Persistence of benthic invertebrates in polluted sediments.
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The successful exploitation of sediments by benthic invertebrates is affected by numerous variables, such as grain size distribution, food availability, predator abundance, competition, and the amount of toxicants (WALLACE & ANDERSON 1978). Hence, species-specific preferences for a distinct type of habitat relate to a series of environmental variables (CUMMINS & MERRIT 1978) that are often interrelated (WALLACE & ANDERSON 1978). These species-specific requirements or tolerances, however, are often specified incompletely, which impedes the interpretation of data on the abundance and distribution of species (TOWNSEND et al. 2000). Answering the questions on the persistence of species in non-disturbed as well as disturbed sediments requires therefore an improved understanding of cause and effect (CHAPMAN et al. 2002).

![Figure 1.1](image)

**Figure 1.1.** Hypothetical response curves for benthic community abundance and biodiversity, and the relative importance of sensitive taxa as pollution concentrations increase (adopted from CULP et al. 2000).

On the community level the hypothetical curves by CULP et al. (2000) (Figure 1.1) provide some guidance to an analyses of disturbance. When the benthic community of the oligotrophic Fraser River was exposed to bleached-kraft pulp mill effluent [PME]) in mesocosm experiments, there was no linear response of the benthic community to different levels of mixed nutrients, organic matter, and toxicants. CULP et al. (2000) suggest that inhibition as well as stimulation may result from different rates of exposure of the benthic community to the PME. At low PME levels, biodiversity of the benthic invertebrate community
increases as rare taxa increase in abundance, because the invertebrates are able to exploit the increase in periphyton due to nutrient enrichment. When the PME levels further increase to the ‘transition zone’ community structure is relatively stable because nutrient enrichment masks low levels of chronic toxicity, but the sensitive species will disappear by the increased toxicant load, and the community will shift to a more mesotrophic species composition. Higher PME levels will lead to further losses of the pollution-intolerant species and the biodiversity of the benthic community will decrease and be dominated by a few, pollution-tolerant, species. Although such studies at the community level can provide a clear description of the effects of combinations of stressors, it still remains difficult to establish to what extent the changes observed are actually caused by the chemical stressors investigated (MALTBY 1999).

Similarly, the absence of species in polluted sediments does not necessarily imply exclusion due to toxicity (CHAPMAN et al. 2002). Other factors such as physical-chemical characteristics (PINDER 1986, ARMITAGE et al. 1995), food availability (PEETERS 2001, VOS 2001), predation (TEN WINKEL 1987, MACCHIUSII & BAKER 1992, BAKER & BALL 1995), or competitive interactions (RASMUSSEN 1985, REYNOLDSON et al. 1994, HADEN et al. 1999) could also participate in their absence. Hence, knowledge of the (combinations of) factors acting on individual organisms is essential in order to understand how populations can persist in polluted sediments, which is the main subject of this thesis. In the following overview, some key processes involved in the regulation of the abundance and distribution of benthic invertebrates will be described.

**FOOD QUANTITY AND QUALITY**

In most aquatic ecosystems, both living and non-living organic matter is available for benthic invertebrates throughout the year, but the relative availability and quality of food sources may be very seasonal (WETZEL 1975, VANNOTE et al. 1980, GOEDKOOP et al. 2000). The diversity of food resources for the benthic community is large and includes: (1) living or decaying animal tissue; (2) living algae, especially diatoms; (3) decomposing vascular macrophytes; (4) fine particulate organic matter; (5) terrestrial leaf litter; and (6) wood (ANDERSON & CUMMINS 1979). The composition and quality of the organic matter is dependent of its source and the degree of degradation and may also depend on the quantity and type of the associated microbial community (bacteria, fungi, and protozoans) (VOS 2001).

Food quality is extremely difficult to define, with different species having different requirements and different, but overlapping, natural diets (PINDER
New developments in analytical chemistry, especially chromatography, have enabled researchers to unravel the chemical composition of (labile) organic material (Åhlgren et al. 1997, Goedkoop et al. 1998, Vos 2001) and tissues of organisms (Goedkoop et al. 2000). Certain individual compounds or compound groups (e.g. amino acids, pigments, fatty acids) were selected, which are unique for specific organism groups, or specific (bio)chemical and ecological processes. These biomarkers as well as the composition of the organic matter are indicative for its state of degradation and help therefore to define the quality of the organic matter.

Field and laboratory studies have shown that bulk organic content of sediments alone is not a good measure for its nutritional value for benthic invertebrates (Goedkoop et al. 1998, Vos 2001). The organisms may be still food limited in organic matter rich sediments if the organic matter is highly degraded and consequently of low nutritive value. Essential compounds like some amino acids, vitamins, and fatty acids, are supposed to play a key role in benthic invertebrate nutrition (Phillips 1984, Marsh et al. 1989, Marsh & Tenore 1990) and are thus much better indicators of nutritional state (food quality). Polyunsaturated fatty acid and pigment content were factors strongly associated with food quality. Many studies observed that food quality is a limiting key factor in regulating benthic communities in the field (Åhlgren et al. 1997, Goedkoop et al. 2000, Peeters 2001, Vos 2001) and that food quality positively influences the performance of individual species in the laboratory (Anderson & Cummins 1979, Ward & Cummins 1979, Sweeney & Vannote 1984, Sweeney et al. 1986, Vos et al. 2000). Knowledge on the role of food quality is therefore crucial to understand the abundance and distribution of benthic invertebrates in polluted sediments. In the present thesis, it is evaluated how individual benthic species respond to differences in food quantity and quality in the sediment in combination with other stressors.

**SEDIMENT POLLUTION**

Contaminants that enter the aquatic environment may end up in the sediment and concentrate in sedimentation areas (Middelkoop & van Haseleven 1999), especially when they are resistant to chemical and/or biological degradation (Beurskens et al. 1993). Due to the strong association of many contaminants with silt, clay, and/or organic particles, sediment is often the final repository (Bierman 1990). Hence, benthic organisms are often in contact with sediments contaminated with a wide variety of sorbed chemicals (e.g. Ankley et al. 1996, Wildhaber & Schmitt 1996, Kovatch et al. 1999), but due to the large number of chemicals it is impossible to analyse all chemicals that may be
Persistenc ee  o f benthi c  invertebrate s  in  pollute d  sediment s present,, and thus, it remains uncertain whether biologically active contaminants are monitored (GIESY & HOKE 1989). Hence, chemical analysis of sediment-bound contaminants as such does not give information on the effects on biota; they give an indication. The interpretation of chemical data is further complicated by unknown effects of combinations of toxicants (GIESY et al. 1988). Moreover, sediments that have been contaminated long may still contain high concentrations of contaminants, but due to aging processes the bioavailable fraction of the contaminants may be low (LANDRUM et al. 1992). The sequestering of both organic and inorganic compounds by organic matter (ABSIIL et al. 1996, MEADOR et al. 1997, STUIFZAND et al. 2000) may influence the bioavailability and hence the toxicity of contaminants (WIEDERHOLM 1984).

Potential toxic effects of contaminants on benthic organisms can be derived from whole-sediment bioassays (see reviews of TRAUNSPURGER & DREWS 1996, INGERSOLL et al. 1995). These whole-sediment bioassays can be performed relatively rapid and simple and have been designed to minimize the effects of natural variability in tested sediments (e.g. US.EPA 2000, OECD 2001). Sediments that are spiked with known concentrations of contaminants can be used to establish dose-effect relationships between chemicals and the responses in the bioassays (e.g. CAIRNS et al. 1984, BORGMANN & NORWOOD 1997, HAGOPIAN-SCHLEKAT et al. 2001, MARSDEN & WONG 2001). These dose-effect relationships may be assessed in relation to physical-chemical variables of sediment toxicity through spiking experiments (e.g. ADAMS et al. 1985, CARLSON et al. 1991, HÖSS et al. 1997, CORREIA & COSTA 2000). Evidently, the responses of benthic invertebrates to sediment pollution, including the apparent lack of response on some occasions (ANKLEY et al. 1994a), is the result of toxicity varying with other environmental conditions (WIEDERHOLM 1984, HARKY et al. 1994a, STUIFZAND et al. 2000).

The relevance of whole-sediment bioassays to observe (toxic) effects of polluted sediment on benthic invertebrates is beyond doubt, but an uncertainty common to all laboratory tests is the relationship between the field and laboratory (BATLEY et al. 2002), because laboratory bioassays lack the level of complexity of the environment in the field (LONG et al. 2001). This thesis therefore tries to relate how responses observed in laboratory bioassays can explain actual effects on local populations.

Biotic interactions

Biotic interactions can have important influences on the abundance and distribution of benthic invertebrates and the flow of energy to higher trophic
levels (e.g. Bustamante & Branch 1996, Menge 1992). Food availability is an important determinant of consumer abundance in natural communities (Hunter & Price 1992, Menge 1992) with resultant changes in prey availability to higher trophic levels, but food availability may also alter competition. Several types of competition among benthic invertebrates have been described: (1) direct interference competition for space; (2) exploitative competition for food; and (3) indirect interference through alteration of the physical environment (Peterson 1980). Competition for food and space (1 & 2) are processes commonly observed in benthic systems and at high densities aggressive encounters enforce spacing and migration of individuals (Mclachlan 1969, Wiley 1981, Sheldon 1984). These competitive effects on individuals are likely to affect the population dynamics of the competing species, which, in turn, may show up as altered species’ distributions (Townsend et al. 2000).

Many benthic invertebrates, especially insects, construct burrows in the sediment (Charbonneau & Hare 1998). Their burrowing, irrigation, and feeding activities redistribute particles and fluids, near the sediment-water interface, also known as bioturbation (Rhoads 1974). Bioturbating benthic invertebrates may counteract the oxygen depletion in the sediment (Aller 1982, Andersen & Kristensen 1991). On the other hand it may also alter the sorption equilibrium of nutrients and toxicants (Granéli 1979a, Petersen et al. 1995), modify sediment properties (e.g. particle size distribution, sediment porosity) (Aller 1982), and, therewith, facilitate increased release and/or bioavailability of sediment-bound nutrients and toxicants.

The role of bioturbation on the release of nutrients from sediment to the overlying water has received considerable attention (Gallepp et al. 1978, Gallepp 1979, Granéli 1979a, 1979b, Gardner et al. 1981, Hansen et al. 1998), but the importance of bioturbation in the transport and availability of sediment-bound contaminants is still poorly understood (Ciarelli et al. 1999, 2000, Goedkoop & Peterson 2003). One consequence of these complex biotic interactions is that any other process leading to alterations in densities or cover of benthic invertebrates can have indirect effects on species not directly involved. Since under ‘natural’ conditions all these factors are closely linked, the prediction of any changes due to sediment pollution cannot be expected to follow simple dose-effect relationships. This thesis therefore attempts to analyze the impact of biotic interactions in the laboratory and tries to verify the findings under natural conditions.
Persistence of benthic invertebrates in polluted sediments

AIM AND OBJECTIVES OF THIS THESIS

This thesis aims to explain the persistence of benthic invertebrate species in polluted sediments. The objectives of the present thesis are:

1) To analyze the responses of two different model species to combinations of food quality and sediment-bound toxicants in laboratory bioassays.
2) To analyze the impact of biotic interactions on the performance of these model species in polluted sediments.
3) To compare the persistence of invertebrate species in polluted sediments in the field with responses of a model species under defined and natural conditions.

Figure 1.2. Sampling locations in floodplain waters of a stretch of the lower River Rhine, the River Waal.
STUDY AREA

The present study is part of the Stimulation Programme System-oriented Ecotoxicological Research (SSEO) of the Netherlands Organization for Scientific Research (NWO), which started in 1999. The most important aims of this program are: (1) to promote scientific knowledge and understanding of the way ecosystems react to a chronic and diffuse exposure of chemicals in sediments and (2) to use scientific knowledge to assist the formulation and implementation of policies with respect to the ecological risks of diffuse pollution. One of the research areas of this stimulation program are the floodplain areas of the River Waal, a stretch of the River Rhine. The floodplain lakes represent a range in properties with respect to the types and levels of contamination and ecological functioning.

During the strong water pollution in the River Rhine in the 1960s and 1970s, many contaminants accumulated in the sediments of the embanked floodplains in the lower reaches (MIDDELKOOP 2000). Although recently deposited sediments contain considerably lower concentrations of contaminants, many floodplain lake sediments are still historically polluted with nutrients, metals, and hydrophobic organic contaminants (MIDDELKOOP & VAN HASELEN 1999). Nutrient-rich water may also stimulate plant growth; and the floodplain lakes tend to be either dominated by macrophytes or by phytoplankton (VAN DEN BRINK 1994). The trophic state of a floodplain lake may influence the supply of food (detritus) to the sediment.

The variable combinations of trophic state and pollution characteristics, make the floodplain lakes an ideal area to study the combined effects of contaminants and available food to benthic invertebrates. For the present thesis seven floodplain lakes located along the River Waal, a stretch of the River Rhine with different trophic state and levels of pollution were selected (Figure 1.2).

TEST ORGANISMS

Selection
The selection of the model species used in the present study was based on the following criteria: (1) it must be possible to keep the organisms in the laboratory under controlled conditions; (2) the organisms must be easy to handle in whole-sediment bioassays; (3) the organisms must, at least part of their life cycle, live in the sediment and feed on sediment-associated organic matter; and (4) the organisms should be different in their sensitivity towards sediment-bound toxicants. Based on the aforementioned criteria two test species were selected:
the midge *Chironomus riparius* (Diptera: Chironomidae) and the mayfly *Ephoron virgo* (Ephemeroptera: Polymitarcidae).

**Ecology of Chironomus riparius**

Chironomids are an important group of invertebrates in many aquatic ecosystems, because they are often the dominant primary consumers and attain extremely high biomass turnover rates (Benke 1998). Since chironomids constitute a major prey category for both invertebrate (Ten Winkel 1987) and vertebrate (Armitage et al. 1995) species their production rates may have a large influence on the community structure (Hooper et al. 2003). Chironomids are a large family of dipteran flies (more than 15,000 different species) that have successfully adapted to a wide range of habitats (Armitage et al. 1995) and their distribution has been well studied (Warwick 1992, Diggins & Stewart 1998). *Chironomus riparius* is a common species in a variety of freshwater habitats throughout Europe and North America (Janssens de Bisthoven 1995).

**Figure 1.3.** Late instar *Chironomus riparius* larva (from Bertrand 1954).

Larvae of *C. riparius* (Figure 1.3) prefer eutrophic or organic enriched waters (Armitage et al. 1995). Furthermore, *C. riparius* larvae have a wide range of ecological tolerance such as to low pH values (Havas & Hutchinson 1982) and low oxygen conditions (Heins 1993). In agreement with its general tolerance to extreme conditions, *C. riparius* is characterized as an opportunistic species (Pinder 1986), quickly invading newly created habitats (Gower & Buckland 1978), and able to exploit habitats where competitors and predators are often excluded (Pinder 1986). This opportunistic behavior is facilitated by the rapid succession of generations (Groenendijk et al. 1996); under controlled laboratory conditions *C. riparius* is able to complete its life cycle within three to four weeks (Groenendijk 1999).

Chironomid larvae pass through four instars, pupate, and then emerge as non-feeding, flying adults (Figure 1.4). Adult females lay their eggs in a gelatinous matrix on the water surface attached to any arbitrary substrate. The first instar larvae are mainly pelagic until a suitable habitat has been found (Oliver 1971). The second to fourth instars often inhabit the upper layer of the sediment, in which they build protective tubes from small particles (Armitage et al. 1995). *C. riparius* larvae build their tubes from detritus, algae, and other sediment
particles, which are joined with its salivary secretions (EDGAR & MEADOWS 1969). The larvae protrude from their tubes in order to feed off the sediment surface surrounding their burrows (RASMUSSEN 1984) and the most commonly reported food ingested by chironomids is detritus (PEREIRA et al. 1982, RASMUSSEN 1984, 1985, PINDER 1986). Consequently, the growth rate of *C. riparius* larvae is often related to the amount of detritus, available as food (RASMUSSEN 1985). Detritus includes all non-living particulate organic matter and its’ associated living non-photosynthetic microorganisms (bacteria, fungi, and protozoans) (CUMMINS 1973) which also serve as a food source.

**Figure 1.4.** Life cycle of *Chironomus riparius*. In which E = the egg stage, I – IV = the four larval instars, P = the pupal stage, and A = the terrestrial imago.

Because of its worldwide distribution, its multivoltine life cycle, and because they are easy to handle in the laboratory, chironomid larvae are widely used test organisms in acute and chronic (whole-sediment) bioassays (HARKEY et al. 1994a, 1994b, KEMBLE et al. 1994, RISTOLA et al. 1996, 1999, BLEEKER 1999, GROENENDIJK 1999, VOS 2001). Recently, chironomids have been successfully used in *in situ* bioassays (SIBLEY et al. 1999, MEREGLI et al. 2000). The *C. riparius* larvae used in the present study originated from a laboratory culture, which has been maintained at the Department of Aquatic Ecology & Ecotoxicology since 1986.

**Ecology of Ephoron virgo**

*Ephoron virgo* is one of the large mayfly species typical for large lowland rivers, it plays an important ecological role as filter feeder of fine organic material and as a food source for fish and birds (GYSELS 1991).
The nymphs of *E. virgo* (Figure 1.5) live on and in the sediments of lakes and slow flowing rivers (GYSELS 1991). The early instar nymphs have no tracheal gills and live freely on the substrate feeding on fine particulate organic matter. Later instars burrow U-shaped tubes in the river sediment (Figure 1.6). By generating wavelike movements with their feathered tracheal gills a water current passes through the tube providing oxygen and food, such as detritus and algae, which are filtered from the water (KURECK 1996).

*Figure 1.5. Late instar Ephoron virgo nymph (from KURECK 1992).*

*E. virgo* was one of the species present in mass numbers at the beginning of the 20th century (ALBARDA 1889), but was observed for the last time in 1936 (MOL 1985). After being extinct in the Netherlands for more than fifty years, they slowly re-colonized the River Rhine since 1991 (BIJ DE VAATE et al. 1992). Fertilized *E. virgo* eggs can easily be collected in the field and kept in the laboratory in artificial diapause (GREVE et al. 1999). It appeared to be a sensitive test organism in acute toxicity tests (VAN DER GEEST et al. 2000a, 2000b, VAN DER GEEST 2001). Recently a 10-day whole-sediment bioassay using newly hatched nymphs of *E. virgo* has been developed (VAN DER GEEST et al. 2001). The *E. virgo* nymphs used in the present study originated from eggs, which were collected from the field in August 2000 and 2001.

**OUTLINE OF THIS THESIS**

In **CHAPTER 2**, the responses of the mayfly *Ephoron virgo* and the midge *Chironomus riparius* to combined toxicant and food input in floodplain lake sediments were assessed in the laboratory. For this study, seven floodplain lakes with different levels of contamination and trophic state were selected and the species-specific preferences for these sediments were assessed using 10-day whole-sediment bioassays with both species and a 28-day emergence experiment with *C. riparius*. Survival, growth, and emergence were related to contaminants (metals, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls) and food quantity and quality (organic matter, chlorophyll a, labile organic matter, fatty acids, and polyunsaturated fatty acids).

The results from **CHAPTER 2** led to the question whether the midge *C. riparius* indeed was tolerant to contamination or whether it takes advantage of the high food levels. Therefore, in **CHAPTER 3**, an attempt was made to discern the
effects of food and a model toxicant, copper, on the midge *C. riparius*. In this study survival, length, and dry weight of the midge *C. riparius* on sediment spiked with different combinations of artificial food and copper were analyzed using 10-day whole-sediment bioassays. In addition, the accumulation of copper in the larvae was determined. Survival, length, and dry weight of *C. riparius* and the copper accumulation were related to both copper and food concentrations.

![Figure 1.6. Life cycle of *Ephoron virgo*. The stages of the short aerial life are: (1) emergence of the sub-imago; (2) imaginal moult of the male; (3) swarming (male); (4) mating; and (5) oviposition of egg masses. The eggs (6) develop within a month (black section) and go in diapause during autumn, winter, and early spring (7, white sections). The nymphs hatch in April (8) the first instar nymphs lack gills and do not burrow; later instars live in U-shaped burrows (9). The active life of this species lasts 3 to 4 months (grey section). (From KURECK & FONTES 1996).](image)

The capacity of *C. riparius* larvae to select sediments differing in food- and toxicant levels are determined in CHAPTER 4. The preference of *C. riparius* for
seven floodplain lake sediments with different levels of contamination and food was assessed using 10-day settling or migration experiments, in which the chironomid larvae could choose between pairs of different sediments. It was analysed whether observations on the selection of a suitable habitat are in concordance with the results obtained from whole-sediment bioassays.

CHAPTER 5 examines the effects of insect larvae reworking polluted and non-polluted sediments. Midges (C. riparius) were added to clean and polluted sediment populated with nymphs of the mayfly E. virgo. Changes in the composition of overlying water and oxygen penetration were observed and the survival and growth of the mayfly were related to chironomid densities, background pollution levels, and water- and sediment characteristics.

To verify the persistence of benthic invertebrates in polluted sediments, benthic community composition was recorded in historically polluted sediments, differing in toxicant level and food quality (CHAPTER 6). Species composition was compared with observations in laboratory and in situ bioassays, with the midge C. riparius as a model species. Survival, growth, and frequency of mentum deformities of the C. riparius larvae were assessed in 10-day in situ and laboratory bioassays. The species composition and outcome of the two bioassays were related to contaminant concentrations (Cd, Cu, and Zn) and food quality levels (chlorophyll a, fatty acids, bacterial fatty acids, and polyunsaturated fatty acids) in the sediment. This enabled us to determine the environmental variables that regulate the distribution of benthic invertebrate species in polluted sediments.

The concluding remarks (CHAPTER 7) discuss the main findings of this thesis and review how the many environmental variables determine the persistence of benthic invertebrates in polluted sediments. Furthermore, it was discussed how the results from this thesis could assist in the formulation and implementation of policies with respect to the ecological risks of diffuse pollution to benthic invertebrates.