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Species-specific responses of two benthic invertebrates explain their distribution along environmental gradients in freshwater habitats

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ABSTRACT

The absence of species in polluted sediments does not necessarily imply exclusion due to toxicity. Other factors, like for instance food availability and oxygen content, could also partly cause 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. In this study species-specific responses of two benthic invertebrate species, the mayfly *Ephoron virgo* and the midge *Chironomus riparius*, to environmental variables were compared. It was assessed how these responses determine the distribution of these species in polluted sediments. Subsequently, it was discussed how these results can assist in the formulation and implementation of policies with respect to the ecological risks of pollution to benthic invertebrates. The present study showed that sediment pollution is likely to act only occasionally as a single selective force reducing the persistence of sensitive species. Yet, it was shown in our studies that the pollution level in some tested sediments limits the persistence of insects with the sensitivity of *E. virgo*. In other cases, however, a combination of conditions is likely to determine their persistence. As shown here for *C. riparius*, sediment pollution drives this species close to intoxication, but a high availability of food enables them to persist very well. The present study provides evidence that pollution levels exceeding current Dutch Negligible Concentrations may pose a detectable ecological effect at least for sensitive benthic invertebrates.

1. Introduction

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 as the absence of species in polluted sediments does not necessarily imply exclusion due to toxicity (Chapman et al., 2002). Other factors, like aforementioned, 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.

During the heavy water pollution in the River Rhine in the 1960s and 1970s, many contaminants accumulated in the sediments of the embanked floodplains of its lower reaches (Beurskens et al., 1993). 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 (Beurskens et al., 1993). Furthermore, the trophic state of the river Rhine floodplain lakes vary widely, influencing the supply of food (detritus) to the benthic community. The floodplain lakes thus represent a range in

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properties with respect to the types and levels of contamination and trophic level. We therefore aim to assess here how species-specific responses to environmental variables of two benthic invertebrate species, the larvae of the midge *Chironomus riparius* and the nymphs of the mayfly *Ephoron virgo*, determine their persistence in these polluted sediments.

Subsequently, it will be discussed how these results could assist in the formulation and implementation of policies with respect to the ecological risks of diffuse pollution to benthic invertebrates.

2. Responses to environmental gradients

C. riparius larvae have a wide range of ecological tolerance such as to low pH values (Havas and Hutchinson, 1982) and low-oxygen conditions (Heinis, 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 and Buckland, 1978), and able to exploit habitats where competitors and predators are often excluded (Pinder, 1986). This opportunistic behaviour is facilitated by the rapid succession of generations (Groenendijk, 1999). *E. virgo* was one of the species present in mass numbers in the large Dutch rivers at the beginning of the 20th century (Albarda, 1889). After being extinct in The Netherlands for more than 50 years, due to the heavy water pollution in the 1960s and 1970s, this species is slowly re-colonizing the River Rhine since the early 1990s (Bij de Vaate et al., 1992). Both species are typical inhabitants of the slow-flowing lower reaches of rivers.

The environmental conditions in these river reaches are often similar to conditions in waters that are more or less stagnant. Therefore, many benthic invertebrates inhabiting slow-flowing river habitats are facultative riverine lake and pond species, which also holds for *E. virgo* and *C. riparius* (Illies and Botosaneau, 1963).

2.1. *Substrate type*

During their development both species live in the sediment where they build tubes. These tubes have several important functions, e.g. protection against predation and disturbance, and acquisition of food and oxygen (e.g. Eriksen, 1968). It is therefore of critical importance for both species to find a substrate in which they can construct a stable tube. Although few benthic invertebrate species are restricted to a specific substrate, most species exhibit distinct preferences for a general bottom type (e.g. mud, sand, gravel). *E. virgo* nymphs preferably burrow their U-shaped tubes in loamy to sandy riverine substrates that contain low amounts of organic material, although their substrate preference is not very strict (Schleuter et al., 1989). This might especially apply to the early instar nymphs that live freely on the sediment, since in two 'clean' sediments substrate grain size (silt-loam vs. sandy-loam) had no effect on survival of the nymphs (De Haas et al., 2002). *C. riparius* larvae on the contrary preferably build their tubes in organic rich sediments in which clay- and silt-sized particles predominate (Pinder, 1986). They may also build their tubes in coarse sand, although they are not able to efficiently construct tubes in the latter substrate (Vos, 2001). Thus, both species prefer different types of substrates and *E. virgo*, preferring loamy to sandy substrate with low organic enrichment, might not be present in the highly organic silt sediments that are preferred by *C. riparius*, and vice versa.

2.2. *Oxygen*

In highly organic sediment, which is preferred by *C. riparius*, oxygen deficits commonly occur, even when the overlying water is well oxygenated. Therefore many benthic invertebrates enhance oxygen uptake by creating currents across boundary layers by ventilatory movements. Burrowing mayfly nymphs beat their gills with a frequency inversely related to the oxygen concentration in their burrow, to maintain a current through their burrow which directly functions in oxygen uptake (Eriksen, 1968). Van der Geest et al. (2001) demonstrated that anoxic and hypoxic conditions had detrimental effects on the survival of early *E. virgo* nymphs, but at oxygen concentrations of 50% or higher, no effects on survival were observed after 4 days of exposure. In contrast to mayflies, *Chironomus* larvae have the ability to synthesise haemoglobin in response to low-oxygen levels (Heinis, 1993). The short-term oxygen storage properties of haemoglobin allow *Chironomus* larvae to alternate periods of feeding with undulatory movements under low-oxygen conditions (Heinis, 1993).

Yet, the increased energy spent on respiration under these low-oxygen conditions may reduce the amount of energy left for growth (Becker, 1987), as was also was observed in field enclosures by De Haas et al. (2005a). Thus, the two model species occupy very different positions in natural oxygen gradients. *E. virgo*, will not be present in highly organic sediments with low-oxygen levels are commonly encountered, while *C. riparius* is particularly fit to cope with such oxygen-depleted sediments.

2.3. *Food quantity and food quality*

C. riparius is frequently encountered in high densities in organically enriched sediments in which food is rarely limiting (e.g. Learner and Edwards, 1966). The most commonly reported food ingested by *C. riparius* is detritus (Rasmussen, 1985; Pinder, 1986), the growth rate of *C. riparius* larvae is, as a consequence, often related to the amount of detritus available (Rasmussen, 1985). Recent studies showed, however, that not the amount of food, but rather the nutritional value of the food determines the growth rates of benthic invertebrates (Vos, 2001). By using (polyunsaturated) fatty acids and chlorophyll *a* as food quality indicators, we concordantly showed that the growth rate of *C. riparius* larvae was higher at sediments with a higher nutritional value (De Haas et al., 2002, 2005a), leading to faster emergence (De Haas et al., 2002). The addition of a highly nutritive food source to natural sediments stimulated growth rates (De Haas et al., 2002, 2004) and accelerated emergence times, especially in sediments that were low in food quality (De Haas et al., 2002).

Because *C. riparius* larvae feed by extending their head and anterior part of the body outside the tube, their foraging areas are restricted to the region immediately surrounding their tube (Rasmussen, 1985). It is therefore of crucial importance for the larvae to settle in an environment with a sufficient amount of high quality food. De Haas et al. (2006) demonstrated that a higher number of larvae settled on substrates with higher food quality compared to substrates with lower food quality. When food availability depletes the larvae may leave their tubes in search for a habitat with higher food availability (De Haas et al., 2005b, 2006). In contrast, we observed that the food quality in the sediments did not lead to detectable differences in survival and growth of *E. virgo* nymphs (De Haas et al., 2002). Also additional feeding with highly nutritious diatoms did not enhance survival or growth (De Haas et al., 2002). Thus the amount of food in the tested sediments was probably saturating for *E. virgo*, in contrast to *C. riparius*. Hence *E. virgo* has a much lower food demand than *C. riparius*. *E. virgo* nymphs may consequently be food limited only under oligotrophic conditions.

The influence of food on the performance of both model species is markedly different and may be explained by their differences in growth after 10 days (~0.2 mm for *E. virgo* vs. ~8 mm for *C. riparius* after 10 days) and life cycle duration. *E. virgo* is restricted to a univoltine life cycle, because of the required temperatures for egg diapause and hatching of the nymphs (Lehmkuhl 1974), whereas, in temperate regions, *C. riparius* has a multivoltine life cycle with 2 to 3 generations per year (Groenendijk, 1999). Even 7 generations per year were observed for *C. riparius* in an organically polluted English river (Learner and Edwards, 1966). In accordance, nymphs of *E. virgo* take much more time to develop, 3 to 4 months (Kureck and Fontes, 1996), compared to *C. riparius*, which is able to complete its life cycle in 3 to 4 weeks (De Haas et al., 2002). Thus, the amount of food needed per unit of time is much higher for *C. riparius* than for *E. virgo*. When the food demands of both species are saturated, *C. riparius* grows much faster and develops far more rapid. This high growth is reached on sediments with a sufficient amount of high quality food and not on sediments with low amounts of high quality food that may sustain the low developmental rate of *E. virgo*.

2.4. Sensitivity to contaminants

Contaminated food might decrease the organisms' performance by e.g. reducing food ingestion rates (Leppänen et al., 1998) and metabolic processes (DePledge, 1998). Some studies observed that *E. virgo* has a high intrinsic sensitivity to contaminants (Van der Geest et al., 2000, 2002), although it showed to be resistant to zinc (Van der Geest et al., 2001).

Sediments with high levels of a mixture of contaminants (metals, PAHs, PCBs, and a variety of unidentified compounds) had detrimental effects on survival and growth of *E. virgo* (De Haas et al., 2002, 2005b). In contrast to *E. virgo*, the amount of toxicants in natural sediments had little effect on the performance of *C. riparius* (De Haas et al., 2002, 2006). This does not imply however that *C. riparius* is tolerant to contaminants. Copper spiked reference sediment caused negative effects on survival, dry weight, and length of the larvae (De Haas et al., 2004), though the median effect concentrations were much higher than the highest copper concentration measured in the natural sediments tested. Addition of highly nutritive food to the sediment improved the physiological state of the larvae, enabling them to put more energy in detoxication or repair mechanisms (Heugens et al., 2001). This was observed as a decrease in sensitivity which coincided with a decrease in copper accumulation with increasing amounts of highly nutritive food (De Haas et al., 2004). Moreover, when the food quality in two sediments was equal a higher proportion of the larvae choose the sediment with the lower contaminant load (De Haas et al., 2006). In addition, it was also observed that in spite of its ability to maintain populations in contaminated sediments *C. riparius* larvae suffered from the high contaminant levels, reflected by a higher incidence of mentum deformities at higher contaminant concentrations in the sediment (De Haas et al., 2005a). It is concluded that both species differ widely in their sensitivity to historically contaminated sediments. The amount of contaminants in most tested sediments exerted toxic effects on *E. virgo* and although high incidences of mentum deformities were induced in *C. riparius*, their development was not impaired.

3. Persistence of benthic invertebrates in polluted sediments

A direct comparison of *E. virgo* and *C. riparius* exposed to the same historically polluted floodplain lake sediments clearly showed a high sensitivity of the mayfly and a certain indifference of the chironomid. This observation is in accordance with the general ranking of these species as 'pollution-sensitive' and 'pollution-tolerant', respectively (De Pauw and Vannevel, 1991). The persistence of *E. virgo* in recently deposited and, hence, relatively clean sediments in the main bed of the River Rhine combined with our observations, confirm that *E. virgo* is able to persist only in sediments with low levels of sediment-bound toxicants, low food levels, and high-oxygen levels. Because of their low growth rates and low food demands they are able to complete their univoltine life cycle on such sediments.

Pollution-tolerant chironomid taxa related to *C. riparius* were observed to persist in the historically polluted floodplain deposits (De Haas et al., 2005a). *C. riparius* itself is able to persist in even more extreme environments, such as the River Dommel, which is both organically enriched and heavily polluted with metals (Groenendijk, 1999). Due to their high growth rates with resultant high food demand they require food rich sediments to complete several life cycles. Combined with their tolerance to low-oxygen levels they can persist in such sediments where oxygen depletion frequently occurs. Absence of more sensitive competing species and predators may lead to continued mass occurrence of *C. riparius* on such sediments.

The different responses of the two model species to several environmental variables indicate that the long-term persistence of these species under specific conditions is bound to a set of characteristics and is unlikely determined by single traits. Therefore, sediment pollution is likely to act only occasionally as a single selective force reducing the persistence of sensitive species. Yet, it was observed that the pollution level in some floodplain lake sediments of the River Rhine is prohibitive for insects with the sensitivity of *E. virgo*. In other cases, however, the combination of conditions is likely to determine the persistence of species in polluted sediments. These combined effects may cause limiting

Table 1 – Standardized concentrations (25% lutum, 10% organic matter) of substances in the floodplain lake sediments of the River Waal measured in September 2000 and sediment quality measures for freshwater sediments (CIW, 2000)

	Sampling locations							Standards	
	D2	G1	G3	O2	3A	3B	D4	NC	MPC
Metals									
Cd	0.26	0.52	0.97	1.20	1.50	2.27	1.77	0.80	12
Cu	20	26	31	41	55	67	57	36	73
Zn	71	146	404	259	424	424	542	140	620
PAHs									
Anthracene	0.01	0.09	0.06	0.05	**0.14**	**0.15**	**0.36**	0.001	0.10
Phenanthrene	0.03	0.04	0.12	0.21	0.37	0.45	**0.89**	0.005	0.50
Fluoranthene	0.07	0.08	0.22	0.38	0.82	0.87	1.59	0.03	3.0
Benz[a]anthracene	0.05	0.04	0.11	0.19	**0.45**	**0.44**	**0.88**	0.004	0.40
Chrysene	0.04	0.05	0.11	0.20	0.45	0.45	0.82	0.11	11.0
Benzo[k]fluoranthene	0.03	0.04	0.07	0.11	0.27	0.27	0.47	0.02	2.0
Benzo[a]pyrene	0.06	0.05	0.14	0.22	0.56	0.53	1.01	0.03	3.0
Benzo[ghi]perylene	0.03	0.04	0.11	0.19	0.40	0.40	0.61	0.08	8.0
Indenopyrene	0.04	0.31	0.14	0.18	0.52	0.48	0.90	0.06	6.0
PCBs									
PCB_{28}	b.d.	b.d.	0.89	0.85	1.85	3.42	3.10	1.0	4.0
PCB_{52}	b.d.	1.09	1.81	2.01	**6.40**	**11.5**	**10.3**	1.0	4.0
PCB_{101}	2.45	1.09	2.79	**4.02**	**12.1**	**20.6**	**16.9**	4.0	4.0
PCB_{118}	1.67	1.33	1.16	1.67	**6.11**	**11.8**	**9.52**	4.0	4.0
PCB_{138}	3.27	1.83	3.87	**5.96**	**15.7**	**24.7**	**21.3**	4.0	4.0
PCB_{153}	0.74	b.d.	0.64	1.00	3.11	5.07	4.29	4.0	4.0
PCB_{180}	2.90	1.26	2.66	**4.19**	**11.8**	**18.5**	**19.0**	4.0	4.0

Metals and ΣPAHs in mg/kg dry weight, PCBs in μg/kg dry weight. NC = negligible concentration; MPC = maximum permissible concentration. Bold numbers indicate exceedance of the MPC for that substance.

conditions for certain species, indirectly promoting other species, as shown here for *C. riparius*. Although sediment pollution drives this species close to intoxication, the high availability of food, caused by the extinction of less tolerant species, enables them to persist very well.

4. Implications for environmental management

Sediment quality is currently classified through simplified sets of chemical standards, which do not reflect the actual risks for biota. Therefore, the current chemical safety standards are compared here to actual contaminant concentrations in floodplain lake sediments and to observations on benthic invertebrates exposed to these sediments in the laboratory.

In The Netherlands, maximum permissible concentrations (MPC) and negligible concentrations (NC) are used to assess the quality of sediments (Table 1) (CIW, 2000). When concentrations in sediments fall below the MPC the risk of the substance is considered to be acceptable. Concentrations in the environment below which adverse effects are considered to be negligible are called NCs (negligible concentrations). Comparing standardized sediment-bound toxicant concentrations with current sediment quality guidelines (SQGs) indicates that in some sediments tested in our studies, the MPCs for some PAHs are exceeded (Table 1), whereas in all sediments several NCs are exceeded, especially those for PAHs. This implies that in three of the seven sediments the concentrations of substances are in ranges that might lead to biological effects. Minor exceedance of the MPC might lead to an increased probability for biota to be affected. In the sediments in which several MPCs are exceeded survival and growth rates of the mayfly *E. virgo* were severely affected (Table 2). And although no effects were observed on survival rates of *C. riparius* larvae in chronic whole-sediment bioassays, a high incidence of mentum deformities was observed in the sediments that exceeded MPCs for some substances (Table 2). Thus, concentrations of sediment-bound toxicants close to

Table 2 – Assessment of sediments with results from whole-sediment bioassays using the effect-classification method according to the TRIAD-method (Maas et al., 1993)

	Control	D2	G1	G3	O2	3A	3B	D4
Exceeded MPCs		No	No	No	Yes	Yes	Yes	Yes
Exceeded NCs		Yes	Yes	Yes	Yes	Yes	Yes	Yes
Effect on survival (%) *E. virgo* [a] (10 days)	86.3%	–	–	±	±	+	+	+
Effect on survival (%) *C. riparius* [a] (28 days)	80.0%	–	–	–	–	–	–	–
Mentum deformities (%) *C. riparius* [b] (10 days)	na	–	–	+	+	+	+	+

[a] Effect classification: –=≤10% mortality (no effect); ±=10%–50% mortality, when significant difference from control (moderate effect); +=≥50% mortality, when significant difference from control (severe effect) (Maas et al., 1993). Data from De Haas et al. (2002).
[b] Effect classification: –=X^2≤7.446 (no effect); +=X^2≥11.479, when significant difference from control (severe effect). Deformities are compared to a fictitious sample of 100 counts with 9% deformities (Maas et al., 1993). Data from De Haas et al. (2005a).

MPC can be responsible for distinct effects on benthic invertebrates. The mortality of the mayflies and the elevated incidence of mentum deformities of the midge were clearly observed also in some of the sediments with concentrations of toxicants below MPCs. Consistently, sensitive benthic invertebrate species, such as mayflies and caddisflies, were observed only in one of the investigated floodplain lakes. The present study provides evidence that pollution levels exceeding current NCs may still pose a detectable ecological effect at least for sensitive benthic invertebrates.

In the Dutch Fourth National Policy Document on Water Management it was proposed that biological effect studies (bioassays) are to be considered for setting environmental sediment quality objectives (CIW, 2000). Whole-sediment bioassays can be performed relatively rapid and simple and take into account the bioavailable fractions of all present toxicants. However, the organisms used in whole-sediment bioassays must have the appropriate sensitivity in order to assess the ecological risk of sediment pollution on the benthic community. The current standard laboratory test organisms used in whole-sediment bioassays, oligochaetes, amphipods, and chironomids (e.g. OECD, 2004), are relatively insensitive to polluted sediments and can, however, be ranked as 'pollution-tolerant' (De Pauw and Vannevel, 1991). Derivation of SQGs from whole-sediment bioassays performed with 'pollution-tolerant' organisms may not be protective for more sensitive species and thus for benthic communities, as shown above. Our study demonstrated that the mayfly *E. virgo* is a promising alternative test organism, better reflecting the actual risk that polluted sediments pose on the benthic community.

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