsea Canal sediments were considerable (VAN KLAVEREN 1989). However, the available data were insufficient to incorporate them into this study. Also, biological factors controlling macroinvertebrate community composition and species abundance, like predation by fish and birds on macroinvertebrates and competition for food or for habitat, might contribute to the unexplained variance.

The contribution of trace metals in structuring macroinvertebrate composition and abundance in the Northsea Canal was about 8% according to this study. Whether this percentage is considered severe or not depends on the targets set in the risk assessment procedure (CALOW 1998) in which assessment endpoints are the actual environmental values to be protected (US ENVIRONMENTAL PROTECTION AGENCY 1992). The observed communities in the Northsea Canal consisted mainly of tolerant species due to the occurrence of the salinity gradient, which constitutes a stressful environment. Truly sensitive species were almost absent. Therefore, the impact of trace metals in the Northsea Canal was probably more related to the loss of sensitive specimens of tolerant species than to a loss of sensitive species.

An attempt was made in this study to link the composition of *in situ* benthic macroinvertebrate community to simultaneously acting multiple stressors. Partitioning of the observed variance in the biological data by partial CCA showed that both common ecological factors and trace metals affected the macroinvertebrate community composition. The impact of sediment-bound trace metals could be quantified and could also be related to shifts in some taxonomic groups. Normalization on organic carbon improved the discrimination of sources of variance, which was attributed to an improved estimate of exposure. Applying the method of variance partitioning in an environment with multiple stressors provided very useful information on the magnitude of anthropogenic impact and is therefore recommended for incorporating in further risk assessment studies.

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CHAPTER 4

EVALUATION OF BIOASSAYS VERSUS CONTAMINANT CONCENTRATIONS IN EXPLAINING THE MACROINVERTEBRATE COMMUNITY STRUCTURE IN THE RHINE-MEUSE DELTA, THE NETHERLANDS

Edwin THM Peeters, Anouk Dewitte, Albert A Koelmans, Joan A van der Velden
and Pieter J den Besten

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> *... was die Dinge an sich sein mögen,*
> *weiß ich nicht und brauche es auch nicht zu wissen,*
> *weil mir doch niemals ein Ding anders*
> *als in der Erscheinung vorkommen kann ...*
>
> *Emmanuel Kant*
> *Kritik der reinen Vernunft, 1781*

Abstract

It is often assumed that bioassays are better descriptors of sediment toxicity than toxicant concentrations and that ecological factors are more important than toxicants in structuring macroinvertebrate communities. In the period 1992 to 1995, data were collected in the enclosed Rhine-Meuse delta, The Netherlands, on macroinvertebrates, sediment toxicity, sediment contaminant concentrations, and ecological factors. The effects of various groups of pollutants (polycyclic aromatic hydrocarbons, trace metals, oil, polychlorinated biphenyls) and of ecological variables on the structure of the macroinvertebrate community were quantified. Ecological factors explained 17.3% of the macroinvertebrate variation, while contaminants explained 13.8%. Another 14.7% was explained by the covariation between ecological variables and contaminants. Polycyclic aromatic hydrocarbons explained a larger part of the variation than trace metals. The contributions of oil and polychlorinated biphenyls were small but significant. Elevated contaminant concentrations were significantly associated with differences in the macroinvertebrate food web structure.

The response in bioassays (*Vibrio fischeri, Daphnia magna, Chironomus riparius*) was susceptible to certain contaminants but also to certain ecological factors. There was a weak correlation between *in situ* species composition and bioassays; 1.9% of *in situ* macroinvertebrate variation was explained by the bioassay responses. This seems to contradict the validity of using bioassays for a system-oriented risk assessment. Possible reasons for this discrepancy might be the manipulations of the sediment before the test and a higher pollutant tolerance of the *in situ* macroinvertebrates. Thus, macroinvertebrate field surveys and laboratory bioassays yield different types of information on ecotoxicological effects, and both are recommended in sediment risk assessment procedures.

Key words: Benthic, Community structure, Correspondence analysis, Pollution, Variance partitioning

Introduction

In sedimentation areas, aquatic sediments form a sink for toxic contaminants due to the adsorption capacity of clay minerals and organic matter and thus pose a potential threat to benthic aquatic biota. In The Netherlands, the southern delta of the rivers Rhine and Meuse has been isolated from the tidal movement of the North Sea since the construction of two dams in 1969 and 1970. This has resulted in increased sedimentation of contaminated solids.

During the period 1992 to 1995, data were collected in various parts of the enclosed Rhine-Meuse delta on benthic macroinvertebrates, contaminant concentrations, sediment toxicity, and bulk sediment characteristics. Assessment of the sediment quality by the triad approach (CHAPMAN ET AL. 1997) showed that the sediment contamination presented risks to the biota (DEN BESTEN ET AL. 1995). Further, multivariate analyses showed that sediment toxicity could indeed explain part of the variation in species composition but also revealed that the species composition was determined more by habitat and sediment characteristics than by sediment toxicity (REINHOLD-DUDOK VAN HEEL AND DEN BESTEN 1999). Although effects of contaminants were indeed observed in these studies, the magnitude of the effects of these substances in relation to each other and to the contribution of other stressors such as habitat variables remained unclear.

Multivariate statistical tools such as principal component analysis, multiple regression, discriminant analysis, and multidimensional scaling can be used to assess the relative importance of different environmental stressors (TER BRAAK 1986, 1990, HAIR ET AL. 1995). Among multivariate techniques, canonical correspondence analysis (CCA) is a direct ordination technique, which can be used to relate species composition directly to measured environmental variables (TER BRAAK 1986, 1990). Techniques like CCA are becoming more important for data handling in ecotoxicological field and laboratory studies (e.g. GOWER ET AL. 1994, VERDONSCHOT AND TER BRAAK 1994, VAN WIJNGAARDEN ET AL. 1995, PEETERS ET AL. 2000).

BORCARD ET AL. (1992) presented a method for partitioning the variation in species abundance data into a spatial and an environmental component using canonical correspondence analysis. This method of variance partitioning was successfully applied to distinguish between variation explained by ecological factors, variation explained by contaminants, variation shared by ecological factors and contaminants, and unexplained variation (PINEL-ALLOUL ET AL. 1996, PEETERS ET AL. 2000). The present study addresses some new aspects in that it includes data from multiple water systems, analyzes macroinvertebrate communities in relation to ecological factors and different groups of contaminants, and analyzes macroinvertebrate communities in relation to the results of laboratory bioassays.

It is generally accepted that it is not the total but the bioavailable concentrations of contaminants that should be related to the effects on biota (e.g. LANDRUM AND ROBBINS 1990, ALLEN 1993, WRIGHT 1995, BERRY ET AL. 1996). Bioassays are assumed to detect the effects of combinations of known and unknown contaminants (REINHOLD-DUDOK VAN HEEL AND DEN BESTEN 1999), which is why they are frequently used in sediment risk assessment, usually as part of the sediment quality triad approach (CHAPMAN ET AL. 1997). However, the relationship between the results of bioassays and *in situ* macroinvertebrate community structure has hardly been studied (e.g. CRANE ET AL. 1995, DAY ET AL. 1995a), nor has its explanatory potential been assessed with direct multivariate methods.

The objectives of the present study were to investigate the relationship between the results of laboratory toxicity tests and *in situ* macroinvertebrate community composition and to quantify the effect of different groups of contaminants on the structure of the macroinvertebrate communities in the enclosed Rhine-Meuse delta. Various statistical analyses were performed on a large database containing information on the distribution and abundance of macroinvertebrates in the enclosed Rhine-Meuse delta as well as on abiotic circumstances and on the results of three different bioassays.

Materials and methods

Sampling, chemical analyses, and bioassays

From 1992 to 1995, field studies were performed in the Rhine-Meuse delta. Sites were chosen to cover the wide range of environmental conditions and provide representative samples of the Meuse-Rhine delta ecosystem. The distances between the samples were such that they could be regarded as independent. This was confirmed by preliminary geostatistical analyses showing that there was no spatial autocorrelation for the main environmental variables and indicating weak correlations for only a few contaminants. In the present study, only those sites were selected for which data were available on *in situ* macroinvertebrates, all chemical analyses, and all three bioassays. Data were available for the Nieuwe Merwede (n=24), Hollandsch Diep (n=46), Dordtsche Biesbosch (n=10), Brabantsche Biesbosch (n=58), and Haringvliet (n=57) water systems (Figure 1). Each site was sampled once in the period 1992 through 1995. At all sites, macroinvertebrates were collected from the top layer of the sediment. Variables measured in the top layer of the sediment at each sampling site included grain-size composition, organic matter content, sediment dry weight, and concentrations of trace metals (arsenic, cadmium, chromium, copper, lead, mercury, nickel, zinc), polychlorinated biphenyls (PCBs), oil, and polycyclic aromatic hydrocarbons (PAHs: acenaphtene, anthracene, benzo[*a*]anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, benzo[*a*]pyrene, chysene,

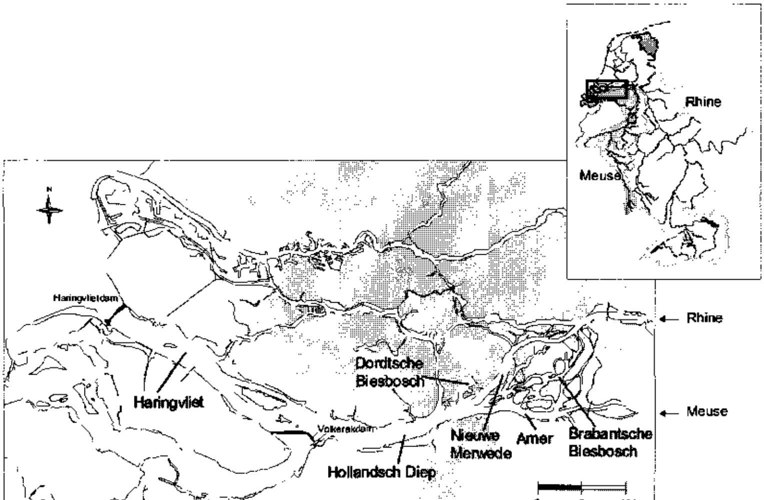

Figure 1: Map of study area.

dibenzo[*ah*]anthracene, fluorene, fluoranthene, indeno(*1,2,3-cd*)pyrene, naphtalene, phenanthrene, pyrene). Additionally, a number of descriptive ecological variables were measured that are considered important for the distribution of macroinvertebrates including current velocity, depth, pH, erosion by shipping, wind erosion, and degree of consolidation of the sediment. Sediment toxicity was assessed by means of three laboratory bioassays. Whole-sediment bioassays were performed with *Chironomus riparius* (chironomid larvae) and pore-water bioassays with *Daphnia magna* (water flea) and the bacterium *Vibrio fischeri*. The results of the bioassays were classified according to DEN BESTEN ET AL. (1995) and the criteria are given in Table 1. A detailed description of the sampling methods, identification of macroinvertebrates, chemical analyses, and bioassays has been published elsewhere (DEN BESTEN ET AL. 1995, REINHOLD-DUDOK VAN HEEL AND DEN BESTEN 1999).

Table 1: Classification of the effects observed in the whole-sediment bioassay with *Chironomus riparius* and in the pore-water bioassays with *Daphnia magna* and *Vibrio fischeri* into three effect classes. NOEC=no observed effect concentration; EC=effective concentration.

Bioassay	Effect	Effect class	Criteria
Chironomus riparius	no effect	0	>75% hatched larvae and mortality/developmental retardation ≤10% and effect on dry weight ≤10%
	moderate effect	1	50-75% hatched larvae or mortality/developmental retardation 10-50% or effects on dry weight 10-25%
	strong effect	2	<50% hatched larvae or mortality/developmental retardation ≥50% or effect on dry weight ≥25%
Daphnia magna	no effect	0	$NOEC_{mortality/reproduction}$ = 100% pore water/elutriate
	moderate effect	1	10% < $NOEC_{mortality/reproduction}$ <100% pore water/elutriate
	strong effect	2	$NOEC_{mortality/reproduction}$ ≤ 10% pore water/elutriate or ≥50% mortality in 100% pore water/elutriate within 48 h
Vibrio fischeri	no effect	0	EC_{20} ≥50% pore water/elutriate
	moderate effect	1	10%< EC_{20} <50% pore water/elutriate
	strong effect	2	EC_{20} ≤10% pore water/elutriate

Statistical analyses

Multinomial logistic regressions were performed to investigate which variables and which species were significantly correlated (likelihood ratio test, $P \leq 0.05$) with the observed results of the three bioassays. The analyses were performed using SPSS for Windows (Chicago, IL, USA).

Prior to the multivariate analyses, abundance data were logarithmically transformed [ln(x+1)]. The option downweighting of rare species in (detrended) correspondence analysis offers the opportunity to give less weight in the calculation to species that are rare in the database. This option was used in all analyses. A preliminary detrended correspondence analysis was performed to assess the length of the gradient. Since this length was found to be moderate to long, a unimodal response model was considered appropriate for this data set (TER BRAAK 1986). A preliminary canonical correspondence analysis (CCA) using all variables as explanatory variables was performed in order to select an appropriate subset of variables. This analysis resulted in a few high variation inflation factors (>20), indicating multicollinearity among some variables (TER BRAAK 1990). The variable with the highest variation inflation factor was omitted from the subsequent analysis. This procedure was repeated until all variation inflation factors were lower than the maximum acceptable value of 20. The rejected variables (latitude, longitude, benzo[*a*]anthracene, benzo[*a*]pyrene, and pyrene) were excluded from all further analyses.

The contribution of the different variables was quantified using partial CCA as proposed by BORCARD ET AL. (1992). Calculating the relative contribution of a subset of variables to the explanation of the observed biological variation is rather complex. Simply calculating the relative contribution through a direct analysis with the subset variables as explanatory variables is not sufficient because this overestimates the contribution due to possible covariance with other variables. This problem can be overcome by means of another analysis in which the effect of the other variables is removed and the remaining variation is related to the subset. To do so, it is necessary to divide the explanatory variables into two subsets. Subset I includes the data with the variables of interest (e.g. PAHs), while subset II includes the data of all other variables. Five different CCA runs were performed to calculate the contributions of the two subsets in explaining the variation in the species data. The five CCA runs were CCA of species data and subset I and subset II as explanatory variables (run 1), CCA of species data constrained by subset I (run 2), CCA of species data constrained by subset II (run 3), the same as run 2 after removal of the effect of subset II (run 4), and the same as run 3 after removal of the effect of subset I (run 5).

The total variation in the species data is called total inertia and is given by the sum of all unconstrained eigenvalues (TER BRAAK 1990). The proportion of variance explained by a set of variables is calculated as the sum of all canonical eigenvalues of a CCA divided by the total inertia. Hence, run 1 provides the overall amount of explained variation. The total variation of the species matrix can be further partitioned as variation explained solely by subset I (run 4), variation explained solely by subset II (run 5), variation shared by subset I and subset II (run 2 minus run 4 or run 3 minus run 5), and unexplained variation (total variation minus run 1).

The above procedure was used to calculate the contribution of all contaminants together as well as the contribution of each individual group of contaminants (PAHs, trace metals, oil, PCBs). This was done for the data set with all data from the study area as well as for the individual river branches. In all analyses, contaminant concentrations and depths were logarithmically transformed. Prior to transformation, contaminant concentrations were normalized to organic matter content to better approximate available concentrations.

In order to test the hypothesis that bioassays provide a better description of the effects of multiple stressors, the above procedure was also used to calculate the proportion of the total variation in the species data that could be explained by the three bioassays together with the ecological factors. The results of the analyses with the bioassays could then be properly compared with those of the analyses performed with the contaminant concentrations.

All (partial) CCAs were performed using the CANOCO program (TER BRAAK AND SMILAUER 1998). The statistical significance of the effect of each set of environmental variables was tested by a Monte Carlo permutation test (TER BRAAK 1990). CANOCO extracts four axes and calculates sample, species, and variable scores for these four axes. The order of the extracted axes is determined by the amount of information they contain. Ordination diagrams (see Figures 2 through 4) visualize the main structure of the multivariate data, usually in two dimensions (the first and second axes). The diagrams are prepared using the calculated sample, species, and variable scores. Sites and species are positioned as points in the diagram, while the variables are represented by arrows. The length of the arrow is a measure of the importance of the contaminant, while the arrowhead points in the direction of increasing influence. Variables can be interpreted in conjunction with the species points in a biplot. The species points can be projected onto a specific arrow to determine the ranking of the taxa according to this variable.

Results

Macroinvertebrate composition

A total of 115 different macroinvertebrate taxa were collected from the soft substrates of the Rhine-Meuse delta, mostly belonging to the taxonomic groups of Oligochaeta, Hirudinea, Bivalvia, Gastropoda, Crustacea, and Diptera. The number of taxa per site ranged from 6 to 54, the number of individuals from 250 to 55,480/m^2. Taxa that occurred in large numbers in all river branches were *Gammarus* sp., *Pisidium* sp., *Potamopyrgus antipodarum, Procladius* sp., Tubificidae with hair setae, Tubificidae without hair setae, and *Valvata piscinalis.*

The majority of the taxa collected, including *Asellus aquaticus, Chironomus* sp, *Cyrnus* sp, *Erpobdella octoculata,* and *Nais* sp, are not restricted to running waters. Typical standing water taxa (e.g. *Anisus vortex, Endochironomus albipennis)* were present as well as a limited number of species characteristic for large rivers e.g. *Corophium curvispinum, Hydropsyche contubernalis, Kloosia pusilla,* and *Limnesia undulata.* A number of exotic species were also present in the delta, including *Branchiura sowerbyi, Corbicula fluminea, Corbicula fluminalis, Corophium curvispinum, Dreissena polymorpha, Dugesia tigrina,* and *Gammarus tigrinus.*

Bioassays

The three bioassays gave different results with regard to the number of sediments that showed an effect (Table 2). Pore-water bioassays with *Vibrio fischeri* revealed effects for only 18% of the sediments and pore-water bioassays with *Daphnia magna* for 48% whereas whole-sediment bioassays with *Chironomus riparius* showed effects for 69% of the sediments. More than 50% of the sediments located in the Hollandsch Diep and Nieuwe Merwede water systems showed an effect in the pore-water test with *Daphnia magna,* while >50% of the sediments from the Brabantsche Biesbosch, Haringvliet, and Nieuwe Merwede systems had an effect in the whole-sediment bioassay with *Chironomus riparius.*

Table 2: Number of sediments in bioassay effect class for the enclosed delta and each individual river branch. See Table 1 for explanation of effect classes. BB=Brabantsche Biesbosch, DB=Dordtsche Biesbosch, HD=Hollandsch Diep, HV=Haringvliet, NM=Nieuwe Merwede.

Bioassay	Effect class	BB	DB	HD	HV	NM	Study area
		58	10	46	57	24	195
Whole-sediment assay with *Chironomus riparius*							
	0	24	6	26	0	5	61
	1	21	2	16	48	11	98
	2	13	2	4	9	8	36
Pore-water assay with *Daphnia magna*							
	0	41	7	14	35	5	102
	1	13	2	16	19	7	57
	2	4	1	16	3	12	36
Pore-water assay with *Vibrio fischeri*							
	0	55	5	25	54	20	159
	1	3	5	19	3	0	30
	2	0	0	2	0	4	6

Table 3 shows that the effect classes of all three bioassays were significantly related to a few toxicants and non-contaminants. Oil, which represents a mixture of compounds, was related to all three bioassays. The *Chironomus riparius* and *Daphnia magna* bioassays were not significantly related to trace metals or polyaromatic hydrocarbons. The results of the *Vibrio fischeri* bioassays were significantly related to the trace metals cadmium and mercury and to acenaphtene.

Table 3: Variables with significant contributions (likelihood ratio test: $P \leq 0.05$) in a multinomial logistic regression with the effect classes of the bioassay as dependent variable and all others as explanatory variables.

Whole-sediment assay	Pore-water assay	
Chironomus riparius	*Daphnia magna*	*Vibrio fischeri*
Ecological variables		
GS[1] fraction < 63μm	Longitude	Organic carbon
Erosion by wind	River branch	Sediment dry weight
River branch		GS fraction <63μm
		GS fraction >210μm
		Erosion by shipping
		River branch
Contaminants		
Oil	Oil	Oil
		Cadmium
		Mercury
		Acenaphtene

[1] GS = grain-size

A limited number of taxa were significantly correlated with the results of the bioassays (Table 4). The number of taxa showing a significant correlation with the pore-water bioassays was lower than the number of taxa showing a significant correlation with the whole-sediment assay. The pore-water bioassay with *Vibrio fischeri* was related to only one species, whereas the *Daphnia magna* bioassay was related to eight species. Some mollusks and some chironomidae had a significant correlation with the whole-sediment *Chironomus riparius* bioassay. Although the bioassay was performed with a species of the genus *Chironomus*, none of the *in situ Chironomus* species showed a significant relationship with the results of the bioassay.

Partitioning of the variance

Approximately 45% of the variation in the species data for the Rhine-Meuse delta was explained by all variables (both ecological variables and contaminants) included in the canonical ordination analyses (Table 5). The ecological factors as a group explained more of the variation in the species data (17.3%) than the contaminants as a group (13.8%). A considerable part of the explained variation was covariation between the combination of ecological variables and contaminants (14.7%). Among the ecological factors, river branch contributed most to the explanation of the biological variation (6.4%) followed by grain size (1.6%) and depth (1.1%). The importance of the factor river branch is in line with the results of the indirect ordination of the species data. Although the contribution of most variables was small (Table 5), the partial CCAs were significant ($P \leq 0.05$; Monte Carlo test) except for current velocity and pH. The contribution of each group of contaminants (PAHs, trace metals, oil, and PCBs) for the whole delta was significant ($P \leq 0.05$; Monte Carlo test). The relative importance of oil and PCBs was rather small, whereas the contributions of PAHs and trace metals were higher.

Table 4: Species with significant contributions (likelihood ratio test, $P \leq 0.05$) in a multinomial logistic regression with the effect classes of the bioassay as dependent variable and the species as explanatory variables.

Whole-sediment assay	Pore-water assay	
Chironomus riparius	*Daphnia magna*	*Vibrio fischeri*
Class Bivalvia	Class Oligochaeta	Class Arachnida
Musculium lacustre	*Psammoryctides barbatus*	*Limnesia maculata*
Class Gastropoda	*Dero digitata*	
Corbicula fluminea	*Uncinais uncinata*	
Valvata cristata	Class Bivalvia	
Valvata piscinalis	*Unio tumidus*	
Litoglyphus naticoides	Class Arachnida	
Class Arachnida	*Piona rotundoides*	
Piona alpicola	Class Insecta	
Piona pusilla	*Caenis horaria*	
Class Malacostraca	*Lipiniella arenicola*	
Corophium curvispinum	*Polypedilum bicrenatum*	
Class Insecta		
Cryptotendipes sp		
Dicrotendipes sp		
Lipiniella arenicola		
Stictochironomus sp		

A partial canonical correlation analysis was performed with macroinvertebrates constrained by contaminants after the effect of the ecological variables had been removed. The resulting species and contaminants distribution pattern for the first two axes is given in Figure 2. The axes constructed are linear combinations of the variables included; hence, the sequence of the taxa is determined by the contaminants. Most contaminant arrows point to the left-hand side, indicating that concentrations increase from the center to the left. The arrows of all trace metals are shorter than those of most PAHs and oil. This indicates that the sequence of the taxa is determined more by PAHs than by trace metals, as was also concluded from Table 5.

Table 5: Partitioning of the variance in the species data in percentages obtained from partial canonical correspondence analyses with contaminant concentrations and ecological factors as explanatory variables. Note that the sum of the variances explained by the individual ecological variables or contaminants does not equal the variance explained by the ecological variables or the contaminants as a group. See Table 2 for explanation of river branch abbreviations. Not significant contributions (Monte Carlo test, $P>0.05$) are italicized.

Source of variation	Delta	River branch		
		BB[1]	HD[2]	HV[3]
Ecological factors	17.3	16.8	15.7	15.6
River branch	6.4			
Current velocity	*0.4*	*1.7*	*2.5*	*0.9*
Organic carbon	0.6	*1.4*	-[4]	-
content	0.6	*0.9*	-	*1.2*
Sediment dry weight	1.6	*3.5*	*3.7*	*3.0*
Grain size	0.6	*1.5*	*2.4*	*1.6*
Consolidation	1.1	*1.9*	3.3	*1.8*
Depth	*0.3*	*1.4*	*1.8*	*1.7*
pH	0.6	*1.5*	*2.0*	*0.0*
Erosion by shipping	0.9	*0.9*	*1.0*	*1.5*
Erosion by wind				
Contaminants	13.8	32.5	31.9	27.6
PAHs	6.7	*14.9*	*12.6*	*12.0*
Trace metals	4.8	13.8	*13.1*	*15.4*
Oil	0.8	*1.6*	-	-
PCBs	0.6	*1.4*	*2.4*	-
Shared by ecological factors and contaminants	14.7	11.9	24.8	13.5
Unexplained	54.2	38.8	27.6	43.3
Total	100.0	100.0	100.0	100.0

[1]: additional variables omitted due to high VIF: chrysene, nickel

[2]: additional variables omitted due to high VIF: chrysene, fluoranthene, indeno(1,2,3-cd)pyrene

[3]: additional variables omitted due to high VIF: zinc, anthracene, benzo[*b*]fluoranthene, benzo[*ghi*]perylene, phenanthrene

[4]: - indicates variables omitted due to high VIF

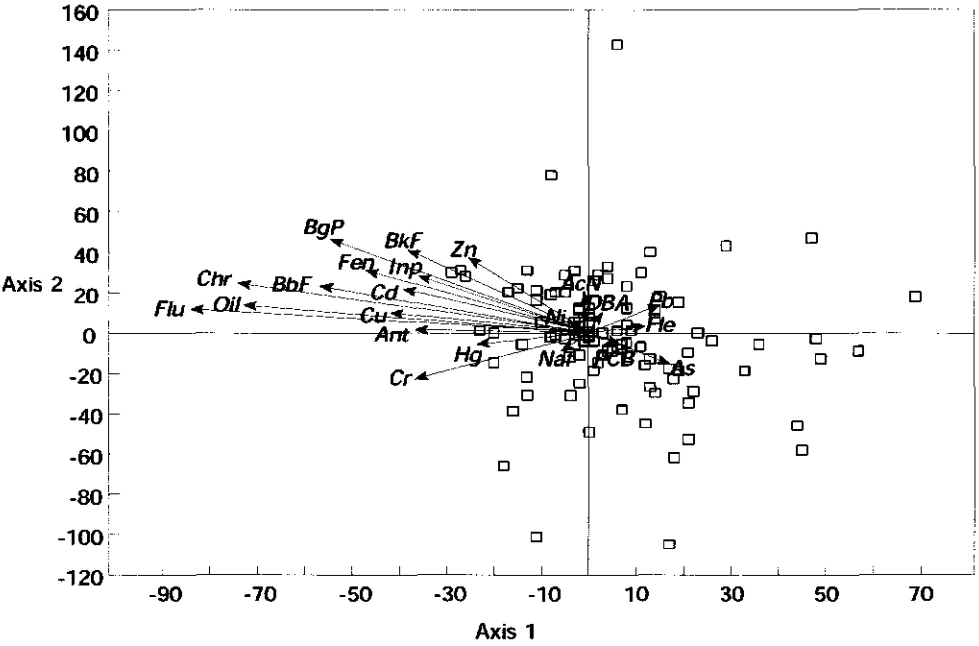

Figure 2: Positions of taxa and contaminants in the ordination diagram for the first two axes of a partial canonical correspondence analysis in which the species data were constrained by the contaminants after the effects of other variables had been removed. Taxa are represented by boxes, contaminants by arrows and their abbreviations: *AcN* = acenaphtene, *ANT* = anthracene, *BbF* = benzo[*b*]fluoranthene, *BgP* = benzo[*ghi*]perylene, *BkF* = benzo[*k*]fluoranthene, *Chr* = chysene, *DBA* = dibenzo[*ah*]anthracene, *Fen* = phenanthrene, *Fle* = fluorene, *Flu* = fluoranthene, *Inp* = indeno(*1,2,3-cd*)pyrene, *Naf* = naphtalene, *As* = arsenic, *Cd* = cadmium, *Cr* = chromium, *Cu* = copper, *Pb* = lead, *Hg* = mercury, *Ni* = nickel, *Zn* = zinc, *PCB* = polychlorinated biphenyls. The length of the arrow is a measure of the importance of the contaminant, while the arrowhead points in the direction of increasing influence.

The positions of the taxa of different taxonomic groups in the ordination diagram are presented in Figure 3. Most bivalve and gastropod taxa are located in the center and the left-hand side of the diagram (Figures 3a, 3b) and are thus associated with elevated contaminant concentrations. Oligochaete and chironomid taxa occurred mainly in the central and right-hand parts of the diagram (Figure 3c, 3d) and are associated with lower contaminant concentrations.

In Figure 4, the positions of the taxa in the ordination diagram are labeled with their trophic relationships as derived from the literature (VERDONSCHOT 1990, MERRITT AND CUMMINS 1996). Carnivores (Figure 4a) and detriti-herbivores (Figure 4b) are positioned throughout the diagram, showing no correlation with contaminant concentrations. Herbivores (Figure 4c) aggregate together in the left-hand part of the diagram and are associated with elevated

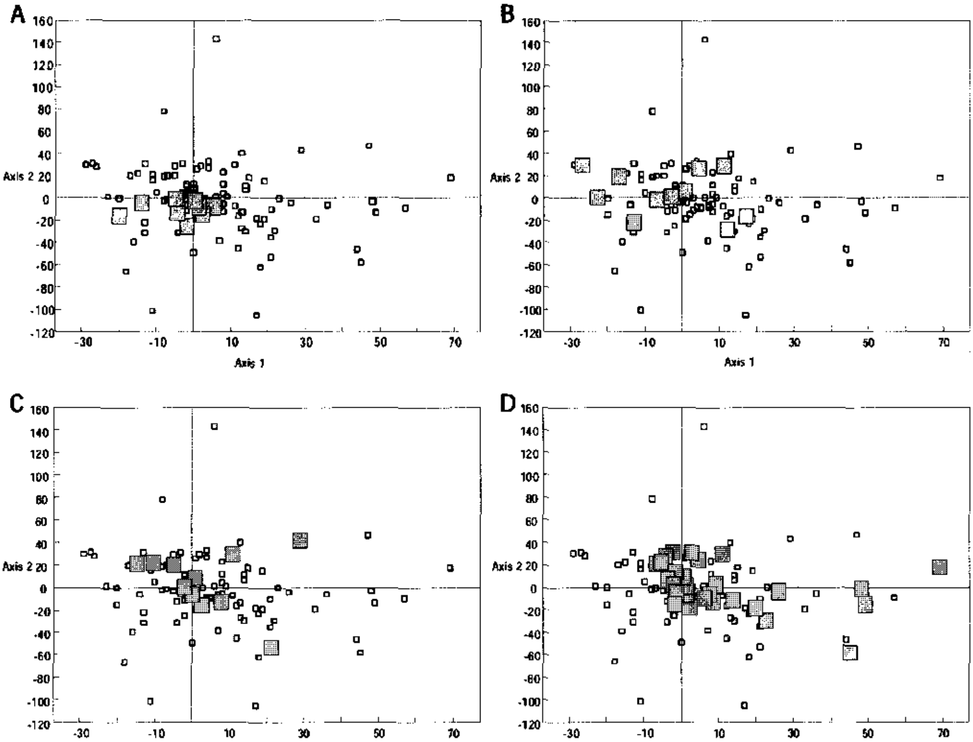

Figure 3: Positions of taxa from selected taxonomic groups in the ordination diagram of a partial canonical correspondence analysis in which the species data were constrained by contaminants after the effects of other variables had been removed. (A) Bivalvia, (B) Gastropoda, (C) Oligochaeta, and (D) Chironomidae. ▨ = taxa belonging to the specific taxonomic group, open squares = other taxa.

contaminant concentrations. Detritivores (Figure 4d) are located more to the right and are therefore associated with lower contaminant concentrations.

Calculating the contributions of the different groups of contaminants was impossible for the Dordtsche Biesbosch and the Nieuwe Merwede because there were too few observations. Compared to the results of the whole delta, the three river branches Brabantsche Biesbosch, Haringvliet and Hollandsch Diep showed a lower percentage of unexplained variance and a similar percentage of variance explained by the ecological factors as a group (Table 5). The contribution of contaminants as a group for these branches was at least double that of the whole delta and much larger than the percentage explained by ecological variables. In all three branches, the contributions of PAHs and trace metals were of the same order of magnitude. These percentages were much higher than those observed for the delta as a whole, but the CCAs were not significant except for trace metals in the Brabantsche Biesbosch. Although the contribution of trace metals and PAHs were in the same order of magnitude for the Brabantsche Biesbosch, only trace metals had a significant contribution. This is due to the lower number of metal than PAH variables included in the analysis.

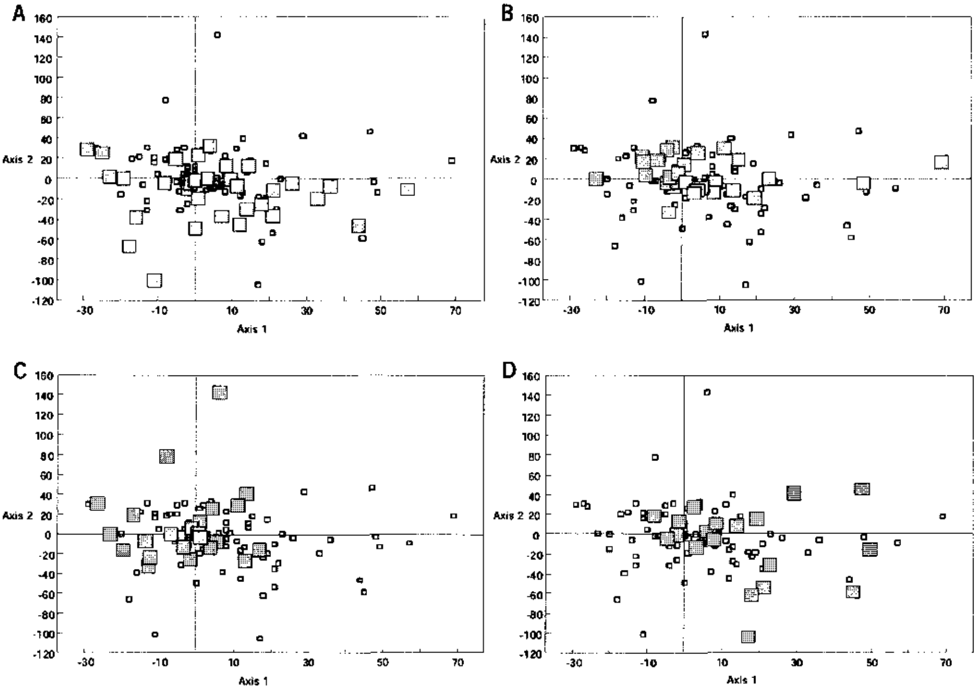

Figure 4: Positions of taxa from selected trophic groups in the ordination diagram of a partial canonical correspondence analysis in which the species data were constrained by contaminants after the effects of other variables had been removed. (A) carnivores, (B) detriti-herbivores, (C) herbivores, and (D) detritivores. ▨ = taxa belonging to the specific functional feeding group, open squares = other taxa.

The variance partitioning from the analyses with the ecological factors (Table 5) and the three bioassays (Table 6) gave different results. The contributions of the bioassays in explaining the variation in the *in situ* macroinvertebrates ranged from 0 to 10%, however, the contribution (1.9%) was only significant for the whole delta. The analyses with the bioassays as explanatory variables resulted in a lower percentage of explained variance than the analyses with measured contaminants concentrations. The contribution of the ecological variables, however, was higher for the analyses with bioassays (Table 6) than for those with measured contaminants concentrations (Table 5). The proportion shared variance on the other hand was higher for the analysis with contaminant concentrations. The results thus indicate that the effect classes of the bioassays correlate less closely with the *in situ* macroinvertebrate variation than the measured contaminant concentrations.

Table 6: Partitioning of the variance in the species data in percentages obtained from partial canonical correspondence analyses with the results of the three bioassays and ecological factors as explanatory variables. Note that the sum of the variances explained by the individual bioassays does not equal the variance explained by the bioassays as a group. See table 2 for explanation of river branch abbreviations. Not significant contributions (Monte Carlo test, $P > 0.05$) are italicized.

Source of variation	Delta	River branch				
		BB	DB[1]	HD[2]	HV[2]	NM[3]
Ecological factors	30.4	27.2		42.2	26.3	53.9
Bioassays	1.9	*5.5*		*5.6*	*4.5*	*10.1*
Daphnia magna	0.5	*1.7*		*2.2*	*1.4*	*3.6*
Chironomus riparius	*0.7*	*2.1*		*1.3*	*2.1*	*3.0*
Vibrio fischeri	*0.4*	*1.3*		*1.5*	*0.1*	*2.3*
Shared by ecological factors and bioassays	1.5	1.4		5.1	3.1	1.1
Unexplained	66.2	65.9		47.1	66.1	34.9
Total	100.0	100.0		100.0	100.0	100.0

[1]: too few observations to calculate variance partitioning

[2]: organic carbon content omitted from analyses due to high VIF

[3]: sediment dry weight omitted from analyses due to high VIF

Discussion

Macroinvertebrate community

Although the water quality of the river Rhine has improved significantly in the last two decades (ADMIRAAL ET AL. 1993), the development of benthic macroinvertebrate communities in this delta is limited by chemical and non-chemical stressors (SMIT AND VAN DER VELDEN 1993). The macroinvertebrate communities from a wide range of environmental conditions in the Rhine-Meuse delta are relatively poor in species composition, and the majority of the taxa collected in the present study are not typical of the downstream parts of large rivers. Despite the severe human impact on these water systems, limited numbers of taxa typical of large rivers are still present. Their presence may be an indication that restoration of these systems may result in communities that are characteristic of lowland rivers.

Main factors

Studies on the littoral macroinvertebrate community in the enclosed Rhine-Meuse delta have shown that geographical zones and grain-size composition were the most important factors influencing species composition (SMIT ET AL. 1994). Although the present study confirms these two variables were the most important among the ecological variables, both made minor contributions to the explanation of the observed variation. The results of the present study also show significant impacts of contaminants, especially PAHs and trace metals. This contrasts with the results of an earlier study based on the same data set, which concluded that ecological factors overruled the minimal impact of the contaminants (REINHOLD-DUDOK VAN HEEL AND DEN BESTEN 1999). In the earlier data analysis, the contribution of the different variables was not quantified but rather was estimated from direct ordinations in which ecological factors and contaminants were used as explanatory variables. Furthermore, the earlier study distinguished four sediment types, and multivariate analyses were performed per sediment type. The present study also differs from the earlier study with respect to contaminant concentrations. Prior to multivariate analysis, contaminant concentrations were normalized to organic matter content to better approximate the bioavailable contaminant concentrations more closely (PEETERS ET AL. 2000). Normalizing contaminant concentrations to organic carbon content yielded a better relationship between the contaminants and the *in situ* macroinvertebrate community structure in the Rhine-Meuse delta.

Bioassays

The present study showed that all three bioassay responses had significant relationships with certain common ecological variables. The effect classes of all three bioassays showed significant relations with oil, but only the pore-water bioassay with *Vibrio fischeri* was significantly related to two trace metals and one PAH. Thus, the three bioassay responses are not only susceptible to pollutants but also correlate with non-pollutant factors. Bioassays are affected by a variety of factors, such as experimental design (NAYLOR AND HOWCROFT 1997), sample storage time (SAE ET AL. 1998), sediment manipulations (DAY ET AL. 1995b), and food addition (HARKEY ET AL. 1994a). The present study clearly shows that the responses in the bioassays are affected by toxicants but also by other field circumstances.

This study showed that the responses in the bioassays with *Daphnia magna* and *Vibrio fischeri* were related to only a few distribution and abundance patterns of *in situ* macroinvertebrate species in the Rhine-Meuse delta. A larger, but still limited, number of *in situ* macroinvertebrate species showed a significant relationship with the responses in the *Chironomus riparius* bioassay. This indicates that whole-sediment bioassays give better results than bioassays with pore-water, which is in line with previously published data (RISTOLA ET AL. 1996, COTE ET AL. 1998). According to HARKEY ET AL. (1994b), the bioavailability of contaminants can be more accurately predicted in bioassays that expose organisms to whole sediments than in those that expose organisms to aqueous representations of whole sediments.

Various species of *Chironomus* were present in the Rhine-Meuse delta and their distribution patterns might be expected to show a significant relationship with the results of the *Chironomus riparius* bioassay. This was not the case, however, and this discrepancy could be explained by the fact that bioassays use specimens from cultured populations.

Furthermore, it is known that *in situ* species can have higher tolerances to contaminants as a result of competition and selection (BLANCK AND WANGBERG 1988, CAIRNS ET AL. 1994, POSTMA ET AL. 1995).

Only a small proportion of the total variation in the macroinvertebrate data could be related to effect classes of bioassays in the present study (1.9% for the Rhine-Meuse delta). This was much lower than the proportion explained using the contaminant concentrations as explanatory variables. This finding contrasts with those by BESSER ET AL. (1996) who found that laboratory sediment bioassays with *Hyalella azteca* and *Chironomus tentans* discriminated more accurately between sites with various degrees of contamination than did characterization of benthic communities. In their study, however, all sites were dominated by oligochaetes, whereas the species variety in the Rhine-Meuse delta was much higher.

The contribution of the ecological variables was higher for the analyses with bioassays than for those with measured contaminant concentrations as explanatory variables. The proportion of the shared variance, however, was higher for the analysis with contaminant concentrations. It seems, therefore, that, in the analyses with bioassay responses as explanatory variables, the effect of the ecological variables are overestimated due to covariation with the contaminant concentrations.

In conclusion, the present study showed that laboratory bioassay responses are weakly correlated with the *in situ* macroinvertebrate community structure. This might be due to the manipulation of the test or to the higher tolerances to pollutants of the *in situ* species. Hence, extrapolating the observed laboratory effects at the species level to the complexity of the field situation at the community level is problematic.

Variance partitioning

The partial canonical correspondence analysis for the whole Rhine-Meuse delta showed that 45.8% of the total variation in the species data could be explained by the variables used in the analysis. Although a large part of the total biological variance was explained, another part remains unexplained that may be due to, e.g., stochastic events, natural fluctuations, and contaminants or stress factors not considered in this study. Biological factors, like predation by fish and birds on macroinvertebrates and competition for food or habitat, might contribute to the unexplained variance. Another reason for the lower explained variance might be that the Rhine-Meuse delta is a complex environment with simultaneously acting multiple stressors, resulting in a community of more tolerant species.

The common ecological variables explained a somewhat larger proportion of the biological variation in the delta than contaminants. The variance shared by the ecological factors and the contaminants was larger than in some other studies (PINEL-ALLOUL ET AL. 1996, PEETERS ET AL. 2000). This means that a substantial part of the explained variation cannot be allocated to a specific source. For the Rhine-Meuse delta, PAHs as a group accounted for 6%, trace metals as a group for 4.1%, and oil for only 0.7% of the observed biological variation. Although these contributions were not very large, they were highly significant. The findings of the present study show that carnivores and detriti-herbivores are hardly affected by sediment contaminants. Detritivores, like oligochaete and certain chironomid taxa, seem to avoid elevated sediment contaminant concentrations, whereas herbivores such as bivalve and gastropod taxa seem to be more tolerant. It seems therefore that sediment-feeding taxa are more affected by sediment contaminants than taxa with other feeding modes. The results clearly indicate that sediment contamination affects the macroinvertebrate food web structure.

The present study found nearly all ecological factors to be significant in explaining some of the observed biological variation in the Rhine-Meuse delta. However, analyses of the individual river branches showed that the contributions of individual variables were mostly not significant, although the contribution of the ecological factors as a group or the contaminants as a group was significant. This might be partially due to the smaller number of samples in the individual analyses. However, the results clearly indicate that the aquatic communities are affected by the combination of all environmental stressors.

The method of variance partitioning could not be applied to all individual river branches because the number of observations was too small. Relatively large numbers of observations are required to calculate the partitioning, which is a disadvantage of the method. The present study showed that the method allows quantification of the impact of different groups of contaminants and may thus be a useful tool for priority setting.

In conclusion, the contribution of different groups of contaminants (trace metals, PAHs, oil) to the structuring of the *in situ* macroinvertebrate community in the Rhine-Meuse delta was quantified using the method of variance partitioning. The results also indicate that sediment contamination significantly affected the macroinvertebrate food web structure. Laboratory bioassays were weaklier correlated with the *in situ* macroinvertebrate composition than chemical measurements. Because field surveys and bioassays reveal different information on ecotoxicological effects, it is recommended both be incorporated in future sediment risk assessment procedures.

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CHAPTER 5

LOGISTIC REGRESSION AS A TOOL FOR DEFINING HABITAT REQUIREMENTS OF TWO COMMON GAMMARIDS

Edwin THM PEETERS and Jean JP GARDENIERS

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> *Man is the measure of all things.*
>
> *Protagoras*
> *Fragment 1, c 480-410 BC*

Summary

Logistic regression predicts the probability of occurrence of a species as a function of environmental variables. This technique was applied to a large data set describing the distribution of two common gammarid species, *Gammarus fossarum* and *Gammarus pulex*, in streams in the Netherlands, to evaluate its usefulness in defining habitat requirements.

A method is presented that derives optimum habitat ranges for environmental variables from logistic regression equations. The calculated optimum habitat ranges, which are related to the maximum likelihood of presence in the field, agreed with habitat requirements and ecological tolerances in the literature.

Single logistic regressions provide good descriptions of the optimum habitat requirements and multiple logistic regressions give insight into the relative importance of each environmental variable. It is the combination that makes logistic regression a valuable tool for constructing habitat suitability indices.

Current velocity, pH, Kjeldahl nitrogen, total phosphorus, ammonium nitrogen, conductivity, width and depth are, in this sequence, the most important environmental variables in predicting the probability of occurrence of *G. fossarum*, whereas current velocity, Kjeldahl nitrogen, pH and depth are the most important variables for the prediction of the probability of occurrence of *G. pulex*.

Introduction

The Law of Tolerance (SHELFORD 1913) states that the occurrence of an organism is bounded by a minimum and a maximum value for any environmental variable, representing the limits of its tolerance. Knowledge of the habitat requirements of organisms expressed as minimum, optimum and maximum values provides very useful information for both water quality management (STATZNER AND SPERLING 1993) and restoration projects (OSBORNE ET AL. 1993).

The Habitat Evaluation Procedure (HEP) is commonly used to define the suitability of habitats (U.S. FISH AND WILDLIFE SERVICE 1980). HEP uses Habitat Suitability Index (HSI) models, which are often derived from qualitative data from the literature. A more quantitative definition of habitats may be obtained by using multivariate statistical methods, but there is a general lack of data quantifying the relationships between species and their habitats (SCHAMBERG AND KROHN 1982).

Regression methods are useful in the analysis of relationships between a response variable and one or more explanatory variables (HOSMER AND LEMESHOW 1989). The advantage of logistic regression is that the probability of occurrence of an event can be predicted as a function of one or more independent variables (JONGMAN ET AL. 1987, HOSMER AND LEMESHOW 1989, TREXLER AND TRAVIS 1993). This has made logistic regression a popular technique in clinical biostatistics (e.g. Van Houwelingen and Le Cessie 1990). Logistic regression is preferred over simple weighted averaging as a practical method for summarizing species' distributions along environmental gradients (Ter Braak and Looman 1986). Stochastic habitat models that combine both biota (species) and their relation with relevant environmental data can be constructed from large data sets by logistic regression (Austin et al. 1984, Huisman et al. 1993). This technique enables prediction of the probability of occurrence of a species as a function of an environmental variable.

Logistic regression has been applied in environmental studies of macrophytes (e.g. Ter Braak and Gremmen 1987, De Swart et al. 1994, Odland et al. 1995), birds (e.g. Ramsey et al. 1994), and freshwater diatoms (Ter Braak and Van Dam 1989, Birks et al. 1990). In the field of freshwater animal ecology this technique has hardly been used. Udevitz et al. (1987) applied logistic regression to mosquito larvae and Eyre et al. (1992, 1993) to water beetles. The aim of the present study, therefore, was to evaluate logistic regression in defining habitat requirements of aquatic macroinvertebrates. The results of logistic regression were compared with data from the literature on the ecology of two species, *Gammarus fossarum* Koch, 1835 and *Gammarus pulex* Linnaeus, 1758.

Materials and methods

Data from 4084 macroinvertebrate samples from running waters in the Netherlands were provided by several regional waterboards. *Gammarus fossarum* and *Gammarus pulex* were selected because they occurred frequently (*G. pulex* in 1425 samples, *G. fossarum* in 397 samples), they were easy to identify and, according to the literature, had different habitat preferences. Information on abiotic circumstances at the sampled sites was incomplete, but fourteen variables had been measured in most of the cases (Table 1).

Logistic regression

Logistic regression falls within the general framework of Generalized Linear Models (GLM) and can be used to analyze the relationship between a binary response variable and one or more explanatory variables (Hosmer and Lemeshow 1989). The 'presence-absence response curve' of a species (Ter Braak and Looman 1986) describes the probability of the species being present, *p(x)*, as a function of a measured environmental variable *x*. The general expression for this probability is:

$$p(x) = \frac{\exp^{\beta_0+\beta_1 x+\beta_2 x^2}}{1+\exp^{\beta_0+\beta_1 x+\beta_2 x^2}} \qquad (1)$$

The parameters β_0, β_1 and β_2 of equation 1 are regression coefficients with β_0 as intercept or constant term. The resulting response curve, the 'Gaussian logit curve' (Jongman et al. 1987), is symmetrical and bell-shaped. When the parameter β_2 becomes zero, the model produces a

Table 1: Environmental variables with numbers of observations, median, minimum and maximum values.

Variable		Number of observations	Value		
			median	minimum	maximum
Current velocity	cm s^{-1}	2438	25	0	200
Width	m	3140	4.00	0.10	40.00
Depth	m	3739	0.40	0.01	5.00
BOD*	mg l^{-1}	1376	3.0	0.1	64.0
Chloride	mg l^{-1}	2042	42	6	498
Conductivity	µS cm^{-1}	1733	547	88	7942
Ammonium nitrogen	mg l^{-1}	2042	0.62	0.01	57.00
Kjeldahl nitrogen	mg l^{-1}	1286	2.50	0.10	68.00
Oxygen	mg l^{-1}	2025	8.40	0.10	27.00
Oxygen saturation	%	2025	78	1	307
Total phosphorus	mg l^{-1}	1737	0.44	0.01	18.00
pH	-	2087	7.3	3.9	9.7
Sulphate	mg l^{-1}	1089	60	9	445
Water temperature	°C	2758	13	0	30

*: biological oxygen demand

sigmoidal increase or decrease in the probability of occurrence. Equation 1 can be transformed to the logit function (2).

$$g(x) = \log\left[\frac{p(x)}{1-p(x)}\right] = \beta_0 + \beta_1 x + \beta_2 x^2 \qquad (2)$$

Equations 1 and 2 can be extended to include more than one variable. The transformation of *p(x)* to *g(x)* results in a linear regression model in which the logit, *g(x)*, is linear in its parameters, may be continuous, and may range from -∞ to +∞, depending on the range of *x* (HOSMER AND LEMESHOW 1989). The maximum likelihood principle is used to estimate the values for the parameters β_0, β_1 and β_2. Estimates of the optimum (*u*) and the tolerance (*t*) can be obtained from the estimates of the parameters β_1 and β_2 (JONGMAN ET AL. 1987):

$$u = \frac{-\beta_1}{2\beta_2} \qquad (3)$$

$$t = \frac{1}{\sqrt{-2\beta_2}} \qquad (4)$$

The likelihood ratio test is applied to assess the significance of the estimated parameters β_1 and β_2 (HOSMER AND LEMESHOW 1989). This is carried out by comparing the predictive value of models including the estimated parameters with those not including them. If the removal of a parameter does not lead to a decrease in the predictive power of the model, the parameter is excluded (Trexler and Travis 1993).

The deviance of a model with only a constant term (= null model) is equivalent to apportioning all of the variation to the random (error) component and is analogous to the total sum of squares in normal linear regression. The deviance of each fitted model is analogous to the residual sum of squares in linear regression. The reduction in deviance (*R*) is used to assess the contribution of a model to the explanation of the variance in the data points:

$$R = \frac{1 - D_1}{D_0} * 100 \tag{5}$$

where R *= reduction in deviance,* D_0 *= deviance of the model without explaining variables and* D_1 = deviance of the model with explaining variables. The deviance from the model with explaining variables (D_1) is always lower than the deviance from the model without those variables (D_0). If D_1 is high, approaching the value of D_0, the reduction in deviance is small. Low values for D_1 will result in high values for the reduction in deviance, which indicate that the logistic regression model fits the data well. If the reduction in deviance is larger than the critical value of chi-square at the 95% level of significance for *n* degrees of freedom (*n* being the number of additional parameters), then the inclusion of that parameter is considered significant.

Data analysis

Logistic regression was applied to the presence/absence data for both gammarid species, using the SPSS-PC software package (Norusis 1992). Separate single analyses were performed for each environmental variable, using logarithmically transformed data when these gave greater deviance reduction. Cases with missing data were left out. Tests were carried out to establish whether the parameters β_1, β_2 from equation 2 differed significantly from zero ($P<0.01$). Probability functions were plotted against the environmental variables. From these plots the values were ascertained at which both species reached the maximum probability (p_{max}) and at which the species no longer occurred ($p_x < 1\%$). The value where p_{max} is reached is equivalent to the optimum *u* from equation 3 for Gaussian response curves.

Logistic regression analysis results in an equation describing the probability of species occurrence as a function of an environmental variable, whereas existing autecological information is usually expressed as ranges of environmental variables. Optimum habitat requirements were calculated from the regression equations to allow a comparison of the results with data from the literature. The optimum habitat requirement was defined as that range of the environmental variable for which the probability divided by the maximum probability was 0.75 or higher. This range was compared with that which can be obtained from the optimum (*u* in equation 3) and tolerance (*t* in equation 4), for $\beta_2 < 0$ (Jongman et al., 1987).

Multiple stepwise logistic regressions were applied in order to determine the minimal adequate set of variables for predicting the probability of occurrence of both species. The relevant terms from the single regressions were used as independent variables. The same procedures and statistical tests were used as within single regressions. Cases with missing data were omitted. Final analyses were undertaken with the minimal adequate set, leaving out one variable per analysis, to assess the relative contribution of the individual variables.

Results

Tables 2 and 3 show the estimated values for the parameters β_0, β_1 and β_2 from equation 2, the maximum probability and the percentage reduction in deviance for *G. fossarum* and *G. pulex*, respectively. In the case of sulphate, the estimated parameters were not significant, so the variance in the data could not be explained by this variable.

Table 2: Estimated values for the parameters β_0, β_1, β_2 from equation (2), maximum probability (p_{max}), and percentage deviance reduction (R) for *Gammarus fossarum*. See Table 1 for units of the variables.

Variable	β_0	β_1	β_2	p_{max}	R
Current velocity	-4.3482	0.0778	-0.0004	0.36	14.3
Width^1	-1.3676	-2.5184	n.s.^{2}	0.76	16.6
Depth^1	-4.4045	-3.4893	-0.3195	0.78	19.8
BOD	-1.7324	-0.1433	n.s.^{2}	0.15	2.0
Chloride^1	-2.7200	7.4004	-4.7918	0.53	22.6
Conductivity^1	-115.618	85.4017	-16.0342	0.13	4.1
Ammonium nitrogen^1	-3.2175	-3.7586	-2.2208	0.16	11.7
Kjeldahl nitrogen^1	-1.8721	-2.7098	-1.7151	0.31	13.7
Oxygen	-25.6799	4.3305	-0.1922	0.21	17.6
Oxygen saturation	-33.6092	0.6390	-0.0031	0.34	20.0
Total phosphorus^1	-2.3570	-1.7586	-2.2420	0.12	4.0
pH	-149.256	35.36360	-2.1070	0.29	18.8
Sulphate	n.s.^{2}	n.s.^{2}	n.s.^{2}		
Water temperature	-3.2996	0.4806	-0.0291	0.21	7.5

^{1} LOG-transformed; ^{2} not significant

The estimated parameter β_2 was not significantly different from zero for the variables width and biological oxygen demand (BOD) for both species and for chloride and ammonium nitrogen for *G. pulex*. The resulting response curve was therefore sigmoidal. For the other variables the response curve was symmetrical and bell-shaped.

For *G. fossarum*, physical factors like current velocity, width and depth, and the chemical water quality variables chloride, oxygen, pH and nitrogen produced a greater reduction in deviance than conductivity, BOD and total phosphorus (Table 2). For *G. pulex*, the greatest reductions in deviance were obtained for BOD, Kjeldahl nitrogen and current velocity (Table 3), but compared to the results of *G. fossarum*, these reductions were much smaller. The reduction in deviance was greater for *G. fossarum* than for *G. pulex* for all variables (except BOD).

The maximum probability varied from 0.12 to 0.78 for *G. fossarum* (Table 2) and from 0.35 to 0.63 for *G. pulex* (Table 3). It was nearly identical for both species as regards oxygen saturation and chloride but probabilities were higher for *G. pulex* than for *G. fossarum* for all other variables except width and depth.

Table 3: Estimated values for the parameters β_0, β_1, β_2 from equation (2), maximum probability (p_{max}), and percentage deviance reduction (R) for *Gammarus pulex*. See Table 1 for units of the variables.

Variable	β_0	β_1	β_2	p_{max}	R
Current velocity	-1.3422	0.0427	-0.0003	0.54	3.5
Width^1	-0.3763	-0.3180	n.s.^{2}	0.48	0.4
Depth^1	-0.8965	-1.1721	-0.5837	0.42	1.1
BOD	0.0924	-0.1472	n.s.^{2}	0.52	3.5
Chloride^1	0.0932	-0.3939	n.s.^{2}	0.52	0.2
Conductivity^1	-8.9822	6.4846	-1.2347	0.39	0.3
Ammonium nitrogen^1	-0.6708	-0.5988	n.s.^{2}	0.63	2.3
Kjeldahl nitrogen^1	0.1149	-0.7898	-0.5493	0.60	3.6
Oxygen	-2.4206	0.3376	-0.0124	0.47	2.4
Oxygen saturation	-2.6058	0.0404	-0.0002	0.35	2.5
Total phosphorus^1	-0.5253	-0.5813	-0.3289	0.43	1.1
pH	-28.7082	7.0268	-0.4328	0.45	2.6
Sulphate	n.s.^{2}	n.s.^{2}	n.s.^{2}		
Water temperature	-1.0700	0.1399	-0.0072	0.40	0.9

^{1} LOG-transformed; ^{2} not significant

Figure 1 shows the probabilities for both species for current velocity and Figure 2 for pH. Although the current velocity ranges in which the species occurred were comparable, the maximum probability was reached at different values (Figure 1). There were similar differences in maximum probability values for current velocity, conductivity, depth, ammonium and Kjeldahl nitrogen, oxygen saturation, water temperature, and total phosphorus (Table 4). Maximum probability values for pH (Figure 2) were similar for both species, but the range of occurrence was much wider for *G. pulex* than for *G. fossarum*. *G. pulex* occurred over a wider range than *G. fossarum* for all variables, except for current velocity (Table 4).

The predicted range of occurrence of *G. fossarum* (Table 4) fell within the observed minimum and maximum values (Table 1), but beyond the maximum value for pH and the minimum values for chloride and water temperature. The predicted ranges of *G. pulex* (Table 4) fell within the observed ranges for current velocity, BOD and oxygen saturation (Table 1). For all other variables, the predicted range was wider than observed.

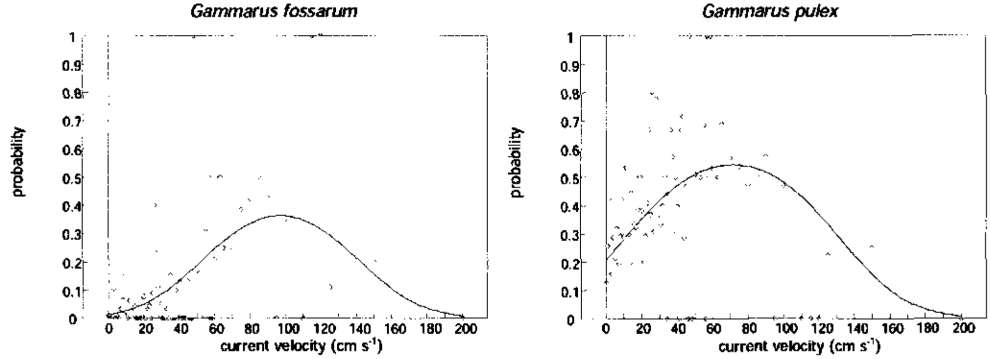

Figure 1: Probability of occurrence of *Gammarus fossarum* and *Gammarus pulex* as a function of current velocity. The squares represent actual frequency of occurrence of species along the gradient.

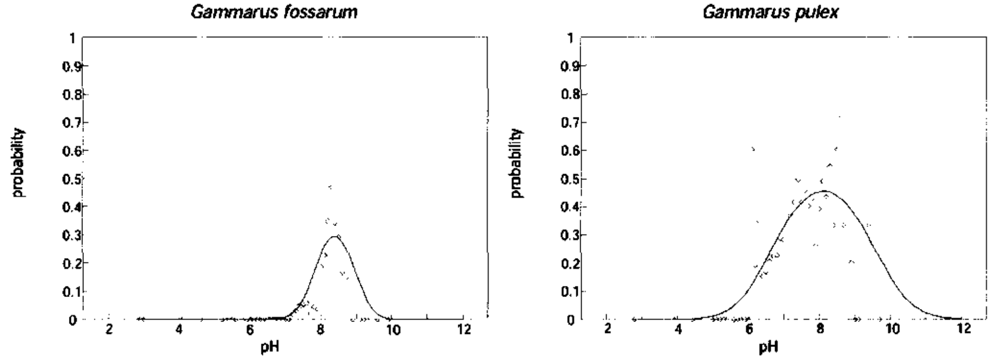

Figure 2: Probability of occurrence of *Gammarus fossarum* and *Gammarus pulex* as a function of pH. The squares represent actual frequency of occurrence of species along the gradient.

The optimum habitat ranges for both species (Table 5) fell within the minimum and maximum observed values, except for the minimum values for width, BOD, chloride and Kjeldahl nitrogen. For BOD and Kjeldahl nitrogen, the minimum values were beneath the detection limit.

The optimum ranges, defined as the intervals in which the probability divided by the maximum probability exceeded 0.75, gave results comparable to the ranges calculated from the optimum u (equation 3) and the tolerance t (equation 4) for those cases in which the estimated parameter $\beta_2 < 0$. This coincidence in values indicates that the method used in the present study is valid for Gaussian response curves and therefore might be applied to sigmoidal response curves.

G. pulex occurred over a wider pH range than *G. fossarum* (Figure 2), and this was also reflected in the derived optimum range (Table 5). The optimal habitat of *G. fossarum* consists of small, shallow streams with high current velocity, high oxygen content and alkaline water with low concentrations of nutrients (Table 5). The optimal habitat of *G. pulex* is found in small,

Table 4: Values for the variables at which the maximum probability (p_{max}) was reached and the total range of occurrence (probability ≥ 1%). See table 1 for units of the variables.

Variable	*G. fossarum*		*G. pulex*	
	p_{max}	range	p_{max}	range
Current velocity	97	0-197	71	0-198
Width	0.1	0.1-24.0	0.1	0.1->40.00[1]
Depth	0.01	0.01-1.33	0.10	0.01->5.00[1]
BOD	1.0	0.1-33.0	0.1	0.1-37.0
Chloride	7	<6[2]-69	6	<6[2]->498[1]
Conductivity	501	170-1620	398	<88[2]->7942[1]
Ammonium nitrogen	0.15	0.01-4.00	0.01	0.01-57.00
Kjeldahl nitrogen	0.16	0.10-8.20	0.20	0.10->68.00[1]
Oxygen	11.3	6.2-16.3	14.0	<0[2]->27.0[1]
Oxygen saturation	103	55-143	90	1-220
Total phosphorus	0.40	0.01-11.75	0.13	0.01->18.00[1]
pH	8.4	6.9->9.9[1]	8.1	4.7->11.6[1]
Water temperature	8.3	<0[2]-21.2	9.8	<0[2]->30[1]

[1]: value higher than observed

[2]: value lower than observed

Table 5: Optimum ranges derived from the logistic regression equations. See Table 1 for units of the variables.

Variable	*G. fossarum*		*G. pulex*	
	I[1]	II[2]	I[1]	II[2]
Current velocity	65-130	62-133	28-114	30-112
Width	0.01-0.22	-[3]	0.01-3.75	-[3]
Depth	0.01-0.04	0.00-0.00	0.02-0.76	0.07-0.83
BOD	0.1-2.4	-[3]	0.1-3.65	-[3]
Chloride	3-14	3-13	6-95	-[3]
Conductivity	328-645	307-691	106-1667	96-1832
Ammonium nitrogen	0.05-0.35	0.05-0.42	0.01-0.12	-[3]
Kjeldahl nitrogen	0.05-0.50	0.05-0.56	0.05-2.14	0.05-1.72
Oxygen	9.88-12.65	9.65-12.88	7.34-19.85	7.26-19.96
Oxygen saturation	92-115	90-116	53-127	51-151
Total phosphorus	0.10-0.98	0.14-1.20	0.01-2.00	0.01-2.23
pH	7.96-8.82	7.90-8.88	7.07-9.16	7.04-9.18
Water temperature	4.8-11.7	4.1-12.4	1.9-17.6	1.4-18.0

[1]: Interval for which p_x/p_{max} exceeds 0.75

[2]: interval covered by $(u-t) - (u+t)$ in case $\beta_2 < 0$ (see equations 3 and 4)

[3]: $\beta_2=0$

relatively shallow streams with moderate current velocity, high oxygen content and alkaline water with relatively low concentrations of nutrients. *G. fossarum* inhabits even smaller and shallower streams with higher current velocity than *G. pulex*.

Only 569 cases were included in the multiple regression analyses as a result of the missing data in the environmental variables. The variables incorporated in the minimal adequate model are given in Table 6 for *G. fossarum* and in Table 7 for *G. pulex*. The full model with all relevant terms gave greater reduction in deviance than the minimal adequate model for both species. The deviance reduction was greater for *G. fossarum* than for *G. pulex*. The model of *G. pulex* had four variables, two of them related to the physical habitat and two to water quality. In the model of *G. fossarum* the same variables were included plus four additional variables. Current velocity was the most important variable for both species, followed by pH for *G. fossarum* and Kjeldahl nitrogen for *G. pulex*. Depth was the less important variable included in the model for both species.

Table 6: Results of the multiple logistic regression for *Gammarus fossarum*. Variables are ordered in increasing deviance reduction (R). See Table 1 for units of the variable.

Model	Without	R
Full model		72.5
Minimal adequat model		62.3
	Current velocity	53.2
	pH	53.3
	Kjeldahl nitrogen	56.1
	Total phosphorus	57.8
	Ammonium nitrogen	57.9
	Conductivity	58.9
	Width	59.4
	Depth	60.5

Table 7: Results of the multiple logistic regression for *Gammarus pulex*. Variables are ordered in increasing deviance reduction (R). See Table 1 for units of the variable.

Model	Without	R
Full model		17.2
Minimal adequat model		13.6
	Current velocity	7.9
	Kjeldahl nitrogen	9.9
	pH	12.4
	Depth	12.6

Current velocity, Kjeldahl nitrogen, pH, and depth were important for predicting the occurrence of *G. pulex* according to the results of the multiple regression analysis (Table 7). Although BOD gave a similar percentage in deviance reduction in the single regression analysis (Table 3), it was not included in the minimal adequate model. On the other hand, depth was included in the model although the single regression gave a moderate deviance reduction compared to the other variables. Current velocity, pH, Kjeldahl nitrogen, total phosphorus, ammonium nitrogen, conductivity, width and depth were included in the minimal adequate model for *G. fossarum*. Chloride and oxygen saturation gave the greatest reduction in deviance in the single analysis for *G. fossarum* (Table 2) but were not included in the minimal adequate model. Total phosphorus and conductivity were included in the model, although they had a relatively low percentage deviance reduction in the single regression analysis (Table 2).

Discussion

Results of the present study found that current velocity was the most important factor for the distribution of both gammarid species. HOLTHUIS (1956), AMBÜHL (1959), NIJSSEN (1963), MEIJERING (1971) and ADAMS ET AL. (1987) also found stream velocity to be one of the limiting factors for the distribution of either *G. fossarum* or *G. pulex*. The present study also found that *G. fossarum* preferred higher current velocities than *G. pulex* and that *G. pulex* could tolerate low flow rates but preferred moderate flow rates. These findings are in accordance with those of MEIJERING (1971) who studied the distribution of both species in the Schlitz area in Germany. In general, the data in the literature confirm the results obtained in the present study with respect to current velocity.

G. fossarum preferred smaller, shallower streams than *G. pulex*; a finding that parallels studies showing that *G. fossarum* usually inhabits the smaller upper reaches of streams, whereas *G. pulex* occurs further downstream (HOLTHUIS 1956, NIJSSEN 1963, MEIJERING 1971, GOEDMAKERS 1972, JANETZKY 1994).

This study showed that *G. pulex* tolerated low pH better than *G. fossarum*, but both species avoided acid conditions. Species of the genus *Gammarus* are regarded as among the most acid-sensitive organisms (SUTCLIFFE 1983, ØKLAND AND ØKLAND 1986), and *G. pulex* does not occur where the pH drops below 5.7 (SUTCLIFFE AND CARRICK 1973, OTTO AND SVENSSON 1983). However, MEIJERING (1971) found *G. pulex* could tolerate pH values down to 4.8, whereas *G. fossarum* was absent from streams where pH dropped below 5.4. In laboratory studies, a pH of 4.7 caused mortality in *G. fossarum* and of 4.5 in *G. pulex* (BREHM AND MEIJERING 1982). The 24-h LC_{50} range for *G. fossarum* was found to be between pH 4.0 and 4.5 (MEINEL ET AL. 1985) and that for *G. pulex* to be between 3.8 and 4.0 (NAYLOR ET AL. 1990). All studies confirm the greater tolerance of *G. pulex* for low pH.

The optimum pH for gammarids is between 7.2 and 7.8 according to SCHUMANN (1928) and between 7.5 and 8.8 according to SCHRIMPFF AND FOECKLER (1985). These accord with the optimum habitat requirements derived from the present study.

Logistic regression showed that *G. pulex* had a greater tolerance for low oxygen concentrations than *G. fossarum*, but that both species inhabited well oxygenated reaches of streams. These results confirm studies showing that *G. pulex* is the more resistant to lower oxygen concentrations (MEIJERING ET AL. 1974, JAHR ET AL. 1980, MEIJERING 1991, JANETZKY 1994).

Optimum ranges obtained in the study reported here for other variables like BOD, ammonium nitrogen, chloride and phosphorus cannot be evaluated because no relevant literature data are available. Ranges presented in the literature are restricted to minimum and maximum values and do not include optima. The optimum ranges for the variables reported here fall within these minimum and maximum values.

Most studies reported in literature were, like this one, based on assessment of distribution data, and some degree of confirmation of present results could therefore be expected. It appeared that under laboratory conditions both gammarid species tolerated pH values where they were never found in field situations. Although some phenomena are confirmed by laboratory studies, for example the greater tolerance of *G. pulex* for low pH, the data on optima and tolerances might be quite different from field studies. These differences can be due to disregarding the effects of other environmental variables and biological phenomena like predation and competition in laboratory studies. This makes validation of the present results with laboratory data less useful. It also is a plea for deriving habitat requirements from field instead of laboratory studies.

The reduction in deviance was greater for *G. fossarum* than for *G. pulex*. Greater reductions in deviance usually indicate a better fit and, since the reduction in deviance was rather low for *G. pulex*, it might be concluded that the logistic regression model was not appropriate for this species. A regression model results in greater reduction of the deviance if the occurrence of a species is restricted to a small part of an environmental gradient, as was the case with *G. fossarum*. However, ecologically tolerant species occur over a wide range of environmental conditions, which automatically results in a smaller reduction of the deviance. Thus, smaller reductions in deviance may indicate that the model is inappropriate. Alternatively, they may indicate that the species has a wide ecological tolerance.

The optimum range for a species was defined as the interval in which the probability divided by the maximum probability exceeds 0.75. Optimum ranges were also calculated from the optimum (u in equation 3) and the tolerance (t in equation 4), provided the resulting response curve is bell-shaped with an optimum (parameter β_2 from equation 2 < 0) (JONGMAN ET AL. 1987). Comparison of the method used in the present study with the latter one showed that the resulting ranges were comparable. This coincidence in values indicates that the method used here is valid for Gaussian response curves and probably also for sigmoidal response curves. The advantage of the method presented here is that it can also be applied in situations where $\beta_2=0$ or $\beta_2>0$.

The present study has shown that, for common aquatic macroinvertebrates, optimum ranges for each variable can be derived from large data sets with missing data using logistic regression. These optima are related to the maximum likelihood of presence in the field. The significance of the estimated parameters in the regression model, the reduction in deviance and the maximum probability reflect the importance of an environmental variable in accounting for the occurrence of a species, but do not determine which variables were the most important. In statistical modeling, the principle of parsimony means that models should have as few variables as possible (CRAWLEY 1993). The minimal adequate model in the present study for *G. fossarum* included eight variables and four for *G. pulex*. The variables included in the models are not necessarily variables that gave greater reduction in deviance in the single regressions. Variables that gave small reduction in deviance in the single analysis were also included. Therefore, no conclusions concerning the relative importance of variables can be drawn from single regressions.

Responses of organisms in space and/or time may be the result of physiological limitations, interactions with other organisms, human interference or other factors (HUISMAN ET AL. 1993). Simple mathematical models describing the observed relationships between a species and an environmental variable are needed and the bell-shaped Gaussian response model is one of the classical and frequently used models (WHITTAKER 1956, GAUCH AND WHITTAKER 1972, ELLENBERG 1983, TER BRAAK AND LOOMAN 1986). Logistic regression, as applied in the present study with linear and quadratic terms, yields results confirmed by literature. However, there are examples from vegetation sciences showing that response curves may be skewed or asymmetrical (AUSTIN 1987, DE SWART ET AL. 1994). The shape of the curve depends on the scale and type of the environmental gradient (AUSTIN 1980) and non-parametric Generalized Additive Models (GAM) might be more appropriate to model such responses. GAMs are useful in an exploratory sense and when the data are too complex for GLMs (YEE AND MITCHELL 1991). However, GLM models are regarded as parsimonious relative to GAM models if there is an equality in the number of terms and degree of freedom (CRAWLEY 1993). An example of the combined use of GLM and GAM is given by HEEGAARD (1997). Investigation is needed to discover whether these GAMs result in a better description of the habitat requirements of aquatic macroinvertebrates than those obtained by logistic regression.

The optima for current velocity, habitat dimensions, pH, and oxygen obtained from the single logistic regressions corresponded with those described in the literature. Differences in tolerance between the two species were also detected. For common aquatic macroinvertebrates, habitat requirements can be derived from large data sets using logistic regression for main factors like current, oxygen, pH, and dimensions that are ecologically meaningful. Multiple logistic regressions provide stochastic models with the minimal set of environmental variables necessary for predicting the probability of occurrence. The relative importance of each variable can also be derived. It is the combination of optimum habitat ranges and the relative importance of the environmental variables that makes logistic regression valuable in constructing habitat suitability indices.

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CHAPTER 6

SHORT- AND LONG-TERM RESPONSE OF THE WATERLOUSE *ASELLUS AQUATICUS* (L.) TO DIFFERENT COMBINATIONS OF CURRENT VELOCITY AND MINERAL SUBSTRATUM

Edwin THM Peeters, Annemarieke M Camu, John AJ Beijer, Marten Scheffer and Jean JP Gardeniers

Submitted

> *Es giebt keinen gefährlicheren Irrthum*
> *als die Folge mit der Ursache zu verwechseln;*
> *Ich heiße ihn die eigentliche Verderbnis der Vernunft.*
>
> *Friedrich Nietzsche*
> *Götzen – Dämmerung, 1888*

Abstract

Experiments were performed to study the effects of current flow and substratum composition on survival, growth and food consumption of the waterlouse *Asellus aquaticus* (L.). Short-term effects of increasing current flow depended on the size of the animal and the type of substratum. Critical current flow for detachment was almost the same on sand as on a polished surface whereas on gravel *A. aquaticus* could withstand higher flow velocities by hiding in interstitial spaces. Long-term experiments with different combinations of current flow and substratum composition showed that flow had a greater effect than substratum on survival and growth. Substratum, however, had a greater influence on the distribution of individuals in the experimental unit. Since growth was reduced at high current flow and no changes in levels of food intake were observed it is concluded that a substantial amount of energy is required for withstanding current at higher flow rates. Furthermore, mortality showed a strong inverse correlation to growth. The interaction of effects of natural habitat characteristics and those of human-induced stressors such as trace metals and pesticides may be better understood using an experimental and modeling approach focusing on energy budgets.

Keywords: energy budgets, interactive, isopoda, multiple stress, scope for growth

Introduction

Aquatic ecosystems are complex systems that are controlled and regulated by various physical, chemical, and biological processes. Therefore, aquatic organisms are exposed to a wide range of stressors that vary spatially and temporally. Frequently, these stressors are interrelated and act simultaneously. For example, current velocity and substratum composition in streams are strongly correlated (e.g. STATZNER 1981; MINSHALL 1984). These two physical factors have a major impact on the distribution of macroinvertebrates (e.g. HYNES 1970; MERRITT AND CUMMINS 1996; PEETERS AND GARDENIERS 1998). Individual effects of current velocity or substratum composition on aquatic macroinvertebrates have been studied intensively (e.g. STATZNER 1981; TOLKAMP 1982; ERMAN AND ERMAN 1984; LOVE AND BAILEY 1992; LAYZER AND MADISON 1995). Substratum acts directly on an organism as a medium for their existence, and indirectly as a major modifier of their environment and thus determines to a large extend the environmental conditions to which organisms are exposed (MINSHALL 1984). Current velocity usually interacts with other ecological variables and induces a direct physical force that determines habitat conditions for benthic invertebrates (WARD 1992).

Recent studies showed that the combined effect of two simultaneously operating stressors may be different from the individual effects. For example, HANAZATO AND DOBSON (1995) and RASMUSSEN (2000) observed that the combined effect of two contaminants exceeds the summed effects of the individual contaminants. Other studies showed that the impact of a contaminant depends on other non-contaminant factors (e.g. WEBER ET AL. 1992; LEMLY 1993; PRESTON ET AL. 1999). Similar results have been obtained in studies that focus on the combined effect different environmental factors (e.g. PORTER ET AL. 1999; RALPH 1999). Susceptibility to stressors seems to be species dependent (e.g. DIAZ ET AL. 1995; WILLIAMSON ET AL. 1999) and may be influenced by the presence or intensity of other stressors in the environment (e.g. FOLT ET AL. 1999; LENIHAN ET AL. 1999). To date, there is still a limited understanding of the complex effect interactions of multiple stressors and the ecological science is still in the first stages of evaluating the effects of multiple stressors on natural communities.

Due to the strong interrelationship between current velocity and substratum composition, it is difficult to disentangle causal links from analyses of field distribution patterns. This can only be reached by performing laboratory experiments in which the two factors are varied systematically in order to determine the strength of their interactions (MINSHALL 1984) and to assess the relative contribution of each factor. However, only a limited number of publications is available nowadays that address the effects of interrelated physical factors on macroinvertebrates (e.g. FELTMATE ET AL. 1986; BARMUTA 1990; LANCASTER AND MOLE 1999).

The objective of this study was to investigate the individual and interactive effects of current flow and substratum composition on survival, growth, and behavior of the waterlouse *Asellus aquaticus* (L.) in laboratory experiments. This species was selected because it is widely distributed throughout Europe, is common in Dutch streams, and its abundance is higher in regulated than in natural, pristine streams. In a pre-experiment, the critical current flow at which the specimens were swept away was investigated with different types of substratum. In one experiment the long-term effects of substratum type were investigated without flow. Long-term individual and interactive effects of current flow and substratum type were investigated in another experiment. Energy budgets were calculated to demonstrate that such an approach could be an interesting tool for interpreting results from laboratory growth experiments.

Materials and methods

Animals

Specimens of *A. aquaticus* were collected in October 1997 from a nearby spring fed pond and brought to the laboratory. They were sorted into three length classes: 4-5 mm (small), 7-9 mm (medium), and 11-12 mm (large). Specimens of these three size classes were used in the pre-experiment. In the main experiments only small specimens were used. 1200 small *Asellus* were divided into lots of 25 specimens for random allocation to experimental compartments. Individuals from 8 lots were preserved immediately in 4% formaldehyde to determine the average length (measured as the length from the top of the head to the end of the pleotelson, thus excluding the antennae and uropods) and pleotelson width. Average length and pleotelson width were not significantly different ($P>0.05$) between the 8 lots and therefore only the length data are presented. The average length at the beginning of the experiment was 4.67 mm (n=200, s.e.=0.04 mm) and the average pleotelson width 1.38 mm (n=200, s.e.=0.02 mm).

Food

Food consisted of discs (∅ 30 mm) punched from autumn-shed poplar leaves (*Populus nigra* L.). Leaves were first kept in a basket under water for 7 days, air dried, and then discs were punched. Discs were dried (80 °C, 24h), weighed, and conditioned by placing them for 3 days in water inoculated with natural water from a pond. Measurements showed that 1 mg dry weight was equivalent to 0.874 mg (n=10, s.e.=0.010 mg) ash free dry weight (550 °C, 2h) and contained 0.503 mg C.

In the main experiments, five discs were placed in each compartment in an upright position using plastic paper clips in order to avoid the creation of a new benthic habitat. Leaves were replaced as they were eaten, with the dry weight of the eaten leaf recorded to provide a total weight of leaf matter consumed in a compartment over the experiment.

Pre-experiment: Short-term effect of current velocity

The short-term effect of current velocity on *A. aquaticus* was studied in an artificial stream (200 x 15 x 15 cm) filled with local groundwater at 18 °C (pH=8.1, conductivity=210 $\mu S\ cm^{-1}$, total N=0.52 $mg\ l^{-1}$, total P=0.07 $mg\ l^{-1}$). Three types of substratum were tested: a smooth, polished surface, sand (∅ 0.25-0.50 mm), and coarse gravel (∅ 8.0-16.0 mm). The water depth was 2.5 cm. Velocity was measured approximately 5 mm above the substratum with an electromagnetic induction velocity meter at the end of the stream. Velocity was increased in steps of 5 $cm\ s^{-1}$ every 20 seconds and a maximum current velocity of approximately 40 $cm\ s^{-1}$ could be generated. Each specimen of *A. aquaticus* was placed on the substratum in the middle of the experimental area, with its head towards the current. After 10 seconds, current velocity was increased and the velocity was recorded when the specimen lost its grip and was swept away. If a specimen was dislodged, but regained a foothold within 5 cm downstream, measurement was continued until it was swept away (at least 50 cm). 25 specimens of each size class were tested individually.

Main experiment A: Long-term effect of substratum without flow

Four aquaria (40 x 40 x 20 cm) were used to investigate the long-term effects of two types of substratum on *A. aquaticus* in the absence of current. Two types of substratum (fine sand ∅ 0.25-0.50 mm; coarse gravel ∅ 8.0-16.0 mm) were assigned at random to the aquaria. Aquaria were filled with groundwater and the water temperature was kept constant at 18 °C. Oxygen levels were constantly monitored and were kept constant. The daily photoperiod was 12 h, simulating spring/early summer conditions in The Netherlands. The experiment lasted for 8 weeks, a period that would allow a measurable growth during the experiment at the selected temperature (MARCUS AND WILLOUGHBY 1978). The experiment was performed in the period December 1997 - January 1998.

The number of individuals seen on the substratum, and on the leaf discs was recorded daily for each aquarium. Dead animals were removed and counted. At the end of the experiment all survivors were collected per aquarium and preserved in 4% formaldehyde. Total length and the width of the pleotelson were measured for each individual.

Main experiment B: Long-term effect of combinations of flow and substratum

In the laboratory, water from one reservoir (400 L) was continually recirculated in eight artificial streams. The reservoir was filled with groundwater, aerated, and kept at a constant temperature of 18 °C. Two different current velocities (3±1 and 9±1 cm s^{-1}) were assigned at random to the streams. Metal grids (5 mm diameter) covered on the upstream side by 0.5 mm nylon mesh, sown onto the sides of the grids were used to create four compartments in each stream. Two types of mineral substratum (fine sand: ∅ 0.25-0.50 mm; coarse gravel: ∅ 8.0-16.0 mm) were randomly distributed between the streams with similar current flow. The daily photoperiod was 12 h. The experiment lasted for 8 weeks and was performed in the period December 1997 – January 1998. Four different regimes with 8 replicates each were tested: sand 3 cm s^{-1}, sand 9 cm s^{-1}, gravel 3 cm s^{-1}, and gravel 9 cm s^{-1}.

The number of individuals found at the front (on the upstream grid), in the back (on the downstream grid), on the substratum, and on leaf discs was recorded daily for each compartment. Dead animals were removed and recorded. At the end of the experiment all survivors were preserved in 4% formaldehyde. Total length and the width of the pleotelson were measured for each specimen.

Calculation of the energy budget

An estimation of the energy budget in terms of ash-free dry weights (AFDW) was made using data from different literature sources. Growth, measured as increase in total length, was translated to mg AFDW using the formulae given by BASSET (1993). Basic respiration by *A. aquaticus*, expressed as oxygen consumption, depends mainly on body dry weight and water temperature (ADCOCK 1982). Temperature and size of *A. aquaticus* used in the present experiments fell within the ranges used by ADCOCK (1982) and therefore his formulae were used to calculate oxygen consumption during the experiment per specimen from length measurements by assuming linear growth in length. Oxygen consumption was then expressed as mg AFDW of food supplied by using conversion factors given by LAMPERT (1984) and under the assumption that carbohydrate was metabolized (LAMPERT 1984). Total leaf consumption per specimen was calculated from the feeding rates. Feeding rates (mg dry weight day^{-1}) were calculated by dividing total leaf consumption by the estimated average number of specimens. Net leaf consumption was estimated using assimilation efficiencies. No published data were available on efficiency coefficients of *A. aquaticus* for poplar leaves. ADCOCK (1982) found an efficiency of 23% for *A. aquaticus* when fed on alder leaves (*Alnus glutinosa* Gertn). This percentage was used to provide a rough estimate the net leaf consumption.

Statistical analysis

Analysis of variance (ANOVA) was performed using SPSS for Windows. GLM Generalized Factorial Procedure was applied to perform Two-way ANOVA with the variables current velocity and type of mineral substratum as independent variables were performed in order to assess the contribution of these variables in explaining the observed variation in the dependent variable. The relative contribution of the factors was assessed following the method described in UNDERWOOD (1997).

Results

Pre-experiment: Short-term effect of current velocity

The average current velocity at which *A. aquaticus* was swept away differed between size classes and between types of substratum (Table 1). *A. aquaticus* could withstand the maximum current flow (40 cm s^{-1}) only on gravel. The maximum velocity at which specimens were swept away for gravel could not be determined because it is higher than the maximum velocity that could be reached with the equipment used. The average velocity at which *A. aquaticus* was swept away on the polished surface and sand was much lower. Although the difference in average velocity between sand and the polished surface was small, it was significant for the smallest (*F*-value=26.451, df=1, *P*=0.000) and largest size class (*F*-value=19.107, df=1, *P*=0.000), but not for the medium size class (*F*-value=1.177, df=1, *P*=0.282).

Table 1: Mean (± 1 s.e.) current velocity (cm s^{-1}) at which three size classes of *Asellus aquaticus* were swept away on three types of substratum.

	Type of substratum		
Size class	Polished	Sand	Gravel
4.5 - 5.5 mm	11.6 ± 0.2	13.2 ± 0.1	> 40.0*
7.5 - 8.5 mm	12.5 ± 0.2	12.1 ± 0.2	> 40.0
11.0 - 12.0 mm	12.0 ± 0.2	13.4 ± 0.2	> 40.0

* Note that the maximum current velocity that could be reached was 40 cm/s.

Main experiment A: Long-term effect of substratum without flow

A. aquaticus grew on both types of substratum (Figure 1a). The increase in length was much more than the standard error of the mean of all specimens collected at the start of the experiment. Total length was significantly higher in aquaria with sand than in aquaria with gravel (Table 2). Specific growth rates (calculated after MARCUS ET AL. 1978) were 1.37% for aquaria with sand and 0.86% fresh weight day^{-1} for aquaria with gravel.

Table 2: Results of the various ANOVAs applied to the data of experiment A. Type of substratum was the explanatory variable.

	ANOVA		
Dependent variable	F-value	df	*P*
Mortality	21.125	1	0.044
Total length	6.102	1	0.017
Total leaf consumption	5.240	1	0.149

The results show that mortality of *A. aquaticus*, calculated as initial number of specimens minus survived specimens, differed between the two types of substratum (Figure 1b). It appeared that the number of dead animals collected during the experiment was lower than the calculated mortality from the survived specimens. Mortality was significantly lower on sand (40%) than on gravel (90%) (Table 2). Although total consumption was higher in aquaria with sand than in aquaria with gravel (Figure 1c), this difference was not significant (Table 2).

Feeding rates were not calculated for aquaria with gravel because reliable estimates of the number of *A. aquaticus* present could not be obtained from the daily observations as specimens could not always be seen. Feeding rates were 0.564 mg dry weight day^{-1} (s.e.=0.059) for aquaria with sand.

Based on the daily observations, the average number of *A. aquaticus* on leaf discs and on substratum was significantly higher in aquaria with sand than in aquaria with gravel. However, this difference was only small when expressed as a percentage of total observed specimens. Approximately 75% of the visible specimens were found on leaf discs and 25% on substratum in both types of substrata (Figure 1d).

A calculation of the energy budget could only be made for aquaria with sand. The majority of the available energy can be allocated to active respiration, approximately 15% to growth, and 42% to body maintenance (Figure 2).

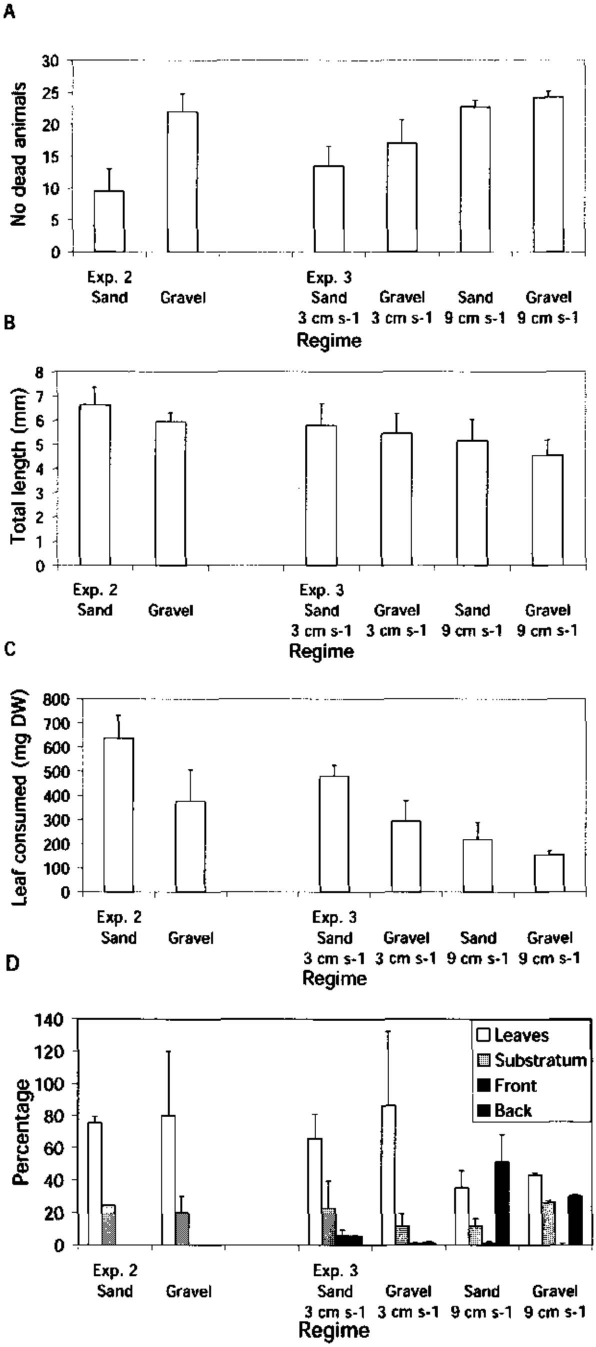

Figure 1: Mean (± 1 s.e.) for A) mortality, B) growth, C) total leaf consumption, and D) positioning of specimen in experiments A and B.

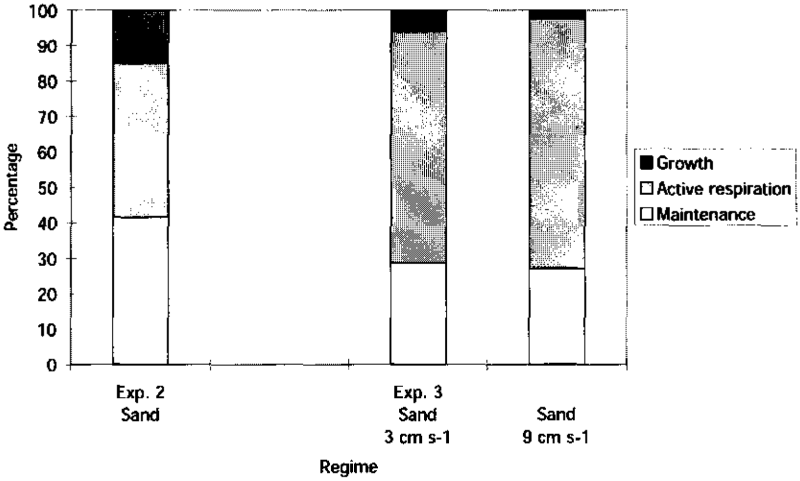

Figure 2: Estimated percentage of efficiently consumed material (AFDW) allocated to body maintenance, growth or active respiration, on sandy substratum.

Experiment B: Long-term effect of combinations of flow and substratum

The various combinations of flow and substratum resulted in differences in growth (Figure 1a). Total length at the end of the experiment increased for all combinations except for gravel with a flow of 9 cm s^{-1} that resulted in a decrease in mean length. Two-way ANOVA showed that both current and substratum were significant but not their statistical interaction term (Table 3). The ANOVA model explained only 11 percent of the observed variation. The relative contribution of current velocity (7%) was somewhat higher than the contribution of substratum (4%).

Table 3: Results of the various ANOVAs applied to the data of experiment B.

		ANOVA			
Dependent variable	Explanatory variable(s)	F-value	df	P	Fraction explained (%)
Mortality	Flow	87.120	1	0.000	69
	Substratum	8.000	1	0.009	5
	Flow*Substratum	1.280	1	0.267	0.8
Total length	Flow	12.586	1	0.001	7
	Substratum	7.246	1	0.001	4
	Flow*Substratum	1.710	1	0.202	0.7
Total leaf consumption	Flow	87.038	1	0.000	53
	Substratum	34.825	1	0.000	21
	Flow*Substratum	8.390	1	0.007	5
Feeding rate (on sand)	Flow	0.340	1	0.569	

Specific growth rates were calculated from total length and were 0.67% fresh weight day^{-1} for sand at 3 cm s^{-1}, 0.10% for sand at 9 cm s^{-1}, 0.38% for gravel at 3 cm s^{-1}, and -0.47% for gravel at 9 cm s^{-1}.

Mortality, calculated as initial number of specimens minus survived specimens, was on both substrate types (sand, gravel) higher in the regimes with a current flow of 9 cm s^{-1} than with flow of 3 cm s^{-1} (Figure 1b). The number of dead animals collected during the experiment was lower than the calculated mortality from the surviving specimens, especially in the regimes with gravel as substratum. Two-way ANOVA showed that both flow and substratum were significant but not their statistical interaction term (Table 3). The ANOVA model explained 75 percent of the observed variation. Current flow contributed more (69%) than substratum (5%).

Total leaf consumption differed between the various combinations (Figure 1c). Two-way ANOVA showed that flow and substratum, as well as their statistical interaction term were significant (Table 3) in explaining the observed variation in leaf consumption. The relative contribution of current (53%) was much higher than the contribution of substratum (21%) and the interaction term (5%).

Feeding rates were not calculated for compartments with gravel because the number of *A. aquaticus* present could not be assessed from the daily observations. Feeding rates for the regime sand 3 cm s^{-1} was 0.694 mg dry weight day^{-1} (s.e.=0.022) and for sand 9 cm s^{-1} 0.649 mg dry weight day^{-1} (s.e.=0.074). This difference was not significant (Table 3).

The variation in the number of specimens observed at different positions of the compartments was significantly explained by both flow and substratum as well as by their interaction term (Table 4). The relative contribution of substratum was higher than current and the interaction term. In the regimes with current flow of 3 cm s^{-1} most specimens were found on the leaf discs, followed by substratum (Figure 1d). In contrast, in the regimes with current flow of 9 cm s^{-1} most specimens were found at the back of the compartment, followed by leaf discs and then substratum. An increase in current velocity thus resulted in a decrease in the number of specimens on leaf discs in favour of the back of the compartment.

Table 4: Explained variation (%) and relative contribution of the independent factors (%) of Two-way ANOVA with average number of specimens as dependent variable.

		Relative contribution of		
Position	Explained variation	Current velocity	Substratum	Interaction term
On leaf discs	87	33	39	15
On substratum	81	23	39	18
Front	62	19	25	17
Back	78	21	44	13

Energy budgets were obtained only for both regimes with sandy substratum because feeding rates could not be calculated for regimes with gravel. The majority of the available energy was allocated to active respiration (Figure 2). Energy allocated to body maintenance was similar for both regimes (approx. 28%) but higher current flow resulted in less energy available for growth (6% for experiments at 3 cm s^{-1} and 2% with flow of 9 cm s^{-1}). In addition, there was a weak but significant correlation between growth and mortality (Figure 3). Increased respiration costs thus resulted in decreased growth and also in higher mortality.

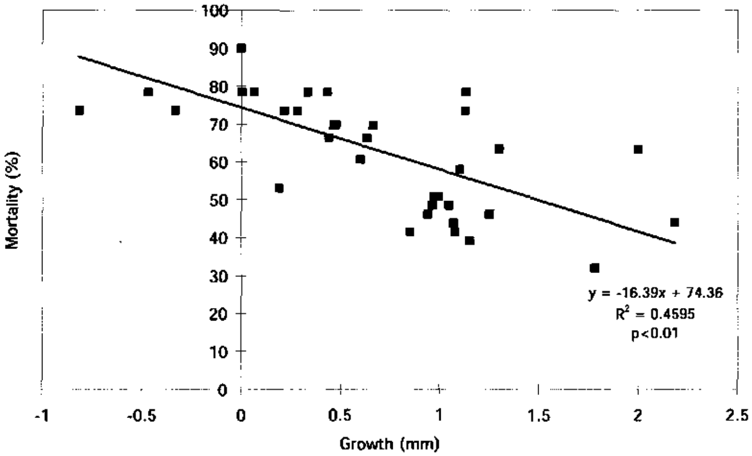

Figure 3: Relationship between growth rates and arcsine transformed mortality. Data were obtained from experiments 2 and 3.

Discussion

The present study clearly shows that the response of *Asellus aquaticus* differed between the various combinations of current flow and type of substratum. Both factors affected growth, mortality, and behavior of *A. aquaticus*. The importance of these two physical factors for benthic macroinvertebrates has already been indicated by many authors (e.g. Minshall 1984, Barmuta 1990, Lancaster and Mole 1999).

The observed specific growth rates in this study ranged from -0.47% (gravel, 9 cm s^{-1}) to 1.37% fresh weight day^{-1} (sand, aquaria). MARCUS ET AL. (1978) and GRAÇA ET AL. (1993) found a strong relationship between growth of *A. aquaticus* and type and quality of food, whereas MURPHY AND LEARNER (1982) showed that growth differed between cohorts of *A. aquaticus*. In the present study, all specimens came from the same overwintering cohort. Food quality was apparently good, as the observed specific growth rate in the regime sand without flow (experiment A) is consistent with other published data for well growing animals (MURPHY AND LEARNER 1982). Since of the same food source was supplied in all regimes, it

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CHAPTER 7

EFFECTS OF BENZO(A)PYRENE AND SIZE OF ORGANIC MATTER PARTICLES ON BIOACCUMULATION AND GROWTH OF *ASELLUS AQUATICUS*

EDWIN THM PEETERS, TABE J DE JAGER, JOHN AJ BEIJER AND ALBERT A KOELMANS

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> *Bodily exercise, when compulsory, does no harm to the body,*
> *but knowledge which is acquired under compulsion*
> *obtains no hold on the mind.*
>
> *Plato*
> *The Republic, c. 428-348 BC*

Abstract

The effects of sediment-bound toxicants to aquatic invertebrates may vary due to differences in bioavailability, food quality or food structure. The equilibrium partitioning theory (EPT) assumes that organic matter content of sediments and not structure of organic matter is relevant for biological effects of polycyclic aromatic hydrocarbons.

To test this hypothesis effects of benzo(a)pyrene (B(a)P) and size of sediment organic matter particles on the bioaccumulation and growth of the waterlouse *Asellus aquaticus* were studied in laboratory microcosms. Sediments and *A. aquaticus* were both sampled in an unpolluted, spring fed pond. The sampled sediment was divided into two portions. From one portion the size of the organic matter particles was mechanically reduced. One set of each sediment fraction (fine and coarse) was spiked with B(a)P and incubated for 3 weeks resulting in a concentration of 70 mg B(a)P per kg sediment. Bioassays of 32 days were performed in a 2 x 2 factorial design with four replicas of each treatment.

The results showed that the growth of *A. aquaticus* was mainly influenced by the size of organic matter particles. Growth was significantly less (27%) on finer sediments than on coarser sediments. The increase in length was 9-14% lower in the spiked sediments, but this difference was not significant. The reduced growth of *A. aquaticus* on finer sediments may be due to a change in the availability and/or quality of food together with a change in feeding behavior.

The coarse and fine spiked sediment types did not differ significantly with respect to the sediment water partition coefficient, the organic carbon water partition coefficient, and the bioconcentration factor. In contrast, the biota to sediment accumulation factors were significantly 15% higher in the cosms with coarse sediments than in cosms with fine sediments. However, this difference is too small to conflict with EPT.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) readily adsorb to inorganic and organic particles in soils and sediments or suspended in water (MÜLLER 1987, KOELMANS ET AL. 1997). The risk of PAH-contaminated sediments for sediment-feeding macroinvertebrates largely depends on PAH bioavailability. Abiotic partitioning between water and sediment and transformation reactions, such as biodegradation and photolysis, determine the actual concentrations of PAHs to which organisms are exposed (VAN BRUMMELEN ET AL. 1998). The partitioning behavior is thus a major factor controlling the abiotic component of bioavailability. Accumulation of PAH in sediments largely depends on the content of organic matter (MÜLLER 1987, VAN HATTUM 1995). The equilibrium partitioning theory (EPT) (DI TORO ET AL. 1991) assumes that the extent of bioaccumulation and biological effects of PAH occur at similar concentrations when normalized to organic carbon content. Therefore, according to the EPT, differences in sizes of organic matter particles do not affect PAH uptake and biological responses, such as mortality and growth rate.

In aquatic environments, uptake of PAHs by organisms may take place via gills or the skin and from dietary sources via the gastrointestinal tract (VAN BRUMMELEN ET AL. 1998). Sediment inhabiting invertebrates may have additional uptake from ingested sediments or from pore-water. Bioaccumulation varies among species and is determined by various characteristics of the organisms, such as size, age and sex, nutritional state, lipid content, metabolism, and feeding behavior (NEFF 1979). Although EPT assumes that exposure route is not significant, LANDRUM AND ROBBINS (1990) observed that for hydrophobic compounds, such as B(a)P, uptake from dietary sources can explain 100% of total residue. It has been observed that selective ingestion of sediment particles of specific size and composition affects contaminant exposure for oligochaetes (MCMURTHY ET AL. 1983) and the amphipod *Diporeia* (LYDY AND LANDRUM 1993). Therefore, it is plausible that the type of ingested food and feeding ecology of species play important roles in bioaccumulation of PAH.

The objective of the present study was to test the EPT-based hypothesis that the size of organic matter particles does not affect the degree of bioaccumulation and biological responses (survival and growth) to contamination with PAH. Therefore, a 2 x 2 factorial laboratory experiment was performed to investigate the effects of two different concentrations of benzo(a)pyrene (0 and 70 mg/kg sediment) and two types of sediments (differing only in the size of organic matter particles) on the growth of the freshwater isopod *Asellus aquaticus* (L.) and on the bioaccumulation in this species.

Materials and methods

Selection of test compound and test species

B(a)P was chosen as test compound because (a) its hydrophobicity implies that uptake via food would be an important route and (b) many sediments in The Netherlands are contaminated with this PAH. Concentrations up to 100 mg B(a)P/kg dry sediment have been observed in suspended solids and sediments of surface waters in the Rhine-Meuse delta (VAN KLAVEREN 1989, VAN HATTUM 1995).

A. aquaticus was chosen as test organism because (a) it is a common species, (b) it accumulates PAHs (CURTO ET AL. 1993), (c) PAH residue levels are preferably measured in species with low metabolic activity (VAN BRUMMELEN ET AL. 1998), and (d) this species showed negligible biotransformation (VAN HATTUM 1995). Furthermore, this deposit-feeding species is an important food source for predatory invertebrates, fish and waterfowl (VAN HATTUM ET AL. 1989) and thus plays an important role in the benthic-pelagic coupling of the food chain transfer of PAH.

Animals

A. aquaticus was collected from a spring-fed pond in January 1998 and brought to the laboratory. For the experiment 400 specimens were collected that had a total length of 5-7 mm each. Total length was measured as the length from the top of the head to the end of the pleotelson, thus excluding antennae and uropods. The collected animals were divided into lots of 20 specimens for later allocation into the experimental set-up. Individuals from three lots (60 in total) were measured immediately to determine the mean total length and wet weight at the beginning of the experiment. Dry weight per lot (70 °C, 24h) was also measured.

Preparation of sediments

Sediments (upper 10 cm) were collected from the same location as *A. aquaticus*. Initial analyses showed that the sediments of this pond were not contaminated with organic contaminants. Different steps were taken in the preparation of the sediments (Figure 1). The sample was sieved through successive sieves of 10 mm and 1 mm mesh size. All material larger than 10 mm, mainly twigs and leaves, was not used in the experiment. The fraction <1 mm was divided in 16 equal parts using a sample divider (Retsch, Dassel, Germany, Type x577). The fraction 1-10 mm was divided in two equal parts on wet weight basis. One part was further divided into eight equal parts, and the other part was mechanically

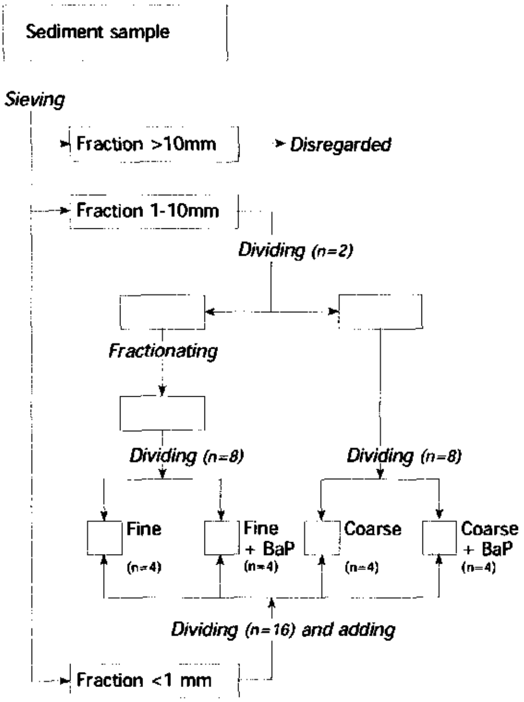

Figure 1. Diagram showing the preparation of the sediments.

fractionated (Heidolph, Type Diax 900) for 5 min to obtain fine material <1 mm. Afterwards, this part was also divided in eight equal parts using the sample divider. The original fraction <1 mm was combined with the original fraction 1-10 mm or with the fractionated fraction 1-10 mm resulting in eight replicates for both 'coarse' and 'fine' sediment type (Figure 1). The >1 mm grain size fraction was nearly absent in the fractionated samples and the organic matter fraction in the different grain size classes increased due to the fractionation, except for the largest grain size (Figure 2). Thus, the sediments differed in the *size* of organic matter particles but not in the nature and the quality of the organic matter fraction.

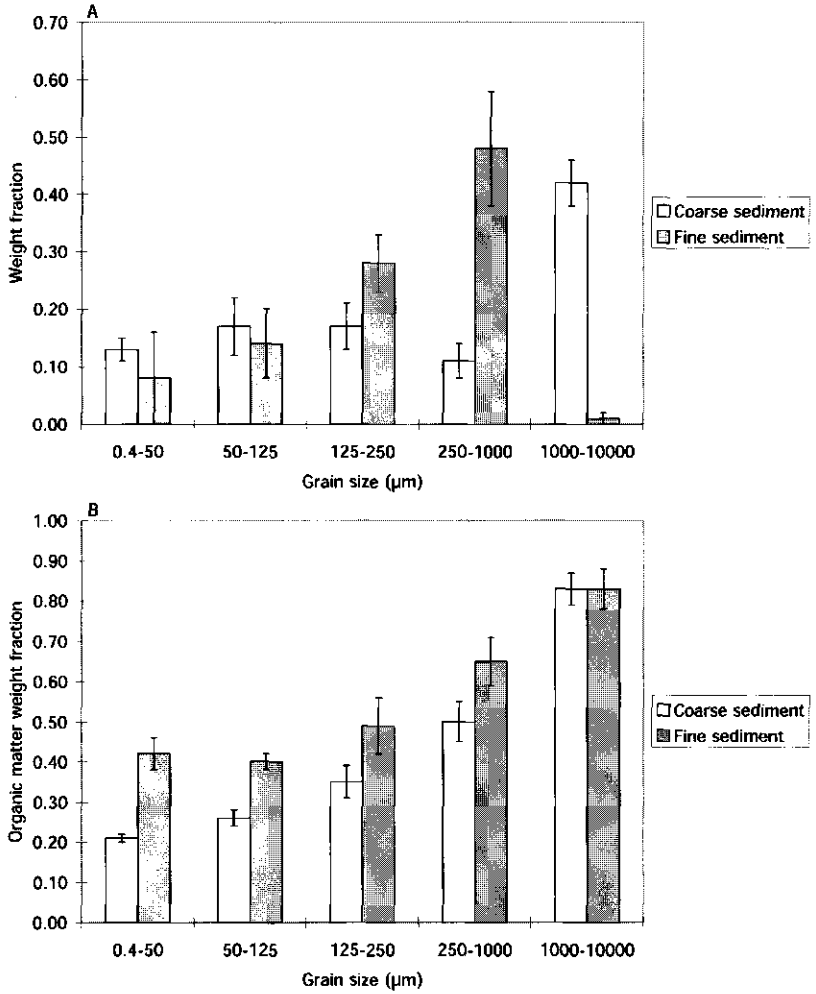

Figure 2: Characterization of the two sediments used in the experiment by (A) average grain size distribution and (B) average fraction of organic matter at the end of the experiment. Error bars indicate standard deviations (n=4).

Spiking sediments

From each sediment type (fine and coarse) four replicates were spiked with a solution of B(a)P (Aldrich, Zwijndrecht, The Netherlands) in acetone while the sediments were thoroughly mixed (Figure 1). All sediments were shaken for 48 h to homogenize B(a)P over the sediment. The spiked sediments were incubated 19 days and shaken manually three times per day, followed by a period of 7 days without shaking. The resultant total contact time of 28 days is supposedly long enough to reach steady-state conditions with respect to PAH partitioning (CORNELISSEN ET AL. 1997). The final B(a)P concentration was 70 mg B(a)P per kg sediment.

Bioassays

The effects of B(a)P and size of organic matter were studied in a 2 x 2 factorial design with four replicas of each treatment (Figure 1). Sixteen glass aquaria (25.5 x 14.5 x 15 cm) were placed in a water bath to maintain a constant temperature of 12 ± 1 °C. Each aquarium was filled with prepared 0.6 L sediment, resulting in a sediment layer of approximately 1 cm. An amount of 3 L well water was slowly added to each aquarium. A daily photoperiod of 8:16 h (light:dark) was maintained with an intensity of 100 $\mu E/m^2/s$. Both temperature and photoperiod simulate late winter/early spring situations in The Netherlands. One week after filling the aquaria with sediment and water, 20 specimens of *A. aquaticus* were released in each aquarium. The experiment finished 32 days later.

Water quality measurements

Temperature, oxygen concentration, pH, and conductivity were measured weekly during the bioassay. Also, weekly samples of 6 ml overlying water were taken to analyze total organic carbon content (OIC, College Station, TX, USA, Model 700 TOC Analyzer).

Sediment analyses

For B(a)P and organic carbon analyses, 20% of the sediment was randomly sampled in each aquarium at the end of the experiment. These samples were freeze-dried for 7 days. The other 80% was used for the determination of grain size distribution and organic matter content. Each sediment sample was sieved through successive sieves of 1 mm, 250, 125, 50, 0 µm mesh size. Each fraction was dried (105 °C, 3 h) and weighted. Ash-free dry weights were determined by heating at a temperature of 600 °C for 3h.

Analysis of B(a)P in water, sediment and A. aquaticus

Tenax (Chrompack, Bergen op Zoom, The Netherlands) was washed three times with acetone and three times with hexane and dried at 105 °C prior to use. Water samples (2 L) were extracted with 0.3 g Tenax in the dark. Tenax was removed from the water, and B(a)P was removed from the Tenax by multiple extraction with hexane. The hexane was reduced to 2 ml under a gentle flow of nitrogen. Samples were stored at -20 °C before analyzing

B(a)P concentrations.

Freeze-dried sediment samples were grinded with a mortar. At least 0.5 g dried sediment, 1 ml demineralized water, and 20 ml NMP (1-methyl-2-pyrrolidinone) were added to a glass beaker. Extraction and destruction were performed in a microwave (CEM, type MDS 2100) at a temperature of 130 °C for 1 h followed by a cooling period of 0.5 h. 2 ml of the extraction liquid was placed in a centrifuge for 3 minutes (10,000 rpm). One ml supernatant was used for B(a)P analysis using a HPLC column (Vydac 5, C18 rev. phase, 250x4.6 mm) followed by combined photo diode array (UV) and fluorescence detection. NOORDKAMP ET AL. (1997) provide a detailed description of the analytical method, including extraction recoveries (97-102%).

At the end of the experiment, isopods were kept in water-only systems without food for 1 day. This period is long enough to defecate (VAN HATTUM 1995). Subsequently, they were freeze-dried and grinded with a mortar. B(a)P extraction was performed as described above with 0.2 ml demineralized water and 6 ml NMP.

Measurements on A. aquaticus

The position of all specimens of *A. aquaticus* in each aquarium was determined each day. The number of specimens in or on the substratum was recorded in each aquarium for 5 minutes. Visibly dead animals were removed from the aquaria. At the end of the experiment total length and wet weight were measured for each specimen. Dry weights were determined per replica.

Statistical analyses

Paired *t* tests were performed to analyze significant differences between treatments over time. The Kruskal-Wallis H test was used as a nonparametric analyses of variance to test for significant differences between the treatments. The null hypothesis (no difference between groups) was rejected when significance was lower than 0.05. All analyses were performed with SPSS for Windows 7.5.

Results

General water quality variables

Temperature (12.4 ± 0.2 °C), oxygen concentrations (9.1 ± 0.9 mg/L), conductivity (179 ± 3 µS/cm), and pH (7.3 ± 0.1) did not show significant differences among the treatments nor over time. Total organic carbon content in the overlying water during the experiment (Figure 3) obviously relates to carbon originating from the sediment. TOC showed significant difference between the treatments and a clear trend in time (ANCOVA: F=1355.4, df=96, $P<0.001$). The carbon loss rate was higher in the course sediments; this can be explained by mineralization or flocculation and subsequent settling to the sediment.

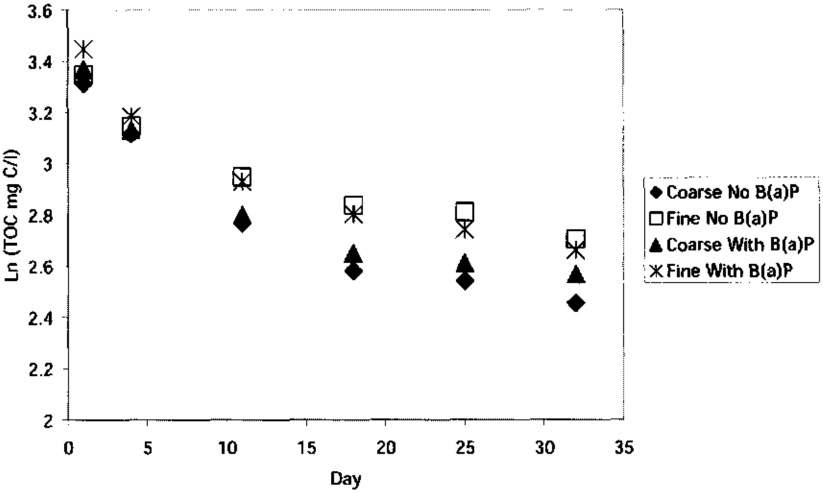

Figure 3: Water TOC concentrations for the four treatments during the experiment (n=4).

B(a)P concentrations and partitioning

Figure 4 shows the B(a)P concentrations in the five grain size fractions for the two spiked sediments. B(a)P concentrations in the smallest grain size class were below detection limits (2 mg/kg sediment on dry weight basis) for both sediment types.

For the coarse sediment type, B(a)P concentrations among the grain size classes (excluding the smallest fraction) was not significantly different (Kruskal-Wallis: H=5.934, df=3, *P*=0.115). This was also the case for B(a)P concentration normalized on organic matter (Kruskal-Wallis: H=4.831, df=3, *P*=0.185). However, B(a)P concentrations among the grain size classes (excluding the smallest fraction) for the fine sediment were significantly different (mg B(a)P/kg sediment: Kruskal-Wallis: H=10.257, df=3, *P*=0.017; mg B(a)P/kg organic matter: Kruskal-Wallis: H=13.059, df=3, *P*=0.005) due to the low concentrations in the largest fraction. Figure 4B shows that normalizing to organic matter content yields a higher variability of B(a)P concentrations. Organic matter normalization obviously does not lead to constant concentrations in the size fractions, as might be expected from EPT.

B(a)P concentrations in the sediment at the start and at the end of the experiment were not significantly different (paired t-test: coarse sediment T=0.91, df=3, *P*=0.432; fine sediment T=2.19, df=3, *P*=0.116). This illustrates that no significant transformation or degradation took place during the experiment.

B(a)P concentrations in the water phase were low (Table 1) and the difference between the sediment types was not significant (Kruskal-Wallis: H=0.527, df=1, *P*=0.468). Although average B(a)P concentrations in the isopods were different between the two sediment types (Table 1), this differences was not significant (Kruskal-Wallis: H=2.000, df=1, *P*=0.157). The calculated partition coefficients are presented in Table 1. The two spiked sediment types were not significantly different with respect to the sediment water partitioncoefficient (K_p:

Kruskal-Wallis: H=0.083, df=1, *P*=0.773), the organic carbon water partitioncoefficient (K_{oc}: Kruskal-Wallis: H=0.333, df=1, *P*=0.564), and the bioconcentration factor (BCF: Kruskal-Wallis: H=0.500, df=1, *P*=0.480). However, biota to sediment accumulation factor (BSAF) was 15% higher in cosms with coarse sediment and this difference was significant (Kruskal-Wallis: H=4.5, df=1, *P*=0.033).

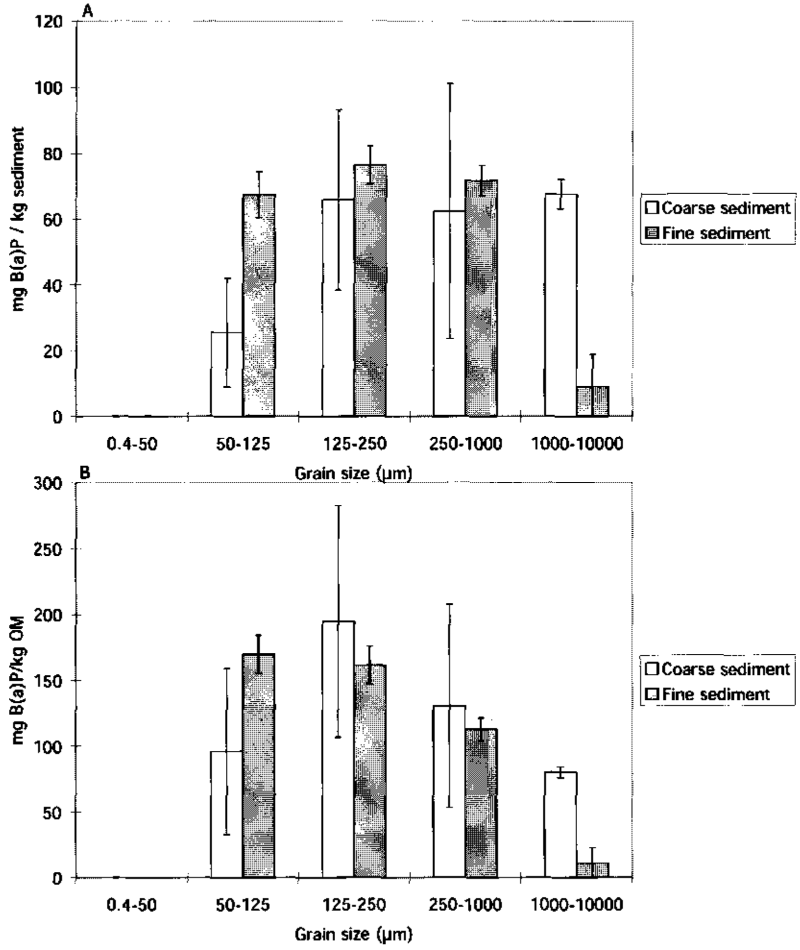

Figure 4: Average B(a)P concentrations per grain size fraction (A) and average B(a)P concentrations normalized to organic matter content (B). Error bars indicate standard deviations (n=4).

Table 1: Summary of experimental conditions and results (all standard deviations relate to quadruplicate measurements, except length and growth of *A. aquaticus*).

Variable	Coarse sediment without B(a)P	Fine sediment without B(a)P	Coarse sediment with B(a)P	Fine sediment with B(a)P
Organic matter (%)	59 ± 9	55 ± 4	59 ± 4	59 ± 8
B(a)P concentrations				
in water (µg/l)			0.0035 ± 0.0013	0.0038 ± 0.0007
in sediment (mg/kg DW)				
start			75.03 ± 5.97	74.93 ± 3.10
end			70.87 ± 10.12	67.91 ± 7.12
B(a)P in Asellus				
µg/g wet weight			13.20 ± 2.06	10.52 ± 0.66
µg/g dry weight			47.16 ± 7.35	37.55 ± 2.37
Partition coefficients				
Log K_p			7.33 ± 0.17	7.25 ± 0.06
Log K_{oc}[a]			7.85 ± 0.17	7.78 ± 0.06
Log BCF[d]			6.60 ± 0.19	6.42 ± 0.11
BSAF[b]			0.66 ± 0.03	0.56 ± 0.10
BSAF[c]			7.87 ± 0.68	6.89 ± 1.80
Performance Asellus				
survival (specimen)	17.0 ± 1.0	14.3 ± 2.8	16.5 ± 0.5	17.0 ± 0.7
% specimen on sediment	54 ± 11	47 ± 16	53 ± 14	50 ± 13
length (mm)	7.16 ± 0.18	6.87 ± 0.19	7.06 ± 0.17	6.76 ± 0.13
growth (% fresh weight / day)	1.33 ± 0.85	0.97 ± 0.81	1.22 ± 0.61	0.86 ± 0.74

[a]: assuming 50 % of organic matter is organic carbon

[b]: calculated as concentration *A. aquaticus* (mg/kg DW)/concentration sediment (mg/kg DW)

[c]: normalized to organisms' lipids (assuming lipid is 2.5 % DW) and sediment organic carbon

[d]: bioconcentration factor

Performance of A. aquaticus

Small differences were observed in survival of *A. aquaticus* (Table 1), but these differences were not significant (Kruskal-Wallis: H=2.52, df=3, *P*=0.472). In general, less than 20% of the specimen died during the experiment. *A. aquaticus* grew in all four treatments and all specimens were much larger than the initial length of 6.07 ± 0.13 mm (Table 1). Isopods held on coarse sediment without B(a)P grew approximately 1.1 mm in 32 days, and the increase in length was 27% less when held on fine sediment. The spiked sediments showed a further 10% reduction in growth. Specific growth rates (Table 1) ranged from 0.86 ± 0.74 (fine sediment with B(a)P), to 1.33 ± 0.85% fresh weight/day (coarse sediment without B(a)P). A Two-way ANOVA showed that length and thus growth was significantly affected by sediment type but not by B(a)P (Table 2). Also the interaction term (B(a)P * sediment type) was not significant, indicating that there were no interactive effects.

Table 2: Results of a two-way ANOVA performed on length data of *A. aquaticus*.

Source	Sum of Squares	df	Mean Squares	F	Sig
Corrected Model	6.569	3	2.190	5.346	0.001
Intercept	12169.136	1	12169.136	29707.511	0.000
B(a)P	.673	1	.673	1.644	0.201
Sediment type	5.753	1	5.753	14.045	0.000
B(a)P* Sediment type	0.005	1	0.005	0.013	0.909
Error	101.589	248	0.410		
Total	12335.369	252			
Corrected Total	108.158	251			

Daily observations on *A. aquaticus* showed that approximately 50% of the specimens were visible on the substratum (Table 1), and there were no differences between the treatments (Kruskal-Wallis: H=7.051, df=3, *P*=0.07). At the end of the experiment it was observed that specimens from the contaminated sediments reacted slower. When placed on their back it took more time to turn back on their feet than specimen from the clean sediments. Because the effect was not quantified, no statistics are presented.

Discussion

B(a)P recovery

The B(a)P concentrations in the spiked sediments were approximately 70 mg/kg and remained constant during the course of the experiment (Table 1). So, during the experiment losses due to degradation, volatilization, photolysis or biotransformation were negligible. However, as these concentrations account for only 67% of the spiked amount, significant losses must have occurred during the spiking and the 28-day B(a)P equilibration phase. These losses occurred prior to the start of the actual experiment, so they do not interfere with the data interpretation.

Abiotic partitioning

The results of this study show that B(a)P concentrations in the size fractions did not converge to similar values when normalized to organic matter (Figure 4). Obviously, the basic premise of EPT that PAHs are rapidly and primarily associated with organic matter fractions does not hold for the present experimental conditions. This may be explained from (a) nonequilibrium in the larger size fractions (CORNELISSEN ET AL. 1997), or (b) B(a)P surface particle size on mediating the effects of B(a)P on growth and survival of *A. aquaticus* could not be addressed because no significant effects of B(a)P on Asellus were detected.

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CHAPTER 8

ANALYZING EFFECTS OF MULTIPLE STRESSORS ON THE GROWTH OF *ASELLUS AQUATICUS*: A MODELING APPROACH

Edwin THM Peeters, Jean JP Gardeniers and Marten Scheffer

in prep

> *It is a capital mistake to theorize before*
> *you have all the evidence.*
>
> *Sir Arthur Conan Doyle*
> *Scandal in Bohemia, The Adventures of Sherlock Holmes, 1891*

Abstract

A bio-energetic model for the waterlouse *Asellus aquaticus* is presented based on published consumption and respiration functions. The model is used to investigate whether effects of multiple stressors on individual organisms can be explained through their effects on energy budgets.

Analyses of data from two laboratory growth experiments with the model indicate that sub-lethal effects of combined stressors such as current velocity, substratum composition, structure of food, and exposure to a toxicant can be understood from the effects of the separate stressors on consumption and respiration.

The energy budget model assumes that food and exposure to benzo(a)pyrene have additive effects on the growth of *A. aquaticus.* The model simulation is in line with the laboratory observations and thus the combined effects are well predicted by the energy budget model. The model also assumes that the combined effects of current flow and substratum type are multiplicative. The model simulation does not fit the experimental observations and in this case the assumption of multiplicative effects seems therefore not valid.

It is also argued that stressors impairing the growth of *A. aquaticus* through a reduction in consumption or through an increase in respiration will tend to have a negative effect on the decomposition rate of organic matter in aquatic systems in which this species is important.

Introduction

Aquatic organisms are exposed to a variety of stressors that may be abiotic (physical and chemical stress) or biotic (competition, predation). Studying multiple stress at different levels of biological organization can give insight into the effects of stressors, their mechanistic bases, and their ecological consequences (MALTBY 1999). Although studies on population and community level may provide a description of the effects of stressors they hardly reveal mechanisms involved. By contrast, studies at the molecular and cellular level may provide detailed information on how chemicals interact with target sites but hardly give any information on the consequences of these effects for higher levels of organization. According to MALTBY (1999) an integrated approach is required in which the understanding of the mechanistic bases of stress responses in individuals is used to predict or interpret their ecological consequences.

Energy budgets and the closely related Scope for Growth (SfG) seem a promising approach for studying the effect of multiple stressors on individual organisms. Scope for Growth is defined as the difference between energy adsorbed from food and that lost via excretion and metabolism (WARREN AND DAVIS 1967). It is usually determined by measuring energy ingested, egested, respired, and excreted. A positive SfG indicates that energy is available for production, while a negative SfG indicates that reserves must be used to maintain the individual (MALTBY 1999).

Several studies showed that various stressors may affect elements of the energy budget of organisms (e.g. KOOIJMAN 2000). For example, ecotoxicological studies have demonstrated that organisms make direct energy costs to resist contaminants by avoidance, exclusion, removal or complexation of the contaminant (CALOW 1989) but also that chemical stressors may impact consumption rate (BASSET 1993, LE BRAS 1987). For other stressors, such as shear stress, competition for food, and predator avoidance it is even more obvious that they have an impact on the energy budget.

The waterlouse *Asellus aquaticus* is a frequently used species in laboratory studies. Many data are available on ecological and ecotoxicological aspects of the species and various publications address the energy budget of this species explicitly (ADCOCK 1982, PRUS 1971, 1972, 1976a, 1976b, 1977). However, so far no attempt has been made to model the response of *A. aquaticus* to multiple stressors.

The aim of this study is to investigate whether effects of multiple stressors can be plausibly explained through their effect on the energy budget. An energy budget model for the waterlouse *Asellus aquaticus* is presented and applied to data from two growth experiments (see chapters 6 and 7) to quantify the relative contribution of the stressors and to analyze the nature of the interaction between the stressors.

Model

The dynamics of body weight, W (J), is described as a function of maximum consumption, C (J d^{-1}); the impact of stressors reducing food consumption, d (-); efficiency for converting food to body weight, e (J J^{-1}); "normal" respiration, R (J d^{-1}); and the impact of stressors affecting respiration, a (-):

$$dW/dt = e*d*C - a*R \tag{1}$$

In general, consumption and respiration tend to be proportional to W^k with $k < 1$ (e.g. STRASKRABA AND GNAUCK 1985). However, the present model uses the linear relationship found by ADCOCK (1982) between consumption (J d^{-1}) and body weight (mg AFDW) for *A. aquaticus* leaving the brood pouch (>1mm) and feeding on alder leaves with a certain energy content, F_E (J mg^{-1}):

$$C=(0.1078+0.1030*W)*F_E \tag{2}$$

It is assumed that the reduction in food intake (*d*) consists of multiplicative effects of toxicants (d_T), and of other aspects not considered explicitly in the model (d_F):

$$d=d_T * d_F \tag{3}$$

The value of *d* is limited to be in the range from 0 (complete reduction) to 1 (no reduction).

Respiration is usually measured through O_2 consumption (µl mg^{-1} h^{-1}) and converted for use in the model to J mg^{-1} d^{-1} (Table 1). It is formulated as a function of weight following Adcock (1982):

$$R=0.4844 * \alpha * W^{\beta} \tag{4}$$

The parameters α and β are temperature dependent and for a temperature of 12 °C α=1.3017 and β =0.6903, for 18 °C α=1.9715 and β =0.7151 (ADCOCK 1982). The effect of stressors on respiration is formulated as multiplicative effects of change in maintenance respiration (a_M) and effects of physical stress (a_P) and toxicological stress (a_T):

$$a = a_M * a_P * a_T \tag{5}$$

The parameters a_P and a_T account for relative increase or decrease in respiration due to physical and toxicological stress respectively.

The complete model can be written as:

$$dW/dt = e\ d_F\ d_T\ F_E\ (0.1078 + 0.1030\ W) - 0.4844\ \alpha\ \ a_M\ \ a_P\ \ a_T\ W^{\beta} \tag{6}$$

Animal body weight is converted to body length (Table 1) and growth rates, G_r (mg d^{-1}), are calculated to be able to check the model against data on growth measurements.

Table 1: Conversions used for the model.

Conversion	Conversion factor	Source
DW Alnus to Joules	1 mg DW Alnus = 22.0259 J	PRUS 1971
O_2 uptake to Joules	1 µL/mg DW/h = 0.4844 J/mg DW/d	
Animal DW to Joules	1 mg DW = 12.3838 J mixed sexes	ADCOCK 1982
Animal DW to length	Ln(DW)=2.71*Ln(length)-4.58	GRAÇA ET AL. 1993

Results

Experiment 1

The model is used to analyze data from a growth experiment with *A. aquaticus* exposed to different combinations of current velocity and mineral substratum (PEETERS ET AL. chapter 6) (Table 2).

Table 2: Measured final length and calculated growth rate of *A. aquaticus* exposed to different combinations of current velocity and mineral substratum. Poplar leave disks were used as a food source. The experiment was performed at 18 °C and lasted for 54 days. Initial length was 4.67 mm.

Type of substratum	Current flow ($cm\ s^{-1}$)	Length (mm)	Growth rate ($mg\ d^{-1}$)
Sand	0	6.63	0.020
Sand	3	5.77	0.008
Sand	9	5.14	0.004
Gravel	0	5.94	0.011
Gravel	3	5.44	0.006
Gravel	9	4.56	-0.001

The initial values of the parameters are given in Table 3.

Table 3: Value of the parameters for the least stressful situation in the first experiment.

Parameter	Initial value	Source
e	0.236	ADCOCK 1982
F_E	24.25 J mg^{-1}	Own measurements
α	1.3017	ADCOCK 1982
β	0.7151	ADCOCK 1982

The least stressful combination (sand, 0 cm s^{-1}) is used to calibrate the consumption parameter (d_F) in such a way that the modeled growth rate equals the observed rate. Assuming that both flow and substratum type affect respiration (a_P) and not consumption, the respiration parameter (a_P) was subsequently adjusted for each experimental treatment in such a way that the modeled and observed growth rates were equal.

The total amount of energy (J) over the experimental period of 54 days is calculated for consumption (T_C), assimilation (T_A) calculated as total consumption multiplied by consumption efficiency, maintenance (T_M), physical stress (T_P), and growth (T_G) for each experimental situation.

To explore the nature of the interaction between the two variables, the effect of the combination 'gravel with 3 cm s^{-1}' is modeled from the single effects and compared with the laboratory results.

The modeled growth rate with the initial parameters was similar to the observed growth rate for the combination sand, 0 cm s^{-1} and therefore the value of the parameter d_F remains 1. Figure 1 shows the calibrated values for the relative increase in respiration due to physical stress (a_P) and in Table 4 the cumulative amounts of energy during the experimental period for the different processes in the model are given.

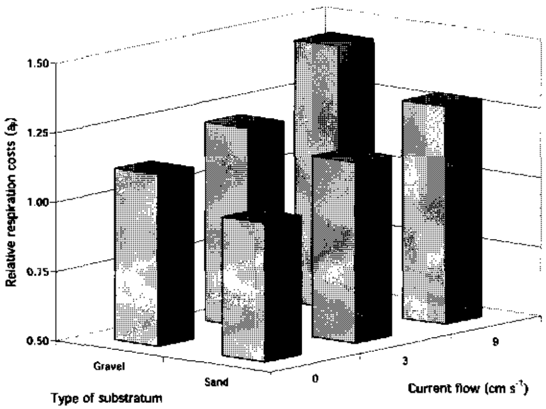

Figure 1: The calibrated relative respiration costs (a_P) for the different treatments in experiment 1.

Table 4: Total amount of energy (in joules) over the experimental period of 54 days for consumption (T_C), assimilation (T_A), maintenance (T_M), extra respiration (T_P) and growth (T_G).

Combination	T_C	T_A	T_M	T_P	T_G
Sand 0 cm s^{-1}	311.59	73.54	60.19	0.00	13.34
Sand 3 cm s^{-1}	276.38	65.22	51.08	7.66	6.48
Sand 9 cm s^{-1}	252.53	59.60	44.42	12.70	2.48
Gravel 0 cm s^{-1}	283.01	66.79	52.86	6.24	7.69
Gravel 3 cm s^{-1}	263.80	62.58	47.63	10.34	4.30
Gravel 9 cm s^{-1}	231.80	54.71	38.21	17.00	-0.50

The tuned parameter a_P ranges between 1.118 and 1.445. For both substratum types, physical stress due to higher current velocity resulted in a higher value for the parameter a_P and as a consequence a higher predicted amount of energy spent to overcome the stress (R_P). At a given current velocity a_P is higher for gravel than for sand (Figure 1). Simulations also show that higher values for the physical stress related respiration (a_P) result in reduced growth accompanied by a lower amount of energy consumed and a lower amount of energy spent for maintenance summed over the 54 day experimental periods (Table 4).

Increasing current velocity from 0 to 3 cm s^{-1} on a sandy bottom resulted in $a_P = 1.150$ whereas changing the type of substratum with no flow resulted in $a_P = 1.118$. Thus changing current velocity from 0 to 3 cm s^{-1} had a greater effect than changing the type of substratum. The combined effect should equal $a_P = 1.150*1.118 = 1.2857$ in case the effects of both stressors are multiplicative. Applying this value in the model resulted in a predicted growth rate of 0.004 mg d^{-1}, which is significantly lower than the observed growth rate of 0.008 mg d^{-1} (Table 2). Thus, the combined effect of both stressors observed in the experiment is smaller than the modeled multiplicative effect.

Experiment 2

The results from a growth experiment with *A. aquaticus* exposed to different combinations of size of organic matter and benzo(a)pyrene are also analyzed (PEETERS ET AL. 2000, chapter 7) (Table 5).

Table 5: Measured final length and calculated growth rate of *A. aquaticus* exposed to different combinations of size of organic matter and benzo(a)pyrene. The experiment was performed at 12 °C and lasted for 32 days. Initial length was 6.07 mm.

Type of organic matter	Benzo(a)pyrene	Length (mm)	Growth rate (mg d^{-1})
Coarse	Without	7.16	0.024
Fine	Without	6.87	0.017
Coarse	With	7.06	0.022
Fine	With	6.76	0.014

The initial values of the parameters used in the model are given in Table 6.

Table 6: Values of the parameters for the least stressful situation in the second experiment.

Parameter	Initial value	Source
e	0.236	ADCOCK 1982
F_E	24.25 J mg^{-1}	
α	1.9715	ADCOCK 1982
β	0.6903	ADCOCK 1982

The least stressful combination (coarse organic matter without B(a)P) was used to calibrate the parameter that describes the energy content of the food (F_E) so that the modeled and observed growth rates were equal. It is assumed that the energy content did not differ between coarse and fine organic matter and that differences in the size of organic matter affects consumption through the parameter d_F. Therefore, the combination fine organic matter without B(a)P was used to calibrate the parameter d_F.

Exposure to contaminants may result in a lower value for the parameters d_T (reduction in food consumption) or a change in a_T (respiration). Which element of the energy budget is affected by exposure to benzo(a)pyrene is not clear and therefore the two possibilities were checked as alternative explanations.

The total amount of energy (J) over the experimental period of 54 days is calculated for consumption (T_C), assimilation (T_A) calculated as total consumption multiplied by consumption efficiency, maintenance (T_M), toxic stress (T_T), and growth (T_G) for each experimental situation.

To explore the nature of the interaction between the two stressors, the effect of the combination 'fine organic matter with benzo(a)pyrene' is modeled from the individual effects and then compared with the laboratory results.

The calibration for this set of experiments resulted in an energy content of the food (F_E) of 18.02 J mg^{-1} for the course organic matter and a reduction in consumption of 6.8% for the fine sediment. Table 7 shows the inferred effects of exposure to benzo(a)pyrene on the elements of the energy budget.

A small relative decrease in the consumption (through d_T) or a small increase in the respiration (a_T) were sufficient to explain the observed decrease in growth rates when *A. aquaticus* was exposed to benzo(a)pyrene.

Table 7 shows that for the fine sediments the value for a_T is slightly higher and for d_T slightly lower than for the coarse sediment. Simulating the combined effects of organic matter type and exposure to benzo(a)pyrene by applying the parameter values from Table 7 resulted in a predicted growth rate of 0.0014 mg d^{-1} for the experimental period of 32 days. Because this value is equal to the observed growth rate (Table 5) the combined effects of food structure and exposure to B(a)P seem simply to be additive.

Table 7: Values for the parameters d_T and a_T to obtain the growth as observed in the experiment for the combinations with benzo(a)pyrene. Cumulative amount of energy (in joules) during 32 days for consumption (T_C), assimilation (T_A), maintenance (T_M), extra respiration due to exposure to B(a)P (T_T), and growth (T_G) are also given.

Combination	d_T	a_T	T_C	T_A	T_M	T_T	T_G
No effect of B(a)P							
Coarse OM	1	0	170.18	40.16	30.34	0.00	9.82
Fine OM	1	0	152.60	36.02	29.08	0.00	6.93
Effect of B(a)P on consumption							
Coarse OM	0.9766	0	163.99	38.70	29.91	0.00	8.80
Fine OM	0.9720	0	146.20	34.50	28.61	0.00	5.89
Effect of B(a)P on respiration							
Coarse OM	1	0.0310	167.94	39.63	29.91	0.93	8.80
Fine OM	1	0.0348	150.41	35.50	28.61	1.00	5.89

Discussion

Although, the behavior of a model may always be an artefact from the mathematical formulation (ROUGHGARDEN 1983), the present results indicate that an energy budget model can be useful to help interpreting the combined effects of multiple stressors on the growth. Simulations give an indication of cumulative effects on growth and consumption over time. A check of the results of predicted effects of multiple stressors against experimental results indicates whether interactions of stressors are simply additive or multiplicative. For example, the energy budget model assumes that food and exposure to benzo(a)pyrene have additive effects on the growth of *A. aquaticus*. The model simulation is in line with the laboratory observations and thus the combined effects are well predicted by the energy budget model. The model also assumes that the combined effects of current flow and substratum type are multiplicative. The model simulation does not fit the experimental observations and in this case the assumption of multiplicative effects seems therefore not valid.

The present model of individual growth does not provide information on the severity of the long-term impact on the population level. For a vital population it is necessary to reproduce within a certain period of time. For a mixed (males and females) population, the mean weight at which reproduction takes place is approximately 2.0 mg DW (WILLIAMS 1960). Furthermore, for a vital population *A. aquaticus* has to reproduce within a period of 2000 degree-days (ØKLAND 1978). These two criteria can be used to calculate the minimal growth rate for *A. aquaticus* to reproduce (experiment 1: 0.015 mg d^{-1}; experiment 2: 0.012 mg d^{-1}). On the basis of the energy budget, all combinations in experiment 2 allow a vital population of *A. aquaticus* whereas in nearly all combinations in experiment 1, the stress offered to *A. aquaticus* is too high for long-term survival of the population. In practice, the species may be able to withstand higher amounts of stress, for example, by increasing its efficiency for converting food to body weight or by a more selective feeding. ADCOCK (1982), for example, found that specimens in a population have higher food conversion efficiencies then individually living specimens.

Although *A. aquaticus* can use different food sources (MARCUS ET AL. 1978) its primary food is decaying organic matter (WILLIAMS 1960). The species, therefore, plays an important role in the decomposition process. The two laboratory experiments show that exposure to stressors may impair growth. The simulations of the experiment with different combinations of current flow and mineral substratum illustrate how stressors affecting respiration have a long-term effect on consumption. Increased stress resulted in a lower amount of energy consumed during the experiment. The energy spent to resist the physical stress cannot be invested in growth and the reduced growth results in a lower food intake because food uptake depends on body weight. The simulations of the experiment in which *A. aquaticus* was exposed to different types of organic sediments and to the chemical stressor benzo(a)pyrene also illustrate that the reduction in the amount of energy consumed, does not depend upon the mode of action of the stressors. Since exposure to stressors affecting elements of the energy budget leads to a decrease in the amount of food consumed in a certain period of time it may well have a negative effect on the decomposition process in the ecosystem.

The results presented in this study illustrate that effects of various types of stressors measured in the laboratory can be well interpreted with an energy budget model. Measurements of energy budgets in short-term experiments correlate well with long-term measures of growth and reproduction (e.g. BAYNE ET AL. 1985, MALTBY AND NAYLOR 1990, MALTBY 1994). A better knowledge of how specific stressors affect elements of the energy budget may therefore enhance the mechanistic understanding of factors that determine animal abundance in the field. Obviously, this requires consideration of more aspects such as predator avoidance behavior and competition for resources, which may be included in an expanded model.

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EPILOGUE

It is quality rather than quantity that matters.

Seneca
Epistles, 4 BC – 65 AD

The primary focus of this thesis is the analysis and quantification of the impact of multiple stressors on aquatic macroinvertebrate species and their communities. The variance partitioning method attributes the biological variation in multivariate field data into different sources. This thesis demonstrates, among others, that the method has been successfully used to distinguish between the effects of food and environmental factors (chapter 2) and to distinguish between the effects of contaminants and other stressors (chapters 3 and 4).

Although the method is very useful in quantifying effects of multiple stressors on communities, it does not in its present form indicate which sites are affected most by the combined effects of simultaneously operating contaminants. Such a procedure could be useful for prioritizing sites for sanitation.

One of the major problems facing ecotoxicology is assessing the ecological relevance of the effects of multiple contaminants. A frequently used endpoint is the protection of a certain fraction of the number of species. Based on such criteria laboratory results from single species experiments have been translated into standards to protect natural communities. It would be a challenge for future research to investigate whether this approach in setting standards could be linked to the variance partitioning method. This could provide a powerful check whether the extrapolation from laboratory to field makes sense.

In contrast to the multivariate analyses of the field data, the laboratory experiments and the energy budget model give insight into the nature of the interaction between stressors. For example, the effects of size of organic matter and exposure to benzo(a)pyrene appeared additive with respect to the growth of *Asellus aquaticus* (chapters 6 and 8). Furthermore, laboratory and model studies also offer better possibilities to analyze non-lethal effects of stressors than studies dealing with multivariate analyses of field observations. The link between the individual and the population is essential for the assessment of the effects of multiple stressors on the population level by the energy budgets of individuals. Especially the combination of experiments and energy budget models seems to offer a valuable tool to analyze and understand non-lethal effects of stressors on individuals and their consequences for the whole population. To increase the value of such an approach, future laboratory studies should focus on the development of response models to stressors whereas future model studies should focus on the link between individuals and populations and between populations and communities.

All studies presented in this thesis indicate that the biological response to a specific (group of) stressor(s) depends at least on the status of all other stressors. For example, the response of the in situ macroinvertebrate communities to food quality differs between water types (chapter 2). The effect of contaminants on in situ macroinvertebrates depends on the amount of organic carbon of the sediment (chapters 3, 4). The analyses of the distribution patterns of two gammarids showed that various stressors are involved in explaining these patterns (chapter 5). Furthermore, the growth of the waterlouse on different types of substratum is affected by the intensity of current velocity (chapter 6) and growth of *A. aquaticus* in laboratory conditions exposed to benzo(a)pyrene differed per sediment type (chapter 7). The modeling results described in chapter 8 indicate that energy budgets may be used to integrate the effects of multiple stressors.

In summary, this thesis demonstrates that effects of multiple stressors can be quantified in laboratory studies as well as in field situations and that energy budgets and scope for growth are powerful tools for improving our understanding of the distribution and abundance of organisms.

SUMMARY

Water is the principal, or the element, of things.

Thales
c 625-545 BC

The structure and the functioning of ecosystems depend on responses to natural and anthropogenic stressors that influence the physiology and behavior of organisms, ecological interactions within assemblages, and ecosystem processes. Nowadays, the impact of human activities has caused major environmental changes, such as climatic shifts, chemical pollution, destruction and loss of habitats, and eutrophication. One of the major negative effects of these impacts is the rapid decline in the world's biodiversity, impairing ecosystem functions such as primary production, carbon and nutrient conservation and cycling, decomposition, and food webs and resilience of ecosystems. A variety of mechanisms can impair ecosystem functioning and in nature organisms are always exposed to several simultaneously operating stressors. Several studies demonstrated that the combined effects of multiple stressors cannot be understood as a simple product of the individual effects. To understand how multiple stressors affect the composition and functioning of ecosystems it is necessary to know their quantitative contributions but also to explore their interactions.

The central theme of this thesis is the quantification of the combined effects of multiple stressors on benthic aquatic macroinvertebrates and communities. This is explored by analyzing databases with field observations, laboratory experiments, and an energy budget model.

The relative contribution of sediment food (e.g. organic matter, carbohydrates, proteins, C, N, polyunsaturated fatty acids) and environmental variables (e.g. oxygen, pH, depth, sediment grain size, conductivity) in explaining the observed variation in benthic macroinvertebrate species composition is investigated (Chapter 2). Soft bottom sediments, water and benthic macroinvertebrates were sampled in several water systems in The Netherlands. The method of variance partitioning is used to quantify the relative contribution of food and environmental variables in structuring the benthic macroinvertebrate community structure.

Approximately 60% of the total variation in the macroinvertebrate community structure could be explained by the variables included in the analyses. The variation in the macroinvertebrate species composition between different water types is primarily related to differences in main environmental variables (e.g. current velocity, dimensions, pH). However, the variance partitioning method shows that food variables also contributed significantly and that the effect of food depends on the intensity of other factors.

The results of the study indicate that the method of variance partitioning is an appropriate tool for analyzing the impact of different groups of variables and thus, contributes to the understanding of the functioning of complex aquatic ecosystems.

The impact of food variables differed between the macroinvertebrate functional feeding groups. Detritivores showed significant correlations with food quantity (organic matter content) and quality (polyunsaturated fatty acids, P, and C/N ratio). A higher content of organic matter usually goes along with lower oxygen concentrations. Therefore, the

observed lower species diversity and not changing macroinvertebrate densities with higher organic matter contents may be due to changes in either food quantity or oxygen concentration. Higher amounts of polyunsaturated fatty acids have a positive effect on the total macroinvertebrate density but not on the total number of taxa. It seems, therefore, that the productivity of benthic macroinvertebrates depends more on food quality than on food quantity.

Macroinvertebrates were also studied along a salinity gradient in the Northsea Canal, The Netherlands, to quantify the effect of trace metals (cadmium, copper, lead, zinc) on community composition (Chapter 3). In addition, two methods for assessing metal bioavailability (normalizing metal concentrations on organic carbon and on the smallest sediment fraction) were compared. Factor analyses showed that normalizing trace metals resulted in an improved separation of trace metals from ecological factors (depth, organic carbon, granulometry, and chloride). The variation in the macroinvertebrate data was partitioned into four sources using partial canonical correspondence analysis: 1. purely ecological factors, 2. purely trace metals, 3. mutual ecological factors and trace metals, and 4. unexplained. Partial CCA applied to total and normalized trace metal concentrations gave similar results in terms of unexplained variances. However, normalization on organic carbon resulted in the highest percentage of variation explained by purely ecological factors and purely trace metals. Accounting for bioavailability thus improves the identification of factors affecting the in situ community structure. Ecological factors explained 45.4% and trace metals 8.6% of the variation in the macroinvertebrate community composition in the ecosystem of the Northsea Canal. These contributions were significant and it is concluded that trace metals significantly affected the community composition in environment with multiple stressors. Variance partitioning is recommended for incorporation in further risk assessment studies.

Data from a study in the enclosed Rhine-Meuse delta were used to test whether bioassays are better descriptors of sediment toxicity than toxicant concentrations and whether ecological factors are more important than toxicants in structuring macroinvertebrate communities (Chapter 4). In the period 1992-1995, data were collected in the study area on macroinvertebrates, sediment toxicity, sediment contaminant concentrations, and ecological factors. The effects of various groups of pollutants (polycyclic aromatic hydrocarbons, trace metals, oil, polychlorinated biphenyls) and of ecological variables on the structure of the macroinvertebrate community in the Rhine-Meuse delta were quantified. Ecological factors explained 17.3% of the macroinvertebrate variation, while contaminants explained 13.8%. Another 14.7% was explained by the covariation between ecological variables and contaminants. Polycyclic aromatic hydrocarbons explained a larger part of the variation than trace metals. The contributions of oil and polychlorinated biphenyls were small, but significant. Elevated contaminant concentrations were significantly associated with differences in the macroinvertebrate food web structure. The response in laboratory bioassays (*Vibrio fischeri, Daphnia magna, Chironomus riparius*) was susceptible to certain contaminants but also to certain ecological factors. There was a weak correlation between in situ species composition and bioassays; 1.9% of in situ macroinvertebrate variation was explained by the bioassay responses. This seems to contradict the validity of using bioassays for a system-oriented risk assessment. Possible reasons for this discrepancy might be the manipulations of the sediment before the test and a higher pollutant tolerance of the in situ macroinvertebrates. Thus, macroinvertebrate field surveys and laboratory bioassays yield different types of information on ecotoxicological effects and both are recommended in sediment risk assessment procedures.

A large data set describing the distribution of two common gammarid species (*Gammarus fossarum* and *Gammarus pulex*) in streams in The Netherlands, was analyzed by logistic regression to evaluate the usefulness of this regression technique in defining habitat requirements (Chapter 5). A method is presented that derives optimum habitat ranges for environmental variables from logistic regression equations. The calculated optimum habitat ranges, which are related to the maximum likelihood of presence in the field, agreed with habitat requirements and ecological tolerances in the literature. Single logistic regressions provide good descriptions of the optimum habitat requirements and multiple logistic regressions give insight in the relative importance of each environmental variable. It is the combination that makes logistic regression a valuable tool for constructing habitat suitability indices. Current velocity, pH, Kjeldahl nitrogen, total phosphorus, ammonium nitrogen, conductivity, width, and depth are in this sequence the most important environmental variables in predicting the probability of occurrence of *Gammarus fossarum*, whereas current velocity, Kjeldahl nitrogen, pH, and depth are the most important variables for the prediction of the probability of occurrence of *Gammarus pulex*.

Experiments were performed to study the effects of near-bed flow and substratum composition on survival, growth and food consumption of the waterlouse *Asellus aquaticus* (L.) (Chapter 6). Short-term effects of increasing near-bed flow depended on the size of the animal and the type of substratum. Critical near-bed flow for detachment was almost the same on sand as on a polished surface whereas on gravel *A. aquaticus* could withstand higher flow velocities because specimens hid in interstitial spaces. Long-term experiments with different combinations of near-bed flow and substratum composition showed that flow had a greater effect than substratum on survival and growth. Substratum, however, had a greater influence on the distribution of individuals in the experimental unit. Since growth was reduced at high near-bed flow and no changes in levels of food intake were observed it is concluded that a substantial amount of energy is required for withstanding shear stress at higher flow rates. Furthermore, mortality showed a strong inverse correlation to growth. The interaction of effects of natural habitat characteristics and those of human-induced stressors such as trace metals and pesticides may be better understood using an experimental and modeling approach focusing on energy budgets.

The effects of benzo(a)pyrene (B(a)P) and size of sediment organic matter particles on the bioaccumulation and growth of the waterlouse *Asellus aquaticus* were studied in laboratory microcosms (Chapter 7). The effects of sediment-bound toxicants to aquatic invertebrates may vary due to differences in bioavailability, food quality or food structure. The equilibrium partitioning theory (EPT) assumes that organic matter content of sediments and not structure of organic matter is relevant for biological effects of polycyclic aromatic hydrocarbons and this was tested in the experiments. Sediments and *A. aquaticus* were both sampled in an unpolluted, spring fed pond. The sampled sediment was divided into two portions. From one portion the size of the organic matter particles was mechanically reduced. One set of each sediment fraction (fine and coarse) was spiked with B(a)P and incubated for three weeks resulting in a concentration of 70 mg B(a)P per kg sediment. Bioassays of 32 days were performed in a 2x2 factorial design with four replicas of each treatment. The results showed that the growth of *A. aquaticus* was mainly influenced by the size of organic matter particles. Growth was significantly less (27%) on finer sediments than on coarser sediments. The increase in length was 9 - 14% lower in the spiked sediments but this difference was not significant. The reduced growth of *A. aquaticus* on finer sediments may be due to a change in the availability and/or quality of food together with a change in feeding behavior. The coarse and fine spiked sediment types did not differ significantly with respect to the sediment water partition coefficient, the organic carbon water partition coefficient, and the bioconcentration factor. In contrast, the biota to sediment accumulation factors were significantly 15% higher in the cosms with coarse sediments than in cosms with fine sediments. However, this difference is too small to conflict with the equilibrium partitioning theory.

A bio-energetic model for the waterlouse *Asellus aquaticus* is presented based on published consumption and respiration functions (Chapter 8). The model is used to investigate whether effects of multiple stressors on individual organisms can be explained through their effects on energy budgets.

Analyses of data from two laboratory growth experiments with the model indicate that sub-lethal effects of combined stressors such as current velocity, substratum composition, structure of food and exposure to a toxicant can be understood from the effects of the separate stressors on consumption and respiration.

The energy budget model assumes that food and exposure to benzo(a)pyrene have additive effects on the growth of *A. aquaticus*. The model simulation is in line with the laboratory observations and thus the combined effects are well predicted by the energy budget model. The model also assumes that the combined effects of current flow and substratum type are multiplicative. The model simulation does not fit the experimental observations and in this case the assumption of multiplicative effects seems therefore not valid.

It is also argued that stressors impairing the growth of *A. aquaticus* through a reduction in consumption or through an increase in respiration will tend to have a negative effect on the decomposition rate of organic matter in aquatic systems in which this species is important.

In summary, this thesis demonstrates that effects of multiple stressors can be quantified in laboratory studies as well as in field situations and that energy budgets and scope for growth are powerful tools for improving our understanding of the distribution and abundance of organisms.

SAMENVATTING

He who knows does not speak
He who speaks does not know.

Lao Tzu
Tao Te Ching, 6th century BC

BENTHISCHE MACRO-EVERTEBRATEN EN MEERVOUDIGE STRESSFACTOREN

DE KWANTIFICERING VAN EFFECTEN VAN MEERVOUDIGE STRESSFACTOREN IN VELD-, LABORATORIUM- EN MODELSTUDIES

De samenstelling en het functioneren van ecosystemen zijn afhankelijk van de reactie op natuurlijke en door de mens veroorzaakte stressfactoren. Al deze factoren beïnvloeden de fysiologie en het gedrag van organismen, de ecologische interacties in gemeenschappen en de processen die in ecosystemen spelen. De van nature aanwezige fluctuaties in abiotische omstandigheden hebben ecosystemen altijd al beïnvloed. De effecten van menselijk handelen op ecosystemen overschrijden tegenwoordig de effecten van de natuurlijke fluctuaties. Dit leidt tot grote veranderingen in het milieu, zoals klimaatsveranderingen, verontreiniging met tal van chemische stoffen en verstoring en vernietiging van de leefomgeving van organismen. Eén van de grootste gevolgen van al deze invloeden is de snelle achteruitgang in de verscheidenheid van organismen ofwel biodiversiteit in de wereld. Het verlies aan biodiversiteit heeft negatieve gevolgen voor het functioneren van ecosystemen zoals bijvoorbeeld voor de primaire productie, de kringlopen van nutriënten en organisch materiaal, de afbraak van organisch materiaal en de samenstelling van het voedselweb. Al die veranderingen kunnen ertoe leiden dat een ecosysteem verschuift van het ene stabiele evenwicht naar het andere.

Ecosystemen staan onder invloed van een grote diversiteit aan stressfactoren en de lijst met potentieel bedreigende fysische, chemische en biologische factoren groeit nog steeds. De effecten en risico's van stressfactoren worden doorgaans per factor onderzocht via veld-, laboratorium- en modelstudies. Echter, in de natuur staan organismen bloot aan vele, tegelijk werkende stressfactoren. Uit onderzoek is gebleken dat het gecombineerde effect van meerdere stressfactoren niet begrepen kan worden als een eenvoudig product van de individuele effecten. Daarom is het noodzakelijk kennis op te bouwen over de kwantitatieve bijdrage van meerdere, tegelijkertijd werkende stressfactoren maar ook over het onderlinge verband tussen die factoren.

Het centrale thema van dit proefschrift is het kwantificeren van de gecombineerde effecten van meerdere, tegelijkertijd werkende stressfactoren op de in de waterbodem levende macro-evertebraten (= ongewervelde, met het blote oog waarneembare waterdieren) en hun gemeenschappen. Daarbij is onderzocht in hoeverre het aantal soorten macro-evertebraten verandert alsmede hun abundantie (= het aantal individuen). De bijdragen van verschillende, simultaan opererende, stressfactoren op de macro-evertebraten zijn onderzocht in veldsituaties (hoofdstukken 2 tot en met 5), onder laboratorium-omstandigheden (hoofdstukken 6 en 7) en met een rekenkundig model (hoofdstuk 8).

Voor het kwantificeren van de bijdrage van verschillende groepen van stressfactoren op de samenstelling en abundantie van de macro-evertebraten in veldsituaties is gebruik gemaakt van een speciale statistische verwerkingsmethode (hoofdstukken 2, 3 en 4). Deze methode, aangeduid als de variantie partitiemethode, maakt het mogelijk om de variatie in de macro-evertebraten gemeenschappen op te splitsen en toe te wijzen aan verschillende stressfactoren. In dit proefschrift is deze methode toegepast om te onderzoeken in hoeverre die bruikbare resultaten oplevert. De uitkomsten van de onderzoeken beschreven in de hoofdstukken 2, 3 en 4 laten zien dat het inderdaad mogelijk is om de effecten van verschillende stressfactoren te kwantificeren.

De variantie partitiemethode is geschikt gebleken om de effecten van de hoeveelheid en de kwaliteit van het voedsel af te zetten tegenover de effecten van andere factoren (hoofdstuk 2). De samenstelling van de macro-evertebraten gemeenschappen uit verschillende watersystemen in Nederland wordt primair in verband gebracht met verschillen in algemene omstandigheden zoals de aanwezigheid van stroming, de dimensies van de watersystemen en de zuurgraad van het water. Dit zijn factoren die samenhangen met het type water. De kwantiteit en de kwaliteit van het voedsel spelen in eerste instantie een minder belangrijke rol. De rol van voedsel blijkt mede af te hangen van de intensiteit van de andere factoren. Analyses van de gegevens uit alleen ondiepe plassen geven aan dat een groter voedselaanbod (meer organisch materiaal) samengaat met een lagere diversiteit aan soorten en een gelijkblijvend aantal organismen. Echter, een grotere hoeveelheid organisch materiaal op de bodem leidt meestal tot een grotere zuurstofvraag van de bodem. De geconstateerde veranderingen in de macro-evertebraten gemeenschappen kunnen dan ook niet eenduidig toegewezen worden aan de hoeveelheid organisch materiaal als bron van voedsel want ook lage zuurstofconcentraties kunnen dergelijke veranderingen teweegbrengen.

Een betere voedselkwaliteit (groter aanbod van meervoudige onverzadigde vetzuren) lijkt geen effect te hebben op het aantal soorten maar wel te leiden tot een groter aantal individuen. De productiviteit van de waterbodemdieren in ondiepe meren lijkt dan ook meer bepaald te worden door voedselkwaliteit dan door voedselkwantiteit. Daar verschillende soorten vissen en vogels macro-evertebraten als bron van voedsel gebruiken, lijkt de draagkracht van ondiepe meren voor deze organismen af te hangen van de voedselkwaliteit voor de macro-evertebraten.

De uitkomsten van de onderzoeken beschreven in de hoofdstukken 3 en 4 geven aan dat met deze methodiek ook de effecten van verontreinigende stoffen op de macro-evertebraten te kwantificeren zijn.

De variatie in de samenstelling van de levensgemeenschappen in het Noordzeekanaal (hoofdstuk 3) blijkt vooral bepaald te worden door reguliere ecologische factoren zoals chloride, diepte en korrelgrootte van het sediment maar zeker ook door zware metalen (cadmium, koper, lood en zink). Zo'n 45 % van de biologische variatie wordt verklaard door de ecologische factoren en zo'n 8 % door zware metalen. De levensgemeenschappen uit het Noordzeekanaal bestaan voornamelijk uit algemene en tolerante soorten. Tolerante soorten zijn organismen die bestand zijn tegen tal van beïnvloedingen. De aanwezigheid van veel tolerante soorten in het Noordzeekanaal wordt voornamelijk veroorzaakt door het verloop in chloride: zout water bij IJmuiden en zoet water nabij het IJsselmeer. Soorten die gevoelig zijn voor allerlei verontreinigingen ontbreken nagenoeg. Het lijkt er dan ook op dat de effecten van de zware metalen in het Noordzeekanaal eerder gerelateerd kunnen worden aan het verdwijnen van de zwakkere exemplaren van de tolerante soorten dan aan het verdwijnen van soorten die gevoelig zijn voor verontreiniging.

Net als in het Noordzeekanaal blijkt de variatie in de samenstelling van de macroevertebraten in de Rijn-Maas delta (hoofdstuk 4) eveneens mede bepaald te worden door de aanwezigheid van verontreinigende stoffen. De aanwezige polyaromatische koolwaterstoffen, polychloorbifenylen, zware metalen en olie bepalen een kleine 14 % van de variatie tegenover zo'n 17 % door de ecologische factoren als korrelgrootteverdeling van het sediment, stroomsnelheid, diepte en zuurgraad. De invloed van de ecologische factoren is in de Rijn-Maas delta veel kleiner dan in het Noordzeekanaal. Dat komt doordat in de Rijn-Maas delta niet sprake is van één dominante factor, zoals het chloride in het Noordzeekanaal. Het onderzoek toont daarnaast dat organismen die de waterbodem als voedselbron gebruiken negatief beïnvloed worden door de verontreinigende stoffen terwijl macroevertebraten met een andere voedingswijze niet of nauwelijks beïnvloed worden. Dit lijkt erop te wijzen dat de opname van verontreinigende stoffen vooral via het voedsel verloopt.

De analyses van de veldgegevens laten duidelijk zien dat de effecten van meerdere stressfactoren weerspiegeld worden in de samenstelling van de macro-evertebraten gemeenschappen. Verschuivingen in zowel de samenstelling van de soorten als in het aantal individuen van soorten kunnen optreden als gevolg van de gecombineerde effecten van stressfactoren. Om meer inzicht te krijgen in de effecten van meerdere stressfactoren op individuele soorten zijn veldgegevens van twee vlokreeftsoorten geanalyseerd (hoofdstuk 5), zijn twee laboratorium experimenten uitgevoerd (hoofdstuk 6 en 7) en is een bio-energetisch model ontwikkeld (hoofdstuk 8).

Uit de analyses van de verspreidingsgegevens van twee soorten vlokreeften in de Nederlandse beken blijkt dat de statistische verwerkingsmethode logistische regressie zeer bruikbaar is voor het definiëren van de eisen die deze organismen stellen aan hun leefomgeving (hoofdstuk 5). Ook geeft de methode inzicht in het relatieve belang van de diverse factoren. De studie toont voorts dat *Gammarus fossarum* voorkomt in kleinere, ondiepere en sneller stromende beken dan *Gammarus pulex*. Dit houdt waarschijnlijk verband met de kleinere tolerante van *Gammarus fossarum* voor lagere stroomsnelheden, zuurstofconcentraties en zuurgraad.

De waterpissebed (*Asellus aquaticus*) is in laboratoriumexperimenten gebruikt om te achterhalen wat de effecten van meerdere, simultaan werkende stressfactoren zijn de overleving en de groei (hoofdstukken 6 en 7). Experimenten met verschillende combinaties van stroomsnelheid en substraatsamenstelling (hoofdstuk 6) tonen dat stroomsnelheid een groter effect heeft op het overleven en de groei dan de substraatsamenstelling. Deze laatste factor heeft aan de andere kant een grotere invloed op de ruimtelijke verdeling van de waterpissebedden in de proefopstelling. De proeven laten zien dat de pissebed slechter groeit bij hogere stroomsnelheden en dat er geen verschil is waargenomen in de hoeveelheden voedsel die de waterpissebedden tot zich nemen. Hieruit kan de conclusie getrokken worden dat een substantieel deel van de opgenomen energie gebruikt is voor het weerstaan van de stress veroorzaakt door de stroming.

In een andere proef is de waterpissebed blootgesteld aan benzo(a)pyreen in combinatie met grof of fijn voedsel (hoofdstuk 7). Het experiment toont dat de groei van de pissebed vooral wordt beïnvloed door de grootte van het voedsel. Normaliter eet de waterpissebed grover organisch materiaal. Het aanbieden van fijn voedsel geeft een aanzienlijke reductie in de groei van de pissebed. Hieruit kan geconcludeerd dat aan het eten van een minder geschikte voedselbron energetische kosten verbonden zijn. Voor de waterpissebed heeft fijn organisch materiaal blijkbaar een lage voedselkwaliteit. De blootstelling aan benzo(a)pyreen leidt eveneens tot verminderde groei, maar de geconstateerde verschillen zijn niet significant.

Voor de waterpissebed is een bio-energetisch model ontwikkeld gebaseerd op bestaande consumptie- en respiratiefuncties (hoofdstuk 8). Toepassen van het model op de gegevens van de eerder genoemde groei experimenten laat zien dat effecten van meerdere stressfactoren via de energiehuishouding geanalyseerd kunnen worden. Het model geeft aanwijzingen dat stressfactoren die de groei van de waterpissebed belemmeren een negatief effect kunnen hebben op de snelheid waarmee het organisch materiaal wordt omgezet. Het maakt daarbij niet uit of de stressfactoren werken via een vermindering in de voedselopname of een verhoging van de respiratie.

Concluderend kan gesteld worden dat de verschillende studies uit dit proefschrift laten zien dat de biologische respons op een specifieke (groep van) stressfactor(en) afhangt van de intensiteit van de stressfactor en van de status van alle andere stressfactoren. Bijvoorbeeld, de respons van de macroevertebraten op voedsel is sterk afhankelijk van het watertype (hoofdstuk 2) en de effecten van verontreinigende stoffen op macroevertebraten zijn onder meer afhankelijk van de hoeveelheid organisch stof in het sediment (hoofdstukken 3, 4). Maar ook de analyse van de verspreiding van twee algemene vlokreeften in Nederlandse beken laat zien dat verschillende stressfactoren betrokken zijn bij het verklaren van deze patronen (hoofdstuk 5). De groei van de waterpissebed in het laboratorium op verschillende substraten blijkt af te hangen van de stroomsnelheid (hoofdstuk 6) en de groei van de waterpissebed blootgesteld aan benzo(a)pyreen hangt mede af de grootte van het organisch materiaal dat als voedsel dient (hoofdstuk 7). Het resultaat van de modelstudie laat zien dat in de energiehuishouding de effecten van meerdere stressfactoren geïntegreerd worden. Tenslotte blijkt uit dit proefschrift dat effecten van simultaan opererende stressfactoren te kwantificeren zijn in laboratorium- en veldsituaties en dat modellen gebaseerd op de energiehuishouding een krachtig instrument kunnen vormen voor het beter begrijpen van de verspreiding en abundanties van soorten.

DANKWOORD

> *There is a time for many words,*
> *and there is also a time for sleep.*
>
> *Homer*
> *The Odyssey, 9th-8th century BC*

Aan alles komt een eind en gelukkig dus ook aan het schrijven van een proefschrift. Een mooi moment om even stil te staan. De fascinatie voor water is mij als klein jochie al aangereikt door mijn pa en ma. Eerst door de onmetelijkheid van de Noordzee, later door het fenomenale schouwspel van watervallen en stroomversnellingen in het buitenland.

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CURRICULUM VITAE

Cogito ergo sum

Rene Descartes
Discours de la méthode, 1637

Edwin Peeters was born in Dongen, The Netherlands, on October 4, 1960. From 1973 to 1979 he attended the J.F. Kennedy Atheneum in Dongen. After his graduation in 1979, he started the study Environmental Sciences at the Agricultural University in Wageningen (WAU). In 1984 he also started to study Philosophy at the Catholic University of Nijmegen. In 1986 he graduated as Master of Science in Environmental Sciences with the specialization Hydrobiology. He also became Bachelor of Science in Philosophy in 1986.

From 1986 onwards he worked at the Aquatic Ecology and Water Quality Management Group (a part of the former Nature Conservation Department), Wageningen University. In the first period of his career he performed several studies in commission of a number of national water board authorities. He and JJP Gardeniers cooperated in the period 1989-1995 in the development of ecological water quality systems that are now commonly used in the daily practice of the Dutch water board authorities. From 1994 onwards he received a permanent position as a researcher and teacher and from 1999 he was also study-counselor for the biology study at Wageningen University. He gave national and international courses on ecological water quality assessment and on multivariate statistical analyses. Parts of his research performed in the latest period are described in this thesis.

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