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Klaas Kaag

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Nicolaas Hendricus Bosco Maria Kaag

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Voor mijn ouders

Voor Henny,
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Samenvatting

Het belang van voedselopname voor de biologische beschikbaarheid van aan sediment gebonden contaminanten voor marien benthische invertebraten

Veel van de contaminanten die aanwezig zijn in het aquatische milieu komen uiteindelijk in het sediment terecht, omdat veel van die contaminanten een sterke binding met deeltjes in het milieu vertonen. Maar waar de waterkwaliteit verhoudingsgewijs gemakkelijk kan worden verbeterd door de bronnen van verontreiniging op te sporen en te elimineren, blijft het sediment langdurig verontreinigd en kan het zelfs als bron van verontreiniging van het aquatische milieu fungeren. Aangezien er geen gestandaardiseerde toxiciteitstoetsen voor sediment beschikbaar zijn, wordt vaak gebruik gemaakt van de evenwichtspartitietheorie om het risico van verontreinigd sediment te schatten. De evenwichtspartitietheorie wordt vooral gebruikt om de risico's van slecht in water oplosbare stoffen zonder lading te bepalen, zoals polycyclische aromatische koolwaterstoffen (PAK's) en organochloorverbindingen, waaronder polychloorbifenylen (PCB's). De evenwichtspartitietheorie maakt het mogelijk gebruik te maken van de ruimschoots beschikbare gegevens met betrekking tot de aquatische toxiciteit van stoffen, en de daarvan afgeleide waterkwaliteitsnormen, om het ecologisch risico van verontreinigde sedimenten te bepalen en om kwaliteitsnormen voor het sediment af te leiden ten behoeve van het beleid.
Twee aannames die ten grondslag liggen aan de evenwichtspartitietheorie zijn belangrijk voor dit proefschrift. Ten eerste veronderstelt de evenwichtspartitietheorie dat er een chemisch evenwicht bestaat tussen de beschouwde milieucompartimenten (in dit geval tussen sediment, (porie)water en organismen). Daardoor is er geen netto transport van contaminanten tussen de compartimenten en kan de concentratie van een stof in een bepaald compartiment berekend worden uit de gemeten concentratie van die stof in een ander compartiment. Hiervoor zijn partitiecoëfficienten nodig, die de verdeling van een stof over twee compartimenten beschrijft als beide compartimenten in evenwicht zijn (zie Figuur 1).
Ten tweede veronderstelt de theorie dat de effectieve concentratie waaraan een organisme wordt blootgesteld voor alle blootstellingsroutes gelijk is als er sprake is van een evenwichtssituatie. Dit houdt in dat de concentratie van een stof (uitgedrukt in de juiste eenheden, zoals het vetgehalte voor PAK's of PCB's) in alle organismen hetzelfde wordt, ongeacht hun levenswijze.
Bij evenwichtspartitie berekeningen wordt derhalve (te) weinig rekening gehouden met de levenswijze van organismen. Gemakshalve wordt er vaak vanuit gegaan dat de organismen blootgesteld worden via water en in het bijzonder poriewater. Maar aangezien er in de veldsituatie waarschijnlijk vaak juist geen evenwicht bestaat tussen de verschillende milieucompartmenten, wordt de feitelijke accumulatie bepaald door de blootstellingsroute, d.w.z. de levenswijze van een organisme. Daarnaast zijn er aanwijzingen dat opname van contaminanten via verschillende blootstellingsroutes (in dit geval via de voedselopname en direct uit water) additief kan zijn, zodat verschillende blootstellingsroutes niet tot dezelfde lichaamsconcentratie leiden, maar opgeteld moeten worden.

Het belangrijkste doel van het onderzoek voor dit proefschrift was om uit te zoeken in hoeverre de blootstelling van marien benthische invertebraten aan aan sediment gebonden contaminanten bepaald wordt door hun levenswijze en in het bijzonder hun manier van eten.

Een tweede doelstelling was om de effecten van aan sediment gebonden contaminanten te onderzoeken en deze effecten zo mogelijk te relateren aan de geaccumuleerde gehaltes van die stoffen, om zo tot een minder variabele schatting van het ecologisch risico te komen, gebaseerd op de biologisch beschikbare fractie in plaats van het totale gehalte van die stoffen in het sediment.

Het onderzoek ten behoeve van dit proefschrift kan gezien worden als een ecologische benadering van een ecotoxicologisch vraagstelling. In Hoofdstuk 1 is, voor de lezers die niet thuis zijn in de ecotoxicologie, een inleiding gegeven in de aquatische ecotoxicologie met de nadruk op verontreinigde sedimenten. Aangezien de biologie en ecologie van de gebruikte proefdieren mogelijk niet bij iedereen bekend zijn, is dit beschreven in het resterende deel van het hoofdstuk.

In Hoofdstuk 2 komt de invloed van de manier van eten op de bioaccumulatie van contaminanten uit sedimenten aan de orde. Drie marien benthische invertebraten, met verschillende levenswijzen en voedingsgewoonten, werden blootgesteld aan verontreinigde sedimenten. Deze sedimenten waren opgebaggerd uit zeehavens en uit de Westerschelde. De dieren werden gedurende een periode van 60 tot 140 dagen blootgesteld in mesocosms op het buitenterrein van TNO. De PAK en PCB gehaltes waren het hoogst in de sediment-etende zeepier, *Arenicola marina*. De concentraties in de zeepier waren gerelateerd aan de concentraties in de onderzochte sedimenten. De gehaltes in de mossel, *Mytilus edulis*, die plankton uit het water filtert, waren daarentegen onafhankelijk van de gehaltes van contaminanten in het sediment. De concentraties in de mosselen reflecteerden juist de concentraties van deze stoffen in het zeewater dat werd gebruikt voor de doorstroming van de mesocosms. In het nonnetje, *Macoma balthica*, dat vooral van het sedimentoppervlak eet, maar ook plankton uit het water kan filteren, werden gehaltes gevonden die tussen die van de mossel en de zeepier in lagen. Door de beschikbaarheid van voedsel (fytoplankton) in het water te manipuleren, kon worden aangetoond dat de opname van contaminanten door het nonnetje gerelateerd is aan de manier van eten, overeenkomstig het verschil tussen zeepier en mossel.

Omdat het eten van sediment een belangrijke opnameroute is voor aan sediment gebonden contaminanten bij de zeepier, kan deze soort een belangrijke rol spelen bij het bepalen van de effecten van verontreinigde sedimenten. Daarom werd een *in vitro* bevruchtingstoets (IVF toets) ontwikkeld, beschreven in Hoofdstuk 3. Voor deze toets zijn zeepieren aan verontreinigd sediment blootgesteld in mesocosms. Begin september, vlak voor het begin van de paaitijd, werden de dieren hieruit verzameld en naar het laboratorium gebracht. De eieren die door de vrouwtjes werden afgezet, werden in schoon zeewater bevrucht met van de mannetjes afgenomen sperma. Nadat de embryo's zich 24 uur hadden ontwikkeld, werden ze gefixeerd en werd het bevruchtingssucces bepaald. De eerste resultaten lieten zien dat blootstelling van de ouderdieren aan verontreinigde havenslib het bevruchtingssucces kan verminderen.

In Hoofdstuk 4 is onderzocht in hoeverre de zeepier bruikbaar is voor het biologisch monitoren van aan sediment gebonden contaminanten in intergetijde gebieden. Van december 1993 tot maart 1995 zijn zes keer zeepieren verzameld op vijf lokaties in de Westerschelde. Van deze lokaties werd verwacht dat ze een gradiënt zouden vertonen in mate van verontreiniging. Ter vergelijking werd een zesde lokatie in de Oosterschelde bemonsterd. De

bedoeling was om het belang van seizoensfluctuaties, ruimtelijke variatie en sexuele ontwikkeling ten aanzien van de geaccumuleerde PAK gehaltes te bepalen. Er werd een duidelijke seizoensgebonden fluctuatie in geaccumuleerde PAK gehaltes waargenomen. Zeepieren vertoonden de laagste PAK gehaltes in maart en de hoogste PAK gehaltes in September, vlak voor de paaitijd. Zoals verwacht namen de PAK gehaltes in de zeepieren af van oost naar west, behalve tijdens de bemonstering in oktober, tijdens de paaitijd. Hoewel de waargenomen seizoensgebonden fluctuatie klaarblijkelijk gerelateerd is aan de voortplantingscyclus van de zeepier, werden geen duidelijke verschillen in gehaltes gevonden tussen rijpe dieren en dieren zonder ontwikkelde geslachtscellen. Het bevruchtingssucces, bepaald met de IVF toets, was niet gerelateerd aan de gevonden verschillen in PAK gehalte.

Om de accumulatie en de effecten van aan sediment gebonden PAK's te kunnen onderzoeken zijn kunstmatige sedimenten gemaakt. De sedimenten werden in twee stappen opgeladen (gespiked) met PAK's met behulp van een nieuw ontwikkelde methode. Met deze nieuwe methode is het mogelijk de grote hoeveelheden testsediment te maken die in de mesocosms nodig zijn. De betreffende PAK wordt eerst geadsorbeerd aan een organische drager (in dit geval gedroogd zeewier, een potentiële voedselbron voor sediment-etende organismen), waarna de opgeladen drager wordt gemengd met zand. Na een stabilisatie periode van enkele maanden, waarin het zeewier wordt afgebroken, is het kunstmatige sediment geschikt om te gebruiken in experimenten.
In Hoofdstuk 5 is de bruikbaarheid van de nieuwe methode om sediment op te laden onderzocht. Daarvoor zijn sedimenten opgeladen met drie verschillende PAK's en een mengsel ervan. De PAK's verschilden voor wat betreft hun affiniteit voor organische stof: fluoreen (logKow ca. 4), fluoranteen (FAN; logKow ca. 5) en benzo(a)pyreen (BaP; logKow ca. 6). Fluoreen was binnen enkele maanden, ruim voordat de sedimenten gebruikt konden worden in experimenten, weer verdwenen. De toegevoegde FAN was na twee jaar verdwenen uit de opgeladen sedimenten en de BaP gehalten begonnen pas na twee jaar af te nemen. Het lijkt er dus op dat deze methode om sedimenten op te laden vooral geschikt is voor sterk aan organische stof hechtende, slecht afbreekbare stoffen, zoals BaP.

Omdat BaP in bovenstaande verkennende experimenten een negatief effect leek te hebben op het bevruchtingssucces van het nonnetje en de zeepier, werden nieuwe sedimenten gemaakt, die werden opgeladen met verschillende BaP concentraties. Dit wordt beschreven in Hoofdstuk 6. Zeepieren en mosselen werden aan de met BaP opgeladen sedimenten blootgesteld, zodat de relatieve biologische beschikbaarheid voor respectievelijk een sediment-eter en een plankton-eter bepaald kon worden. Met behulp van de IVF toets werd vervolgens het bevruchtingssucces van de zeepier bepaald. De BaP gehaltes in de mossel waren duidelijk gerelateerd aan de BaP gehaltes in de sedimenten. Dit duidt erop dat de aan de kunstmatige sedimenten toegevoegde BaP meer biologisch beschikbaar is dan contaminanten in veldsedimenten gewoonlijk zijn. In Hoofdstuk 2 bleek immers dat de contaminanten in uit het veld verzamelde sedimenten niet beschikbaar waren voor mosselen. De BaP gehalten in de zeepieren waren echter ca. twee keer zo hoog als in de mosselen, hetgeen nogmaals laat zien hoe belangrijk de manier van eten is voor de accumulatie van contaminanten. Deze hoge BaP gehaltes in de zeepieren resulteerden echter niet in duidelijke effecten op het bevruchtingssucces.

In Hoofdstuk 7 zijn de resultaten gezamenlijk bediscussieerd, om conclusies te kunnen trekken. Vergeleken met in laboratoriumtoetsen gebruikte methodes om sedimenten op te laden, was het opladen van BaP met de nieuwe methode niet zo efficiënt als naar het totale gehalte in sediment gekeken werd. De terugwinst van BaP was echter vrijwel gelijk aan de terugwinst van organische stof, zodat de uiteindelijke BaP concentraties vrijwel gelijk waren aan de beoogde concentraties als ze werden gecorrigeerd voor het organisch stof gehalte in het sediment. Gesuggereerd wordt dat de sedimenten efficiënter met BaP en andere contaminanten zouden kunnen worden opgeladen door de stabilisatie periode te verlengen, met name de periode voordat de waterverversing wordt gestart. Daarnaast kan mogelijk efficiënter worden opgeladen door gebruik te maken van al gedeeltelijk afgebroken organisch materiaal in plaats van vers zeewier als organische drager. De aan de sedimenten toegevoegde PAK's zijn meer biologisch beschikbaar dan contaminanten in veldsedimenten, een verschijnsel dat al vaker is opgemerkt. Factoren die hier mogelijk een rol bij spelen zijn verschillen in sediment samenstelling, de ouderdom van de verontreiniging en, voor PAK's, de oorsprong van de verontreiniging. Behoudens het verlies gedurende de stabilisatie periode, bleven gehalte en biologische beschikbaarheid van BaP gedurende tenminste twee jaar stabiel, zonder tekenen van veroudering. Dit maakt het mogelijk om lange termijn experimenten uit te voeren met de opgeladen sedimenten.

De experimenten toonden duidelijk aan dat de manier van eten van marien benthische invertebraten een uitgesproken invloed heeft op de accumulatie van aan sediment gebonden contaminanten. Sediment-etende organismen accumuleren meer dan plankton-etende organismen. Omdat blootstelling aan poriewater waarschijnlijk geen belangrijke rol speelt bij de accumulatie van aan sediment gebonden contaminanten door de onderzochte organismen, werd geconcludeerd dat de hoge concentraties die gevonden werden bij sediment-etende dieren veroorzaakt werden door de inname van het verontreinigde sediment. Door het bijzondere chemische milieu in het spijsverteringskanaal, die de binding van contaminanten aan het sediment beïnvloedt, en doordat de hoeveelheid organisch materiaal afneemt door vertering, kan de opname van contaminanten uit ingeslikt sediment groter zijn dan wordt verondersteld in de evenwichtspartitietheorie.

Alhoewel het er in een eerste onderzoek met verschillende PAK's op leek dat BaP een negatief effect zou kunnen hebben op het bevruchtingssucces van de zeepier, kon dat uiteindelijk niet bevestigd worden. Het is mogelijk dat de zeepier, of in ieder geval het bevruchtingssucces zoals dat bepaald wordt met de IVF toets, toch niet zo gevoelig is voor BaP. Ook de IVF methode zal verbeterd moeten worden, gezien de grote variatie in bevruchtingssucces tussen individuele vrouwtjes binnen een behandeling.
Het is echter een gegeven, dat een organisme dat een bepaalde stof goed accumuleert, niet noodzakelijkerwijs ook een goed organisme is om de effecten van die stof te bestuderen. Geconcludeerd werd daarom dat de zeepier een geschikt organisme is om de biologische beschikbaarheid van aan sediment gebonden contaminanten mee te bepalen. Gelijktijdige blootstelling van mosselen maakt het mogelijk te bepalen in hoeverre transport van aan sediment gebonden contaminanten naar de waterkolom optreedt. Als dergelijke accumulatie studies gecombineerd worden met effect studies, gebruik makend van gevoeliger soorten en/of gevoeliger parameters, kan het milieurisico van verontreinigde sedimenten niet alleen gerelateerd worden aan de totale gehaltes van contaminanten in het sediment, maar ook aan de biologisch beschikbare fractie daarvan.

Summary

The role of feeding in the bioavailability of sediment-bound contaminants to marine benthic invertebrates

The sediment is the final sink for many contaminants entering the aquatic environment, due to the strong association of many chemicals with environmental particles. Whereas water quality may be relatively easily improved by eliminating the sources of contamination, sediments remain contaminated and may act as a source of contaminants to the aquatic system for many years. Due to the lack of standardised sediment toxicity tests, the equilibrium partitioning theory is regularly applied as it enables the use of the large set of aquatic toxicity data available and the water quality criteria derived from these data in order to be able to assess the risk of contaminated sediments to the environment and to establish sediment quality criteria for regulatory purposes. The equilibrium partitioning theory is especially used for the risk assessment of neutral lipophilic compounds, such as PAHs and PCBs. Two assumptions underlying the equilibrium partitioning theory are important for this thesis. Firstly, the equilibrium partitioning theory assumes that the environmental compartments considered (i.e. sediment, (pore)water and biota) are at chemical equilibrium. As a consequence, there are no net fluxes of contaminants between compartments and the concentration of a chemical in a specific compartment can be predicted from the measured concentration in another compartment, using a partition coefficient (see Figure 1). Secondly, the equilibrium partitioning theory assumes that when all compartments are at chemical equilibrium, the effective exposure concentration experienced by an organism is the same, regardless of the exposure route. This implies that, at equilibrium, all organisms attain the same body residue level (normalised to a relevant fraction, e.g. body lipid content for PAHs or PCBs), regardless of their mode of life. However, since conditions of equilibrium may not be common in the field, the actual body residue levels of an organism will depend on the exposure route, viz. the mode of life of an organism. Furthermore, it has been suggested that uptake through different exposure routes (specifically uptake through ingestion and direct uptake from water) may be additive.

The main purpose of this study was to investigate the role of the mode of life and, more specifically, the mode of feeding in determining the exposure of marine benthic invertebrates to sediment-bound contaminants. A second aim was to study the effects of sedimentary contaminants and to relate these effects to internal concentrations, in order to provide a less variable risk estimate, based upon the bioavailable fraction of the contaminant instead of the total concentration in the sediment.

The research in this thesis may be seen as an ecological approach to an ecotoxicological problem. In Chapter 1, for readers not familiar with ecotoxicology, an introduction to aquatic ecotoxicology is given, with emphasis on contaminated sediments. Since the biology and ecology of the test species may not be known to anyone, these are introduced in the remainder of Chapter 1.

In Chapter 2, the importance of the mode of feeding for the bioaccumulation of contaminants from sediments was assessed. Three marine benthic invertebrates, with different feeding habits, were exposed to contaminated sediments dredged from harbour areas and from

the Western Scheldt estuary. The animals were exposed in outdoor mesocosms for periods of 60 to 140 days. Body residue levels of PAH and PCB were highest in the sediment feeding lugworm, *Arenicola marina*, and were related to the levels of these contaminants in the test sediments. The body residue levels in the suspension feeding mussel, *Mytilus edulis*, on the other hand, appeared to be independent of the contaminant levels in the sediment, but reflected instead the contaminant concentrations in the surface water used to refresh the mesocosms. Intermediate body residue levels were found in the facultative deposit feeding baltic tellin, *Macoma balthica*. By manipulating the availability of food in the water column, it could be shown that the uptake of contaminants in *M. balthica* was related to its mode of feeding.

Since ingestion is the major uptake route of sediment-bound contaminants for the lugworm, *A. marina*, this species is an important candidate for the assessment of the effects of contaminated sediments. To this end, an *in vitro* fertilisation assay was developed, as is described in Chapter 3. Lugworms were exposed to contaminated sediments in outdoor mesocosms and were brought to the laboratory just before the beginning of the spawning period in September. Spawned eggs were fertilised in clean sea water and the fertilisation success was assessed after 24 h of development. The first results indicated that parental exposure to contaminated harbour dredged sediment might reduce the fertilisation success.

In Chapter 4, the suitability of *A. marina* for the biomonitoring of sediment-bound contaminants in intertidal areas was explored. During a period of more than a year, lugworms were sampled six times at five locations along a presumed contamination gradient in the Western Scheldt area and at a reference location in the Eastern Scheldt, in order to assess the importance of seasonal fluctuations, spatial variation and sexual development to PAH residues. A clear seasonal pattern of PAH body residue levels was observed, with the lowest levels occurring in March and the highest levels occurring just prior to the spawning season in September. The body residue levels of PAH clearly reflected the expected contamination gradient, with the exception of the samples collected during the spawning season. Although the observed seasonal pattern is apparently related to the reproductive cycle of *A. marina*, no clear differences in body residue levels were found between animals with or without developed gametes. The fertilisation success, determined with the *in vitro* fertilisation assay, was not affected by the observed differences in body residue levels between stations.

In order to be able to assess the accumulation and effects of sediment-bound PAH, artificially prepared sediments were spiked with PAH using a newly developed, two step spiking procedure. This procedure enables the preparation of the large amounts of test sediments needed in the marine mesocosms. The PAH is first adsorbed to an organic carrier (i.e. the food of sediment feeding organisms), after which the spiked carrier is mixed with sand. In Chapter 5, the suitability of this spiking procedure was tested, using three PAHs with a different affinity for organic matter: fluorene (logKow ca. 4), fluoranthene (FAN; logKow ca. 5) and benzo(a)pyrene (BaP; logKow ca. 6). Fluorene disappeared from the spiked sediments within a few months, well before the sediments could be used for experimentation. FAN had disappeared from the spiked sediments after two years and BaP concentrations only started to decline after two years. The spiking procedure is, thus, mainly suitable for very lipophilic, degradation resistant compounds like BaP.

Since BaP seemed to have a negative effect on the fertilisation success of *M. balthica* as well as that of *A. marina*, new artificial sediments were prepared and spiked with a concentration series of BaP, as described in Chapter 6. *A. marina* and *M. edulis* were exposed to these sediments in order to assess the relative bioavailability to a sediment feeder and a suspension feeder respectively. The fertilisation success of *A. marina* was assessed using the *in vitro* fertilisation assay, in order to establish the effects of BaP and to relate these effects to internal concentrations of BaP in *A. marina*. The body residue levels of BaP in the suspension feeding *M. edulis* were clearly related to the concentrations of BaP in the sediments, showing that the spiked BaP was more readily available for uptake than contaminants in field sediments. The body residue levels of BaP in the sediment feeding *A. marina* were ca. two orders of magnitude higher than those in *M. edulis*, demonstrating the importance of the mode of feeding. However, no effects were found on the fertilisation success of *A. marina*.

In Chapter 7, the combined results are discussed and conclusions are drawn. Compared to most laboratory spiking procedures, the spiking efficiency of BaP was relatively low. The recoveries of BaP were, however, nearly identical to the recoveries of organic matter, resulting in organic matter normalised concentrations that were very close to the nominal concentrations. It was suggested that the spiking efficiency of BaP and other contaminants might be improved by extending the stabilisation period, especially the period without water refreshing, and by using decomposed organic matter (litter) instead of fresh macroalgae as an organic carrier.
The spiked contaminants were more readily available for uptake than contaminants in field sediments usually are, a phenomenon regularly reported. The reasons for this may be differences in sediment composition, the age of the contamination and, for PAH, the source of the contamination. After an initial loss during the stabilisation period, the concentration and bioavailability of BaP remained stable for at least 2 years, without signs of ageing, enabling long-term studies with the spiked sediments.

The experiments clearly showed that the feeding habits of marine benthic invertebrates have a pronounced influence on the uptake of sediment-bound contaminants, with sediment feeding organisms accumulating contaminants to much higher levels than suspension feeding organisms. Since exposure to pore water probably does not play a significant role, it was concluded that these higher concentrations are the result of ingestion of the contaminated sediment. Due to the chemical environment in the gut and the digestion of organic matter, the uptake of contaminants through ingestion may be higher than is predicted by the equilibrium partitioning theory.

Although the preliminary screening of PAH indicated that BaP might have a negative effect on the fertilisation success of *A. marina*, this could not be confirmed. The reasons for this may be the insensitivity of *A. marina*, the insensitivity of the fertilisation endpoint, or the insensitivity of the *in vitro* fertilisation assay, due to the high level of variation in the fertilisation success between individual females. Recognising that a good accumulator is not necessarily also the best species for the assessment of effects, it was concluded that the lugworm, *A. marina*, is a suitable species for assessing the bioavailability of sediment-bound contaminants. Simultaneous exposure of the mussel, *M. edulis*, may be applied in order to assess whether direct transfer of sediment-bound contaminants to the water column occurs. Combining these assays with effect studies, using more sensitive endpoints or more sensitive

species, may yield risk estimates that are not only related to bulk chemistry, but also to the available fraction of the relevant chemicals.

Contents

Chapter

Introduction

1. Introduction

1.1. Ecotoxicology: an overview

Environmental contamination is closely linked to anthropogenic activities. Although some contaminants (such as heavy metals and PAH) may enter the environment through natural processes, the rate and the scale at which contaminants are brought into the natural environment by human activities are such that many organisms do not have the opportunity to either avoid contamination or adapt to it. Together with other anthropogenically induced disturbances (such as those related to deforestation, urbanisation, fisheries, agriculture, etc.), this may result in the extinction of local populations and even complete species at a rate previously unseen (Wilson, 1988).
The negative effects of contamination on the environment were recognised well before the Second World War (e.g. Dawson, 1935 and references therein), but research was generally aimed at the beneficial use of the toxic properties of chemicals, e.g. when applied as anti-fouling agents, or pesticides (e.g. Barnes & Stanbury, 1948; Cowell, 1976; Scholten, 1995). It was not until the 1960's that ecotoxicology became firmly established. The publication, in 1962, of Rachel Carson's book "Silent Spring" proved to be a major turning point in the way chemicals were regarded. During the years following, causal relationships were established between the occurrence of organochlorine chemicals in tissues and the population decline of seals and predatory birds (Ratcliffe, 1967; Jensen *et al.*, 1969; Ratcliffe 1970; Koeman & Van Genderen, 1972; Newton, 1979; Peakall, 1993). Moreover, the devastating effects of some major oil spills and other accidents affecting the coastal environment and human health aroused the public awareness and released major governmental funds (Cowell, 1976; Goldberg, 1992). As a result of the increased research effort and the implementation of more adequate legislation, the last two decades saw a gradual improvement in the water quality of most industrialised countries.
Nevertheless, accidents still occur regularly. The wreck of the oil tankers Exxon Valdez (1989), Braer (1993) and Sea Empress (1996) shook the world, as did the huge oil spill during the Gulf War in 1991. From Dutch coastal waters, the wreck of the coaster Nordfrakt, loaded with lead concentrate (1992), and the loss of a container with the carbamate pesticide Apron+ (1994) may be mentioned. Seen in a broader perspective, such incidents are, however, of minor importance. Even during the heyday of major tanker incidents in the 1970's, these accidents contributed less than 30% to the annual emissions of petroleum hydrocarbons to the marine environment (Evers *et al.*, 1997). The majority of contaminants entering the marine environment cannot be traced to a single source. Most are not derived from accidents, but from regular use and abuse, resulting in a world wide distribution. Accumulation of the more persistent chemicals has even been observed in such remote areas as the Arctic and Antarctic (Bacon *et al.*, 1992; Barrie *et al.*, 1992; Hargrave *et al.*, 1993; Tanabe *et al.*, 1994; MacDonald & Brewer, 1996).

Contaminants entering the aquatic environment may finally end up in the sediment and concentrate in sedimentation areas, especially when they are resistant to chemical or biological degradation. Due to the large surface area provided by sediment particles and the affinity many contaminants have for organic carbon, sediments act as an important sink for aquatic

contaminants (Shiaris, 1989). Sediments characteristically contain many different contaminants, since they are influenced by a multitude of sources. This is especially so for marine sediments, which are the final sink for most persistent contaminants. Contaminants may enter the marine environments through the following general pathways:

1. directly, from activities in coastal and marine areas, such as industrial and harbour activities, shipping and offshore oil and gas exploration;
2. from riverine and other freshwater runoff, including many land-based sources;
3. through atmospheric deposition.

The relative importance of the different pathways and the sources they integrate determine which contaminants may be present. Contaminants typical for many marine sediments are heavy metals, tributyltin, organochlorines and polycyclic aromatic hydrocarbons (PAH).

Once in the sediment, degradation of organic contaminants ceases or is considerably slowed down. This is mainly caused by the oxygen requirements of the degradation process, limiting degradation to the water column and the well oxygenated surface layer of the sediment (DeLaune *et al.*, 1981; Cerniglia, 1991; Wilcock *et al.*, 1996).
Contaminated sediments occur widely in coastal waters, due to the concentration of human activities in coastal areas and along the main waterways. Contaminated marine sediments are virtually beyond recovery and have to remain where they were deposited until they are washed away or covered with new layers of sediment. Most contaminated freshwater sediments may be recovered by dredging in order to restore the quality of the ecosystem. However, due to the enormous costs involved, such operations are mostly restricted to areas with a high natural or recreational value and to maintenance dredging in order to ensure a certain minimum depth in canals and harbour areas. In the latter case, the restoration of ecosystem functions is not the (primary) objective.

The disposal of these dredged materials poses a special problem. Dumping these at another site only displaces the problem and unnecessarily threatens other (parts of) ecosystems, whereas cleaning and/or confined disposal are very expensive. A compromise between these extremes is the confined disposal (or treatment) of the most heavily contaminated dredged materials and dumping, usually offshore, of the lightly contaminated materials, which are thought not to pose a risk to the environment. Such a 'division' is, for instance, implemented in the Netherlands, where 4 classes of contaminated sediments are discerned (Table 1). Sediment in classes III and IV are considered to be heavily contaminated, class IV to such an extent that remedial actions should be taken. The sediments in these classes should be stored in confined disposal facilities or be subjected to remediation treatments. Sediments in classes I and II are considered to be only moderately contaminated and may be mostly dumped at disposal sites at sea (Min. V&W, 1994). There isn't usually much discussion about which sediments are lightly contaminated, or which are heavily contaminated. However, there is a wide grey area of moderately contaminated sediments in between for which additional testing is needed in order to establish a more reliable estimate of the risk to the environment. Disposal options are not always obvious, especially for the sediments in class II. The class limits are determined by the concentrations in the sediment, which are related to the toxicity of the individual compounds, and not by direct toxicity assessment. Toxic effects may, however, occur due to the combined

effect of chemicals, being individually below apparent threshold levels (Stronkhorst *et al.*, 1996).

Table 1: Lower levels of some individual and sum chemicals for 4 classes of contaminated sediments as are used in the Netherlands. Values in mg.kg^{-1} standard sediment (containing 10% organic matter and 25% lutum) (from: Min. V&W, 1994)

Chemical	Class I	Class II	Class III	Class IV
Cadmium	0.8	2	7.5	12
Zinc	140	480	720	720
PCB-153	0.004	0.004	0.03	
sum 7 PCB			0.2	1
Benzo(a)pyrene			0.8	
sum 10 PAH	1	1	10	40

In the early days of ecotoxicology, research was generally aimed at effects occurring in the field and specific tests were developed in order to establish cause and effect relationships. Such a defensive approach was justified in a period when negative effects were observed all over the world and the causes were still unknown. When, around 1970, environmental issues finally entered the political agenda, the need arose for standardised, predictive tests, which could aid in the assessment of the possible negative effects of chemicals before those effects would occur in the environment and, preferably, before those chemicals were released into the environment (Koeman, 1992). The OECD has now published guidelines on 10 toxicity test procedures, using algae, *Daphnia*, fish (3 tests) micro-organisms, birds (2 tests), earth worms and plants (OECD, 1993). The emphasis is clearly on aquatic tests (6 out of the 10), because of the ease of testing and the fact that most chemicals can be found in water. Although several species are recommended for most tests and the use of additional species is not prohibited, marine species are clearly underrepresented. Considering the final fate of most chemicals, the absence of guidelines for sediment toxicity tests is striking and may be attributed to the difficulties experienced in standardising test procedures and the discussion regarding the use of whole sediment tests, or pore water and/or elutriate tests. Moreover, the importance of contaminated sediments has been greatly underestimated for a long time (Van der Kooij *et al.*, 1991).
Although the standardisation of test procedures was initiated in order to enable the prediction of the environmental effects of chemicals, it primarily facilitates comparisons between chemicals (i.e. their relative toxicity), while yielding only a qualitative estimate of the environmental risk (Solbé, 1993; Cairns, 1995; Taub, 1997; Landis *et al.*, 1997). Extrapolation of these data to field situations is, therefore, surrounded by many uncertainties. Nevertheless, data obtained from standardised toxicity tests are regularly used in ecological risk assessment, even with regard to sedimentary contaminants, because it is the most extensive data set on ecotoxicological effects. Uncertainties are often coped with by the application of safety factors (Van Leeuwen *et al.*, 1992). This is a practical solution, enabling at least some protective measures to be taken. Scientifically, however, further research, addressing contaminated sediments and the effects at the level of populations and communities, is definitely needed.

In order to assess whether environmental policy has the desired effects and as a warning indicating negative effects, natural ecosystems are (more or less) regularly monitored.

Chemical monitoring assesses the levels of contaminants in environmental compartments (water, sediment, soil, air, biota). However, it is impossible to analyse for all of the chemicals that may be present and, thus, it remains uncertain if the correct contaminants are monitored. Moreover, the concentrations of contaminants often tell us little about their effect on the environment; they merely give an indication. Supplementary information may be obtained by biological monitoring, for which several techniques are available nowadays, including the assessment of contaminant levels in indigenous or transplanted organisms and effects tested with laboratory assays, or assessed at the level of populations and communities in the field (Hopkin, 1993; Kramer, 1994). Ultimately, it is the effects exerted by contaminants that are of concern. However, since effects may often not be easily distinguished from natural variation in the field and, more importantly, monitoring should give a warning before effects really occur, the monitoring of chemical concentrations in different environmental compartments is invaluable. Suspension feeders, such as mussels and oysters, have long been used to monitor chemicals in marine waters, supplementing or even replacing direct chemical analyses, and procedures are well described (De Kock & Kramer, 1994; O'Connor *et al.*, 1994). Sediment contamination is mostly monitored by direct chemical analysis, sometimes supplemented with the sampling of a variety of benthic and epibenthic species, including suspension feeders (Duinker *et al.*, 1983; Langston, 1986; Szefer & Szefer, 1990; Burt & Ebell, 1995; Van Bavel *et al.*, 1995; Adami *et al.*, 1997; Muhaya *et al.*, 1997).

1.2. Risk assessment of contaminated sediment

Sediment is the final repository for many contaminants, due to the strong association of many chemicals with environmental particles (Bierman, 1990). Whereas water quality can be relatively easily improved by eliminating the sources of contamination, contaminated sediments have to be removed and treated or disposed of (Di Toro *et al.*, 1991). Moreover, contaminated sediment may act as a source of contaminants to the water column for many years after the original source has been eliminated (Hoke *et al.*, 1997). Understanding the environmental fate of contaminants in sediments is, therefore, important.

Mackay introduced fugacity modelling in order to predict the distribution of chemicals over environmental compartments (Mackay, 1979; Mackay & Paterson, 1981; 1982). Fugacity is the tendency of a substance to move from compartments with a high fugacity to compartments with a lower fugacity, in order to attain thermodynamic equilibrium. When all compartments are at equilibrium with each other, there is no net transport between compartments and concentrations in a specific compartment can be predicted from measured concentrations in other compartments. In Figure 1, a simplified scheme for aquatic systems is given. When equilibrium is attained, the ratios between compartments (the partition coefficients) are constant. The ratio between biota and water is called BCF (bioconcentration factor). The ratio between biota and sediment is often called BSAF (biota to sediment accumulation factor), but is also known as BAF, AF, PF or ARS. The ratio between particulate matter (incl. sediment) and water is known as the Kd. A considerable improvement in the model predictions can be obtained by normalising tissue concentrations to lipid content (Chiou, 1985) and sediment concentrations to organic carbon content (Di Toro *et al.*, 1991). Moreover, the organic carbon normalised partition coefficient for organic chemicals between sediment and water, Koc, appeared to be reasonably well correlated with the partition coefficient between octanol and water, Kow (Pavlou & Weston, 1984; Di Toro *et al.*, 1991).

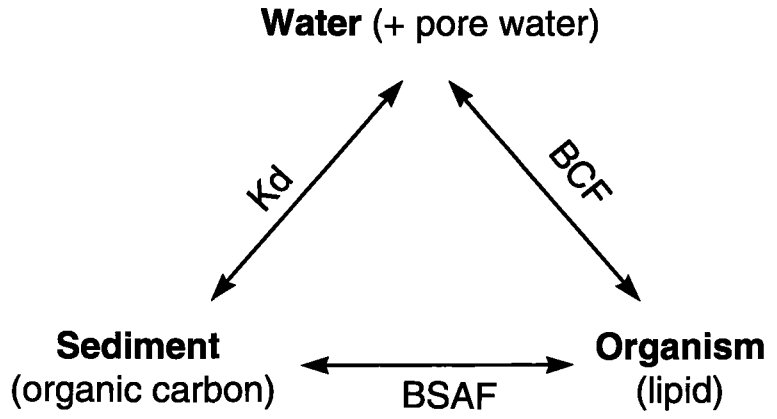


Figure 1: Schematic representation of the relationships between contaminant concentrations in (pore)water, sediment organic carbon and organism lipid.

The predictable partitioning of chemicals between different environmental compartments under conditions of equilibrium was recognised as a useful tool in order to establish sediment quality criteria by using water quality criteria (Pavlou & Weston, 1984). A comprehensive review of the backgrounds of what has become known as the equilibrium partitioning theory, in establishing sediment quality criteria, was given by Di Toro *et al.* (1991). When a system is in equilibrium, an organism receives an equivalent exposure from each phase. The route of the exposure is not important. Aquatic toxicity data, established in water-only exposures, may, therefore, be used to evaluate sediment toxicity by calculating the equivalent porewater concentrations. Conversely, for regulatory objectives, water quality criteria may be used in order to establish sediment quality criteria. Nevertheless, it should be applied with caution, as the equilibrium approach, like any model, is a very simplified representation of the environment. Often, the predictions are in good correspondence with observations (Di Toro, 1989; Di Toro *et al.*, 1991; Hoke *et al.*, 1994; Webster & Ridgway, 1994; Hoke *et al.*, 1995a), but not always (Landrum & Faust, 1991; Landrum *et al.*, 1994; Boese *et al.*, 1995; Iannuzzi *et al.*, 1995; Chapman, 1997). As such, the equilibrium approach is a valuable tool in the initial screening of contaminated sediments and is a welcome aid with respect to establishing environmental criteria for sediments, as it makes use of the relatively large body of aquatic toxicity data. More importantly, the assumptions of the equilibrium partitioning theory can be tested and the model can give guidance in environmental research as to which processes are the most important in the understanding of the environmental fate of contaminants.

A basic assumption of the equilibrium partitioning approach is that the environmental compartments considered are in chemical equilibrium. This is probably rarely true in the field (Mackay, 1979; Bierman, 1990; Suffett *et al.*, 1994). Furthermore, it is recognised that contaminants in field sediments are often less available (chemically and biologically) than contaminants spiked to experimental sediments (Oliver, 1985; Varanasi *et al.*, 1985; Readman *et al.*, 1987; Boese *et al.*, 1997; Ten Hulscher *et al.*, 1997; Thompson, 1997). As a result, Koc's are higher and do not yield a clear relation with the Kow anymore. Many organic chemicals

become more strongly associated with the sediment after prolonged contact times (Karickhoff & Morris, 1985; 1987; Landrum *et al.*, 1992; Alexander, 1995). The nature of this association remains unclear as yet, but entrapment in micropores and integration within the organic matter may be important processes (Pignatello & Xing, 1996; Cornelissen *et al.*, 1997; Hatzinger & Alexander, 1997; Ten Hulscher *et al.*, 1997).
The situation may even be more complicated with regard to PAHs. Petrogenic PAHs, which originate from oil and oil products, behave like most organic chemicals and Koc's in freshly deposited sediments are comparable to those measured in the laboratory. A considerable fraction of PAH in field sediments may, however, have a pyrolitic origin (i.e. they are derived from the combustion of oil, wood, etc.) and are strongly associated with soot particles. These PAHs have a very high Koc and their bioavailability is generally thought to be extremely low (Prahl & Carpenter, 1983; Readman *et al.*, 1984; 1987; McGroddy & Farrington, 1995; Meador *et al.*, 1995b; Gustafsson *et al.*, 1997). PAH may, however, be released from soot particles by solvents (Akhter *et al.*, 1985; Püschel & Calmano, 1995).

In the equilibrium approach, aquatic toxicity data are compared with predicted porewater concentrations, because under the condition of equilibrium the actual exposure route is not important (Karickhoff & Morris, 1987; Swartz *et al.*, 1990; Di Toro *et al.*, 1991). This is commonly, but not correctly, interpreted as porewater being the main exposure route for benthic species. In natural systems, the mode of life of the organisms is an important factor determining the actual exposure (Adams, 1987; Bierman, 1990; Thomann *et al.*, 1992). Direct uptake from ingested sediment particles may be especially of importance, as digestion of the organic matrix may provide an additional source for uptake, even when equilibrium seems to exist (Knezovich *et al.*, 1987; Suffett *et al.*, 1994; Boese *et al.*, 1995; Gagnon & Fisher, 1997). For the evaluation of sediment toxicity, it is, therefore, crucial to address the biology, and the feeding preferences especially, of the test species (Harkey *et al.*, 1994a; Kukkonen & Landrum, 1995b; Meador *et al.*, 1995b; Luoma, 1996; Kane Driscoll & Landrum, 1997).

1.3. Aspects of the biology of the test species

For the research presented in this thesis, three benthic invertebrate species were chosen that are markedly different in their modes of feeding. The lugworm, *Arenicola marina*, is a sub-surface bulk sediment feeder; the mussel, *Mytilus edulis*, is an epibenthic suspension feeder; the baltic tellin, *Macoma balthica*, is an infaunal surface deposit feeder, which, under certain conditions, may also collect a significant portion of its food particles from the water column. Therefore, with respect to feeding habits, *Macoma* takes an intermediate position between the other two species. These three species occur in abundance in the intertidal areas of Dutch coastal waters. As such, they are not only important chains in the ecological processes, but it also ensures that they can be collected in sufficient quantities for experimental purposes. A short biological and ecological characterisation of the species will be given in the following sections.

Arenicola marina (L.) (Annelida, Polychaeta)

Lugworms, of which *A. marina* is probably the best studied species, occur world wide in coastal areas. *A. marina* can be found in most intertidal areas of the northern hemisphere, north of the 20°C summer isotherm (Wells, 1963). It is the only species of lugworm found in Dutch coastal waters.

In the Dutch part of the Wadden Sea, the highest densities (>20 ind.m^{-2}) of adult lugworms can be found at ca. 80 cm below the mean tidal level (MTL) (Beukema & De Vlas, 1979). Juveniles are usually found at much higher densities at or above MTL in so-called 'nursery areas', if suitable areas are present. Only very limited numbers of juveniles are found between the adults. If adults are absent, high densities of juveniles may be found locally below MTL (Beukema & De Vlas, 1979; Farke *et al.*, 1979; Farke & Berghuis, 1979b). *A. marina* is only present in very low densities in the upper regions of the subtidal parts of the Wadden Sea (Dekker, 1989), although it may be predominantly subtidal in colder areas (Wells, 1963).

A. marina prefers rather sandy sediment, in which it constructs a "U" shaped burrow (Beukema & De Vlas, 1979). The burrow consists of a "J" shaped gallery, in which the worm lives and a head shaft through which water is pumped upward (see Figure 2). The gallery extends to a depth of 20 to 30 cm and then bends for another 10 to 15 cm in a horizontal direction. The walls of the gallery are impregnated with the worm's secretions (Wells, 1966), so that they will not collapse. Near the surface, the gallery narrows into the tail shaft at the top of which a characteristic pile of faecal castings is deposited. A typical head shaft is a descending column of sand, often indicated by a shallow, saucer-like depression at the sediment surface. The worm feeds from the lower end of the head shaft by ingesting all particles that stick to its proboscis (Baumfalk, 1979b; Rijken, 1979). In this manner, finer material is gradually removed and deposited at the surface with the faeces and the coarse material (>400µm, depending on the size of the worm) remains at the deepest part of the burrow. In densely populated locations, the coarse material may form a definite layer (Wells, 1966; Cadée, 1976; Baumfalk, 1979b). The sediment above this layer is intensively bioturbated (Cadée, 1976). Although *A. marina* may migrate to another location when conditions are unfavourable, the adult lugworms are usually very sedentary, moving only the position of the head shaft when food is depleted locally (Rijken, 1979). In densely populated locations, the new head shaft will be somewhere close to the faecal coil and may even be shared with other individuals (Rijken, 1979). In sparsely populated areas the new head shaft may be created beyond the old one, sometimes resulting in galleries that extend for nearly 1 meter in a horizontal direction (pers. obs.). As a result of the regular pumping of water to ventilate the burrow, the material in the head shaft and the wall of the gallery is well oxygenated and the yellow colour is clearly visible against the surrounding grey or black sediment. The food of *A. marina* probably consists of bacteria and benthic diatoms, supplemented by some detrital material (Rijken, 1979).

A. marina gains most weight in the first two years of its life. After that, somatic growth continues at a slower rate and most energy is expended on maintenance and reproduction (Beukema & De Vlas, 1979). In coastal populations where recruitment occurs regularly, the average weight is very stable and seasonal weight changes are more obvious (Beukema & De Vlas, 1979). The lowest individual weights are observed in the winter period, from December to February. The weight then increases until it reaches an apparent plateau in

summer (June-August). During exceptionally warm summers, a temporary decline in the average weight may be observed (viz. 1976, De Wilde & Berghuis, 1979). From September to early December, the weight decreases rapidly (Beukema & De Vlas, 1979; De Wilde & Berghuis, 1979). In early November, the period between the two spawning periods in the Dutch Wadden Sea (see below), a small increase in average body weight may even be observed (De Wilde & Berghuis, 1979). The weight changes in autumn are, therefore, closely related with the spawning season.

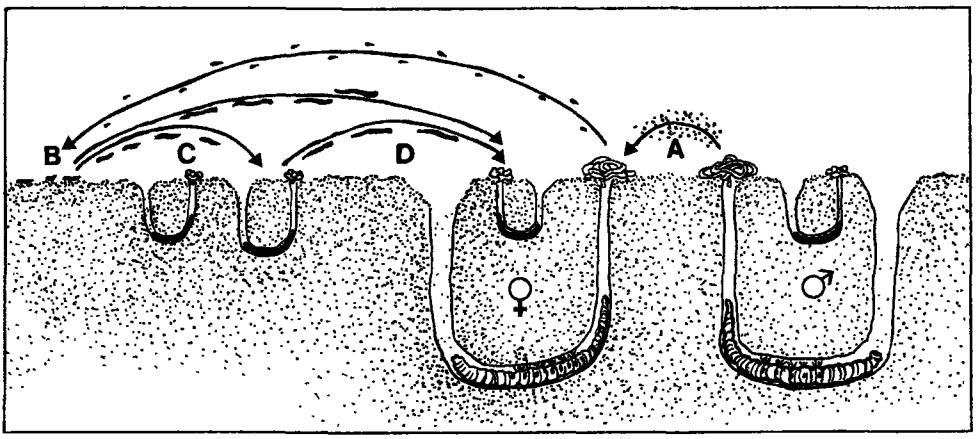


Figure 2: Pattern of migration of the lugworm A. marina. *A: the eggs are fertilized in the burrow of the female; B: the early larvae migrate shorewards and settle on undisturbed surfaces covered with a diatom film; C: the postlarvae (Benham stages) migrate downshore to nursery areas or settle directly between the adults on the tidal flats; D: juveniles from the nursery areas migrate into the adult areas (Adapted from Farke & Berghuis, 1979a).*

In *A. marina*, the gametes proliferate in the gonads, but most of the development occurs while they are floating in the coelom. From April onwards, increasing numbers of spermatogonia and small oocytes (20-60 μm) can be found in the coelomic fluid. During June, oocytes enter the vitellogenic stage (Rashan & Howie, 1982) and rapidly grow to a mature size of at least 150 μm, while the spermatogonia develop into discs of mature spermatozoa, called morulae. In the morulae, the spermatozoa are cytoplasmically connected to a central mass of cytoplasm, called the cytophore, while their tails point to the outside (Pacey & Bentley, 1992a). The sex of individual worms may be assessed by visual inspection during this stage. As the spawning season approaches, no new gametes are formed and the coelom is filled with sperm morulae and mature sized eggs (Pacey & Bentley, 1992a; Rashan & Howie, 1982). After spawning, gametes (sperm morulae, oocytes and earlier developmental stages) may be retained in the body cavity of some individuals, but during the winter period these are probably resorbed (De Wilde & Berghuis, 1979; Bentley & Pacey, 1989). Before spawning can occur, the gametes undergo a final maturation step which is under endocrine control. The eggs simultaneously progress from the late prophase of the first meiosis to metaphase and are subsequently taken up by the nephridia and shed (Howie, 1961b; Meijer, 1979; De Wilde & Berghuis, 1979). The

gametic development of the sperm is complete, but the sperm morulae have to dissociate before the spermatozoa can be spawned (Newell, 1948; Howie, 1961a; Howie, 1963). The sperm morulae are probably not dissociated simultaneously, as it has been observed that males often do not spawn completely (De Wilde & Berghuis, 1979). In contrast to the females, the coelomic fluid of mature males contains active sperm between the morulae (Bentley, 1985) and sperm taken from the body cavity may gain activity after dilution in sea water (Howie, 1961a). Sperm extracted directly from the body cavity can, therefore, be used in *in vitro* fertilisation assays, as is described in Chapter 3.

Physiologically, the spawning is initiated by hormones released from the prostomium (for a review, see Bentley & Pacey, 1992). The presence of such hormones was first established by Howie (1961a; 1961b; 1963) and 30 years later the fatty acid 8,11,14-eicosatrienoic acid was identified as the principal hormone inducing spawning in male *A. marina* (Pacey & Bentley, 1992b). A second, as yet unidentified, hormone is involved in initiating spawning in *A. marina* females (Watson & Bentley, 1997). The identification of maturation hormones could be helpful to the improvement of the *in vitro* fertilisation technique described in Chapter 3. Preliminary experiments with the male maturation hormone 8,11,14-eicosatrienoic acid in order to induce spawning did not yield satisfactory results, however. It is not clear whether this is due to technical inexperience or to genetical differences between British and Dutch lugworms. Addition of 8,11,14-eicosatrienoic acid to sperm in suspension did result in the breakdown of the sperm morulae and largely increased the motility of the spermatozoids, but in some preliminary experiments using this 'activated sperm' fertilisation rates were not improved compared to untreated sperm.

In the Dutch Wadden Sea, the main spawning period for *A. marina* is between early September and late October, when 60-80% of the population spawns. A second, more confined period, occurs in late November/early December (De Wilde & Berghuis, 1979). The Dutch population, therefore, shows characteristics of continental populations which spawn mainly in September-October and of British populations which spawn from late October to early December (Duncan, 1960). The differences in the characteristic spawning period may have a genetic background, which would imply that two genetically distinct populations occur intermixed in the Dutch Wadden Sea (Duncan, 1960; De Wilde & Berghuis, 1979; Cadman & Nelson-Smith, 1990). However, the genetic variation between several populations sampled along the mainland European coast from France to Norway was found to be very low (Hummel *et al.*, 1997a) and it seems reasonable, therefore, to assume that ecological factors determine this segregation. The proportion of the population spawning during the second period in the western Wadden Sea, is nearly the same size as the proportion of juvenile recruitment into the adult population found by Beukema & De Vlas (1979). This could mean that the juveniles that enter the reproducing population for the first time are not able to acquire full sexual maturity in time to join the first spawning period.

Although the spawning of *A. marina* is confined to a relatively short period of time, it remains unclear by which environmental cues it is initiated. A relationship with spring or neap tides was suggested by Newell (1948) and Duncan (1960), whereas Howie (1959) stressed the importance of a sudden decrease in ambient temperatures. Lugworms collected in the Dutch Wadden Sea did not respond to a drop in temperature, but temperatures below 15°C at the end of November seemed to be a prerequisite for spawning, which occurred about the time of the

full moon (Farke & Berghuis, 1979a). During the *in vitro* fertilisation assays performed for this thesis, which were all conducted during the first spawning period, no indications were found for a pronounced influence by temperature or by the lunar cycle. The spawning commences at varying time intervals after sampling, depending on the stage of maturity of the experimental population, and continues with a varying number of females spawning each day, until most of the females are spent. The first spawning period in the Dutch Wadden Sea is rather diffuse, extending for more than 2 months, while the second period is more epidemic, being completed within a fortnight. The spawning in the latter period is probably triggered by some environmental cue, in order to ensure that the larvae are able to develop into stages capable of surviving the coming winter period. Larvae produced in the first period probably have sufficient time to reach a winter hardy stage and strong environmental cues are not necessary. Maturation in the first spawning period may be controlled by general growth processes, related to the availability of food. Spawning may become more synchronised since mature animals that are ready for spawning respond to pheromones released by other individuals that spawn at random, because they are so ripe that any changing factor could induce spawning (Duncan, 1960). Such a pheromone, 2-ethyl-1-hexanol, seems to be present in the spawning water of males (Hardege *et al.*, 1996).

Males usually spawn at low tide, the sperm forming puddles on the surface. The sperm is washed away with the incoming tide, resulting in optimal, but spatially variable, sperm concentrations in the water of the first waves sweeping the surface (Williams *et al.*, 1997). The sperm may then be drawn into the female burrow, where fertilisation takes place, by the pumping activity of the females. Pumping activity was shown to be maximal directly after the burrows are submerged (Baumfalk, 1979a) and it is also stimulated by pheromones released by spawning males (Hardege *et al.*, 1996). The actual fertilisation success may vary between 0 and 80%, with a mean of 40-60% (Williams *et al.*, 1997). As the eggs will remain viable for at least 2 days at low temperatures (Farke & Berghuis, 1979a), additional fertilisation may occur during subsequent tides.

The eggs develop in the burrow of the female. Newly hatched larvae and the early stages with up to 3 chaetigerous segments are able to swim by ciliary movements, enabling them to leave the burrow, helped by the pumping activity of the female. During the time when larvae are present in the burrow, which may last for 3 to 4 weeks, the females do not feed, whereas males resume feeding 2 to 3 days after spawning (Farke & Berghuis, 1979a). In the laboratory, the first swimming trochophore larvae were observed 4 days after fertilisation. Settling larvae constructing tubes of mucus may be found within 1 week after fertilisation (pers. obs.). The early larvae that have left the burrow are washed shorewards with the tides and settle in tubes of mucus on sandy sediments covered with a thin diatom film. These diatom layers can be found high in the intertidal area (Farke & Berghuis, 1979b). Here they remain until they have reached a length of 4-9 mm. From January to March, these postlarvae (or Benham larvae, since they were described in detail by Benham, 1893) can be found swimming, while migrating to deeper water (Beukema & De Vlas, 1979; Farke & Berghuis, 1979b). The young lugworms, now approx. 6 months old, either settle directly between the adults, or in the so-called 'nursery areas', where they remain until the following winter when they definitely settle between the adults, depending on the local situation (Farke *et al.*, 1979). This migrational sequence is depicted in Figure 2. The pattern of migration may be more complicated when additional habitats are available, such as mussel beds and seagrass meadows (Reise, 1985). In the Dutch Wadden Sea, an average yearly recruitment of juveniles into the adult population of 20% was

observed, which, at an observed average mortality rate of 22%, would be sufficient to keep the population almost stable (Beukema & De Vlas, 1979). The seaward migration of juveniles (and probably also of adults) seems to be driven by low temperatures. During the 1970's, which were characterised by a sequence of several mild winters, juveniles migrated only short distances. As a consequence, the population density at offshore sampling stations in the Dutch Wadden Sea (>3 km from the coast) showed a continuous decrease, while the populations within 3 km from the shore remained stable (Beukema *et al.*, 1978). The annual mortality rate of ca. 20% in the adult population, suggests that *A. marina* may live for at least 5 or 6 years (Beukema & De Vlas, 1979). An even higher life span may be deduced from the fact that after 7 years without any obvious recruitment, (some) adults were still present at the above mentioned offshore stations (Beukema *et al.*, 1978), although immigration of adults would have gone unnoticed.

Lugworms (mainly *A. marina*; occasionally *A. cristata* and *Abarenicola pacifica*) have only been used to a limited extent in ecotoxicological research. Accumulation and effects of contaminants have been studied in laboratory experiments using lugworms, but only a few have incorporated exposure to contaminated sediments (Lyess, 1979; Augenfeld, 1980; Oakley *et al.*, 1980; Augenfeld *et al.*, 1982; Miramand *et al.*, 1982; Parrish *et al.*, 1989; Weston, 1990). Some studies addressed the influence of sediment reworking by lugworms on the fluxes of contaminants into and out of the sediment (Garnas, 1977; Gordon *et al.*, 1978; Marquenie *et al.*, 1985a; O'Neill *et al.*, 1985; Loring & Prosi, 1986; Davey *et al.*, 1990; Doyle & Otte, 1997; Kure & Forbes, 1997). On the other hand, the feeding rate of lugworms, measured as cast production, may be reduced due to the presence of contaminants in the water or the sediment (Schoor & Newman, 1976; Rubinstein, 1978; 1979; Augenfeld, 1980) and this has successfully been incorporated into a test programme for the assessment of the quality of field sediments (Thain *et al.*, 1996). Due to their abundance and their importance as a prey item, lugworms have been sampled during field surveys in intertidal areas (Holme, 1978; Goerke *et al.*, 1979; Packer *et al.*, 1980; Duinker *et al.*, 1983; Essink, 1989; Morris *et al.*, 1989; Hidding & Bouwhuis, 1991) and formed an important member of the macrozoobenthic community present in field experiments (Crapp *et al.*, 1971; Levell, 1976; Rachor, 1984; Farke *et al.*, 1985; Matthiessen & Thain, 1989). Their intimate relationship with the sediment and their importance as a member of the macrozoobenthic community of the Dutch Wadden Sea were the main reasons for incorporating the lugworm, *A. marina*, into ecotoxicological mesocosm studies performed by TNO in Den Helder (Kuiper *et al.*, 1983; Marquenie *et al.*, 1985a; 1985b; 1986; Bowmer, 1987; Scholten *et al.*, 1987; Jenner & Bowmer, 1990; Bowmer *et al.*, 1991; 1993; Dekker *et al.*, 1993; Scholten *et al.*, 1994).

Mytilus edulis L. (Mollusca, Bivalvia)

Mussels of the genus *Mytilus* occur world wide in temperate and boreal seas of the northern and southern hemispheres. Much confusion exists about the taxonomic status of the species in the genus. Systematics were originally based solely on shell morphology, which is enormously plastic (Seed, 1968). The use of biochemical techniques reduced the number of currently recognised species to 6 (Gosling, 1992a;1992b). Only *M. edulis* sensu stricto, a species which is found in most coastal areas of the North Atlantic, is known to occur in Dutch coastal waters. Apart from the as yet unresolved systematics of *Mytilus*, the different species

also show a remarkable flexibility in life history traits in response to variations in environmental conditions. The following treatise on the biology of *M. edulis* is, therefore, necessarily very general and is largely based on some extensive reviews, supplemented with some specific information regarding the mussels in Dutch coastal waters.

M. edulis typically occurs in intertidal habitats, but it can also be found subtidally. Although the environmental conditions for growth are very good subtidally, the subtidal distribution of *M. edulis* is severely limited by predation (Seed & Suchanek, 1992). In the commercial mussel beds in the Dutch Wadden Sea, mussels are regularly collected and predators (mainly the sea star *Asterias rubens*) removed (Dekker, 1989). The upper limits of the distribution of *M. edulis* are determined by their capability to withstand extreme temperatures and desiccation. The exact height may vary from year to year, depending on the occurrence of extreme weather conditions, and from place to place, depending on the amount and quality of the available food, which is needed to supply the energy required for metabolism during periods of starvation, e.g. during aerial exposure (Seed & Suchanek, 1992).

Mussels are usually found in dense aggregations, a mode of life made possible by their typical wedge shaped shells and the neotenial retainment of the larval byssus threads into adult life (Morton, 1992). Living in such densely packed aggregations provides stability against wave action and probably also affords some protection against predators (Morton, 1992; Seed & Suchanek, 1992). Although the infaunal species of tidal flats may be smothered by the presence of dense mussel aggregates, mussels beds are a suitable habitat, providing food and shelter, for many species that would otherwise not have been able to survive in intertidal areas (Dittman, 1990; Seed & Suchanek, 1992; Ramón, 1996). Additionally, being the main food item for many species, the presence of large mussel stocks may release predation pressure on other species (Beukema, 1993a). Due to their tendency to aggregate, mussels are very heterogeneously distributed over the area they inhabit. During a survey of the Dutch Wadden Sea, mussels were found on 25 out of 99 transects. Nevertheless, they contributed ca. 25% to the total biomass, more than any other species (Beukema 1976). In the shallow subtidal areas of the Dutch Wadden Sea mussels even contributed as much as 40% to the total macrozoobenthic biomass (excl. culture plots), although they were found at only 2% of the sampled locations. This may be a result of the presence of commercial mussel beds in the area. Mussels in culture plots are regularly fished up for removal of predators and redistribution in optimal densities (Dekker, 1989). The abundance of mussels in the Wadden Sea is, however, very variable. Mussel beds in exposed parts appear to be highly sensitive to storms. Therefore, persistent mussel beds can only occur in sheltered areas (Nehls & Thiel, 1993). Even these permanent mussel beds may, however, show considerable annual variations in density, due to the variable recruitment (Beukema *et al.*, 1978; Beukema, 1982).

Mussels are very efficient suspension feeders, removing particles down to a size of 2-3 µm with 80-100% efficiency from the inhaled water (Seed & Suchanek, 1992). The main food constituent is phytoplankton, mainly diatoms, while unpalatable particles are rejected as pseudofaeces (Smaal, 1997). Due to their high numbers and the large amounts of water they process, mussels play a central role in the processing of coastal and estuarine material (Dame & Dankers, 1988; Smaal *et al.*, 1986). Mussels are quite flexible in their reproductive strategy. In some populations, a conservative strategy is followed, with spawning in spring, thus enabling the developing larvae to exploit the spring phytoplankton bloom characteristic for temperate

waters. Populations growing under particularly favourable culture conditions may be rather opportunistic in their reproductive strategy and show a reproductive period starting in early spring and continuing into autumn. During this protracted reproductive period, gametogenesis is repeatedly initiated in response to the continuous availability of food (Seed & Suchanek, 1992). It is not clear which factors initiate the onset of spawning. A variety of environmental cues has been reported, involving changes in temperature and salinity and physical forces (Seed & Suchanek, 1992). In the Dutch Wadden Sea, the spawning of *M. edulis* usually takes place at the end of May and in June (Honkoop & Beukema, 1997). In general, it might be that ripe individuals will respond to a variety of cues, initiating a synchronising cascade, as the presence of gametes in the water stimulates other ripe mussels to spawn. The larval development is also very flexible. Fertilisation takes place in the water and is relatively insensitive to variations in environmental conditions. Larval development is more sensitive, being optimal at temperatures of around 20°C and a salinity of 25-30‰. Under favourable conditions, larval development is fast. The free-swimming stage is known as a veliger larvae. During certain times of the year, these veligers can dominate the plankton community. Development into pediveliger larvae, i.e. those ready for settlement and subsequent metamorphosis, may be completed within 20 days. However, when conditions are marginal and a suitable substrate for settlement is not encountered, it may also last for more than 6 months. After metamorphosis, the young mussels, now called plantigrades, may exhibit a second pelagic phase, the bysso-pelagic or byssis drifting phase. During this stage, which may last for another 2 months, until they reach a shell length of 2-2.5 mm, they detach from the original substrate and, often in several consecutive steps, settle on the adult beds (Lutz & Kennish, 1992). Altogether, it is not surprising that many mussel populations are characterised by sporadic, often unpredictable pulses of recruitment throughout the year (Seed & Suchanek, 1992).

Mussels are probably among the best known and most utilised organisms in environmental research. This is partly due to their importance as a shellfood, their widespread occurrence and the ease of collection and handling. Another factor encouraging their use is that mussels are important marine fouling organisms and had been subjected to toxicity testing well before environmental concern emerged. A wide array of endpoints has been tested under laboratory, field and semi-field conditions at cellular, individual, population and community levels of organisation, including mortality, growth, physiology, biochemistry, histopathology, cytology, genotoxicity, behaviour and stress responses (Roberts, 1976; Bayne *et al.*, 1979; Cunningham, 1979; Sheffrin *et al.*, 1984; Moore *et al.*, 1987; De Zwaan & De Kock, 1988; Viarengo & Canesi, 1991; Widdows & Donkin, 1991; Bakke, 1992; Livingstone & Pipe, 1992; Widdows & Donkin, 1992; Widdows, 1993; Baldwin & Kramer, 1994; Eertman & De Zwaan, 1994; Hose, 1994; Salazar & Salazar, 1995). Mussels process large volumes of water during suspension feeding and may accumulate many contaminants, heavy metals as well as organics, to levels well above ambient concentrations. Mussels are, therefore, regularly used in monitoring programmes, either passively, by sampling natural populations, or in active biological monitoring, by suspending them in baskets or nets (Roberts, 1976; Coombs & George, 1978; Goldberg *et al.*, 1978; Cunningham, 1979; Pries *et al.*, 1984; De Kock, 1986; Cossa, 1989; Ernst, 1992; Widdows & Donkin, 1992; Rainbow & Phillips, 1993; De Kock & Kramer, 1994; O'Connor *et al.*, 1994; Salazar & Salazar, 1995). Starting with anti-fouling research in the early 1950s, the mussel has been in use as an important organism for (eco)toxicological research at TNO Den Helder for over 40 years (De Wolf, 1972; De Kock, 1983a; Pries *et al.*, 1984; De Kock & Van het Groenewoud, 1985; De Kock, 1986; De Kock &

Scholten, 1987; De Zwaan & De Kock, 1988; Kramer *et al.*, 1989; Wrisberg, 1991; Baldwin & Kramer, 1994; De Kock & Kramer, 1994; Scholten, 1995; Kaag *et al.*, 1998).

Macoma balthica (L.) (Mollusca, Bivalvia)

Species of the tellinid genus *Macoma* are widely distributed in northern seas. *Macoma balthica* is a characteristic species of shallow water communities of the North Atlantic. It can be found from subpolar regions in the north of Scandinavia, Russia and Canada to, approximately, the south of France and the north of Spain in Europe and the coast of North Carolina in the U.S. (Thorson, 1957; Bachelet, 1980; Meehan, 1985). However, considerable genetic variation was found between different geographic areas (Väinölä & Varvio, 1989).

M. balthica is particularly abundant in intertidal areas, but can also be found subtidally to a depth of ca. 10 m and sometimes more (Thorson, 1957; Eisma, 1966; Beukema, 1976; 1982; Dekker, 1989). In the Dutch Wadden Sea, the average density of *M. balthica* was more than 100 ind.m^{-2} in the early 1970's, resulting in an average biomass (in ADW) of 2.2 g.m^{-2}, more than 8% of the total macrozoobenthic biomass (Beukema, 1976). Apart from an increase in the biomass, due to eutrophication, observed in the western Wadden Sea, yearly fluctuations in biomass are very low in this area (Beukema *et al.*, 1993). The subtidal distribution of *M. balthica* is less well known. In the subtidal parts of the Wadden Sea, an average biomass (outside commercial mussel beds) of 1.5 g ADW. m^{-2} was found, contributing nearly 6% to the total biomass (Dekker, 1989). Along the Dutch coast, the densities were generally below 50 ind.m^{-2} in 1964, although local densities could exceed this number (Eisma, 1966). During the 1970's densities ranging from 20 to 70 ind.m^{-2} were found in the North Sea off Terschelling (Beukema & De Vlas, 1989). At present, densities along the Dutch coast are high enough to enable some commercial exploitation of this species.

The feeding behaviour of *M. balthica* is very variable. Morphologically, it is a typical surface deposit feeder. It lives up to 10 cm below the sediment surface, depending upon age and temperature, and uses its long and flexible siphon to browse the sediment surface, sometimes leaving clear feeding marks (Hulscher, 1973; Reading & McGrorty, 1978; Ratcliffe *et al.*, 1981). Behavioural studies and analyses of the stomach contents, however, showed that suspension feeding might be important under certain circumstances (De Wilde, 1975; Hummel, 1985; Ólafsson, 1986). Active suspension feeding may prevail on poor sandy substrates (Ólafsson, 1986), but passive suspension feeding is thought to occur, because material from the sediment surface is whirled up into suspension before it is sucked up (Hulscher, 1973; Hummel, 1985; Kamermans, 1992). Although most organic carbon sources may be used as food by *M. balthica*, living algal cells probably have the highest nutritional value. Consequently, growth is only observed during periods characterised by a high algal production in the overlying water. Growth does not occur during periods when *M. balthica* relies solely on deposit feeding, even when benthic productivity is high, because then the bentic diatoms are mixed with much less nutritive organic material (Hummel, 1985; Beukema & Desprez, 1986). Being a species with a rather northerly distribution, the growth of *M. balthica* is further restricted by its sensitivity to high temperatures, being possible only between ca. 4°C and 16°C (De Wilde, 1975; Beukema *et al.*, 1985). Prolonged exposure to temperatures of 20 to 25°C may even be deleterious (De Wilde, 1975), although short term exposure (6 h) to temperatures over 30°C are tolerated

(Ratcliffe *et al.*, 1981). As seen above, within the constraints set by temperature, growth is largely determined by the availability of suitable food sources. Thus, in the Dutch Wadden Sea, growth is only observed during spring, starting in February/March and ceasing in late June/early July, but being particularly fast from April to June. Body weight continuously decreases during summer and autumn, resulting in a loss of 50-70% of the weight gained during spring. In the more southerly parts of its geographical distribution, feeding conditions may, however, facilitate a second growth period in autumn (Beukema & De Bruin, 1977; Bachelet, 1980; Beukema *et al.*, 1985; Beukema & Desprez, 1986). During its first year, *M. balthica* reaches a weight of 20 mg ADW, which is facilitated by the relatively high tolerance of the spat to high environmental temperatures, enabling them to continue feeding throughout summer and autumn (Beukema & De Bruin, 1977; Ratcliffe *et al.*, 1981). After their first year, *M. balthica* shows an average yearly weight increase of ca. 10 mg ADW, decreasing slightly with age, reaching a weight of ca. 60 mg ADW at an age of 5 to 6 years, which is near to the maximum age observed on the tidal flats of the Dutch Wadden Sea (Lammens, 1967; Beukema & De Bruin, 1977). Spatial differences in growth rate may occur due to differences in local conditions. At the Balgzand in the western Wadden Sea, an interaction was found between primary production (increasing with tidal height; determining food quantity) and immersion time (decreasing with tidal height; determining food availability), resulting in maximum growth at stations located 2 to 4 km from the shore, these being immersed ca. 60 to 80% of the time (Beukema *et al.*, 1977).

In the Dutch Wadden Sea, gonadal development of *M. balthica* starts in July and August. As feeding has ceased by this time, the animal is fully dependent on reserves stored during the previous growing period. The first ripe gametes may be observed in December, while maturation is completed in April (De Wilde & Berghuis, 1978). Lammens (1967) observed that the spawning of mature animals occurred when temperatures on the tidal flats rose above 10°C, a temperature which indeed seemed to be critical (De Wilde, 1975) and apparently delimits the distribution of *M. balthica* in southern directions (Beukema & Meehan, 1985). A rapid increase in temperature seems especially to stimulate spawning in mature animals and at the same time speeds up maturation in others, whereas only a limited number of animals can be stimulated when the temperature rises at a rate lower than $2°C.h^{-1}$. In the laboratory, spawning is usually not completed once only, as individuals may be forced to spawn a number of times when temperature shocks are applied for several days in a row (De Wilde & Berghuis, 1978). Lammens (1967) suggested that spawning in the subtidal populations off the Dutch coast would occur later in the year, because the temperature of the North Sea water rises more slowly than the temperature on the tidal flats. De Wilde & Berghuis (1978) argued that spawning would be poor anyway in these populations, since the temperature rises too slowly to initiate spawning in a sufficient number of individuals for a successful fertilisation to occur in the water. The latter view seems to be supported by Beukema & De Vlas (1989) who observed that the abundance and size distribution of year classes of *M. balthica* in the North Sea off Terschelling were strongly related to the distance to a presumed source area in the Wadden Sea and the occurrence of winter conditions promoting seaward migration. After a temperature shock is applied, spawning commences after ca. 45 min and is completed within the next 2 hours. This implies that spawning will occur on the tidal flats during the incoming tide, thereby ensuring that the gametes are retained within the intertidal area (De Wilde & Berghuis, 1978). During the spawning period, the growth of *M. balthica* may be temporarily arrested, because the gametes may comprise up to 25% of the body weight (De Wilde & Berghuis, 1978; Harvey

& Vincent, 1989). The larval development of *M. balthica* is rapid. Shortly after spawning, initial settlement occurs in the low intertidal, where the highest densities are observed during May. Subsequently, however, the abundance of the spat decreases as the juveniles migrate to the uppertidal (nursery areas), where conditions for growth are more favourable and predation is less. During the winter and early spring (January to April), the juveniles again migrate seawards and secondary spatfall is observed among the adults. This results in a rather uniform distribution of individuals aged 1 year and older on all tidal flats and in the adjacent subtidal area (Beukema & De Vlas, 1989; Armonies & Hellwig-Armonies, 1992; Beukema, 1993b). This winter migration seems to be stimulated by low temperatures, resulting in recruitment occurring mainly in the nearshore areas after mild winters, with substantial recruitment into the central parts of the Wadden Sea and into the North Sea occurring only after cold winters (Beukema *et al.*, 1978; Beukema & De Vlas, 1989; Beukema, 1993b).

Compared to *Mytilus*, *Macoma* species have only participated to a limited extent in environmental research. Still, *M. balthica*, *M. nasuta* and, incidentally, *M. inquinata*, *M. calcarea* and *M. carlottensis* have been used in ecotoxicological studies more or less continuously for the past 30 years. More than 40% of these were field studies, assessing the effects of oil pollution and oil combatment on existing populations (Woodin *et al.*, 1972; Thomas, 1977; Van Bernem, 1982; Elmgren *et al.*, 1983; Rachor, 1984; Humphrey *et al.*, 1987) and the accumulation of contaminants, mostly heavy metals, sometimes oil or organic contaminants, with respect to the availability of sediment-bound contaminants and the suitability of *Macoma* for use in monitoring programmes (Shaw & Wiggs, 1980; Boehm *et al.*, 1982; Duinker *et al.*, 1983; Bryan *et al.*, 1985; Cain & Luoma, 1985; De Giulio & Scanlon, 1985; Luoma *et al.*, 1985; Shaw *et al.*, 1986; Foster & Wright, 1988; Johns & Luoma, 1990; Szefer & Szefer, 1990; Goede *et al.*, 1993; Hummel *et al.*, 1997b).
In laboratory experiments, much effort has been directed at establishing the importance of dietary uptake of contaminants from sediment and suspended particulate matter. This showed that, relative to dissolved contaminants, particulate-bound contaminants may contribute significantly to the accumulation of more lipophilic organic contaminants (Roesijadi *et al.*, 1978; Varanasi *et al.*, 1985; Foster *et al.*, 1987; Boese *et al.*, 1990; Lee *et al.*, 1990; Ferraro *et al.*, 1991; Pruell *et al.*, 1993; Boese *et al.*, 1995; 1996) and to the uptake of several, but not all, heavy metals (Luoma & Jenne, 1976; Ray *et al.*, 1981; Crecelius *et al.*, 1982; Harvey & Luoma, 1985a; 1985b; Brown, 1986; Decho & Luoma, 1991; Absil, 1993; Decho & Luoma, 1994; Luoma *et al.*, 1995; Decho & Luoma, 1996). Practical experience with *Macoma balthica* at TNO Den Helder dates back to the early 1970s, when this deposit feeder was used in field and laboratory studies in order to assess the bioavailability of sediment-bound contaminants in relation to exposure conditions and sediment characteristics (De Kock, 1975; De Kock & Dinneen, 1979; De Kock, 1983b; Marquenie, 1985). Subsequently, *Macoma* was used in mesocosm studies assessing the bioavailability of contaminants in dredged materials and fly-ash (Marquenie *et al.*, 1985a; 1985b; Jenner & Bowmer, 1990). Being an important member of the macrozoobenthic community of the Dutch Wadden Sea, *M. balthica* was also incorporated into mesocosm studies assessing the effects of dredged materials and oil pollution and oil combatment on simulated intertidal communities (Kuiper *et al.*, 1983; 1984; Scholten *et al.*, 1987; Dekker *et al.*, 1993; Scholten *et al.*, 1994).

1.4. Aim of the study

According to the equilibrium partitioning theory, ecotoxicological effects of sediment contamination can be predicted from the concentrations of toxicants in the pore water. Although the predictions of the equilibrium partitioning theory are often fairly good, demonstrating that it may be a powerful tool in the assessment of contaminated sediments, they are not always correct. Taking the wrong decisions, for instance with regard to the disposal of dredged materials, may be very expensive in an economical as well as in an ecological sense. Much research is currently aimed at the chemical behaviour of contaminants in sediments, resulting in more accurate and site-specific partition coefficients between sediment and (pore)water. Less attention is, however, given to the biological characteristics of species that might influence the actual route of exposure. This discrepancy may in part be caused by the fact that nowadays the view seems to be broadly held that benthic organisms are primarily exposed to sedimentary contaminants via the porewater. This may, however, not be true for more lipophilic contaminants. Firstly, even when the sediment and pore water are in equilibrium with each other, deposit feeding may result in additional uptake of sediment bound contaminants, due to digestion of the organic matrix and/or the chemical properties of the digestive fluids influencing the solubility of the contaminants (e.g. Mayer *et al.*, 1996). Secondly, equilibrium between sediment (and porewater) and overlying water may be observed only rarely (Suffett *et al.*, 1994). Benthic suspension feeders will be mainly exposed to the overlying water and not to the porewater. Moreover, as is seen with *M. balthica*, presumed deposit feeders should sometimes be regarded as suspension feeders too.
The first purpose of this study was, therefore, to investigate whether differences in the mode of life, viz. feeding preferences, might result in different levels of contaminants accumulated.

Body residue levels provide a measure of the bioavailable fraction of sediment-bound contaminants and are, thus, more closely related to the internal effect concentration than environmental concentrations (McKim & Schmieder, 1991; De Kock & Bowmer, 1993; Luoma, 1996; Penttinen *et al.*, 1996; Van Straalen, 1996).
A second aim of this study was, therefore, to study the effects of sedimentary contaminants in relation to internal concentrations.

Fertilisation success was chosen as the main effect parameter, because reproduction is an important parameter in the population dynamics of species (Levin *et al.*, 1996). Although reproduction has been incorporated into chronic bioassays with a number of species, fertilisation assays have mainly been performed with echinoderms (e.g. Pagano *et al.*, 1985; Dinnel & Stober, 1987; Wynberg *et al.*, 1989). For practical reasons, the effect studies were conducted with organisms also used in the bioaccumulation studies. It was recognised, however, that good accumulators are not necessarily the most sensitive species and that clear dose-response relationships might not be found. Nevertheless, the use of the same species seemed justified, because little is known about the relative sensitivity of marine benthic invertebrates and the species concerned play an important role in the Wadden Sea ecosystem.

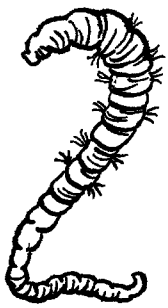
The research concentrated on three species with different modes of feeding: the lugworm, *Arenicola marina*, the mussel, *Mytilus edulis*, and the baltic tellin, *Macoma balthica*. In the first instance, 'naturally' contaminated sediments were used, containing an unspecified

suite of contaminants. The analyses were focused on organic contaminants, because these were thought to yield the most clear differences, due to their low solubility and their consequently high affinity for sediments. Polycyclic aromatic hydrocarbons (PAHs) received the most attention, as they occur world wide in coastal and estuarine sediments, are often the critical compounds determining disposal options in dredged sediments and are continuously produced and emitted into the environment.

1.5. Outline of the thesis

During the 1980's, several mesocosm studies with marine benthic organisms were carried out at TNO Den Helder, in which the bioavailability of sediment-bound contaminants was assessed. In a number of these studies, none of which has been fully published in the scientific literature, the effect of size fractionation on the bioavailability of contaminants was addressed. In Chapter 2, some of these studies are evaluated with regard to the accumulation of organochlorines and PAHs by *A. marina*, *M. edulis* and *M. balthica*. Only the 'naturally' polluted sediments (i.e. not spiked, nor subjected to remediation treatments) were considered in order to avoid variation originating from the treatments. Additionally, the feeding conditions for *M. balthica* were manipulated, accidentally as well as on purpose, in order to show the effect of the mode of feeding on the accumulation route within a single species and thus eliminating differences in the accumulation potential that might exist between species from different taxonomic groups. The accumulation patterns showed the importance of the mode of feeding in determining the uptake of sediment-bound contaminants by benthic invertebrates, with the highest levels found in *A. marina*, the species with the most intimate relationship with the sediment. Therefore, during the remainder of the study, the emphasis was placed upon *A. marina*. An *in vitro* fertilisation assay was developed, in order to asses the effect of contaminated sediments on the reproductive potential of *A. marina* (Chapter 3), because a large fraction of the organic contaminants may accumulate in the lipid rich gametes. The development of this IVF assay was initiated by Dr. Tim Bowmer (Bowmer *et al.*, 1991). In Chapter 4, the suitability of *A. marina* for the biomonitoring of sedimentary PAHs is explored. As the environmental chemistry of PAHs in sediments is not fully understood as yet, further research was conducted in the controlled environment of mesocosms. The procedures currently used for the spiking of sediments in laboratory experiments were not suitable for the spiking of the large amounts of sediment needed in the mesocosms. A new spiking procedure was, therefore, developed and tested with three PAHs with a different Kow (Chapter 5). Subsequently, sediments were spiked with a concentration series of benzo(a)pyrene (BaP). The bioavailability of BaP to *A. marina* and *M. edulis* was tested over a period of 2.5 years, together with the effect of BaP on the fertilisation success of *A. marina* (Chapter 6). Finally, in Chapter 7, the results are discussed in relation to the aims of this thesis.

Chapter



Comparison of contaminant accumulation in three species of marine invertebrates with different feeding habits

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with E.M. Foekema, M.C.Th. Scholten and N.M. van Straalen

2. Comparison of contaminant accumulation in three species of marine invertebrates with different feeding habits.

Abstract

In order to assess the importance of the mode of feeding for the bioaccumulation of contaminants from sediments, three marine benthic invertebrates, with different feeding habits, were exposed to contaminated sediments in outdoor mesocosms. Residue analyses were carried out for several polychlorinated biphenyls and polycyclic aromatic hydrocarbons after exposure periods of 60 to 140 days. It was shown that sediment ingestion is a major uptake route for the sediment feeding lugworm, *Arenicola marina*, and for the facultative deposit feeding baltic tellin, *Macoma balthica*. Residues in the filter feeding mussel, *Mytilus edulis*, appeared to be independent of contaminant concentrations in the sediment. The difference between deposit and filter feeding bivalves was confirmed in experiments involving the baltic tellin, with differences in the food availability in the overlying water. A simple linear regression model was used to describe contaminant concentrations in sediment feeding invertebrates as a function of concentrations in sediment. A correction for the accumulation from water was made by subtracting the concentrations in filter feeders. It was concluded that chemical equilibrium partitioning alone is not sufficient for the assessment of the risks of contaminated sediments to sediment feeding invertebrates, but that feeding habits should also be considered.

2.1. Introduction

Generally, only dissolved fractions of lipophilic contaminants are considered to be bioavailable to aquatic organisms, irrespective of their mode of life. As a consequence of this, the risk that contaminated sediments pose to aquatic animals is estimated by using equilibrium partitioning coefficients to calculate contaminant concentrations in the pore water and in the water overlying contaminated sediments (Di Toro *et al.*, 1991). Bioconcentration factors are used to calculate concentrations in the animals and aquatic LC50 values are used to estimate the toxicity of the sediment.
Laboratory experiments, however, have shown that ingestion of sediment particles can be a major route for the uptake of lipophilic contaminants (Fowler *et al.*, 1978; Landrum & Scavia, 1983; Landrum, 1989), and that uptake from different sources is additive (Jarvinen & Tyo, 1978). In combination, this may result in higher levels of accumulation compared to exposure to contaminated water alone. Assessments relying on equilibrium partitioning alone, therefore, may lead to an underestimation of the risk posed by contaminated sediments.
When sediment ingestion is a major additional uptake route for lipophilic contaminants, it should result in different levels of bioaccumulation of contaminants for species which differ in their dependency upon sediment for food. To this end, a review was made of a series of (yet unpublished) experiments, originally undertaken as ecotoxicological tests to assess the bioavailability of contaminants in dredged materials prior to disposal. A comparison was made between contaminant accumulation in three marine benthic invertebrates, differing in their mode of feeding and their direct body contact with the sediment: the lugworm (*Arenicola*

marina), a bulk sediment feeder living deep in the sediment, the blue mussel (*Mytilus edulis*), an obligate filter feeder living on or above the sediment, and the baltic tellin (*Macoma balthica*), a facultative deposit feeding bivalve living in the sediment. As the baltic tellin and the mussel are both protected from direct contact with the sediment by their shells, differences in accumulation should reflect differences in the importance of their modes of feeding (Knezovich *et al.*, 1987).
In two additional experiments, the food availability for the baltic tellin was manipulated in the waterphase, in order to assess the difference between suspension and deposit feeding. An overview of the experiments is given in Table 2.

2.2. Material & methods

Exposure systems

The bioassays with contaminated sediments were executed in 2.2 m^3 mesocosms situated at Den Helder naval harbour, The Netherlands. Mesocosms were used, in order to provide realistic exposure conditions. A 25 cm layer of test sediment was covered by 50 cm of seawater, which was continually refreshed with Wadden Sea water at a rate of ca. 40 $L.h^{-1}$, replacing the water in the mesocosms after ca. two days. The water was also aerated, in order to achieve complete mixing.

Sediments

'Naturally' contaminated sediments were dredged from harbour areas (Rotterdam, Delfzijl), the Western Scheldt estuary (along a pollution gradient: Zandvliet, Bath, Valkenisse and Konijnenschor) and the western Wadden Sea. The sediments were applied to the mesocosms directly after collecting. Sediments used in succesive experiments, remained in the mesocosms under experimental conditions.

In one of the experiments, sediment was spiked with benzo(a)pyrene (BaP). This was done by adding BaP, dissolved in DMSO, to dried and milled sea lettuce (*Ulva sp.*) which was wetted again to make a slurry. The slurry with BaP was then mixed with organically poor North Sea sand. The spiked sediment had a nominal organic matter content of 1% (based on dry weight) and a BaP content of 4 $mg.kg^{-1}$ dry organic matter. After one year of ripening, these values were 0.8% and 2.5 $mg.kg^{-1}$ respectively. The details of the spiking procedure will be reported elsewhere.

Test animals

Lugworms (*Arenicola marina*) were collected at Mokbaai, Texel, by a professional baitcollector. Usually, 150 to 250 lugworms were introduced into each mesocosm.
Mussels (*Mytilus edulis*) were collected from buoys in the North Sea, or from commercial mussel beds in the Wadden Sea or in the Eastern Scheldt. Mussels were selected so that they were all within the same size (age) class. In each mesocosm, with the exception of experiment 6 (see below), 150 to 200 mussels were suspended in the water column in a small basket according to the procedures for active biological monitoring, given by De Kock & Kramer (1994).
Baltic tellins (*Macoma balthica*) were collected from tidal flats in the Wadden Sea, near Den Helder. Approx. 250 baltic tellins were usually introduced into each mesocosm.

Table 2: Overview of experiments. The successive experiments are numbered; treatments within experiments are denoted with a letter. Treatments in experiment 6 were replicated. In experiment 2 treatments were not replicated, but can be interpreted as such, because differences are related to presence (2a and 2b) or absence (2c and 2d) of mussels. Treatment 2a is the same as 1d and was used for comparison with 2b-d, which were executed simultaneously. The specific locations in the Western Scheldt estuary are ordered from west (least contaminated) to east (most contaminated).

Experiment	Test period	Species combinations	Sediment origin	specific location (+year of collection or experiment previously used in)	Chemical analyses
1a	April-June 1984	lugworm+mussel+baltic tellin	Western Scheldt	Konijnenschor (1984)	PCB
1b				Valkenisse (1984)	PCB
1c				Bath (1984)	PCB
1d				Zandvliet (1984)	PCB-PAH
2a (=1d)	April-June 1984	lugworm+mussel+baltic tellin	Western Scheldt	Zandvliet (exp 1)	PCB(-PAH)
2b		mussel+baltic tellin		Zandvliet (exp.1)	PCB
2c		baltic tellin		Zandvliet coarse fraction	PCB
2d		baltic tellin		Zandvliet fine fraction	PCB
3a	May-Oct 1986	lugworm	Wadden Sea	Balgzand (1986)	PCB-PAH
3b			Rotterdam Harbour	Botlek harbour mouth (1985)	PCB-PAH
4a	Oct-Dec 1986	lugworm+mussel	Wadden Sea	Balgzand (exp.3)	PCB-PAH
4b			Rotterdam Harbour	Botlek harbour mouth (exp 3)	PCB-PAH
5a	June-aug 1990	lugworm	Wadden Sea	Napoleon Dam (1990)	PCB-PAH
5b			Rotterdam Harbour	Botlek harbour mouth (exp.3,4)	PCB-PAH
5c				Botlek harbour basin (1989)	PCB-PAH
5d			Delfzijl harbour	harbour canal (1989)	PCB-PAH
6a-b	April-May 1994	baltic tellin	artificial	spiked with BaP (1993)	BaP
6c-d		mussel+baltic tellin			BaP

Sample preparation and chemical analyses

Upon termination of the exposure, lugworms and baltic tellins were held in clean water overnight in order to allow them to release their gut contents. The bivalve tissues were removed from their shell with a solid titanium knife. Tissues were homogenized in acid/acetone rinsed glass pots with a homogenizer (Ultra-turrax) equipped with solid titanium blades. The pots were sealed with teflon-lined lids and stored at -20°C until analysis.

Since in the earlier experiments lipid content of the test animals was not routinely measured, all contaminant levels are expressed on the basis of ash-free dry weight (ADW). Dry weight was determined by oven drying ca. 1 g of the samples for 16 h at 150°C (experiments 1-4, cf. Table 2), or for 48 h at 110°C (experiments 5-6). These two procedures gave comparable results. Ash weight was determined by ashing dried samples in a muffle furnace for 4 h at 600°C (tissues) or 450°C (sediments). The percentage of the ash-free material of the sediments was used as an estimate of the percentage of organic matter present in the samples.

Tissue samples for organochlorine analysis were digested by pepsin, then both tissue and sediment samples were steam distilled under acid conditions with hexane. The extracts were cleaned over aluminium oxide and dried over sodium sulphate. Finally, the extracts were analysed by capillary gas chromatography using an electron capture detector. Sediment samples for PAH analysis were extracted by the 'soxhlet' method and concentrated. Tissue samples were hydrolized with NaOH and extracted with hexane, after which the extract was cleaned-up over aluminium oxide and concentrated. Both types of samples were taken up in methanol and were analysed by reverse phase high pressure liquid chromatography (RP-HPLC), followed by fluorescence detection or UV absorption detection.

Overview of experiments

An overview of the experiments is given in Table 2. The exposure period varied between 56 and 140 days. In experiments 1 to 5, the bioavailability of several contaminants to benthic invertebrates was tested using the 'naturally' contaminated sediments. Originally, these experiments were designed to assess the effect of sediment remediation treatments (washing, size fractionation) on the bioavailability of contaminants. For this article, untreated sediments only are considered, with the exception of experiment 2. The treatments were not replicated. Experiment 2 was performed simultaneously with experiment 1, using the most contaminated Western Scheldt sediment (Zandvliet). In order to assess the influence of bioturbation by the lugworm on contaminant remobilization, an extra mesocosm was installed in which only mussels and baltic tellins were exposed (2b). In other mesocosms, the coarse and fine fraction of Zandvliet sediment (obtained by wet sieving) were applied. Only baltic tellins were exposed to these sediments (2c and 2d). Although the sediments were treated, the results are important for the understanding of the results of the other experiments. The Zandvliet treatment of experiment 1 (1d) was used for comparison (2a).

Sediment spiked with benzo(a)pyrene was used instead of 'naturally' contaminated sediments in experiment 6. In this experiment 470 baltic tellins were exposed, either with or without 1200 mussels in the same mesocosm, in order to assess the influence of the mussel as a competitor for food in the waterphase on the bioavailability of contaminants to the baltic tellin. The mussels were suspended in 12 baskets above the sediment (see above). The exposure systems were replicated.

2.3. Results

Differences between test species

In all experiments where the species were exposed simultaneously, residues of PCBs and PAHs were highest in lugworms, intermediate in baltic tellins and lowest in mussels. The only exception was the least contaminated Western Scheldt sediment (Konijnenschor). Lugworms and baltic tellins exposed to this sediment showed lower levels of accumulated PCBs (PAHs were not measured in this case).

The accumulation of PCB 28 in lugworms and mussels relative to the sediment concentration is represented in figure 3 for data from experiments 1, 3, 4 and 5. As can be seen, levels in mussels were fairly constant, irrespective of the concentration in the test sediment. The average level in mussels is indicated by the broken line. The accumulation in lugworms on the other hand, was clearly related to the concentration in the sediments. There was, however,

considerable variation in accumulated levels. Much of this variation might have been caused by differences in the sampling date relative to the reproductive cycle of the lugworms and to analytical errors in experiment 1, causing relatively high detection limits (concentrations below the detection limit were considered to be zero). For the other PCB congeners similar observations were made.

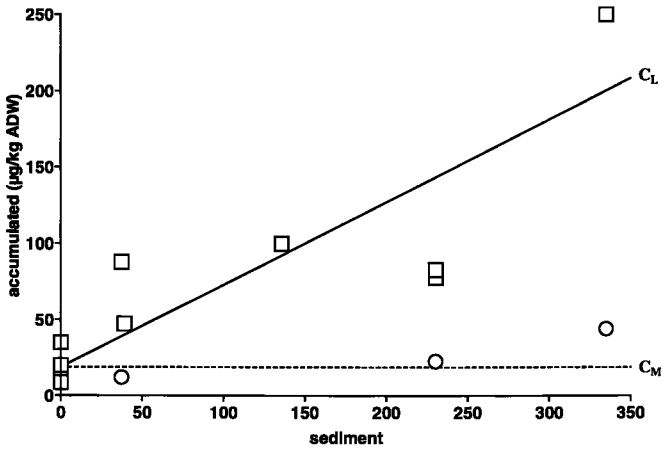


Figure 3: Residues of PCB-28 in lugworms (squares) and mussels (circles), after exposure to sediments with different levels of PCBs. Concentrations in µg.kg^{-1} organic matter. The broken line (C_M) is the average level in mussels. The solid line (C_L) is the calculated level in lugworms, with C_M as background concentration.

In order to obtain an idea of the difference in accumulation between mussels and lugworms, a linear relation was fitted through the lugworm data, using the following equation:

$$C_L = C_M + bC_S$$

in which C_M is the concentration in mussels, considered as background concentration, C_L is the concentration in the lugworm, C_S is the concentration in the sediment and b is the accumulation factor from sediment.
This accumulation factor b is comparable to the commonly used AF, the concentration in the animal divided by the concentration in the sediment (Lake *et al.*, 1990), but with a correction for background levels due to accumulation from the overlying water.

The December values (experiment 4) were left out in order to reduce some of the variability, since after the reproductive season levels in lugworms were lower than during the summer (experiments 1-3 and 5) due to contaminant release with the lipidrich gametes. Sediment concentrations were estimated when these were below the detection limit (experiment 1). These values were estimated as follows: zero for the least contaminated sediment (Konijnenschor), half the detection limit for sediment collected at Valkenisse and equal to the detection limit for sediment collected at Bath. The fitted relation between sediment

concentration and concentration in the lugworm is given as a solid line in Figure 3 for PCB 28.

Table 3: Accumulation factors (±s.e.) for lugworms based on the slope of the regression to sediment concentrations, corrected for average levels (±s.e.) accumulated in mussels. Data from experiments 1, 2, 3 and 5.

PCB congener	Accumulation Factor	concentration in mussels
44	0.307±0.195	23.4±4.5
28	0.541±0.089	18.9±6.4
180	0.569±0.123	11.7±1.7
52	0.595±0.208	40.2±10.5
138	0.904±0.189	81.2±8.8
153	1.175±0.210	98.4±6.6

Calculated accumulation factors for PCB congeners measured in experiments 1-3 and 5 are given in Table 3. Unfortunately not enough data were available for the calculation of accumulation factors for more compounds. Accumulation factors were calculated for all the compounds measured in the most contaminated Western Scheldt sediment tested (Zandvliet, experiment 1), in order to allow some comparison between different compounds. These accumulation factors (Table 4) were higher than the average based on several experiments (see Table 3). In general, the accumulation factors for PCBs were higher than those for PAHs. Plotting the accumulation factors, as given in Table 4, against the logKow (Figure 4) reveals that the maximum AF for PAHs was found at a logKow of ca. 5 and for PCBs at a logKow of ca. 6.

Interactions between test species

The results of experiment 2 are represented graphically in Figure 5. In this figure, the PCB congeners are sorted according to increasing concentrations in the lugworm, which contained the highest residue levels. The data for the lugworm are the same as in Table 4. The lowest residue levels were found in mussels. As no influence of the presence or absence of lugworms on accumulation in mussels was observed, the levels in the mussels were averaged over the two mesocosms 2a and 2b. The presence of lugworms had no clear effect on accumulation in baltic tellins either. Therefore, these data were also averaged. There was only a slight increase in the accumulated levels of most PCBs in baltic tellins exposed to the fine sediment fraction obtained by wet sieving as compared to those exposed to the coarse sediment fraction, even though the contaminant concentration in the fine sediment fraction was considerably higher than in the coarse fraction and the untreated sediment. As accumulation in both these treated sediments (2c and 2d) was low, these data were also averaged.

Table 4: Accumulation factors (AF) for lugworms exposed to the Zandvliet sediment in experiment 1, corrected for levels accumulated in mussels exposed in the same mesocosms. Compounds in order of increasing logKow [from Hawker & Connell, 1988; Meador et al., *1995].*

		concentration in mg.kg^{-1} ADW in			
Compound	**logKow**	**sediment**	**lugworms**	**mussels**	**AF**
PCB 15	5.30	137	250	27	1.62
PCB 28	5.67	37	88	12	2.03
PCB 44	5.75	77	98	20	1.02
PCB 52	5.84	62	190	23	2.68
PCB 49	5.85	46	120	17	2.22
PCB 70	6.20	107	120	44	0.71
PCB 87	6.29	43	99	15	1.96
PCB 101	6.38	121	310	58	2.08
PCB 138	6.83	155	330	76	1.64
PCB 153	6.92	173	420	98	1.86
PCB 180	7.36	91	130	13	1.29
Anthracene	4.54	520	390	15	0.72
Phenanthrene	4.57	1243	1200	22	0.95
Pyrene	5.18	1786	3100	230	1.61
Fluoranthene	5.22	1831	1600	17	0.86
Benzo(a)anthracene	5.70	3166	2000	81	0.61
Benzo(a)pyrene	6.10	4522	570	16	0.12
Benzo(k)fluoranthene	6.40	1447	370	24	0.24

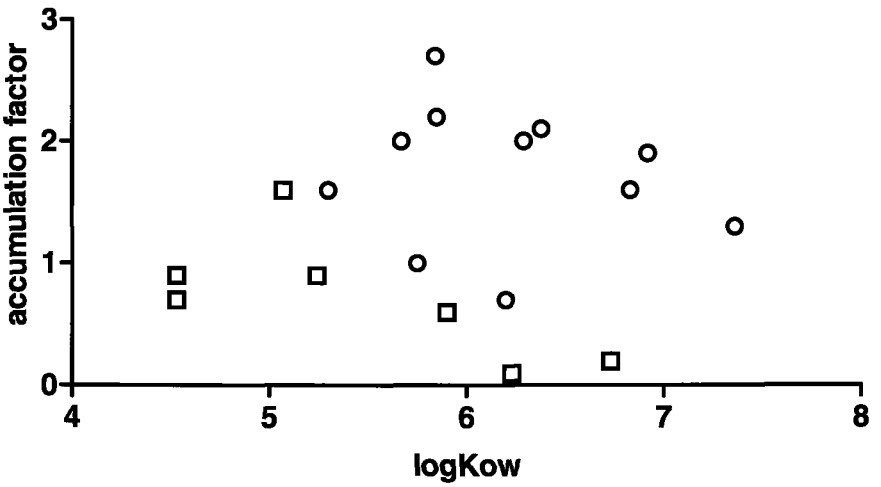


Figure 4: Accumulation factors (AF) for lugworms plotted against logKow for PAHs (squares) and PCBs (circles). Data according to Table 4.

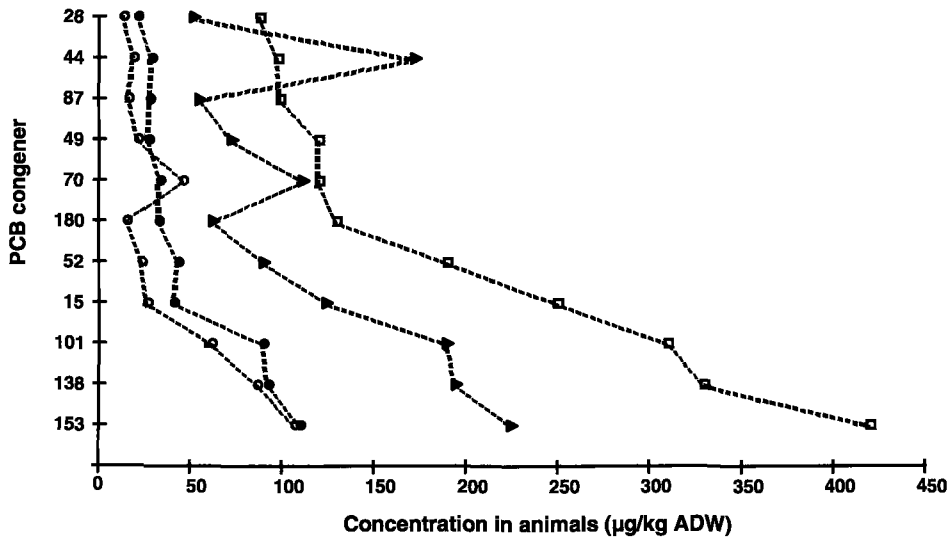


Figure 5: Accumulation of PCBs in baltic tellins exposed without mussels (dots), or together with mussels (triangle), compared to accumulation in mussels (circles) and lugworms (squares).

As can be seen, residue levels in baltic tellins were between those found in mussels and lugworms, but the presence of mussels in the same experimental systems determined the actual levels found. Exposed without mussels, baltic tellins accumulated PCBs to the same low levels as mussels, but when mussels were present, accumulation in baltic tellins increased considerably, although the residue level remained lower than that in lugworms for most PCBs.

The influence of the presence of mussels on the bioaccumulation of baltic tellins was confirmed in experiment 6. Due to the filtration activity of the mussels (6c and 6d), no phytoplankton bloom developed during the experiment. The water remained clear and the chlorophyl-a concentration varied between 1 and 4 $mg.L^{-1}$. The BaP concentration in the mussels remained low throughout the experiment (on average 11.4 $mg.kg^{-1}$ ADW), while the concentration in the baltic tellins increased (Figure 6). The concentrations in the mussels were comparable to the concentrations measured in experiment 1 (see Table 4). In figure 6, the results for the replicates of this treatment are averaged. The results for the baltic tellins exposed in the mesocosms without mussels are represented separately in Figure 6 as the two mesocosms developed asynchronously. In mesocosm 6a, the phytoplankton did not develop until May, while in mesocosm 6b this already started in April, both reaching levels of more than 100 $\mu g.L^{-1}$ chlorophyl-a. Initially, the baltic tellins in those two mesocosms accumulated BaP to the same levels as baltic tellins exposed together with mussels, but when the phytoplankton levels rose the BaP levels started to decrease.

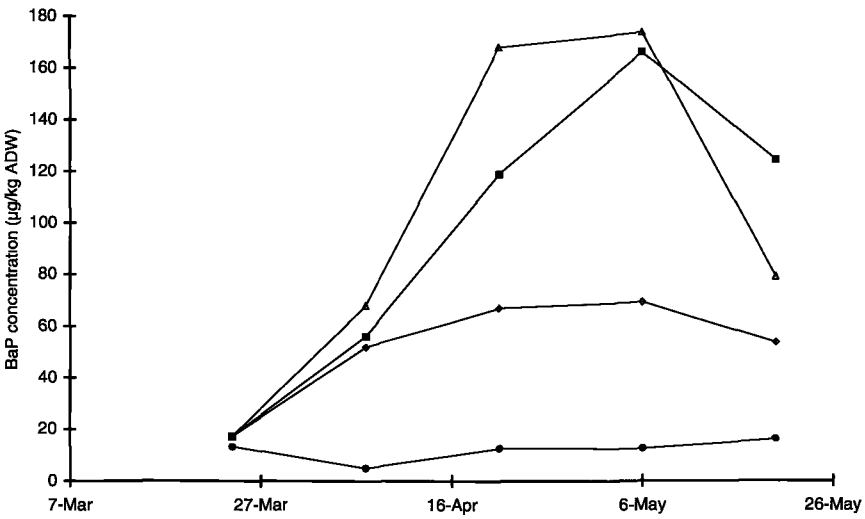


Figure 6: Accumulation of benzo(a)pyrene in baltic tellins exposed with (solid square) or without mussels (6a: triangle; 6b: diamont), compared to the accumulation in mussels (dots).

2.4. Discussion

The mesocosm experiments clearly showed that accumulation of sediment-bound lipophilic contaminants depends on the feeding mode of the animals studied. The highest levels were found in the sediment feeding lugworm, the lowest levels in the filter feeding mussel, and intermediate levels in the facultative deposit feeding baltic tellin. These differences in accumulation are due to a difference in exposure, rather than a difference in elimination rates. This is obvious from the fact that mussels may accumulate lipophilic compounds to very high levels when exposure is high. Levels of more than 200 mg PCB153.kg^{-1} ADW have been found in the Western Scheldt (Bergman, 1993). The results of the manipulation experiments with the baltic tellins (experiments 2 and 6) also show that the lower levels of accumulation found in the suspension feeding animals used in these experiments are not an intrinsic property of the species, but are related to the compartment they were feeding in.

The question as to whether the increased accumulation caused by the more intensive contact with the sediment is a direct result of ingesting the sediment particles, or is due to the contact with the pore water can not be answered directly as yet, as no measurements of the pore water were performed. There is however, some indirect information, indicating that the role of the pore water is relatively small. Firstly, the lugworm lives in a burrow lined with mucus, which might act as a 'molecular sieve' (Aller, 1983). The burrow is ventilated with overlying water, which is also used to loosen the sediment in the head shaft before it is ingested (Wells, 1945). Together, these two processes will have a strongly diminishing effect on the influence of pore water.

Secondly, Winsor *et al.* (1990) reported that only 4% of the water used for ventilation by *Macoma nasuta*, a North American relative of the baltic tellin, is pore water.

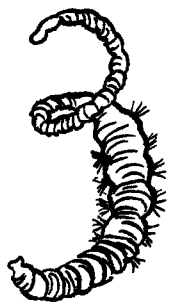
As the concentrations observed in the mussels remained fairly constant irrespective of the levels in the sediment, they probably reflected the quality of the inflowing water, which originated from the Marsdiep between Den Helder and the island of Texel. Concentrations of PCBs in mussels exposed to the inflowing water during experiment 1 in 1984 were comparable to the concentrations in mussels exposed in the mesocosms. PCB levels were lower in mussels collected off the coast of Den Helder in September 1988 (Bergman, 1993). This difference between 1984 and 1988 might have been caused by a general decline in PCB levels in Dutch coastal waters during this period. A decrease in PCB levels in mussels sampled along the Dutch coast between 1971 and 1987 was reported by Scholten (1995). This decrease continued in the period 1989 - 1992, as was established in an active biological monitoring programme by the Dutch government (Heesen, 1995). A similar trend was observed in sediment cores in the Baltic (Broman *et al.*, 1994).

Accumulation in lugworms is definitely related to concentrations in the sediment. However, the variation in accumulated levels is considerable. As yet, the sources of this variation are not fully understood. As explained in the results section, analytical errors played a role at the lower sediment concentrations, causing relatively high detection limits. Another factor which might have played a significant role is the time of year in which the different experiments were performed (Scholten, 1995). In general, levels appeared to increase during the gamete formation from April to August, after which levels declined again as an increasing number of spent individuals occured in the population. In mesocosm experiments with spiked sediments, a similar seasonal cycle of the lipid content was found, but the lipid content varied less than the levels of contaminants (Kaag, unpubl.). Normalization of tissue concentrations for lipid content reduces only part of the variation therefore. Seasonal variation in PCB content has also been observed in mussels (on wet weight as well as on lipid weight), but highest levels were found in February (Hummel *et al.*, 1990), as this species is a spring spawner. Research on seasonal variation in accumulated levels of PAH in field sampled lugworms is ongoing. Seasonal variation cannot explain all the variation observed. Sediment characteristics will have had some influence also. Lipophilic contaminants are mainly bound to the organic and fine fraction of the sediments, which is also the fraction of the sediment selected by sediment feeding organisms (Lee *et al.*, 1990; Harkey *et al.*, 1994). The lugworm is able to ingest particles up to 2 mm in diameter, but usually selects particles between 3 and 400 mm (Cadée, 1976). Data on particle size distribution of the tested sediments are not available and no relation with organic content (expressed as ash-free dry weight) was found. Weston (1990) found increased accumulation of benzo(a)pyrene in the Pacific lugworm, *Abarenicola pacifica*, exposed to sediments with a very low organic carbon content (<1%), which he attributed to increased feeding rates. Lake *et al.* (1990) also found lower accumulation factors in sediments with a higher organic content. However, feeding rates of the lugworm *Arenicola marina* increased with increasing food content (Cadée, 1976). Due to these uncertainties, the exact nature of the relation between sediment concentration and accumulation in the lugworm cannot be established. Therefore, a linear relation was assumed, in order to be able to estimate accumulation factors. Residue levels in mussels (or other filter feeders) can be used to correct for the contribution of uptake from water to the total residue level, as residue levels in these organisms do not appear to be influenced by the contaminant level in the sediment.

Different compounds have various accumulation factors, which appeared to be related to the logKow. Accumulation factors increased with increasing logKow, until a maximum was reached. At higher logKows (>5-6) the accumulation decreased again. Similar patterns were found for PAHs (Landrum, 1989) and for PCBs (Oliver, 1984; 1987; Landrum *et al.*, 1989). There was, however, considerable variation in accumulation factors at any specific logKow, as is illustrated by the relatively low accumulation factors for PCB44 and PCB70 (Table 4 and Figure 4). This might be related to structural differences between congeners, causing differences in uptake/elimination kinetics or biotransformation, but neither molecular surface area nor volume (Opperhuizen *et al.*, 1988), nor the placement of the vicinal H-atoms (Boon *et al.*, 1994) seem to offer a suitable explanation.
However, as the maximum accumulation factor for PAHs and PCBs was found at a different logKow (ca. 5 and ca. 6 respectively), it is clear that the logKow is not the only factor determining the accumulation factors. Differences in molecular size and structure may result in different rates of accumulation and elimination, but the manner in which different compounds are bound to the sediment matrix may also be influenced.

In conclusion, it can be said that the risk posed by contaminated sediments to benthic invertebrates cannot be assessed by using chemical equilibrium partitioning alone. Life history traits, such as food source and feeding habits also have to be taken into account, especially when contaminants with a logKow of 5-6 (showing the highest accumulation potential) are considered.
Although the importance of sediment ingestion for contaminant levels in benthic animals has been clearly established, it is not yet possible to quantify this uptake process for different modes of feeding. Using a bulk-sediment feeding organism, such as the lugworm, to assess bioavailability represents a realistic scenario for contaminated sediments. Comparison with filter feeders, such as mussels, will give an impression of the relative importance of sediment contamination compared to water quality. Deposit feeding bivalves, such as baltic tellins, are less suitable for the assessment of the risk of contaminated sediments, as their exposure varies with their feeding habits, which are determined by the food availability in the overlying water.

Chapter



A new approach for testing contaminated marine sediments: fertilisation success of lugworms following parental exposure

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1994

with E.M. Foekema and C.T. Bowmer

3. A new approach for testing contaminated marine sediments: fertilisation success of lugworms following parental exposure.

Abstract

A sediment bioassay is being developed using several marine benthic invertebrates to assess the effects of parental transfer of contaminants to the gametes. In this preliminary study, the emphasis was placed on developing methods for the *in vitro* fertilisation of lugworm, *Arenicola marina*, oocytes. Lugworms exposed to contaminated sediments in outdoor mesocosms were brought to the laboratory, just before the beginning of the spawning period. The reliability of an *in vitro* fertilisation procedure was tested by varying several parts of the method. Main results are that eggs and embryos may be physically damaged by cleaning over a sieve. However, as no negative effects were observed when leaving eggs and sperm together for 24 h, the sperm need not be washed off until the embryo's are preserved for further examination later on. A first, incomplete screening of the effects of contaminated harbour dredged sediments indicated some effect on the reproductive success.

3.1. Introduction

Background

In the marine environment, many toxicants are adsorbed or bound to the suspended matter in the water column, which settles in areas of low turbulence. Marine benthic organisms in sedimentation areas may therefore be chronically exposed to these toxicants. Recent work has shown that the bioavailability of lipophilic toxicants in the sediment cannot always be explained by the equilibrium partitioning constant between the organic fraction of the sediment and the interstitial water; ingestion and digestion of the sediment may also play an important role (Boese *et al.*, 1990; Weston, 1990; Opperhuizen, 1991). Previous (unpublished) studies of dredged materials in model ecosystems (mesocosms) at our laboratory revealed that the exposure of benthic invertebrates to lipophilic compounds, estimated from the internal concentrations, increases with the intensity of sediment contact of the species, which is dependent on feeding and burrowing behaviour. In order of increasing intensity of sediment contact these are: the edible cockle (*Cerastoderma edule*), the baltic tellin (*Macoma balthica*) and the lugworm (*Arenicola marina*).

Additionally, the contaminants were found to accumulate more strongly in the lipid rich eggs of the lugworm, *Arenicola marina*, compared to the rest of the body (Scholten, 1995). Allocation of contaminants in eggs was also found by other workers, i.e. in the blue mussel, *Mytilus edulis* (Hummel *et al.*, 1989; 1990) and in the calanoid copepod *Acartia tonsa* (McManus *et al.*, 1983). This poses the question as to whether or not accumulation of lipophilic contaminants in reproductive tissues has an influence on the reproductive success. To this end, an *in vitro* technique was developed to test the fertilisation success of lugworms following parental exposure to contaminated sediments. Based on a literature survey and observations made during earlier experiments, a basic procedure for the *in vitro* fertilisation method was set up (Figure 7). This procedure was tested by varying several parts of the method and some

preliminary tests with contaminated sediments were carried out to investigate the applicability in ecotoxicological studies.

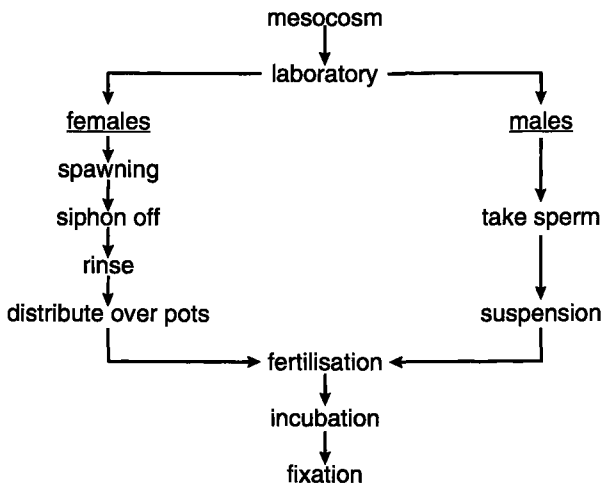


Figure 7: Scheme representing the steps in the basic procedure for the in vitro *fertilisation of the lugworm.*

The lugworm

The lugworm, *Arenicola marina*, is probably the most important polychaete worm in the biological economy of the intertidal zone of the Wadden Sea. The total biomass of lugworms amounts to ca. 20 percent of the total macrozoobenthos, more than the biomass of all other polychaetes combined (Beukema, 1976; Dekker, 1989). The population is stable, as the yearly recruitment of ca. 20 percent equals the average annual mortality (Beukema & de Vlas, 1979). Lugworms live in a 20 to 40 cm deep, J-shaped burrow. At the bottom they create a funnel, from which they ingest all sediment particles smaller than 2 mm. The food consists of bacteria, benthic diatoms, and detritus (Rijken, 1979; Grossmann & Reichardt, 1991) which are digested, while indigestible parts are deposited at the sediment surface. Lugworms in the Dutch Wadden Sea recirculate a 6 to 7 cm thick layer of surface sediment per year, on average (Cadée, 1976).

Egg production can start as early as February. Vitellogenesis (yolk synthesis) begins in mid-June. By the end of August or early September up to 90 percent of the eggs are approaching mature size (Howie, 1984).

3.2. Material & methods

The mesocosms

Lugworms were exposed to contaminated and reference sediments in circular outdoor mesocosms, each with a volume of 2.2 m^3. A 25 cm deep layer test sediment was constantly covered by 50 cm of seawater, with a salinity of ca. 29‰. Tides were not implemented. Instead the water was continuously refreshed at a rate of 40 $L.h^{-1}$, sufficient to replace the water in the

mesocosms in ca. two days. The water was also aerated, to improve circulation within the system.
The lugworms were introduced to the systems in May, before vitellogenesis commenced. In the second week of September, just before the main spawning period in Dutch waters starts, the animals were collected and returned to the laboratory.

Basic procedure for IVF procedures

In the laboratory, males and females were separated (in ripe animals eggs are visible through the body wall as small yellowish spheres, while the sperm appears as a amorphous yellowish mass). Each individual female was placed in a 2 L plastic container, containing a 2 cm layer of their original test sediment. The sediment is necessary, as the animals will not survive without it. The eggs are normally laid within the burrow and stay there until the swimming trochophore larvae leave it. Therefore, the sediment layer should not be too thick in order to be able to collect the spawned eggs. Using 2 cm of sediment, sufficient amounts of eggs are layed upon the sediment surface, discernible as yellow patches of several thousands of eggs, which can be easily collected.
Males were usually held in groups in a larger container with their original test sediments. All containers were supplied with slowly running seawater.

At present the spawning of the lugworms cannot be induced artificially. Neither can the eggs be removed from the body cavity, as the onset of maturation occurs immediately prior to spawning (Howie, 1961). Thus this event had to be awaited.
When freshly laid eggs were observed, they were siphoned off, flushed through a 250 mm sieve to remove large debris and collected in a 100 mm sieve, through which smaller sediment particles could pass. The eggs were then brought into suspension in seawater and distributed over 100 ml fertilisation pots. For all test combinations replicates were prepared. Sperm was taken directly from the body cavity of the males with a fine syringe. The needle was inserted obliquely through the body wall so as not to damage the gut or major blood vessels. A suspension of 30 drops of sperm in 100 ml of seawater was prepared, using the sperm from ten different males.
For fertilisations, 1 ml of this suspension was added to the fertilisation pots containing the eggs, after which the pots were filled up with sea water to 100 ml. This gave a dilution factor of ca. 10,000 relative to the original concentration in the body cavity of the males. After adding the sperm, the fertilisation pots were incubated in a slowly rotating (<100 rpm) incubator at 15°C, a normal temperature in the Dutch Wadden Sea during the spawning season.
After a fertilisation period of two hours the sperm was washed off over a 100 mm sieve. The fertilisation pots were then filled up to 100 ml again with fresh sea water and returned to the incubator. After 24 hours the embryos were concentrated using a 100 mm sieve and then preserved in 4 percent neutral buffered formalin for later examination.

Variations in the procedure

A. Storage of the eggs

In the basic procedure the females were checked for signs of spawning every 8 hours. To test the effect that a less intensive observation frequency might have on egg quality, part of the spawned eggs were left on the sediment for later use. Freshly spawned eggs were also stored in seawater with and without aeration, to assess the effect of storage on fertility. However, when

freshly laid eggs are compared to stored eggs in this manner, the sperm suspension is either a different one, or if the same, also older. Freshly made sperm suspensions were preferably used.

B. Rinsing of the eggs

Rinsing of the eggs was carried out twice during the procedure, once after the spawned eggs were collected and once to wash away the sperm. The effect of rinsing was tested by comparing the single pair of rinsings of the basic procedure with a tenfold repeated rinsing over the sieves at both steps.

C. Fertilisation time

The fertilisation period was varied from 30 min to 24 hours (i.e. the sperm was not washed away until fixation in formaldehyde). This was carried out not only to establish an optimal fertilisation period, but also to assess the possibility of skipping the steps in which the sperm is washed off. If successful the fertilisation period could possibly be extended to the whole incubation period of 24 hours.

D. Sperm dilution

The sperm dilution factor was varied from 1000, using 10 ml of suspension, to 1,000,000, using 0.01 ml of suspension, in order to avoid potential polyspermy.

Eggs and embryos

The eggs of the lugworm are biconcave with a diameter of ca. 180 mm when ripe (Meijer, 1979). Within a few hours after fertilisation several stages of dividing eggs can be observed. Under normal experimental conditions nearly all fertilised eggs have developed into the blastula/gastrula stage after 24 h. Swimming trochophore larvae are encountered 72 h after fertilisation and after 96 h, the larvae elongate and settle to the bottom. At this stage however, a large *in vitro* mortality occurs, irrespective of treatment, probably due to lack of sediment, food, and/or dermal stimuli.

In order to assess fertilisation success, eggs were preserved 24 h after fertilisation and counted under a light microscope at 200x magnification. At this point of development, fertilised eggs have usually reached the blastula stage and are just commencing gastrulation. They are round to slightly oval, with a rough surface. Unfertilised eggs have a smooth surface and a light centre, which is caused by the nucleus. Eggs or embryos deviating from this description were considered to be abnormal. Abnormal unfertilised eggs were usually dented, showing lateral depressions in the cytoplasm, while abnormal embryos mostly showed severely unequal division and sometimes a very elongated shape. Embryos with severely unequal division had one big cell, nearly the size of an unfertilized egg, and a clump of very small cells at one side, instead of a normal first division as was described by Child (1900), Ashworth (1904) and Newell (1948). Normally 200 to 400 eggs were counted. The results were expressed as the percentage of fertilised eggs of the total number counted.

Sediment treatments

The adult lugworms were exposed to harbour dredged sediments and reference sediments. Two of the harbour sediments originated from the Rotterdam petrochemical harbour area. The first was dredged in the harbour basin and was moderately contaminated with PAHs

and PCBs (Σ21PAH: 59.7 and Σ8PCB: 3.5 $mg.kg^{-1}$ dry organic matter respectively[#]). The other was dredged near the harbour entrance and was less contaminated (Σ21PAH: 30.9 and Σ8PCB: 0.8 $mg.kg^{-1}$). Both sediments had an organic content of ca. 12 percent. Another harbour sediment was dredged in the harbour of Delfzijl, a small industrial harbour in the Wadden Sea. This sediment, with an organic content of ca. 6 percent, was lightly contaminated with PAHs (Σ21PAH: 14.2 and Σ8PCB: 0.2 $mg.kg^{-1}$). A relatively clean Wadden Sea sediment, with an organic content of less than 3 percent was used as a reference sediment (Σ21PAH: 10.7 and Σ8PCB: 0.3 $mg.kg^{-1}$). This is referred to as the *mesocosm control.* For the fertilisations, freshly collected males from the Wadden Sea were also used. These are referred to as the *field control.*

3.3. Results

Variations in the procedure

A. Storage of the eggs

Leaving the eggs on the sediment for another 8 h after they were found did not have an effect on the fertilisation rate. Neither could an effect on the percentage of abnormally developing embryos be observed (Table 5).

Table 5: Fertilisation percentage comparing eggs used immediately after discovery with eggs used after storage for another 8 h. The same sperm suspension was used with fresh eggs and stored eggs for fertilisations marked with an asterisk (*). Otherwise a new sperm suspension was prepared.

fertilisation	fresh	stored on sediment	stored in water	stored in aerated water
1*	99.7	89.1		92.3
2*	99.1	99.2		97.8
3	97.7	74.2		
4	97.5	97.9		
5	95.0	96.4		96.7
6	92.2	95.2	91.1	
7	82.2	80.3		
8*	73.9	60.9		97.2
9	58.9	75.7		
10		96.4	88.5	
11		93.6	78.3	

Storing the eggs in aerated water until later use increased the fertilisation success, although in one experiment an unusually large portion of abnormally developing embryos was encountered in both replicates (ca. 80 percent compared to 1-4 percent with eggs from the same batch used fresh or stored on the sediment).
Without aeration the fertilisation success seemed to be slightly depressed.

[#] Σ21PAH and Σ8PCB is the sum of a standard list of PAH compounds (phenanthrene, anthracene, fluoranthene, pyrene, 3,6-dimethyl phenanthrene, benzo(b)fluorene, benzo(a)anthracene, chrysene, benzo(e)pyrene, benzo(j)fluoranthene, perylene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(aj)anthracene, dibenzo(al)pyrene, benzo(ghi)perylene, dibenzo(ah)anthracene, indeno(123cd)pyrene, 3-methylcholanthrene and anthanthrene) and PCB congeners (28, 49, 52, 87, 101, 118, 138, 153 and 180) routinely analysed at TNO.

B. Rinsing eggs

The effect of repeated rinsing was only be tested once, with the eggs laid by a female exposed to Delfzijl harbour dredged sediment. The eggs were fertilised with sperm freshly taken from field control males.
Using the basic procedure, the total fertilisation was more than 97 percent. Repeated rinsing before fertilisation reduced total fertilisation to 70 percent. Of the unfertilised eggs ca. 67 percent were dented, compared to the 2 percent or less normally encountered. Repeated rinsing after fertilisation to wash off the sperm, did not reduce the total fertilisation. However, only ca. 20 percent of the embryos developed normally, compared to at least 50% after extra washing before fertilisation and with standard procedures.

C. Fertilisation period

The fertilisation percentage generally increased with increasing length of the fertilisation period. Sometimes maximum fertilisation was reached within one hour, while on other occasions, even an 8 h fertilisation period resulted in a considerably lower fertilisation percentage, compared to a 24 h fertilisation period. The largest differences in fertilisation success were encountered between 30 min and 2 h fertilisation time. Important for the procedure however, is that the percentage of abnormally developing embryo's did not increase with an increased exposure to the sperm suspension.

D. Sperm dilution

As might be expected a more concentrated sperm suspension resulted in higher fertilisation percentages. The highest fertilisation success was observed with a sperm dilution factor of 1000, while a sperm dilution factor of 10,000 resulted in a slightly lower fertilisation success. Fertilisation success was significantly lower when a dilution factor of 100,000 was used, but a prolonged fertilisation period could compensate for this to a great extent. When a dilution factor of 1,000,000 was used however, the fertilisation percentage was too low to be compensated for by a prolonged fertilisation period (Table 6). Higher sperm concentrations did not have a negative effect on the development of the embryos. The percentage of abnormally developing embryos was similar in all sperm dilutions. Polyspermy was not observed.

Applicability in ecotoxicological studies

The objective of the experiments was to establish experimental conditions and limitations for an *in vitro* fertilisation procedure, and not the study of the effect of exposure to different types of sediment. However, an analysis of the dataset on differences between parental sediment exposure, can indicate the applicability of the method to study the effects of contaminated sediments.

Table 6: Fertilisation percentage at different sperm dilutions and fertilisation periods.

fertilisation	fertilisation period in h	sperm dilution 1000	sperm dilution 10,000	sperm dilution 100,000	sperm dilution 1,000,000
1	0.5		78.2		1.9
2	2	81.5	65.8	65.3	
3	2	76.2	54.7	18.9	10.7
4	2	36.1	30.1	9.5	2.4
1	2		97.9		51.7
5	2		97.3	91.5	
6	2		96.3	77.5	
7	2		91.2	69.2	
8	2		85.8	28.5	
9	2		82.2	26.0	
10	2		74.6	52.0	
11	2		58.9	10.4	
12	6.5	99.9	99.6	92.3	
13	6.5		95.1	83.6	
14	6.5	84.1	68.8	39.5	8.2
2	24	92.2	92.5	86.1	

In table 7 the average percentage fertilized eggs is given for crossings performed with a sperm dilution of 10,000 and a fertilisation period of 2 h.
For the most relevant crossings, where both parents were exposed to the same sediment, the differences in fertilisation success are significant ($p<0.001$; G-test, Sokal & Rohlf, 1981). The other data suggest that the male lugworms are most susceptible.

Table 7: Fertilisation percentage of crossings with a sperm dilution factor of 10,000 and a fertilisation period of 2 h. The number of crossings is given between brackets.

sediment exposure males→ / females↓	field or mesocosm control	Rotterdam Botlek harbour bassin	Rotterdam Botlek harbour entrance	Delfzijl harbour
mesocosm control	**88.1(15)**	52.9(3)	97.2(1)	55.4(3)
Rotterdam Botlek harbour bassin	86.0(12)	**80.9(6)**		71.8(3)
Rotterdam Botlek harbour entrance	88.7(11)			95.4(1)
Delfzijl harbour	87.9(14)			**46.6(5)**

3.4. Discussion

IVF procedures

The *in vitro* fertilisation technique is fairly simple, but labour intensive, because during several weeks, the female lugworms need to be checked for signs of spawning. However no negative effects were observed when leaving the eggs on the sediment a little longer, compared to using them immediately. It seems therefore sufficient to check for spawning once a day. At present, this spawning cannot adequately be induced artificially and in the laboratory occurs simultaneously with spawning in the field population. This indicates that spawning occurs independent of variations in temperature, water quality, etc. (see also Howie, 1984). Egg collections, therefore, must be continued until a sufficient number of tests are performed, or spawning ceases.
Some steps in the procedure require special attention. Eggs and embryo's seem to be sensitive

to mechanical damage. Handling should therefore be reduced as much as possible and when necessary be performed with great care. As no negative effects (such as polyspermy) were observed when the eggs were exposed to sperm for prolonged periods of time, it is best not to wash the sperm suspension off until after 24 h, when the eggs are preserved for later examination. Additionally, to ensure a high enough fertilisation rate in control treatments, the sperm dilution should be between 10,000 and 100,000. The guideline for the *in vitro* fertilisation tests, as modified based on the results obtained, is summarised in Table 8.

Table 8: Guideline for the in vitro fertilisation with lugworm gametes, obtained from adults exposed to contaminated sediments.

- adults are exposed to test sediments in mesocosms
- they are introduced in April/May and collected again in the second week of September, just before the natural spawning season commences
- ripe females are placed in 2 litre containers with 2 centimetre of their original sediment
- they are checked daily for signs of spawning
- spawned eggs are siphoned off and flushed very gently through a 250 mm sieve onto a 100 mm sieve to clean them
- clean eggs are gently brought into suspension and decanted into 100 ml fertilisation pots
- 3 drops of sperm are taken from 10 males of each test sediment
- the sperm is suspended in 100 ml of seawater
- between 0.1 and 1 ml of sperm suspension is added to the eggs the fertilisation pots
- the pots are filled up to 100 ml with seawater and are incubated for 24 hours at 15°C
- the eggs are preserved in 4% neutral buffered formalin until fertilisation success is counted
- fertilisation success is counted under a light microscope at 200x magnification
- basically the difference between fertilised eggs (smooth discoidal with a light centre) and embryo's (a hollow blastula/gastrula with a rough surface and without the light centre, often slightly oval) is easily assessed
- sediment exposure is tested by adding sperm to eggs in the following combinations:
  a) eggs of control females are fertilised with sperm suspensions made up from males exposed to all the test sediments and the control sediment. For each type of suspension another combination is made.
  b) eggs of females exposed to test sediments are fertilised with sperm suspensions made up from control males and from males exposed to the same test sediment as the female
- each combination is duplicated

At this stage no attention is given to the presence of abnormally developing embryo's, as the meaning of these is as yet not clear. They might be the result of physical damage imposed on the developing embryos, as was seen in the experiment where the eggs were rinsed several times after fertilisation (3.1d). Considering some other experiments we performed, it is not to be expected that these damaged embryo's will develop into larvae, as the proportion of normally developing embryo's after 24 h is comparable to the proportion of trochophore larvae after 3 d.

Applicability in ecotoxicological studies

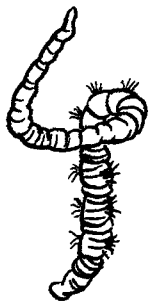
A first screening of the available data on fertilisation success performed under similar conditions showed the IVF procedure seems to be sensitive enough to discriminate between different types of sediment. Further research is needed to explain why the sediment effect was most clearly shown in the response to the exposure of male lugworms to contaminated sediments.

The differences between sediment treatments could not, however, be directly related to the level of contaminants in the sediments. This is partly due to the fact that the experiments were

set up to test several steps in the IVF technique, but other sediment characteristics (texture, organic carbon content) may have played a significant role. Additionally, only PAHs and PCBs were analysed, while the Delfzijl harbour sediment is suspected to contain specific organic pesticides as well as trace metals related to local chemical industries.
Future research is aimed at clarifying the influence of different contaminants on fertilisation success of the lugworm using spiked sediments and at the use of the IVF procedure in biomonitoring of effects at contaminated field stations.

A major advantage of the method presented is the natural exposure regime. The test animals are not only exposed under semi-field conditions, but also, as in the field, the adults are exposed to the contaminated sediments, while the reproductive potential is tested in a relatively clean water column. Often methods for testing sediment toxicity are derived from water column toxicity tests and are adapted for sediment testing, by using elutriate or pore water exposure (Burton & Scott, 1992). When eggs or larvae are used, they are obtained from 'clean' adults, and exposed themselves, because early life-stages are usually more sensitive compared to adults (Casillas *et al.*, 1989; Meador *et al.*, 1990). Our method takes into account that sediment bound contaminants may accumulate in the adult before they are being transferred to the gametes, which are the internally exposed. Therefore, the presented test method may be a useful contribution to risk assessment systems for contaminated marine ecosystems.

Chapter



Factors affecting PAH residues in the lugworm, *Arenicola marina*, a sediment feeding polychaete

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with M.C.Th. Scholten and N.M. van Straalen

4. Factors affecting PAH residues in the lugworm, *Arenicola marina,* a sediment feeding polychaete

Abstract

Being a sediment feeder, the lugworm, *Arenicola marina,* could be a suitable candidate organism for the biomonitoring of sediment-bound contaminants in intertidal areas. Since lugworms are only rarely used in environmental monitoring, little is known about the natural variation in their body residue levels. In this study, the importance of seasonal fluctuations, spatial variation and sexual development to PAH residues in lugworms were assessed. During a period of 15 months, lugworms were sampled along a contamination gradient in the Western Scheldt estuary in the Netherlands. A clear seasonal pattern in the body residue levels of PAH was observed, with lowest levels occurring in March and highest levels just prior to the spawning season in September. During the spawning season the body residue levels showed a marked decrease. Although this seasonal pattern is apparently related to the reproductive cycle of the lugworm, no clear differences in body residue levels were found between animals with or without developed gonads. The contamination gradient, present in the estuary, was clearly reflected in the body residue levels of PAH. The expected gradient of internal concentrations was, however, absent in October, when the spawning period was not yet finished. The variation in lugworm body residue levels was smaller than the fluctuations in sediment PAH levels. It can be concluded that the lugworm is a suitable organism for the biomonitoring of sediment-bound contaminants, provided that attention is paid to the reproductive cycle of the species.

4.1. Introduction

Estuaries are important sinks for hydrophobic contaminants in river discharge. To assess trends of environmental quality in these ecosystems, monitoring of contaminant levels in water and sediment is conducted on a large scale in coastal areas around the world. In addition to the measurement of residues in the abiotic environment, monitoring programs often include determination of residues in selected organisms. Such measurements will provide an indication of the bioavailability of contaminants and thus of the potential to exert effect at biological levels (Goldberg *et al.*, 1978; De Kock & Kramer, 1994). Eventually, the levels of contaminants (whether they are ambient or internal) are not of primary concern. The health of an ecosystem is related to the effects of contaminants, which are, however, very difficult to assess in field research (McIntyre, 1984). An important perspective for monitoring body residue levels in organisms is that these can be more easily related to internal effect concentrations and so provide a measure of risk (De Kock & Bowmer, 1993; Van Straalen, 1996).

Many organisms have been used for biomonitoring in the marine environment. Suspension feeding bivalves, such as the mussel, *Mytilus edulis,* are often used, because they are sedentary and are able to process large amounts of water (O'Connor *et al.*, 1994). Since suspension feeders primarily filter the water, residues in their body reflect water quality, rather than

sediment quality. In addition to mussels, benthic invertebrates may be used to monitor sediment quality. In this study we investigate the possibility for using the lugworm, *Arenicola marina*, to monitor sediment quality.
A. marina is a bulk sediment feeding polychaete worm, which lives in a U-shaped burrow. During the passage of the sediment through the digestive tract, a fraction of the contaminants desorbs from the sediment. This fraction would not be available to organisms when only the sediment/water exchange is considered (Mayer *et al.*, 1996). Thus, it was found in bioaccumulation studies that lugworms accumulated organic contaminants to higher concentrations than filter feeding bivalves (Chapter 2). As in *Mytilus edulis*, the capacity of lugworms to biotransform organic contaminants is probably very limited (Kane Driscoll & McElroy, 1996). Lugworms, therefore, appear to be suitable organisms for the biomonitoring of sediments in intertidal areas. However, since lugworms are only rarely used in environmental monitoring, little attention has been given to natural variation in body residue levels.
The main purpose of this study was to assess the importance of seasonal fluctuations and differences related to sexual development for contaminant residues in lugworms in relation to spatial variation. Polycyclic aromatic hydrocarbons (PAH) were chosen, because they represent one of the major contaminant groups trapped in estuarine sediments (Brunk *et al.*, 1997). In the study area (Western Scheldt, SW Netherlands), PAH loads are relatively high compared to other Western European estuaries (Van Zoest & Van Eck, 1990). In addition to the determination of the body residues, the fertilisation success of lugworms collected at some of the sampling stations was assessed using an *in vitro* fertilisation assay.

4.2. Material & methods

Samples were taken at five stations in the Western Scheldt estuary and at a reference station in the Eastern Scheldt near Yerseke (Figure 8). In contrast to the Eastern Scheldt, the Western Scheldt estuary is one of the most contaminated estuaries in Western Europe (Stronkhorst, 1993), with pollution loads that are higher than those of the rivers Rhine and Meuse (Van Zoest & Van Eck, 1990; Van Eck & De Rooij, 1993). Polluted fresh water is mixed with relatively unpolluted sea water from the North Sea, resulting in an East to West gradient of decreasing pollution (Zwolsman *et al.*, 1996; Van Zoest & Van Eck, 1990; 1993). In addition there are possible local sources of pollution, such as industrial activity near Terneuzen on the southern border of the estuary, near stations 4 and 5, a nuclear power plant at Borsele, near station 6 and dredging activities.
Sampling of lugworms took place in December 1993, March, June and October 1994 and February/March 1995. Large animals (more than ca. 1.5 g fresh weight) were taken, as these were easiest to collect. In December 1993 and October 1994 sediment samples were taken from the top 5 cm and five samples for each station were pooled for analyses.
In the laboratory, the lugworms were placed overnight in sea water without sediment. This allowed them to empty their gut, without eliminating PAH (Kukkonen & Landrum, 1995a). The following morning, the animals were blotted dry and stored at -20°C before further processing. At least 20 individuals from each station were pooled for analysis. In October, when the sexes could be distinguished, females, males and undifferentiated individuals (usually 10 or more) were pooled separately for each station. After thawing, tissues were homogenised using an Ultra-turrax homogeniser and subsamples were taken for analysis of PAH, lipid and ash-free dry-weight.

Dry weight was determined by oven drying ca. 1 g of the samples at 110°C for 48 h. Ash weight was determined by ashing dried samples in a muffle furnace for 4 h at 600°C (tissues) or 450°C (sediments). The percentage of the ash-free dry-weight of the sediments was used as an estimate of the percentage of organic matter present in the samples. We realize that the loss on ignition may overestimate the actual organic carbon content. Although ashing at 450°C does not remove carbonates, the loss of water from clay minerals will erroneously contribute to the weight loss (Dankers and Laane, 1983). This error will not be very significant since the sediments sampled were very sandy, but a more important source of error may be the combustion of the soot fraction in the sediments (Chapman, 1997). Analytical techniques for the routine analysis of the soot content of sediment samples have recently been published (Gustafsson *et al.*, 1997). Nevertheless we assume that a comparison of the stations is still possible.

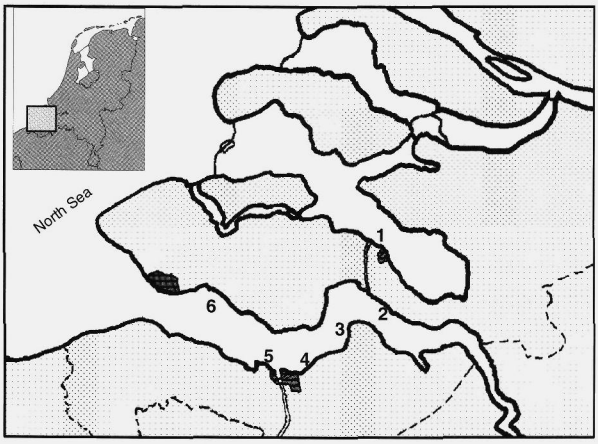


Figure 8: Map of the study area in the southwestern part of the Netherlands. Sampling stations: Yerseke (1) in the Eastern Scheldt and Den Inkel (2), Knuitershoek (3), Terneuzen-East (4), Terneuzen-West (5) and Borssele (6) in the Western Scheldt.

Total lipids were analysed gravimetrically, by the slightly modified method of Folch *et al.* (1957) and Böttcher *et al.* (1959) described in TNO (1980). Homogenised tissue samples (1-2 g) were successively extracted with mixtures of chloroform-methanol (1:1), chloroform-ethanol (2:1) and methanol-chloroform-water (48:5:47). After each step, the samples were centrifuged and the fluid fraction was collected. Finally, the chloroform phase was dried at 60°C in pre-weighed beakers and the residue weighed.
Sediment samples for PAH analysis were extracted by shaking the samples with acetone and hexane and concentrated. Ca. 25 g of the sediment sample was first thoroughly mixed with 50 ml acetone for 10 min and then with 50 ml hexane for another 10 min. Finally 100 ml deionized water was mixed into the sample. After separation of the hexane and acetone/water phases, a 10 ml subsample of the hexane phase was dried under a nitrogen stream and taken up in acetonitrile. This procedure was validated with NBS Standard Reference Material No. 1941 (Organics in marine sediment), yielding an average recovery of 92.4±15.7% relative to the

certified concentrations. Tissue samples were hydrolysed with 4M NaOH for 3 h at 60°C. The hydrolised samples were extracted with hexane for 2 h, after which the extract was cleaned-up over a column with sodium sulphate and 10% deactivated aluminium oxide. The cleaned extract was dried under a nitrogen stream and then taken up in acetonitrile. This procedure could not be validated due to the absence of suitable reference materials. As an internal standard, 6-methylchrysene was added to the samples. Recoveries were usually acceptable (>90% for sediments; >80% for tissues), but in some cases lower recoveries were found. No corrections for recovery were, however, made. The samples were analysed by reverse phase high performance liquid chromatography (RP-HPLC), with fluorescence detection. The response of the detector was calibrated with a certified NBS PAH standard mixture (Standard Reference Material No. 1647).

For the *in vitro* fertilisation (IVF) assay, lugworms were collected at stations 1 and 6 in September 1993 and at stations 2 and 6 in September 1994. The IVF assay was performed according to the procedures described in Chapter 3. In summary, the females were placed individually in small containers with a 2 cm layer of sediment from the station where they were collected. When eggs were observed, these were collected and rinsed. Sperm was taken from the body cavity of males collected at the same station as the spawning females and was diluted with sea water. Diluted sperm was added to the eggs and after 24 h incubation, the fertilised eggs were fixed in neutral buffered formalin and the fertilisation success was assessed under a microscope. For statistical analyses, the fertilisation percentages were transformed using the arcsine transformation in order to meet the assumptions of analysis of variance. When these assumptions could not be met (Bartlett's test of homogeneity of variances), the Wilcoxon two sample test was used (Sokal & Rohlf, 1969).

4.3. Results

Table 9: PAH levels in sediments in December 1993 and October 1994 in mg.kg^{-1} organic matter. <: values were below the detection limit (corrected for % organic matter). For station numbers see Figure 8.

		December 1993					
	Station	1	2	3	4	5	6
	%OM	1.1	1.5	1.4	1.2	1.6	0.7
Phenanthrene		4.20	8.20	12.46	2.89	3.15	<5.72
Anthracene		0.96	2.50	3.82	0.97	0.83	<0.36
Fluoranthene		12.71	18.37	20.91	7.89	7.46	<1.79
Pyrene		20.40	41.01	33.37	10.53	10.45	<5.01
Benzo(a)anthracene		5.95	11.77	12.26	3.55	3.98	<1.07
Chrysene		5.83	11.95	12.06	5.13	3.32	<2.15
Benzo(b)fluoranthene		5.83	12.84	8.24	3.42	2.99	<0.36
Benzo(k)fluoranthene		2.80	5.88	4.22	1.58	1.44	<0.36
Benzo(a)pyrene		5.25	1.21	8.64	3.29	2.65	0.43
Indeno(123cd)pyrene		4.55	9.81	4.82	2.63	1.99	<2.15
Σ 10		68.48	123.54	120.81	41.88	38.26	0.43

Sediment

The concentrations of PAH in the sediment were dominated by pyrene and fluoranthene, while other PAHs were present in lower concentrations (Table 9). In December 1993, there was a clear gradient in sediment PAH levels in the Western Scheldt estuary from station 2 in the East to station 6 in the West. In October 1994 this gradient was less clear but still present. The sediment PAH levels at reference station 1 in the Eastern Scheldt were comparable to sediment PAH levels at stations 4 and 5 in the Western Scheldt. Sediment PAH levels were considerably higher in December 1993, compared to October 1994. At station 6 the total PAH concentration could not be established in December 1993. Most of the individual PAHs were below the detection limits, which in this case were relatively high due to the low amount of organic matter in the sample.

Lugworms

Most of the PAHs present in the sediments were also detected in the lugworms (Table 10). The PAH concentrations in the lugworms were dominated by fluoranthene and pyrene, although in March 1995 these were below the detection limit in some samples. Relatively low levels of anthracene and indeno(123cd)pyrene were found. Compared to the sediments, PAH concentrations in lugworms were relatively low, resulting in biota-to-sediment accumulation factors (body lipid to sedimentary organic matter) ranging between 0.01 and 0.1.
The body residue levels of PAH in lugworms sampled in the Western Scheldt estuary (stations 2-6) showed a gradient of PAH levels similar to the sediment, with the highest levels in lugworms sampled in the Eastern parts of the estuary and the lowest levels in the Western part (Table 10). In October 1994 this gradient was not so clear, mainly due to the high PAH levels observed in lugworms sampled at station 5 and especially at station 6 (Table 11). The data on PAH in lugworms are summarised in Figure 9, which shows the average sum of 10 PAH for all five samplings. The gradient from station 2 in the east to station 6 in the west is clearly visible. The body residue levels at reference station 1 were among the lowest from each sampling and showed little variation.

Tabel 9 continued

		October 1994					
	Station	**1**	**2**	**3**	**4**	**5**	**6**
	%OM	**1.5**	**0.6**	**0.8**	**0.5**	**2.0**	**1.4**
Phenanthrene		1.33	<1.36	<1.00	<1.67	1.01	<0.56
Anthracene		0.44	0.68	0.50	0.28	0.36	0.28
Fluoranthene		3.89	5.00	3.83	1.67	2.61	2.43
Pyrene		3.01	4.32	3.17	1.39	1.96	1.87
Benzo(a)anthracene		1.68	2.50	1.33	0.83	1.01	0.93
Chrysene		1.68	2.95	1.50	0.56	1.09	0.56
Benzo(b)fluoranthene		2.21	3.64	2.00	1.11	1.96	1.78
Benzo(k)fluoranthene		0.89	1.82	1.00	0.56	0.87	0.84
Benzo(a)pyrene		2.12	3.64	2.00	0.83	1.67	1.50
Indeno(123cd)pyrene		1.77	2.95	1.33	<0.56	2.10	1.78
Σ 10		19.03	27.50	16.67	7.22	14.64	11.96

Table 10: PAH levels in lugworms in mg.kg^{-1} lipid. The data for October 1994 are shown separately in Table 11. For each sample, the lipid content is given as a percentage of the fresh weight. <: values were below the detection limit (corrected for % lipid). Station 5 was not sampled in March 1994. For station numbers see Figure 8.

		station					
		1	2	3	4	5	6
December 1993	**% lipid**	**1.03**	**1.13**	**1.01**	**1.19**	**0.98**	**1.11**
Phenanthrene		0.39	0.50	0.73	0.22	0.55	0.39
Anthracene		0.07	0.10	1.68	0.02	0.06	0.04
Fluoranthene		2.08	1.92	2.40	0.45	1.06	0.75
Pyrene		1.73	2.71	2.50	0.41	1.21	1.12
Benzo(a)anthracene		0.24	0.60	0.89	0.08	0.19	0.09
Chrysene		0.56	1.42	1.43	0.14	0.59	0.39
Benzo(b)fluoranthene		0.40	0.99	0.84	0.18	0.48	0.48
Benzo(k)fluoranthene		0.15	0.35	0.32	0.06	0.15	0.12
Benzo(a)pyrene		0.14	0.60	0.51	0.07	0.20	0.17
Indeno(123cd)pyrene		<0.12	<0.11	0.18	<0.11	<0.16	<0.09
Σ 10		5.74	9.20	11 48	1.63	4.51	3.57
March 1994	**% lipid**	**0.85**	**0.97**	**0.96**	**1.18**		**0.86**
Phenanthrene		0.62	0.33	0.34	0.71		0.67
Anthracene		0.07	0.05	0.04	0.05		0.03
Fluoranthene		2.10	1.60	0.91	1.66		0.87
Pyrene		1.59	2.78	1.65	2.63		0.93
Benzo(a)anthracene		0.25	0.46	0.25	0.53		0.17
Chrysene		0.52	1.06	0.62	1.28		0.48
Benzo(b)fluoranthene		0.47	0.82	0.71	0.75		0.36
Benzo(k)fluoranthene		0.16	0.25	0.20	0.24		0.10
Benzo(a)pyrene		0.19	0.46	0.36	0.42		0.13
Indeno(123cd)pyrene		<0.21	<0.21	<0.20	<0.14		<0.17
Σ 10		5.98	7.81	5.09	8.27		3.74
June 1994	**% lipid**	**0.97**	**0.92**	**1.12**	**1.53**	**1.16**	**1.06**
Phenanthrene		0.44	0.44	0.35	0.46	0.39	0.74
Anthracene		0.05	0.09	0.07	0.07	0.08	0.06
Fluoranthene		2.05	2.13	1.54	1.73	0.94	1.10
Pyrene		1.87	3.10	2.43	3.04	1.20	1.02
Benzo(a)anthracene		0.15	0.56	0.36	0.40	0.30	0.07
Chrysene		0.48	1.53	1.17	1.38	0.69	0.62
Benzo(b)fluoranthene		0.73	1.63	1.37	1.64	1.03	0.71
Benzo(k)fluoranthene		0.23	0.63	0.52	0.60	0.33	0.20
Benzo(a)pyrene		0.26	1.05	0.70	0.86	0.43	0.27
Indeno(123cd)pyrene		<0.11	0.41	0.26	0.31	0 29	0.12
Σ 10		6.27	11.57	8.77	10.48	5.68	4.90
March 1995	**% lipid**	**0.86**	**1.03**	**0.92**	**1.22**	**0.73**	**0.71**
Phenanthrene		0 60	0.36	0.33	0.28	0.40	0.38
Anthracene		0.06	0.09	0.06	0.06	0.06	0.04
Fluoranthene		1.15	2.04	1.10	<0.02	0.79	<0.04
Pyrene		1.22	<0.02	2.13	<0.01	1.58	<0.02
Benzo(a)anthracene		0.18	1.02	0.53	0.56	0.40	0.25
Chrysene		0.31	1.34	0.68	0.86	0.67	0.54
Benzo(b)fluoranthene		0.24	1.51	0.61	0.96	0.42	0.68
Benzo(k)fluoranthene		0.09	0.57	0.22	0.28	0.16	0.23
Benzo(a)pyrene		0.17	1.23	0.52	0.63	0.35	0.43
Indeno(123cd)pyrene		0.10	0.19	0.06	0.06	0.16	0.07
Σ 10		4.13	8.35	6.25	3.69	4.99	2.61

Table 11: Body residue levels of PAH in lugworms in October 1994 for females (F), males (M) and non-gravid animals (?) in mg.kg^{-1} lipid. <: values were below the detection limit (corrected for % lipid).

station	1			2			3		
sex	F	M	?	F	M	?	F	M	?
PAH % lipid	2.20	0.79	0.73	1.86	0.73	0.75	1.64	1.02	0.97
Phenanthrene	0.69	0.71	0.64	0.38	0.96	0.99	0.67	0.37	0.61
Anthracene	0.04	0.04	0.05	0.07	0.07	0.07	0.07	0.08	0.06
Fluoranthene	<0.01	1.14	1.40	0.97	1.64	1.73	1.83	1.20	0.47
Pyrene	<0.01	1.14	1.53	1.61	2.47	2.80	2.56	2.20	0.45
Benzo(a)anthracene	0.18	0.13	0.21	0.39	0.63	0.45	0.45	0.48	0.21
Chrysene	0.30	0.42	0.35	1.24	2.06	1.83	1.46	0.78	0.79
Benzo(b)fluoranthene	0.62	0.79	0.55	0.75	1.37	1.59	1.16	1.51	0.64
Benzo(k)fluoranthene	0.19	0.20	0.19	0.31	0.58	0.40	0.47	0.52	0.26
Benzo(a)pyrene	0.28	0.32	0.31	0.59	0.96	0.93	0.61	0.87	0.31
Indeno(123cd)pyrene	0.09	0.15	0.10	0.21	0.45	<0.11	0.36	0.17	<0.06
Σ 10	2.38	5.03	5.30	6.51	11.18	10.79	9.64	8.16	3.80

station	4			5			6		
sex	F	M	?	F	M	?	F	M	?
% lipid	2.81	1.06	0.94	1.03	1.10	0.49	2.38	1.66	0.22
Phenanthrene	0.53	0.94	0.79	0.94	0.71	0.94	0.54	0.51	2.04
Anthracene	0.11	0.09	0.03	0.08	0.07	0.06	0.04	0.07	0.17
Fluoranthene	1.07	1.32	0.95	2.43	1.46	1.65	1.96	2.29	2.25
Pyrene	1.60	1.98	1.06	2.62	1.82	1.49	2.17	0.96	<0.51
Benzo(a)anthracene	0.39	0.43	0.21	0.48	0.37	0.22	0.24	0.31	1.07
Chrysene	1.35	1.60	0.84	1.65	1.52	1.04	0.98	0.90	2.28
Benzo(b)fluoranthene	1.00	1.13	0.57	1.36	1.50	1.02	1.66	1.02	4.52
Benzo(k)fluoranthene	0.39	0.43	0.21	0.52	0.44	0.33	0.53	0.29	1.30
Benzo(a)pyrene	0.68	0.74	0.34	0.64	0.64	0.43	0.31	0.55	1.01
Indeno(123cd)pyrene	0.28	0.32	<0.06	0.39	0.31	<0.12	0.18	0.23	0.66
Σ 10	7.40	8.99	5.01	11.11	8.83	7.18	8.60	7.14	15.30

The body residue levels varied over the year. They were generally lower in winter and higher in summer/early autumn, but the exact pattern and magnitude differed between stations (Table 10). The seasonal changes are illustrated in Figure 10 for station 2, for which extra data were available from two additional samplings, in September and October 1994. This figure shows that PAH levels increased during spring and summer. After September, the PAH levels showed a marked decrease, which slowly continued throughout the winter.
In October, females, males and non-gravid animals were analysed separately (Table 11). PAH levels differed substantially between the three groups, but no consistent pattern was present. The individual PAHs also showed considerable variation.

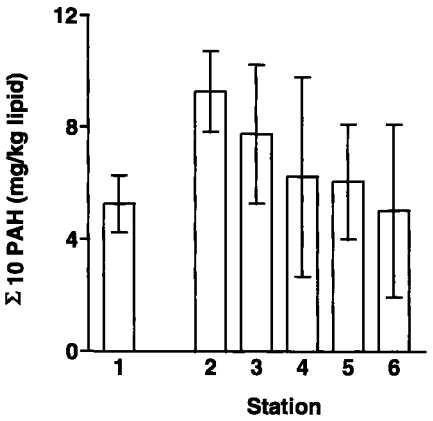


Figure 9: Mean body residue levels of PAH (sum of 10) in lugworms at different stations, averaged over all sampling dates. Error bars indicate standard deviations. For station numbers see Figure 8.

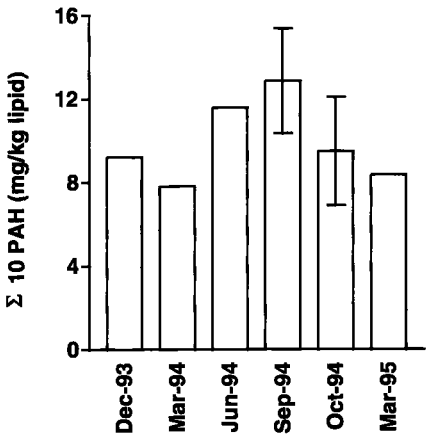


Figure 10: Seasonal pattern of body residue levels of PAH (sum of 10) in lugworms sampled at station 2 in the Western Scheldt. Values for September and October are averages of females, males and non-gravid animals; error bars indicate standard deviations.

Fertilisation success

The results of the IVF assays are shown in Figure 11. The fertilisation success of individual female lugworms is presented. At reference station 1, the fertilisation percentage ranged between 80 and 100%. The fertilisation success of females sampled at station 6 in 1993 was not significantly different from station 1 (Anova, $p>0.05$). Compared to 1993, the average fertilisation success was significantly lower in 1994 (Wilcoxon $p<0.001$). At the station with the highest PAH levels (station 2), the fertilisation success was significantly higher than that at station 6 in the same year (Anova, $p<0.05$). In Figure 11 it can be seen that the reduced fertilisation success in the populations tested is not caused by a general decrease in the fertilisation percentage for all female lugworms. It is due rather to a higher incidence of individual females within the population showing a reduced fertilisation percentage, while at the same time individual females with nearly 100% fertilisation success remain present in the population.

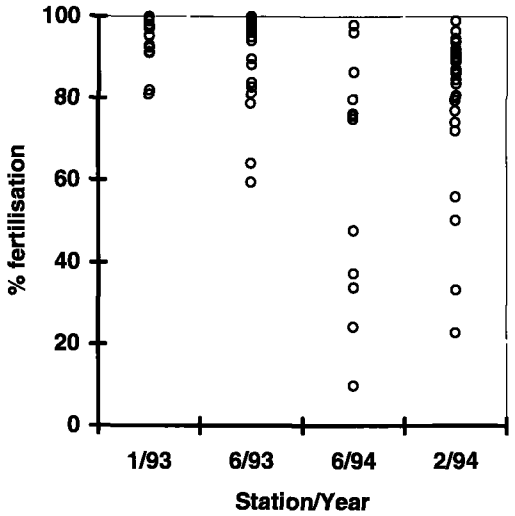


Figure 11: Fertilisation success in standardised in vitro *assays of lugworms sampled at station 1 and 6 in 1993 and at station 2 and 6 in 1994. The fertilisation percentages for individual females are shown. See Figure 8 for a map of the study area.*

4.4. Discussion

The PAH levels in the sediments were dominated by fluoranthene and pyrene, and the concentrations of other compounds were lower. A fluoranthene/pyrene ratio larger than 1 or a phenanthrene/anthracene ratio smaller than 10 indicate that the PAH originated from combustion processes (Benlahcen *et al.*, 1997). For the sampling in December the ratio phenanthrene/anthracene ranged from 2.2 to 3.8 (3.1±0.6), indicating that the PAH in the sediments were predominantly of pyrolitic origin. The ratio fluoranthene/pyrene ranged from 0.4 to 0.7 (0.6±0.1), which indicates that petrogenic PAH also contributed to sediment contamination. In October only the ratio fluoranthene/pyrene could be calculated. As it ranged from 1.2 to 1.3 (1.25±0.05) it also indicated a predominance of pyrolitic PAH in the sediments. The dominance of pyrolitic PAH in the sediments may hamper the comparison between stations, because the PAH may be associated for a large part with the soot fraction in the sediments, which was not assessed separately. However, because the ratios fluoranthene/pyrene and phenanthrene/anthracene show very little variation between stations, we assume that the soot content will be very similar too and a comparison between stations remains possible.
As was anticipated, PAH levels in the sediments were highest in the inner part of the estuary and decreased in a downstream direction, resulting in a clear gradient. Unexpectedly, PAH levels in the sediment at the reference station in the Eastern Scheldt were relatively high, which could be due to the presence of a local source of PAH, such as the nearby harbour at Yerseke.

Sediment PAH levels were considerably higher in December 1993 than in October 1994. Although pooling of five subsamples might not be sufficient to reduce local variations in sediment chemistry (Essink *et al.*, 1989), it is unlikely that local variability can explain this

decline, considering the magnitude of the difference and the fact that it was observed at five of the six sampling stations. Similar seasonal fluctuations have been observed in concentrations of PAH in estuarine water and suspended matter (Bouloubassi & Saliot, 1991). Estuaries are dynamic systems, in which sedimentation and erosion alternate due to wave-action and variations in run-off (Essink *et al.* 1989). As a consequence, the sediment surface, and its PAH levels with it, show spatial and temporal variation (Stainken *et al.*, 1983). Additionally, the sediment surface may be disturbed by human activities. Bait digging and tracks left by cockle fishing were observed at station 4. Since the body residue levels of lugworms sampled in March 1994 were comparable to the body residue levels in March 1995, the exposure levels have probably remained more or less the same, despite considerable fluctuations in total sediment concentrations during the year.

Seasonal fluctuation

In contrast to the sediments, the body residue levels at most stations increased from winter to summer and autumn, followed by a subsequent decrease towards the next winter. This seasonal pattern in accumulated levels of PAH is strongly correlated with the reproductive cycle of the lugworm (Mayes & Howie, 1985). Body residue levels were lowest during the winter months, when no reproductive activity occurs. Increased body residue levels during summer have been attributed to higher environmental temperatures, causing increased uptake rates (Landrum, 1988). However, Jovanovich and Marion (1987) showed that depuration rather than uptake of anthracene was enhanced by higher temperatures in the brackish water clam, *Rangia cuneata*. Uptake and depuration were strongly related to the reproductive cycle of the clams. The uptake rates were highest in August, when the gonad index was largest, while depuration increased during spawning in autumn. In mussels, which spawn in spring, the highest levels of organochlorines were found during the winter (Hummel *et al.*, 1990; Lee *et al.*, 1996). This supports the importance of the reproductive cycle. Apparently, spawning is an important mechanism for the loss of hydrophobic contaminants in several species. Concentrations seem to go through a maximum before spawning. Since the time of spawning differs between species, the seasonal pattern of contaminant concentrations is also different.

Relationship with sex and spawning

Our study did not reveal consistent differences between PAH body burdens of different sexes and between gravid and non-gravid animals. The accumulation and elimination kinetics of females, males and non-gravid animals may play an important role. After exposure to No. 2 fuel oil, the concentration of naphthalenes in males of the polychaete, *Neanthes arenaceodentata,* decreased rapidly after transfer to clean sea water. In (gravid) females, however, the naphthalene concentration remained high until spawning. In post-spawning females, the levels of naphthalenes were close to the detection limit, while in the zygotes the levels of naphthalenes were as high as in the gravid females and remained so until yolk reserves were mobilised during the development of trochophore larvae (9 d) into 18-segment juveniles (21 d) (Rossi & Anderson, 1977).

Differences may also exist among non-gravid lugworms, as the population may contain non-reproducing animals as well as spent males and females. Non-reproducing individuals occur in low numbers, varying between years and between populations (Howie, 1959; De Vooys, 1975; De Wilde & Berghuis, 1979). Since the fraction of non-reproducing animals in a population is usually small, this cannot explain the differences in PAH residues. The difference in behaviour between spent males and females may be of more importance. It is known that lugworms show

enhanced growth after spawning. This appears as an increase in the average weight of the adult population just after the main spawning period (De Wilde & Berghuis, 1979). Males resume feeding within 2 d after spawning, but females do not feed for a period of 3-4 weeks after spawning, in order to protect their larvae which develop within the burrow (Farke & Berghuis, 1979a). To what extent these factors actually have an influence on the observed body residue levels cannot as yet be stated. Analyses should be specifically directed at differences in contaminant levels between gametes and the rest of the body and at males and females before and after spawning.

Local variation

Even between neighbouring populations of *A. marina*, differences may occur in the characteristic spawning time (Duncan, 1960). *In vitro* fertilisation assays showed that the spawning of females collected at station 6 occurred one or two weeks later than the spawning of females collected at stations 1 or 2. Additionally, there may be a difference in spawning characteristics between young lugworms (1 year old) and older lugworms (2 or more years old). In the Dutch Wadden Sea, spawning in older lugworms is generally confined to a short period at the end of September and early October and partly again in early November, while the spawning of young lugworms occurs during an extended period from August to November (De Vooys, 1975). However, since mostly large animals were sampled in the present study, the latter factor will not have been of importance.

Since the sources of variation between individuals, as discussed above, are largely related to the actual period of spawning (September-November) differences in the characteristic spawning period may have caused the differences in the body residue levels observed at the sampling stations, which are not related to the actual differences in exposure. This may explain the absence of the pollution gradient in lugworms sampled in October 1994, while this gradient was present during all other samplings.

Effects of PAH on fertilisation success

It was not surprising that in 1993 no difference in fertilisation success was found between stations 1 and 6, since PAH levels in lugworms at both stations were low. Considering the differences in PAH levels, in the lugworms as well as in the sediments, the fertilisation success was expected to be lower at station 2 than at station 6, either in response to the PAH level, or in response to other contaminants occurring along the same gradient.

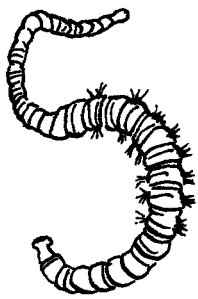
Instead, the fertilisation success at station 2 turned out to be higher than the fertilisation success at station 6. Some local disturbance may have caused the low fertilisation success in the lugworms at station 6 in 1994. This station is near to the cooling water outlet of a nuclear power plant. In the absence of more detailed information on yearly and local fluctuations in fertilisation success it may also be true that the difference between 1993 and 1994 and between stations reflects a natural range of variation.

4.5. Concluding remarks

The body residue levels of PAH in lugworms sampled along a pollution gradient in the Western Scheldt estuary decreased from East to West on most sampling dates, in accordance with what was expected from the characteristics of the estuary and contaminant levels of sediments sampled at the same stations. The fact that concentrations of PAH in the lugworms were not directly proportional to the concentrations in the sediments shows that the use of sediment-feeding benthic animals in monitoring programs may have an added value, compared to the analysis of sediments alone. Lugworms showed a seasonal fluctuation in body residue levels of PAH, which was correlated with the reproductive cycle of the species. The sampling of lugworms for the purpose of environmental monitoring should, therefore, be performed in a restricted period relative to the reproductive cycle. From the present results it is obvious that at least the period from the end of August to November should be excluded. However, since there is considerable individual variation in the rate of vitellogenesis (Mayes & Howie, 1985), differences in the duration of the vitellogenic period may occur between populations and between years. The best period for sampling lugworms would therefore be between December and May.

As yet, the fertilisation success of the lugworms could not be related to the contamination gradient. More research is needed in order to gain insight into the natural variation in fertilisation success and the sensitivity of the method.

Chapter



The effects of PAH-loaded sediments on the fertilisation success of two marine benthic invertebrates

with M.C.Th. Scholten

5. The effects of PAH-loaded sediments on the fertilisation success of two marine benthic invertebrates

Abstract

Sediment quality criteria are generally derived from aquatic toxicity data, using the equilibrium partitioning theory. This method underestimates the actual bioavailability of contaminants to sediment feeding organisms. Therefore, a mesocosm-scale bioassay was developed, in which marine benthic invertebrates were exposed to sediments artificially spiked with fluorene, fluoranthene (FAN) and benzo(a)pyrene (BaP) and their reproduction success was determined after chronic exposure. In order to spike the large amounts of sediment used in mesocosm studies, a new spiking procedure was developed in which the PAH is first adsorbed onto an organic carrier (i.e. the food of sediment feeding organisms), after which the spiked carrier is mixed with sand.
Fluorene disappeared from the spiked sediments within a few months and FAN within two years, while the level of BaP only started to decrease after two years. The spiking procedure is, thus, mainly suitable for very lipophilic, degradation resistant compounds like BaP.
Exposure to BaP spiked sediments resulted in a reduced fertilisation success in baltic tellins (*Macoma balthica*). Contrary to previous experiments, no reduction of the fertilisation success was found when lugworms (*Arenicola marina*) were exposed to a harbour dredged sediment with relatively high BaP levels. This could be explained by the very low bioavailability of PAHs in this sediment and the finding that the fertilisation success appeared to be related to the body residue level of BaP in female lugworms, rather than to the total concentration in the sediment. It is proposed, therefore, to use sediment feeding animals, like the lugworm, to assess the actual bioavailability of contaminants in sediments and to relate the resulting body residue levels to ecological effects.

5.1. Introduction

In order to evaluate disposal options for dredged materials, sediment quality criteria are being developed, often based on the results of ecotoxicological experiments (Ross & Munawar, 1994). However, testing of sediment-bound contaminants is difficult, due to the complex interactions of the contaminants with the sediment and the various ways in which infaunal organisms are exposed to the contaminants. Alternatively, quality criteria for the water phase can often be based on a large body of aquatic toxicity data, as testing methods, at least for water soluble aquatic contaminants, are relatively well developed and internationally standardised. In the Netherlands, sediment quality criteria were principally derived from water quality criteria using equilibrium partitioning theory, assuming that sediment dwelling organisms accumulate chemicals via the interstitial water (Van der Kooij *et al.*, 1991). The validity of the use of equilibrium partitioning to derive sediment quality criteria from aquatic data is, however, being questioned. Model calculations with a small deposit feeding amphipod (Landrum & Robbins, 1990), as well as mesocosm studies with a polychaete and two bivalves (Chapter 2), indicate that sediment ingestion can be a major direct source of sediment-bound

contaminants, due to the mobilisation of contaminants during digestion (Mayer *et al.*, 1996). In this case, the equilibrium partitioning model will underestimate body burdens. There is, therefore, a need for the development of sediment bioassays with sediment feeding animals, in order to gain insight into the actual risk that contaminated sediments pose to the marine benthic environment.

In this paper, we report on the development of a mesocosm scale bioassay using sediments artificially spiked with polycyclic aromatic hydrocarbons. PAHs were chosen because they are an important group of contaminants in harbour dredged materials. Due to their lipophilic nature and their resistance to biodegradation, most PAHs, especially those with three or more aromatic rings, tend to accumulate in the sediment and in sediment feeding invertebrates. Few aquatic toxicity data for PAHs are available, making the approximation of sediment quality criteria based on aquatic toxicity relatively weak.

The lugworm, *Arenicola marina*, was used as the main test organism. The lugworm is a bulk sediment feeder and is, therefore, intensively exposed to sediment-bound contaminants. A recently developed *in vitro* fertilisation procedure (Chapter 3) was used to assess the effect of chronic exposure of the adult lugworms on fertilisation success. For comparison, lugworms were also exposed to a harbour dredged sediment. Additionally, the baltic tellin (*Macoma balthica*, a deposit feeding bivalve) was exposed to the spiked sediments.

5.2. Material & Methods

The experiments were conducted in circular outdoor mesocosms with a volume of 2.2 m^3. A ca. 20 cm deep layer of test sediment was constantly covered by 50 cm of sea water, with a salinity of 29 ‰. Tides were not implemented; instead the water was continuously refreshed at a rate of 40 $L.h^{-1}$, sufficient to replace the water in the mesocosms in ca. 2 days. The water was also aerated, in order to improve circulation within the mesocosm.

Several methods have been proposed for the spiking of sediments with chemicals for ecotoxicological studies (e.g. Lee *et al.*, 1989; Hill *et al.*, 1993). A common feature of these methods is that they were developed for laboratory tests on a limited scale. In order to be able to spike the mesocosms, each containing nearly 600 kg of sediment, a new method for spiking large amounts of sediment had to be developed.
Within sediment, lipophilic contaminants are preferentially bound to the organic fraction (Lee *et al.*, 1990; Harkey *et al.*, 1994a). This principle was used to create test sediments artificially spiked with three different PAH compounds. The test chemicals were first added to an organic carrier, after which the loaded carrier was mixed with sand.

Sea lettuce (*Ulva sp.*) was used as an organic carrier. The material was collected along the shores of the Wadden Sea in October 1992. It was dried with hot air for 1 week and then milled in a hammer mill. The resultant powder had an organic matter content (measured as ash-free dry-weight, ADW) of 55%. Sand with a low organic matter content (ca. 0.5%) was dredged from the Marsdiep between Den Helder and the island of Texel.

The sediment was prepared in January 1993 by mixing 1 ml of dimethylsulfoxide (DMSO), containing the selected PAH, through 800 g 'sea lettuce powder', which was wetted with 2 L of sea water. The resulting thick suspension was then mixed with 20 kg of sand in a cement mixer. This procedure was repeated until 600 kg of sand was loaded with the PAH. After one mesocosm was filled, all materials were cleaned before the next mesocosm was filled with sediment loaded with another PAH. The loaded sediments contained 1.5% organic matter directly after preparation (0.5% from the sand and 1% added). This was slightly higher than the highest organic matter content of 1.36% observed during lugworm sampling in the Dutch and German Wadden Sea (unpublished results).

After being filled with the test sediment, sea water was pumped into the mesocosm until the layer of sediment was just submerged. The degradation of the sea lettuce could commence in the wet sediment, but the sea lettuce particles could not drift out of the sediment. After 2 weeks, the pumps were started and the water was continuously refreshed.

Sediments were dosed to a nominal concentration of 4 $mg.kg^{-1}$ dry organic matter (which amounts to 26 $\mu g.kg^{-1}$ dry sediment) of either fluorene, fluoranthene or benzo(a)pyrene, a mixture of 12 $mg.kg^{-1}$ of all three PAHs, or with DMSO (solvent control) only. Since only a limited number of mesocosms was available, the treatments, as such, were not replicated. Instead, sediments loaded with one PAH were considered to be replicates of the control treatment for the other two PAHs, while the sediment loaded with the mixture was used as a replicate for the sediments loaded with a single PAH. This seems to be a valid construction, since the effects of combinations of PAHs are usually believed to be strictly additive (Verhaar *et al.*, 1992). In addition, at the time of the determination of the fertilisation success (September 1994), the levels of fluorene and fluoranthene were no longer different between treatments and controls, so the effects were dominated by benzo(a)prene (see below).
The concentrations used, were based on the benzo(a)pyrene concentration (3.15 $mg.kg^{-1}$) measured in a sediment dredged from the Botlek petrochemical harbour, Rotterdam. In previous experiments, lugworms showed a reduced fertilisation success after exposure to this sediment (Chapter 3). For comparison, in 1994 a Rotterdam harbour dredged sediment, originating from the same site as the one previously used, was applied in a sixth mesocosm. This harbour sediment, which originally had an organic matter content of ca. 11%, was mixed with Wadden Sea sand until the organic matter content was less than 3%.

Over a 3 year period, prior to November 1995, sediment samples were regularly analysed for the dosed PAH, generally at the same time as the animals exposed to the sediments were sampled. Five sediment cores were taken and pooled for analyses from each mesocosm. Lugworms (*Arenicola marina*) and baltic tellins (*Macoma balthica*) were exposed to the sediments for periods of 3 to 6 months. At the end of each exposure period, the animals were sampled for chemical analysis. A minimum of 10 animals per mesocosm was pooled for chemical analyses. In their respective reproductive seasons, baltic tellins and lugworms were transferred to the laboratory in order to study the effect of PAH on the fertilisation success, using *in vitro* fertilisation (IVF).

For the IVF experiments, mature lugworms were brought into the laboratory in September 1994, where they were kept individually in small containers until the females spawned. Sperm was then taken from five males exposed to the same sediment in order to

fertilise the eggs. The eggs were preserved in 4% neutral buffered formalin after an incubation period of 24 h. Details of the IVF procedure for lugworms can be found in Chapter 3.

The IVF procedure for baltic tellins is not yet fully protocollized. In general, the procedure for fertilisation was based on the procedure for lugworms, with adaptations from Timmermans *et al.* (1996). As the spawning of baltic tellins is triggered by rising temperatures in spring (De Wilde & Berghuis, 1978), spawning can be induced by applying temperature changes. For the IVF experiments, baltic tellins were sampled from the mesocosms when the water temperature approached 10°C (a critical temperature with respect to the onset of spawning in the Dutch Wadden Sea, De Wilde & Berghuis, 1978). In the laboratory, the baltic tellins were held at a temperature of 6°C. To induce spawning, the animals were cleaned with tap water and left to dry for 5 to 30 minutes. Then they were individually placed in beakers with relatively warm water (room temperature, ca. 20°C). After 1 to 2 hours, the water was poured off and replaced with colder water (12°C) for another 1 to 2 h. The temperature changes were repeated if considered necessary.
When animals had spawned they were removed from the beaker and the eggs or the sperm were stored in water at ca. 12°C until the IVF was performed (always within a few hours on the same day). To fertilise the eggs, 1 or 5 ml of the sperm suspension was added to the eggs. At time intervals of up to 40 h (1.5, 2.5, 17, 24 and 40 h after fertilisation), subsamples were taken in order to examine the development of the embryos. Samples were preserved in 4% neutral buffered formalin.

Ash weight of the sediments was determined by ashing dried samples in a muffle furnace for 4 h at 450°C. The ash-free content of the sediments (ADW) was used as an estimate of the percentage of organic matter present in the samples.
Total lipids were analysed using a slight modification of the method of Folch *et al.* (1957) and Böttcher *et al.* (1959), as described in TNO (1980).
Sediment samples for PAH analysis were extracted by shaking the samples with acetone and hexane, and concentrated. Ca. 25 g of the sediment sample was first thoroughly mixed with 50 ml acetone for 10 min and then with 50 ml hexane for another 10 min. Finally, 100 ml deionized water was mixed into the sample. After separation of the hexane and acetone/water phases, a subsample of the hexane phase was dried under a nitrogen stream and taken up in acetonitrile. This procedure was validated with NBS Standard Reference Material No. 1941 (Organics in marine sediment), yielding an average recovery of 92.4±15.7% relative to the certified concentrations. Tissue samples were hydrolysed with 4 M NaOH for 3 h at 60°C. The hydrolised samples were extracted with hexane for 2 h, after which the extract was cleaned-up over a column with sodium sulphate and 10% deactivated aluminium oxide. The cleaned extract was dried under a nitrogen stream and then taken up in acetonitrile. This procedure could not be validated due to the absence of suitable reference materials. As an internal standard, 6-methylchrysene was added to the samples. Recoveries were usually 90-100% for sediments and 60-85% for lugworms. No corrections for recovery were made. The samples were analysed by reverse phase high performance liquid chromatography (RP-HPLC), with fluorescence detection. The response of the detector was calibrated with a certified NBS PAH standard mixture (Standard Reference Material No. 1647).

Differences between treatments were tested with analysis of variance and, when appropriate, followed by *a posteriori* tests for differences between means (Student-Newman-

Keuls). Fertilisation percentages were transformed using arcsine transformation, in order to meet the assumptions of analysis of variance. These assumptions could not be met in the case of the fertilisation success of lugworms (Bartlett's test of homogeneity of variances). These data were analysed with a non-parametric test (Kruskal & Wallis) (Sokal & Rohlf, 1969).

5.3. Results

The development of artificially spiked sediments

As temperatures rose during the spring of 1993, the degradation of sea lettuce in the sediments accelerated. This was accompanied by anoxia, increased ammonium concentrations and the development of sulphur bacteria on the sediment surface. In July (6 months after the spiking), this breakdown of easily degradable compounds ceased and the sediments regained their normal appearance.

Nearly half of the total organic matter was lost during the first months. After that, the organic matter content stabilised at ca. 0.8% of the dry sediment, i.e. somewhat higher than the 0.5% of the Wadden Sea sand used.
Fluorene disappeared very quickly from the sediments. In March '93 (6 weeks after the spiking), the concentrations were ca. 2.5 $mg.kg^{-1}$ organic matter in the sediments spiked with this compound and close to the detection limit in the other three sediments. After ca. 5 months (June '93), fluorene concentrations were below the detection limit in all sediments. Therefore, fluorene was not further analysed and the mixture was considered to be composed of fluoranthene (FAN) and benzo(a)pyrene (BaP).

Table 12: Concentrations (in $\mu g.kg^{-1}$ dry sediment) of fluoranthene (FAN) and benzo(a)pyrene (BaP) in artificially contaminated sediments. Organic matter is expressed as average percentage ash-free dry-weight (ADW). Sediments to which the measured PAH compounds were added are given in bold. n.a = not sampled

		nominal	March '93 surface	March '93 deep	June '93	September '94	May '95	November '95
% ADW (±st.dev)		**1.5**	**1.14 ±0.08**	**0.93 ±0.15**	**0.87 ±0.10**	**0.78 ±0.09**	**0.71 ±0.11**	**0.82 ±0.09**
treatment	**analysis for**							
control	FAN	0	20	22	21	19	27	4
FAN	**FAN**	**26**	**50**	**64**	**68**	**24**	**16**	**14**
BaP	FAN	0	23	30	n.a.	20	6	2
mixture	**FAN**	**26**	**61**	**90**	**41**	**29**	**5**	**7**
control	BaP	0	7	<0.134	7	9	12	10
FAN	BaP	0	8	6	10	11	6	13
BaP	**BaP**	**26**	**38**	**30**	**25**	**34**	**16**	**16**
mixture	**BaP**	**26**	**33**	**35**	**29**	**23**	**14**	**13**

In March '93, the concentrations of FAN and BaP (Table 12) were not significantly different between the sediment surface and deeper sediment (Anova, FAN: $p>0.05$; BaP: $p>0.05$). Therefore, mixed sediment samples were taken in later samplings.

There was a significant interaction between the spiking of the sediments and the sampling date for FAN (Anova, $p<0.05$) as well as for BaP (Anova, $p<0.01$). This is explained by the fact that the initial differences in sediment concentrations due to the spiking (Table 12) decreased in time and had disappeared after 19 months (FAN) and 28-33 months (BaP).

Bioaccumulation

The level of fluorene accumulated by the baltic tellins sampled in June '93 still reflected the loading of the sediments, even though the actual levels in the sediments themselves no longer showed differences. Fluorene levels were below the detection limit (ca. 0.18 $mg.kg^{-1}$ lipid) for baltic tellins exposed to the control sediments, while 0.90±0.25 mg $fluorene.kg^{-1}$ lipid was found in baltic tellins exposed to the fluorene loaded sediments. No significant differences in fluorene levels were found in the lugworms exposed from July to September. The fluorene concentrations in the lugworms (2.65±1.60 $mg.kg^{-1}$ lipid) more or less represent the background level, which is much higher for lugworms than for bivalves.

Table 13: Accumulation of fluoranthene (FAN) and benzo(a)pyrene (BaP) in lugworms ($mg.kg^{-1}$ lipid) exposed to artificially contaminated sediments. In 1994 lugworms were also exposed to a Rotterdam harbour dredged sediment. Males were not found in all treatments in July '94. Sediments to which the measured PAH compounds were added are given in bold. FAN = fluoranthene; F = females; M = males; ? = non-gravid individuals

Treatment	Analysis for	September '93			March '94	July '94			September '94		
		?	F	M	?	?	F	M	?	F	M
Control	FAN	1.0	2.3	1.1	3.3	2.3	2.5	2.8	1.7	1.8	1.0
FAN	**FAN**	**1.3**	**26.8**	**16.0**	**7.5**	**5.1**	**16.9**	**-**	**5.9**	**13.0**	**6.3**
BaP	FAN	1.6	3.3	1.6	3.2	3.4	4.6	3.0	1.6	3.1	2.5
Mixture	**FAN**	**7.6**	**12.8**	**11.1**	**14.4**	**3.4**	**1.5**	**-**	**0.5**	**8.7**	**9.7**
Rotterdam	FAN					5.0	17.2	-	8.6	7.4	9.7
Control	BaP	0.3	0.2	0.3	0.3	0.4	0.5	0.6	0.3	0.3	0.4
FAN	BaP	0.2	0.3	0.3	0.3	0.2	0.6	-	0.3	0.4	0.2
BaP	**BaP**	**7.9**	**6.1**	**9.4**	**6.4**	**8.5**	**2.6**	**5.7**	**5.4**	**11.3**	**7.2**
Mixture	**BaP**	**6.6**	**6.0**	**7.4**	**4.8**	**7.0**	**4.3**	**-**	**3.8**	**6.0**	**5.1**
Rotterdam	BaP					3.2	4.8	-	3.6	0.7	1.5

Lugworms exposed to spiked sediments accumulated significantly more FAN (Anova, $p<0.001$) and BaP (Anova, $p<0.001$) compared to lugworms exposed to the control sediments (Table 13). The body residue levels of BaP were not significantly different between September 1993 and September 1994 (Anova, $p>0.05$). As would be expected considering the decrease in sediment FAN levels, the body residue levels of FAN showed a significant decrease in September 1994, compared to September 1993. However, the significance was weak (Anova, $p<0.10$), because the body residue levels of FAN in lugworms exposed to spiked sediments were still raised relative to the control exposures. There was a significant difference in FAN levels between females, males and non-gravid lugworms, especially when exposed to FAN-

loaded sediments (Anova, $p<0.05$). Such differences between females, males and non-gravid animals were not significant for the body residue levels of BaP.

Table 14: Accumulation of fluoranthene and benzo(a)pyrene in baltic tellins (μg.kg^{-1} lipid), sampled in June '93 and March '95. Sediments to which the measured PAH compounds were added are given in bold.

Treatment	Analysis for	baltic tellins '93	baltic tellins '95
Control	fluoranthene	0.67	0.32
Fluoranthene	**fluoranthene**	**1.41**	**0.25**
Benzo(a)pyrene	fluoranthene	0.38	0.51
Mixture	**fluoranthene**	**1.33**	**0.13**
Control	benzo(a)pyrene	49	83
Fluoranthene	benzo(a)pyrene	24	74
Benzo(a)pyrene	**benzo(a)pyrene**	**475**	**509**
Mixture	**benzo(a)pyrene**	**299**	**437**

In baltic tellins (Table 14), there was a significant interaction between the exposure to the sediments and the sampling date for the accumulation of FAN (Anova, $p<0.01$). Exposure to FAN spiked sediments resulted in significantly higher body residue levels compared to the control in 1993, whereas this difference had disappeared in 1995. Regarding the accumulation of BaP, no such interaction was observed. The body residue levels of BaP were significantly raised relative to the control exposures (Anova, $p<0.01$) and were similar in both years (Anova, $p>0.05$).

In 1994, lugworms were also exposed to a Rotterdam harbour sediment. Although the level of FAN and BaP in this sediment was considerably higher than that in the artificially spiked sediments (FAN 250 and BaP 167 μg.kg^{-1} dry sediment, organic matter 2.39%), this was not reflected in the levels accumulated by the lugworms (Table 13).

Fertilisation success

For the baltic tellin, spawning of females as well as males could be induced by temperature changes. The number of responding individuals differed for each treatment, but was not related to the treatments themselves, but rather to the length of time they were left drying after they were washed with tapwater. The highest response (10-11 spawning animals out of 30) was found in animals exposed to FAN and the mixture, which were collected first, while only 2 responded from the BaP treatment, which were collected last. A fertilisation assay was performed, for which eggs from females exposed to the same sediment were pooled. Histological examination of the remaining animals showed that only a few were still gravid. Most animals were either spent, or had resorbed their gametes.
The fertilisation percentages increased with time, to a maximum at ca. 17-24 hours after fertilisation. However, the fertilisation percentage started to decline in some test beakers at 24 h after fertilisation. As this was obviously caused by imperfections of the culture method, further analyses were performed with the fertilisation percentages at 17 h.
For the BaP treatment, from which only one relatively small male could be forced to spawn, the sperm concentration used was 4 to 7 times lower compared to the other treatments. The eggs

were, therefore, separately fertilised with either 1 or 5 ml of the sperm suspension. This resulted in differences in fertilisation percentages less than 5% for all treatmenst. The results for the fertilisation with 1 or 5 ml sperm suspension were, therefore, considered to be replicates. The fertilisation success for the BaP treatment was significantly (Student-Newman-Keuls, $p<0.05$) lower than for the other treatments (Figure 12).

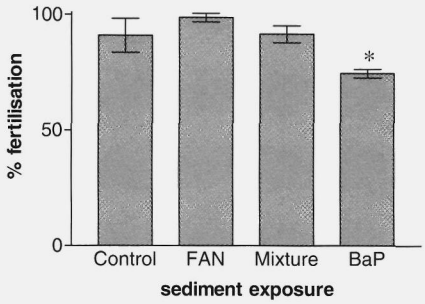


*Figure 12: Mean fertilisation success (2 replicates) of baltic tellins exposed to artificial sediments loaded with benzo(a)pyrene (BaP), fluoranthene (FAN), or a mixture of both. Error bars indicate standard deviations *: Mean fertilisation percentage is significantly lower than mean fertilisation percentage after exposure to other sediments (SNK, $p<0.05$).*

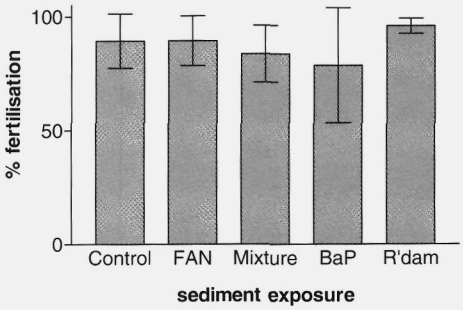


Figure 13: Mean fertilisation success of lugworms exposed to artificial sediments loaded with benzo(a)pyrene (BaP, n=12), fluoranthene (FAN, n=10), or a mixture of both (n=9), and to Rotterdam harbour dredged sediment (R'dam, n=9), compared to a control sediment (n=13). Error bars indicate standard deviation.

In total, 53 fertilisations were carried out with the lugworms. The average fertilisation percentage of lugworms exposed to the control sediment was 89.3%, varying between 60 and 100%. The fertilisation success was not significantly different between treatments (Kruskal & Wallis, $p>0.05$, Figure 13).

The fertilisation success is plotted as a function of the internal BaP concentration of females in Figure 14. Additional data from a previous experiment using Rotterdam harbour sediment were also included in the figure. Figure 14 shows that in lugworms with high body burdens of BaP, the number of individuals with a reduced fertilisation success is higher.

5.4. DISCUSSION

The present research describes the development of a new procedure for the testing of sediment-bound contaminants. The scale of the assays prompted the development of a novel spiking procedure, in which the contaminants are first added to the organic fraction, after which the spiked organic fraction is mixed with a base sediment. An advantage of this new procedure

compared to traditional spiking procedures in which whole sediments are spiked, is that the contaminants are directly added to the food-source of sediment feeding animals.

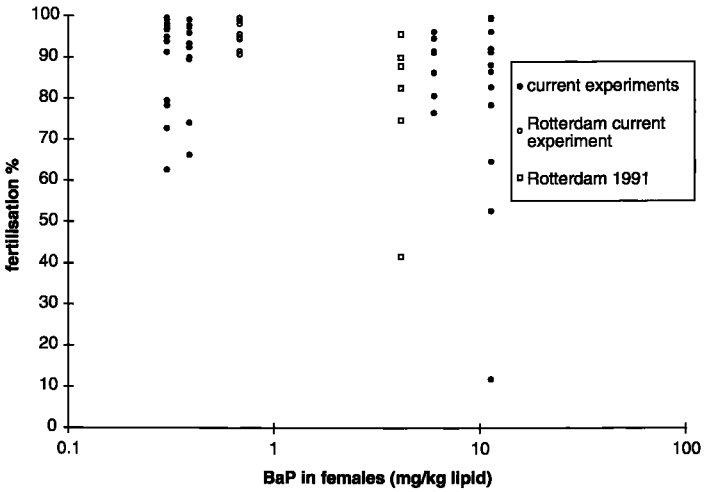


Figure 14: Relation between internal benzo(a)pyrene load in females and the fertilisation percentage, for lugworms exposed to artificially loaded sediments (dots), Rotterdam harbour dredged sediment during this experiment (circles) and to Rotterdam harbour dredged sediment during previous experiments (squares). The corresponding level of BaP in females from the 1991 experiments, was recalculated from Bowmer (1987), using ash-free dry-weight and lipid content as found in this study.

For a first screening, three PAHs differing in lipophilicity were used in order to assess the suitability of the spiking procedure for contaminants with a different affinity for sediments. The stability of the three PAHs was directly related to their logKow: fluorene disappeared within 4 months, whereas benzo(a)pyrene (BaP) levels were still slightly raised after nearly 3 years. The rate at which the PAH levels decreased is comparable to the half-lives calculated for PAH in "naturally" contaminated forest soils by Van Brummelen *et al.* (1996), but is probably faster than in natural marine sediments, in which biodegradation is mainly restricted to the oxygenated sediment surface (DeLaune *et al.*, 1981; Cerniglia, 1991). In the mesocosms, biodegradation may be enhanced by the bioturbation caused by the lugworms (Gardner *et al.*, 1979), which were held in relatively high densities with a limited sediment depth.

Exposure of the animals to the contaminated sediments resulted in increased levels of accumulated PAH compared to the control exposure. In accordance with the observations in Chapter 2, body residue levels of the PAH were higher in the bulk sediment feeding lugworm, compared to the facultative deposit feeding baltic tellin.
It is not clear whether the differences observed in PAH levels between gravid and non-gravid lugworms have any biological significance. The highest body residue levels of fluoranthene (FAN) were found in females, followed by males and non-gravid animals. These differences were more variable and not significant for BaP. The differences may be a reflection of the

variability in body residue levels between individuals within a treatment. However, part of the body burden may be eliminated through the spawning (cf. Hummel *et al.*, 1990), resulting in differences in body residue levels between gravid and non-gravid animals. Moreover, males and females may have different accumulation and elimination characteristics (Rossi & Anderson, 1977; based on fresh weight) and show a different post-spawning behaviour (Farke & Berghuis, 1979a), which might result in differences between the non-gravid animals themselves. Further research is needed in order to clarify whether the observed differences are meaningful.

Compared to previous experiments using similar harbour sediments (Bowmer, 1987), body residue levels of PAH were relatively low in lugworms exposed to the Rotterdam harbour sediment. This may have been caused by the mixing of the original harbour sediment with sand in order to decrease the organic matter content (McGee *et al.*, 1993), or it may be the result of ageing of the sediment (Alexander, 1995) as it was used in other experiments prior to those reported here, in which negative effects on reproductive parameters of bivalves were observed (Timmermans *et al.*, 1996).

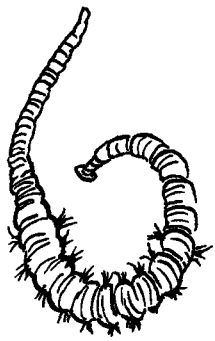
There appeared to be a significant effect by BaP on the fertilisation success of baltic tellins. However, exposure to the mixture sediment with similar BaP concentrations did not result in effects on the fertilisation success. Caution should, therefore, be exercised when interpreting these results. The IVF tests with baltic tellins are not fully developed yet and only a limited number of fertilisations was performed, especially with BaP exposed animals.
No significant effects of either BaP or FAN were observed on the fertilisation success of lugworms. However, when the individual fertilisation results are plotted against the BaP level in females, it can be seen that differences between treatments seem to exist (Figure 14). Although the majority of females tested showed a good fertilisation success (>80%) in all treatments, the incidence of females showing a reduced fertilisation success increased with increasing levels of BaP. This effect is reinforced when the results of exposure to Rotterdam harbour sediments (in this and previous experiments) are added to the plot. This is rather suggestive with respect to the effects of BaP, since the harbour sediments contain a complex mixture of contaminants which may act on the fertilisation success. It does, however, demonstrate that contamination may not merely result in a general decline in fertilisation success, but rather in an increased incidence of individuals in which reproduction may fail as well.

Lugworms may be protected against the direct effects of BaP due to the immobilisation of this contaminant in the lipid reserves, from which it will not be released in any quantity until the lipids themselves are mobilised. If the kinetics of diaromatic hydrocarbons in the polychaete *Neanthes arenaceodentata*, as reported by Rossi & Anderson (1977), are representative of the kinetics of BaP in lugworms, this would happen 4 to 5 days after fertilisation, when the swimming trochophore larvae transform into settling worms. Unfortunately, this transition is accompanied by a large mortality in all treatments, which renders a quantitative analysis impossible.

The results of this experiment should be seen in the light of the establishment of sediment quality criteria. The Dutch Maximum Permissible Concentration (MPC) for benzo(a)pyrene in sediment is 2.5 $mg.kg^{-1}$ dry "standard sediment", with 10% organic carbon

(Min. VROM, 1991; Min. V&W, 1994). This MPC is based on a calculated aquatic no observed effect concentration, divided by an assessment factor of 10, and using equilibrium partitioning (Van de Meent *et al.*, 1990; Van de Meent & Toet, 1992). Using the correction formula given (Min. VROM, 1991), the MPC for the sediments used in the present experiments is estimated to be 0.5 $mg.kg^{-1}$ dry sediment.
In this experiment, significant effects were found using artificial sediments with a benz(a)pyrene concentration of ca. 0.03 $mg.kg^{-1}$ dry sediment. Even though not all organic matter will comprise organic carbon, this concentration is lower than the MPC and equals the target value of 25 $\mu g.kg^{-1}$ dry sediment (Min. VROM, 1994). This indicates that caution should be exercised when using aquatic toxicity data and equilibrium partitioning to calculate safe levels for sediments, especially where lipophilic and persistent compounds such as benzo(a)pyrene are concerned. In order to estimate the actual risk of contaminated sediments, true sediment feeding animals, like the lugworm, should be used to assess the bioavailability of the contaminants present. Effects related to these internal concentrations may be assessed by a fertilisation assay such as that performed in this study, or by other sediment toxicity tests, even with different organisms. Considering the effects observed and the stability in the spiked sediments, BaP seems to be a suitable model contaminant for further research using an extended range of concentrations.

Chapter



Bioaccumulation and ecotoxicity to lugworms by benzo(a)pyrene spiked to sediments in a mesocosm experiment

with M.C.Th. Scholten and N.M. van Straalen

6. Bioaccumulation and ecotoxicity to lugworms by benzo(a)pyrene spiked to sediments in a mesocosm experiment

Abstract

Field contaminated sediments typically show a large variation in contaminant bioavailability, thereby making it difficult to assess the risk these sediments pose to the environment. Contaminant body burdens in sediment feeding invertebrates reflect the bioavailable fraction of sediment bound contaminants and may, therefore, be more closely related to environmental effects, provided that the associated effects are known. The sediment feeding lugworm, *Arenicola marina*, and the suspension feeding mussel, *Mytilus edulis*, were exposed to artificially prepared sediments, which had been spiked with BaP in mesocosms. An *in vitro* fertilisation assay was used in order to assess whether the resultant body residue levels were able to reduce the fertilisation success of the lugworm. The body residue levels of BaP in the mussel were clearly related to the levels of BaP in the sediment, showing that the spiked contaminants were more readily available for uptake than contaminants in field sediments. The body residue levels of BaP in the lugworm were one to two orders of magnitude higher than in the mussel, demonstrating the importance of sediment feeding. No effects were found on the fertilisation success. This may be caused by insensitivity of the IVF assay, but also by insensitivity of the lugworm, as it is probably not able to metabolise BaP into more toxic products. *A. marina* could thus be a suitable indicator of the bioavailable fraction of sediment bound contaminants, which could induce effects in more sensitive species.

6.1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) occur worldwide in marine ecosystems (Mix, 1984) and due to their lipophilic nature they have a strong tendency to sorb to suspended matter which will eventually sedimentate. Sediments, therefore, are a final sink for PAHs in the marine environment, especially for the more persistently high molecular weight PAHs with four or more aromatic rings (Stebbing & Harris, 1988; Shiaris, 1989). The risk that these sediment-bound PAHs pose to benthic invertebrates depends on their bioavailability. The bioavailability of PAHs, however, cannot simply be predicted from PAH levels in sediments. PAHs from different sources have very different sorption characteristics. Pyrogenic PAHs, usually present in soot particles, are much less bioavailable than PAH directly originating from oil or oilproducts (Readman *et al.*, 1987; McGroddy & Farrington, 1995; Gustafsson *et al.*, 1997). Moreover, PAHs become less bioavailable after prolonged contact times with the sediment (Alexander, 1995). On the other hand, direct ingestion of sediment will result in higher body residue levels in sediment feeding organisms compared to suspension feeding organisms (Knezovich *et al.*, 1987; Suffett *et al.*, 1994; Chapter 2). In order to circumvent differences in the bioavailability of PAHs between different sediments and organisms with a different mode of feeding, the risk of sediment-bound contaminants should be related to body residues. This allows for a better estimate of the toxicity inside an organism, provided that the contaminant is not metabolised (McKim & Schmieder, 1991; McCarty *et al.*, 1992; Van Straalen, 1996).

In a previous paper, a newly developed method for spiking marine sediments with PAHs was described (Chapter 5). The method appeared to be especially suitable for long-term studies on the accumulation and effect of very lipophilic PAHs with a logKow of 4 or more. The results of this study also suggested benzo(a)pyrene might have a negative effect on the reproductive success of sediment feeding invertebrates. Little is known about the direct toxic effects of BaP to benthic invertebrates. Studies with aquatic organisms indicate that BaP shows little direct toxicity within its natural solubility range (Mekenyan *et al.* 1994), as was predicted in QSAR studies (Veith *et al.*, 1983). However, an LC50 very close to the solubility limit in saline water can be inferred from Swartz *et al.* (1995) and, thus, effects may occur as a result of the combined exposure to water and sediment.
Sediments spiked with a series of BaP concentrations were prepared in order to study the accumulation and effects of BaP on some important marine benthic invertebrates from Dutch coastal waters, which, as far as we know, are unable to metabolise BaP to a significant extent. The results of a study, in which the accumulation of BaP in mussels and lugworms was assessed after exposure to the BaP-spiked sediments in mesocosms are presented in this paper. An *in vitro* fertilisation assay was used in order to assess whether the resultant body residue levels were able to reduce the fertilisation success of the lugworms.

6.2. Material & methods

Artificial sediments were prepared in January/February 1995 following the procedure described in Chapter 5. Dried and milled seaweed, *Gracilaria*, was mixed with coarse sea sand (grain size more than 60% >300µm) with a low (0.2%) organic matter content. The seaweed was collected along the shores of the Wadden Sea in November 1994 and had an ash-free dry-weight (ADW) of 51.9±2.3%. The nominal organic matter content (measured as ADW) of the artificial sediment was 1.01%. Benzo(a)pyrene, dissolved in DMSO, was spiked to the seaweed before this was mixed with the sand.
The artificially prepared sediments were stored in mesocosms with just enough seawater to be submerged, in order to enhance degradation of the seaweed. After two weeks, the pumps were started and the water was continuously refreshed and aerated.
The sediments were dosed with concentrations close to 0, 1.8, 6, 18, 60 and 180 mg benzo(a)pyrene per kg dry organic matter. The control was dosed with DMSO only. The nominal concentrations, including the background concentration in the sea sand, are given in Table 15. As the aim was to establish the concentration-response relationship, the set-up of the experiment followed a regression design. Rather than allocating the limited number of mesocosms to replicates of a few treatments, it was decided to maximise the number of exposure concentrations and to use no replicates.

Lugworms, *Arenicola marina*, and mussels, *Mytilus edulis*, were exposed to the sediments during the winter of 1995/1996 and the summer of 1996 (see also Table 16) in order to assess accumulation, survival and growth. After the exposure in summer 1996, lugworms were also used in an *in vitro* fertilisation (IVF) assay, in order to assess the influence of BaP on the fertilisation success.
To ensure natural mixing of the artificially prepared sediments, lugworms were introduced in

the summer of 1995. These lugworms were removed and not further analysed in September 1995, before newly collected lugworms were introduced for the exposure during the winter.

The lugworms were spread out on the sediment surface and were left to find their own preferred position. The mussels were suspended above the sediment in baskets, according to the procedures for active biological monitoring (De Kock & Kramer, 1994). The baskets were positioned perpendicular to the water inflow on each side of the mesocosm.

For chemical analyses, a single subsample was taken from pooled samples from each treatment at each sampling date. From the sediments, 5 randomly taken cores were pooled. At least 10 lugworms and all of the mussels were pooled. A subsample was taken after the pooled sample was thoroughly homogenised. The lugworms were left in clean sea water overnight in order to purge their gut, before being processed for chemical analyses.
Previous research indicated that there might be a sex related difference in accumulation characteristics (Chapter 5). For an assessment of the relationship between accumulated BaP and fertilisation success, only females were used for chemical analyses in September 1996. In March, sexes were not analysed separately because at that time of the year they cannot be discriminated from each other.

The variation of the BaP concentrations among the lugworms, mussels and sediments within the same treatment was estimated by analysing multiple samples within a treatment. From the sediment containing the highest BaP concentration, 5 sediment cores were analysed separately. The lugworms were randomly divided into 5 groups of 10 individuals each. Each group was analysed separately. Too few mussels from the mesocosm containing the sediment with the highest BaP concentration survived the severe frost of December 1996. Therefore, mussels were sampled from the mesocosm containing the sediment with the second highest BaP concentration. The surviving mussels from each basket were divided into two groups of nearly equal size and the resulting four groups were analysed separately. The coefficients of variation for the BaP concentration in the sediment (organic matter normalised) and in the lugworms and the mussels (lipid normalised) were 16.6%, 17.1% and 18.2% respectively. This was less than the (expected) differences between treatments and comparable to the variation between replicated mesocosms measured in previous experiments (10-30%, unpublished results). Pooling of the samples for chemical analysis, as described above, seems justified, therefore.

The ash weight of the animal tissues was determined by ashing dried samples in a muffle furnace for 4 h at 600°C. The ash weight of the sediments was determined by ashing dried samples in a muffle furnace for 4 h at 450°C. The ash-free content of the sediments was used as an estimate of the percentage of organic matter present in the samples. Since a temperature of 450°C is well below the temperature at which carbonates will volatilize (Hirota & Szyper, 1975) and the artificially created sediment was nearly free of clay, it is assumed that this simple method gives a reasonable estimate of the fraction of organic carbon present in the sediments.
Total lipids were analysed gravimetrically, using a slight modification of the method of Folch *et al.* (1957) and Böttcher *et al.* (1959), as described in TNO (1980). Homogenised tissue samples (1-2 g) were successively extracted with mixtures of chloroform-methanol (1:1), chloroform-ethanol (2:1) and methanol-chloroform-water (48:5:47). After each step, the samples were centrifuged and the fluid fraction was collected. Finally, the chloroform fraction was separated

by centrifugation and dried at 60°C in pre-weighed beakers. The residue was then weighed. Sediment samples for PAH analysis were extracted by shaking the samples with acetone and hexane, and concentrated. Ca. 25 g of the sediment sample was first thoroughly mixed with 50 ml acetone for 10 min and then with 50 ml hexane for another 10 min. Finally, 100 ml deionized water was mixed into the sample. After separation of the hexane and acetone/water phases, a subsample of the hexane phase was dried under a nitrogen stream and taken up in acetonitrile. This procedure was validated with NBS Standard Reference Material No. 1941 (Organics in marine sediment), yielding an average recovery of 92.4±15.7% relative to the certified concentrations. Tissue samples were hydrolysed with 4 M NaOH for 3 h at 60°C. The hydrolised samples were extracted with hexane for 2 h, after which the extract was cleaned-up over a column with sodium sulphate and 10% deactivated aluminium oxide. The cleaned extract was dried under a nitrogen stream and then taken up in acetonitrile. This procedure could not be validated due to the absence of suitable reference materials. As an internal standard, 6-methylchrysene was added to the samples. Recoveries were usually 90-100% for sediments, 70-100% for mussels and 60-85% for lugworms, except for the lugworms exposed during the winter, which showed recoveries <40%. No corrections for recovery were, however, made. The samples were analysed by reverse phase high performance liquid chromatography (RP-HPLC), with fluorescence detection. The response of the detector was calibrated with a certified NBS PAH standard mixture (Standard Reference Material No. 1647).

For the IVF assays, mature lugworms were sampled from the mesocosms and cultured in the laboratory in September 1996, where they were kept individually in small containers until the females spawned. Sperm was then taken from five males exposed to the same sediment in order to fertilise the eggs. The eggs were preserved in 4% neutral buffered formalin after an incubation period of 24 h. Details of the IVF procedure for lugworms can be found in Chapter 3.

For the analysis of the effects of treatment and time (sampling), a two-way analysis of variance without replication was used. For the regression analyses, the concentrations were transformed using the common logarithm. A linear regression model was fitted through the data in order to test the significance of the regression of accumulated BaP against the sediment concentrations. Differences between slopes were tested with analysis of variance and between intercepts with analysis of covariance. Differences between intercepts were further analysed using Hochberg's GT2 method, estimated with approximate comparison intervals due to Gabriel. The fertilisation percentages were analysed with analysis of variance, after arcsine transformation. All methods are described in Sokal & Rohlf (1995).

6.3. Results

Sediment chemistry

At the first sampling, the organic matter content of the sediments was ca. 40% of the value expected on the basis of organic matter addition (i.e. 1%). This is comparable to the previous experiment, in which ca. 50% of the organic matter expected was recovered in the first sampling (Chapter 5). No further decrease was observed at subsequent samplings (Anova, $p>0.05$). The organic matter content was not significantly different between treatments (Anova, $p>0.05$).

In the control treatment and in the sediment with the lowest BaP concentration, the actual (organic matter normalised) concentrations were higher than the nominal concentrations, due to the net increase of BaP in the control and a much smaller decrease in the lowest concentration, compared to the higher concentrations. In the other treatments, bulk sediment concentrations were ca. 40% of the expected value, resulting in organic matter normalised concentrations that were very close to the nominal concentrations (Table 15). Within each treatment, the BaP levels were not significantly different between samplings (Anova, $p>0.05$), indicating that the concentrations of BaP in the sediments had remained stable within the period of observation. The average value of the three measurements was used for a comparison between concentrations in the sediment and organisms.

Table 15: Benzo(a)pyrene concentrations in spiked sediments from three samplings after the spiking in February 1995. A: Concentrations in $\mu g.kg^{-1}$ dry sediment. B: Concentrations in $mg.kg^{-1}$ organic matter.

A					
Nominal Feb '95	**Sept '95**	**March '96**	**Sept '96**	**Average**	**±st.dev**
0.47	1.64	6.29	2.10	3.34	2.56
18.2	15.3	16.5	17.4	16.4	1.0
59.5	32.5	35.9	18.8	29.1	9.0
177	98.4	52.2	57.3	69.3	25.3
591	300	293	134	242	94
1770	513	1081	647	747	296
B					
Nominal Feb '95	**Sept '95**	**March '96**	**Sept '96**	**Average**	**±st.dev**
0.24	0.30	1.40	0.55	0.75	0.57
1.99	3.74	4.23	4.09	4.02	0.25
6.08	6.38	9.20	4.94	6.84	2.16
17.8	22.9	21.7	15.3	20.0	4.1
58.7	83.3	73.2	33.2	63.3	26.5
175	128	245	210	195	60

Accumulation

Mussels and lugworms were exposed to the sediments for a period of several months during the winter of 1995/1996 and during the summer of 1996. The results are summarised in Table 16. BaP concentrations in tissues reflected the BaP concentrations in sediment. Lugworms exposed to the dosed sediments accumulated BaP to levels 10 times higher than mussels in winter and 40 to 100 times higher in summer.

In order to be able to compare the accumulation characteristics of the two species used and to assess the difference between the two exposure seasons, the concentrations in the organisms were regressed against the concentration in the sediment, using linear regression with the common logarithm of the concentrations.

*Table 16: BaP concentrations in mussels (*Mytilus*) and lugworms (*Arenicola*) after exposure during the winter of 1995/1996 and the summer of 1996. Sediment concentration in mg.kg^{-1} organic matter, average of 3 samplings. Tissue concentrations in mg.kg^{-1} lipid.*

	Mytilus		*Arenicola*	
sediment	**winter**	**summer**	**winter**	**summer**
0.75	-	0.02	0.05	0.24
4.02	0.12	0.07	1.49	7.03
6.84	0.50	0.23	4.78	15.6
20.0	2.20	1.28	21.1	50.2
63.3	3.75	4.29	38.8	160
195	32.8	10.5	292	888
lipid%	0.89±0.10	1.85±0.19	1.27±0.17	3.00±0.50
ADW%	9.40±0.58	17.12±1.40	8.97±0.49	14.79±0.44

- no data.

The slopes of the four regressions were not significantly different (Table 17a). The similarity of the slopes indicate that the fraction of BaP available to the suspension feeding mussel, was directly proportional to the fraction available to the sediment feeding lugworm, irrespective of season. This fraction seemed to increase with increasing sediment concentration since the common slope of the regressions was 1.36, which suggests that the bioaccumulation factor increases with increasing concentrations of BaP in the sediment.

Table 17: Regression statistics for the accumulation of BaP. All concentrations were logarithmically transformed. A: analysis of variance for the regressions of slopes of BaP in animals versus BaP in sediment; B: analysis of covariance for BaP in animals, using BaP in sediment as a covariable.

A	**Source of variation**	**df**	**SS**	**MS**	**F_s**
	among slopes	3	0.1485	0.0495	0.68ns
	within	15	1.0908	0.0727	
	Fcrit.05(3,15)=3.29				
B	**Source of variation**	**df**	**SS**	**MS**	**F_s**
	adjusted means	3	10.7558	3.5853	72.6632***
	error	18	0.8881	0.0493	
	Fcrit.001(3,18)=15.4				

As the slopes were not significantly different, an analysis of covariance was performed, which showed that there was a significant difference between the adjusted means of the regression lines (Table 17b). Unplanned comparison indicated that mussels exposed during the winter, or during the summer, accumulated similar levels of BaP. Female lugworms exposed during summer accumulated significantly higher levels of BaP compared to non-gravid lugworms exposed during winter and both accumulated significantly higher levels of BaP than did the mussels (Figure 15).

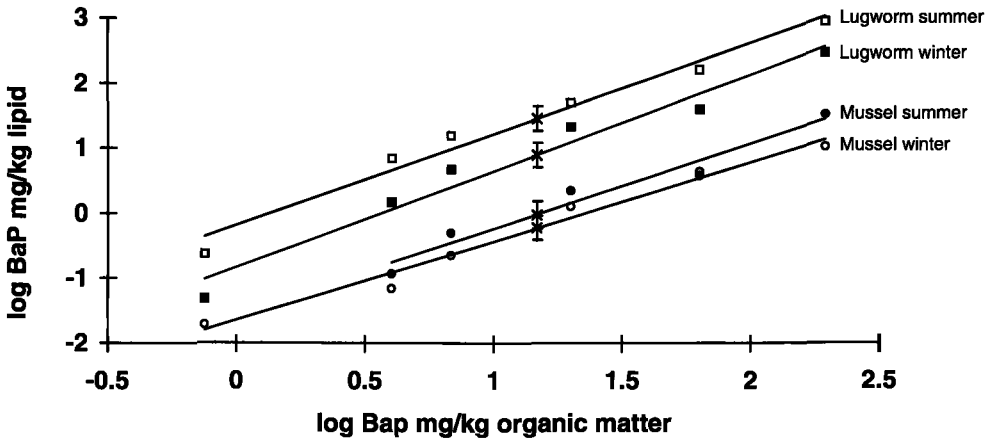


Figure 15: Regression of BaP concentration accumulated ($\mu g.kg^{-1}$ lipid) versus BaP concentration in sediment ($\mu g.kg^{-1}$ organic matter) for lugworms (squares) and mussels (circles), after exposure during winter (filled) and summer (no fill). The adjusted means are indicated with **x**, *the error bars show the approximate comparison intervals due to Gabriel. Overlapping intervals indicate that the adjusted means are not significantly different (Hochberg's GT2 method).*

Fertilisation assays

Although differences occurred in the number of animals surviving and their weight, especially after the severe winter of 1995/1996 during which the mesocosms were partly frozen, these effects were not related to BaP concentrations.

For the *in vitro* fertilisation assays with the lugworms, 274 females were collected from the mesocosms on 10 and 11 September 1996 (34 to 54 per treatment). During the following week, 61 females (21 to 24% of the number per treatment) spawned and the eggs were fertilised. The results of the *in vitro* fertilisation assays are shown in Figure 16, in which the fertilisation success of individual females is plotted against the BaP level accumulated by females. The fertilisation success was highly variable, varying from 0% to nearly 100%, with the lowest as well as the highest values found in females exposed to the highest BaP concentration. The fertilisation success was, therefore, not significantly different between treatments (Anova, $p>0.05$).

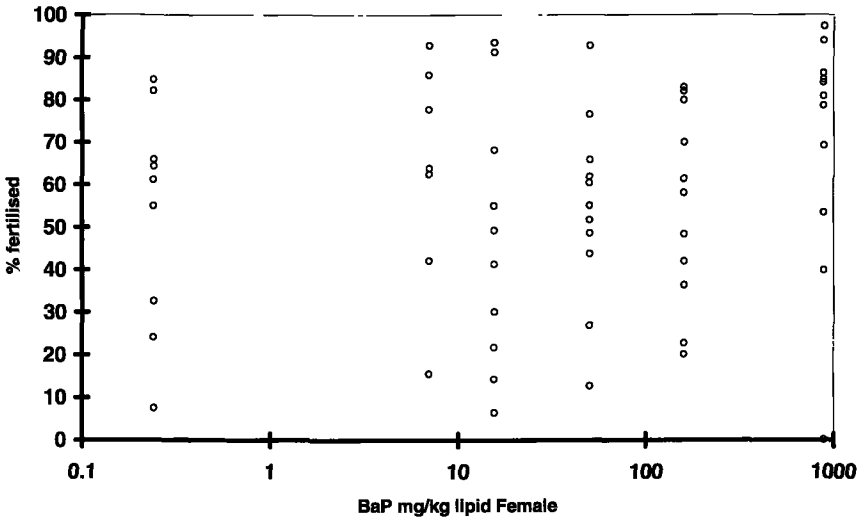


Figure 16: Fertilisation success, as measured with in vitro fertilisation assays with lugworms exposed to BaP spiked sediments for nearly 4 months, in relation to the body residue levels of BaP in the females.

6.4. Discussion

Sediment chemistry

The final BaP concentrations in the spiked sediments were rather low compared to laboratory studies, in which usually 60-100% of the added compound is recovered (Boese *et al.*, 1995; Hoke *et al.*, 1995b; Murdoch *et al.*, 1997; Boese *et al.*, 1997), although lower recoveries (40-50%) have also been reported (Douglas *et al.*, 1993; Hoke *et al.*, 1995b; Barber *et al.*, 1997). It has been suggested that these low recoveries might be due to the irreversible binding of the added compound to the organic carbon in the sediment, rendering it unavailable for the extraction method used (Hoke *et al.*, 1995b; Barber *et al.*, 1997). This would correspond with the fact that the recovery of added compounds are usually lower when the spiked sediment is allowed to equilibrate for some weeks. In our experiment, BaP was added to the sediments together with organic matter. Only ca. 40% of BaP as well as organic matter was recovered at the first sampling after ca. 7 months. This finding suggests that BaP was primarily lost due to water refreshment, together with the (dissolved and suspended) organic matter with which it was associated.

Accumulation

As was expected, the concentration of BaP in the lugworms was considerably higher than the concentration of BaP in the mussels, reflecting the closer association of the lugworms with the sediment. However, the BaP concentration in the mussels increased with increasing levels of BaP in the sediment in the same way as was observed for the lugworms, as was indicated by the similarity of the regression slopes. Contaminants in field contaminated sediments were only bioavailable to mussels, exposed in the same type of mesocosms as were used in this study, to a very limited extent (Chapter 2).Therefore, the bioavailability of the spiked contaminant appears to be higher than the bioavailability of contaminants in field contaminated sediments. Differences between spiked and field contaminated sediments were also found in experiments using BaP, PCB and DDT (Varanasi *et al.*, 1985; Murdoch *et al.*, 1997; Boese *et al.*, 1997). It is thought that organic contaminants in sediments are present in two fractions, a resistant and a reversible fraction (Di Toro *et al.*, 1982; Cornelissen *et al.*, 1997). The resistant fraction increases with prolonged contact times of the contaminant with the sediment (Alexander, 1995; Carmichael *et al.*, 1997), resulting in a lower bioavailability of contaminants in field sediments, compared to spiked sediments. Effects of ageing were not observed within the experimental period reported. Concentrations of BaP were the same following either the winter or the summer exposure for mussels. For lugworms, the concentrations of BaP were even higher following the summer exposure, compared to the preceding winter exposure. This is probably due to the fact that only mature females were analysed, in order to establish a relationship between the body burden and the fertilisation success (see below). Body residue levels of organic contaminants fluctuate during the year, in close relationship with the reproductive cycle, and as a result, the highest body residue levels can be found at the onset of the spawning season (Hummel *et al.*, 1990; Lee *et al.*, 1996; Chapter 4). Effects of ageing may occur when the prepared sediments are older than the 2.5 years in this experiment. In a previous experiment, BaP levels decreased after 3 years (Chapter 5).

Effects

Bioaccumulation increased with increasing levels of BaP in the sediment, as was indicated by the slope of the regressions (Figure 15), which were larger than 1. An explanation could be that the resistant fraction of BaP is not a constant proportion of the total BaP concentration, but rather a fixed amount, determined by the availability of binding sites in the sediment. Another explanation, not excluding the first, would be that the increasing accumulation is caused by sublethal effects of BaP, affecting the physiology and resulting in increased uptake (Landrum *et al.*, 1991).

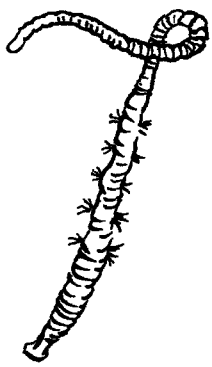
Although previous research suggested that exposure to BaP might reduce the fertilisation success of lugworms (Chapter 5), this could not be confirmed in the present experiments. On the basis of the previous research, it was suggested that effects on fertilisation success would appear as an increased incidence of individuals with reduced fertilisation success at high body burdens of BaP. In the current experiment we found an unusually large variation in the fertilisation success of lugworms exposed to the control sediment (see Figure 16), with fertilisation percentages ranging from ca. 5-85%, instead of being consistently above 80% as in the previous experiments. It is not known whether this was due to experimental factors or whether it is within the natural range of variations for this species. However, such a large basic variation will mask any effects of the treatments, unless they are very large indeed. However, large effects were not expected. McCarty (1991) suggests a lethal body burden for nonionic

hydrophobic compounds of 2-6 μmol.g^{-1} fresh weight and a chronic value ca. 10 times lower. Normalisation to lipid content results in critical body burdens of 40-160 μmol.g^{-1} lipid (Van Wezel *et al.*, 1995). In the lugworms exposed to the highest BaP concentration, a body burden of ca. 0.1 μmol.g^{-1} fresh weight, or 3.5 μmol.g^{-1} lipid was found; which is well below the suggested chronic values. Effects due to the exposure to BaP, if any, would therefore be very small and might, indeed, be physiological effects. The ability of bivalves to withstand extreme environmental conditions (low temperatures) was clearly reduced as a consequence of exposure to BaP spiked sediments (Kaag *et al.*, 1998). Additionally , BaP will mostly be immobilised in the large lipid fraction of the eggs, from which it will probably not be liberated until the larva changes from a swimming trochophore into a settling small worm after a few days (compare Rossi & Anderson, 1977). As yet, the development into this stage cannot be quantitatively assessed.

Conclusions

The spiking method used is a good alternative for the preparation of contaminated sediments for use in larger scale, long term experiments with persistent contaminants (logKow>4). After an equilibration period of ca. 6 months, the concentrations of BaP remained stable for ca. 2 years, enabling experiments comprising several seasons. No effects of BaP on the reproductive success of the lugworm, *Arenicola marina*, were observed. The lugworm might, therefore, be used in combination with sediment feeding organisms that do have the capacity to metabolise BaP and other PAHs. The latter organisms will be affected by the toxic metabolites, but will not show increased body burdens (cf. Kane Driscoll & Landrum, 1997), whereas the lugworm remains largely unaffected while its body burdens may give an indication of the bioavailable fraction of the compound.

Chapter



Discussion and conclusions

7. Discussion and conclusions

The central aim of this study has been to investigate the role of the mode of life, and more specifically the mode of feeding, in determining the exposure of benthic invertebrates to sediment-bound contaminants. This theme was addressed in Chapters 2, 5 and 6 and will be discussed in section 7.2.
A second aim was to study the effects of sedimentary contaminants and to relate these effects to internal concentrations, in order to provide a less variable risk estimate than direct chemical analysis of the sediment. This issue was addressed in Chapters 3, 4, 5 and 6 and will be discussed in section 7.3.
The characteristics and performance of the newly developed sediment spiking procedure will be discussed in section 7.1.
The thesis will be concluded in section 7.4.

7.1. Sediment spiking procedure

The marine mesocosm systems used in this thesis were developed by TNO in the 1980's and have been used and improved over the past 15 years. Although details are still amenable to improvement, this will merely affect the ease of maintenance and not their functioning. The mesocosms are filled with a 20 to 25 cm thick layer of test sediment, covered with a 50 cm layer of water. The water is continuously refreshed at a rate of $2\%.h^{-1}$, sufficient to replace the entire volume approximately every two days. The sea water is pumped from the North Sea at the harbour entrance and is filtered over a sandbed before use. Aeration is added near the bottom to ensure circulation of the water and periwinkles (*Littorina littorea*) are introduced to graze macroalgae from the inner walls of the mesocosms. Several species, among which *A. marina*, *M. edulis* and *M. balthica*, were extensively tested in bioaccumulation studies using natural sediments, dredged materials, treated dredged materials, fly-ash and drill cuttings. The results of these experiments have been published in several reports and articles (Marquenie *et al.*, 1985a; 1985b; 1986; Bowmer, 1987; 1989; Bowmer & Scholten, 1990; Jenner & Bowmer, 1990; Bowmer *et al.*, 1991; 1993; 1994; Foekema *et al.*, 1998; Kaag *et al.*, 1998).
For the research presented in this thesis, a new method for spiking sediments is described in Chapters 5 and 6 and was partly used in Chapter 2. The techniques currently used for spiking (organic) contaminants to sediments in laboratory experiments are not suitable for use in mesocosm experiments. Typically, the contaminant is spiked to the whole sediment, either directly, or by coating it to the wall of a glass jar before the sediment is added. The sediment is then thoroughly mixed, usually by rolling on a roller bank for a number of days (Lee *et al.*, 1989; Hill *et al.*, 1993; Stewart & Thompson, 1995; Murdoch *et al.*, 1997). These procedures are not feasible for the mesocosms, as each mesocosm contains ca. 600 kg dry sediment, which cannot be processed in vessels on a roller bank. Therefore, a two-step procedure was developed (Chapter 5), based on the observation that, within the sediment, organic contaminants are predominantly associated with the organic fraction, which is the food source of sediment feeding organisms (Lee *et al.*, 1990; Harkey *et al.*, 1994a). First, the organic contaminant, in this case PAH, was spiked to an organic carrier using DMSO as a solvent, and then the spiked

organic carrier was mixed with sea sand with a low organic content. The choice of the organic carrier is an important issue, since it is supposed to be the food source of the sediment feeding organisms. Ideally, natural organic matter, in the form of deposited detritus, should be used. This mainly consists of fine organic particles with an abundant microflora and can be used immediately. It is, however, difficult to find sufficient amounts of contaminant free detritus or organically rich sediments and, therefore, macroalgae (sea weed) was used, which can often be found in autumn floating in the sheltered waters of the Dutch Wadden Sea at the lee side of dikes. The green macroalgae, *Ulva sp.*, and the red macroalgae, *Gracilaria verrucaria*, were used as organic carriers. Macroalgae are not suitable as a direct food source for the organisms used in the experiments (Rijken, 1979) and the artificial sediment had thus to mature for some months in order to facilitate decomposition of the algal fragments. During this period, lugworms were added to provide a natural, and very intensive, means of reworking the sediment. These lugworms were removed before experimentation commenced.

When fluoranthene was spiked to sediments using the above described procedure, the concentrations were initially >100% of nominal concentrations, but later decreased to background levels (Chapter 5). The final concentrations of BaP were, however, 40% (Chapter 6) to 70% (Chapter 5) of nominal concentrations and remained stable. In laboratory experiments, using conventional spiking procedures, spiking efficiencies of 40 to >100% may be observed, with recoveries commonly being >70% (Landrum *et al.*, 1991; Douglas *et al.*, 1993; Boese *et al.*, 1995; Hoke *et al.*, 1995b; Stewart & Thompson, 1995; Barber *et al.*, 1997; Boese *et al.*, 1997; Ciarelli *et al.*, 1997; Murdoch *et al.*, 1997). The relatively low recoveries found in the mesocosms may have been due to the use of fresh macroalgae as an organic carrier. It has been shown that a fresh macrophyte (viz. *Zostera*) possesses different sorption characteristics for fluoranthene compared to organic matrices that have been subject to decomposition, resulting in higher concentrations in the pore water (DeWitt *et al.*, 1992). Although initially high recoveries of fluoranthene were found in the mesocosms, the later decrease in fluoranthene concentrations may have been caused by the low sorption of fluoranthene to the macroalgae, causing it to be washed out of the sediment by the bioturbation and irrigation (see section 7.2) of the sediment by the lugworms. Furthermore, fluoranthene may desorb from the macroalgae during decomposition, resulting in additional losses. However, these processes cannot explain the behaviour of BaP in the mesocosms. Most BaP was typically lost in the first few days after water refreshment was started (Kaag *et al.*, 1997) and the concentrations remained stable for a period of 2 to 3 years, with only little additional loss during the period in which the decomposition of the macroalgae occurred (Chapter 5 and 6). An important factor determining the spiking efficiency of BaP in the mesocosms may, therefore, be the length of the stabilisation period between spiking and the commencement of water refreshing, especially since most laboratory tests are static. A period of at least one month is suggested in order to facilitate optimal equilibration of the spiked contaminant between sediment organic matter and water (Boese *et al.*, 1997). In the mesocosms, water was added after two weeks of stabilisation. A longer period might result in increased sorption of BaP to the organic matter. The fact that the losses of BaP were nearly the same as those for organic matter, suggests that BaP is predominantly washed out of the mesocosms in association with dissolved and suspended organic matter. A longer stabilisation period, without water refreshing, might facilitate increased settlement of suspended organic matter to the sediment.
Due to the close association of BaP with the organic matter, the final sediment concentrations were very close to the nominal concentrations when they were normalised to organic matter

content. In that respect, the recoveries of BaP were very good. The spiking efficiency of less lipophilic compounds, such as fluorene and fluoranthene, might be improved by using a more decomposed organic carrier (litter), in order to eliminate losses due to the decomposition of the macroalgae.

A marked difference was observed in the availability of contaminants from 'naturally' contaminated field sediments as were used in Chapter 2 and spiked sediments as were used in Chapter 6. This is best illustrated with the clear dose dependent accumulation of BaP by mussels as was seen in Chapter 6, whereas the concentrations of PCBs in mussels exposed to the field contaminated sediments were mainly determined by the quality of the inflowing water (Chapter 2). This might be caused, in part, by differences in the sediment composition of the artificial sediment used for spiking and the field sediments. It is a well known phenomenon that spiked contaminants are more readily available for uptake than contaminants in field sediments (Oliver, 1985; Varanasi *et al.*, 1985; Readman *et al.*, 1987; Boese *et al.*, 1997; Ten Hulscher *et al.*, 1997; Thompson, 1997). The reduced bioavailability of organic contaminants in field sediments, compared to spiked sediments, has been attributed to ageing (Karickhoff & Morris, 1985; 1987; Landrum, 1989; Landrum *et al.*, 1992; Alexander, 1995). Additionally, a considerable fraction of the PAH in field sediments may not be available for uptake, because it is associated with soot particles (Prahl & Carpenter, 1983; Readman *et al.*, 1984; McGroddy & Farrington, 1995; Gustafsson *et al.*, 1997). Differences in sediment composition and the age of the contamination may also have resulted in the variable accumulation of PCBs in lugworms exposed to field contaminated sediments (Chapter 2), compared to the clear dose dependent accumulation of BaP from spiked sediments (Chapter 6).

The two-step spiking procedure has not yet been thoroughly compared to the whole sediment spiking procedures used in laboratory assays. As is seen in laboratory spiking experiments, the contaminants spiked to the sediments in the mesocosms are more readily available for uptake than contaminants present in field collected sediments. A preliminary study, using the amphipod *Corophium volutator*, however, indicated that compared to whole sediment spiking, BaP might be more strongly bound to the sediment when spiked using the two-step procedure.

7.2. Mode of feeding

The equilibrium partitioning theory facilitates the use of aquatic toxicity data in calculating sediment quality criteria and the assessment of the ecological risks of contaminated sediments. Two assumptions, underlying the use of equilibrium partitioning, are of particular interest to this thesis:

1. The environmental compartments considered are at chemical equilibrium.
2. When all environmental compartments are at equilibrium, the effective exposure concentration experienced by an organism is the same regardless of exposure route.

The conditions of equilibrium are necessary in order to enable calculations with the equilibrium partitioning theory. However, these conditions may not be common in the field (Mackay, 1979; Bierman, 1990; Suffett *et al.*, 1994). When equilibrium is not present, uptake will be determined by the actual route of exposure, which, as a result of the second assumption,

cannot be assessed in equilibrated experiments (Karickhoff & Morris, 1987; Di Toro *et al.*, 1991). In order to assess the importance of the mode of life, accumulation of PAHs and PCBs in the mussel, *M. edulis*, and the lugworm, *A. marina*, exposed in the same systems was compared (Chapter 2 and 6). This clearly showed the marked influence of the mode of life on exposure: the concentrations in the lugworm being much higher than those in the mussel. Similar differences were found between the oligochaete, *Tubifex sp.*, and the suspension feeding bivalve, *Dreissena polymorpha*, in static freshwater mesocosms (Kaag *et al.*, 1998). By manipulating the availability of food to the baltic tellin, *M. balthica*, it could be shown that these differences are primarily related to differences in feeding habits (Chapter 2).

Until now, it has only been shown that feeding habits may explain differences in final body residue levels between organisms feeding from the sediment and organisms feeding from the overlying water. Within the sediment, exposure may still be determined by the concentration of the contaminant in the pore water, which may be much higher than in the overlying water. Pore water concentrations were not measured in the mesocosms, but the importance of pore water relative to overlying water may be calculated for the confined space of the experimental mesocosms. For the experiments with spiked PAH, ca. 600 kg of sand was applied to each mesocosm. At samplings during the course of the experiments, the sediments were found to contain ca. 18% water by weight, yielding a volume of 108 L water within the sediment. Lugworms pump water through their burrows in a tail to head direction. The net pumping rate (i.e. the amount of water leaving the feeding funnel) was estimated to vary from 10 $ml.h^{-1}$ at 5°C to 50 $ml.h^{-1}$ at 18°C for a lugworm with a fresh weight of ca. 4 gr (Baumfalk, 1979a). On average, ca. 150 lugworms were present in each mesocosm, pumping 1.5 to 7.5 $L.h^{-1}$, and thus refreshing the total sediment water volume each 72 h (at 5°C) to 15 h (at 18°C), whereas the overlying water in the mesocosms was replaced each 48 h by pumps (see section 7.1). Although this calculation is only approximate, it probably gives a minimum estimate of the refreshment rate due to pumping by the lugworms. Firstly, because the pumping rate used is rather low, higher pumping rates, varying from 60 to >400 $ml.h^{-1}$ have been reported (Krüger, 1964; Toulmond & Dejours, 1994; Riisgård *et al.*, 1996). Secondly, lugworms do not refresh the total volume of sedimentary water, but only the water present in the feeding funnel, which has a typical diameter of only 5 mm (Rijken, 1979).
Therefore, it may be concluded that the pore water to which sediment feeders are exposed is refreshed at such a rate that the contaminant concentration in the pore water will be equal or nearly equal to the contaminant concentration in the overlying water and that the additional uptake from feeding is determined by the ingestion of food.

Several mechanisms may be responsible for uptake through the ingestion of sediment. Belfroid *et al.* (1996) distinguish redistribution and digestion. For redistribution, the uptake processes in the gut are essentially the same as on the outside of the organisms. Increased uptake due to feeding is the result of the continuous ingestion of fresh sediment (and pore water), whereas the pore water surrounding the organisms is rapidly depleted. It is questionable whether this mechanism plays an important role, at least with respect to the organisms considered in this thesis. As has been discussed above, the sediment in the feeding funnel of *A. marina* is irrigated so heavily that the concentrations in the pore water will be the same as in the overlying water. Similarly, *M. balthica* processes large amounts of overlying water when sucking up food from the sediment surface (see Chapter 1). For its North American relative, *M. nasuta*, it was shown that pore water was of only minor importance compared to the overlying

water and contaminants were mainly taken up from ingested sediment particles (Winsor *et al.*, 1990; Boese *et al.*, 1990).
Through digestion, the amount of organic matter in the ingested sediment is reduced. As a result, the uptake will increase, because the concentration of contaminants in the remaining organic matter increases. This view is supported by observations that organic carbon normalised contaminant concentrations in the faeces are higher than in the bulk sediment (e.g. Boese *et al.*, 1996).
Additionally, a third mechanism, which may be called solubilisation, may play a role. As it is part of the digestive process, the net result is not easily distinguished from digestion, but the mechanism itself is quite different. Due to the digestive fluids produced, the chemical conditions in the gut are markedly different from those in the surrounding sediment. This may result in an increased solubility of contaminants, as was shown by Mayer *et al.* (1996), although in their experiments some *in vitro* digestion may have occurred also. The extent of solubilisation will vary between species, according to the strength and composition of the digestive fluid. In bivalves, gut pH decreases to ca. pH5 during digestion and even pH3 during intracellular digestion, resulting in increased solubility of metals and PAH (Wijayaratne & Means, 1984; Decho & Luoma, 1991; Simkiss, 1995). In lugworms, pH changes do not occur in the gut, but the digestive fluid has surface active properties, which may result in an increased solubility of PAH (Plante & Mayer, 1994; Grimberg *et al.*, 1995; Mayer *et al.*, 1996), while metals may be released in a bioavailable form by complexation with amino acids, e.g. copper by histidine (Chen & Mayer, 1998).

Both digestive mechanisms, digestion and solubilisation, will result in a higher uptake of chemicals than the equilibrium partitioning theory predicts, explaining the additivity of uptake from food sources (Knezovich *et al.*, 1987; Suffett *et al.*, 1994, Boese *et al.*, 1995; 1996; Gagnon & Fisher, 1997). The magnitude of this additional uptake is not easily predicted, as it depends on the feeding rate, gut residence time, digestive efficiency, particle selectivity and the mode of feeding. Moreover, these factors may not only vary between species, but also within species and even within individuals. Additional variation may result from differences in chemical properties between different compounds, which cannot be fully explained by differences in octanol/water partitioning (Chapter 2).
An indication of the additional uptake of contaminants through ingestion may be obtained by comparing the observed BSAF (tissue lipid to sediment organic carbon) to a theoretically predicted maximum BSAF of 1.7 to 2 (Lake *et al.*, 1987; Boese *et al.*, 1995; 1996). In *Macoma nasuta*, the BSAFs for most PCB congeners spiked to sediments were higher than the predicted maximum, even after correction for feeding selectivity, mostly being ca. 2 times the predicted maximum BSAF (Boese *et al.*, 1995; 1996). In deposit feeding *M. balthica*, body residue levels remained lower than in the lugworm *A. marina*, as is illustrated in Figure 17, indicating that higher BSAFs may be expected in *A. marina* and other bulk sediment feeders. Based on exposure alone, even higher BSAFs may be expected in selective deposit feeders, especially when the bulk of the contaminant is associated with the preferred sediment fraction (Harkey *et al.*, 1994a; 1994b; Kukkonen & Landrum, 1995b; 1996), although the final outcome will be determined by their digestive properties.

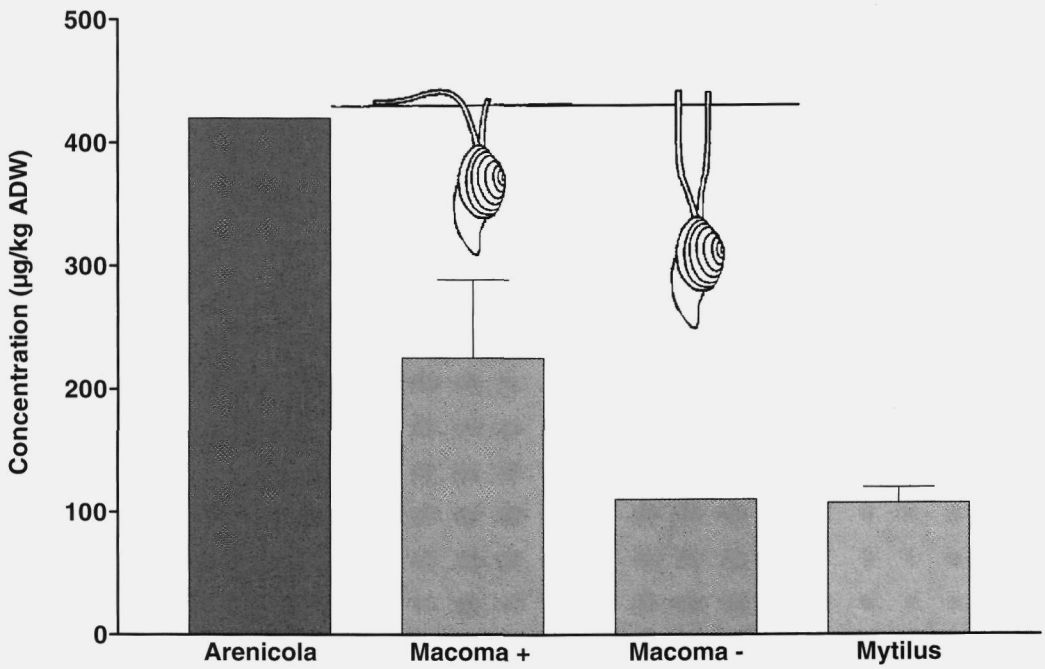


*Figure 17: Bioaccumulation of PCB-153 by the lugworm (*Arenicola marina*), the baltic tellin (*Macoma balthica*) and the mussel (*Mytilus edulis*) after exposure to sediment dredged from the Western Scheldt in mesocosms. Macoma +: deposit feeding* M. balthica *exposed together with* M. edulis. *Macoma -: suspension feeding* M. balthica *exposed without* M. edulis.

7.3. Effects

As with the spiking procedure, the *in vitro* fertilisation (IVF) assay was a newly developed technique (Bowmer *et al.*, 1991), which was technically refined in the course of the research for this thesis (Chapter 3). Its sensitivity relative to other endpoints, other species and with regard to different contaminants, however, has not been established yet.
Generally, it is thought that early life stages are the most sensitive stages in the life cycle of many organisms and, consequently, standardised early life stage tests for fish have been developed and adopted for regulatory testing (Woltering, 1984; Middaugh *et al.*, 1993; OECD, 1993). Ideally, early life stage tests should start at, or shortly after fertilisation and last until the juveniles start feeding, but for practical reasons shorter test durations are often used (Solbé, 1993). Fertilisation is the starting point of larval development and has been successfully used as an endpoint in ecotoxicological research using sea urchins (Adams, 1983; Pagano *et al.*, 1985; McGibbon & Moldan, 1986; Dinnel & Stober, 1987; Wynberg *et al.*, 1989). The early larval development of fish has been studied extensively (see Woltering, 1984; Middaugh *et al.*, 1993 for a review). Bivalve and echinoderm early larval development has regularly been

incorporated into ecotoxicological testing (Castagna *et al.*, 1981; Klöckner *et al.*, 1985; Ozretic & Krajnovic-Ozretic, 1985; Den Besten, 1991; Widdows, 1993; Pinto *et al.*, 1995). Early larvae of the lugworm *Arenicola cristata* have been tested for their sensitivity to organotin compounds (Walsh *et al.*, 1986).
Fertilisation and the early larval development of lugworms is mainly influenced by the quality of the overlying water and, therefore, has no direct relevance for the evaluation of contaminated sediments. Fertilisation success may, however, be used as a measure for the reproductive success of the adults, which are predominantly exposed to sediment bound contaminants (see section 7.2). Reproductive success seems to be one of the most sensitive parameters when testing adult fish (Woltering, 1984) and is an important parameter in the population dynamics of species (Levin *et al.*, 1996). The IVF assay with *Arenicola marina* represents a realistic exposure scenario, with adults living in contaminated sediment and fertilisation taking place in clean water. Potentially, the procedure may be extended by incorporating larval development and the settlement of larvae on "nursery" areas, as the basic requirements for the culturing of larvae and the settlement of juveniles are known (M.G. Bentley, Univ. St. Andrews, Scotland, pers. com.; Farke & Berghuis, 1979a; 1979b).

Initially, the IVF assay was used to assess the ecotoxicity of harbour dredged sediments. Compared to a reference sediment, obtained from the Dutch Wadden Sea, the fertilisation success of lugworms exposed to the harbour dredged sediments was significantly depressed (Chapter 3). However, a clear relationship with concentrations of analysed contaminants (PAHs, PCBs) could not be established. Usually, 'naturally' contaminated sediments contain a suite of different contaminants, originating from a variety of sources. The effects could be caused by contaminants that were not analysed, with a different bioavailability, or contaminants acting in combination. Moreover, the harbour dredged sediments were more fine grained and organically enriched, compared to the reference sediment, which was more typical of the sediment *A. marina* naturally inhabits. However, compared to the reference sediment, growth and maturation of *A. marina* were not retarded in harbour sediments (Bowmer *et al.*, 1991). Bioavailability probably plays an important role in determining the ecotoxicity of field contaminated sediments. Exposure of *A. marina* to a weathered harbour sediment did not result in any effects on the fertilisations success, although the PAH concentrations were even higher than in a comparable sediment used for the experiments described in Chapter 3. Analysis of the accumulation of BaP, however, showed that the bioavailability of contaminants in this weathered sediment was considerably lower than in the freshly dredged sediments previously used (Chapter 5).
In order to circumvent any interference from the sediment composition with the interpretation of test results and to enable the establishment of dose effect relationships for single compounds, lugworms were exposed to artificial sediments (see section 7.1). In a screening test, using fluorene, fluoranthene (FAN) and benzo(a)pyrene (BaP), the suitability of the spiking procedure for compounds with a different lipophilicity was assessed (Chapter 5). This test indicated that BaP might have a negative effect on the fertilisation success of *A. marina* and *M. balthica*. Subsequently, *A. marina*, for which the IVF procedure was technically the most developed, was tested on an extended series of BaP concentrations. Although BaP was readily accumulated, no effects on the fertilisation success were observed, however (Chapter 6). As is discussed in Chapter 6, it may be that the body residue levels of BaP in the lugworms were not high enough to exert toxic effects, since they were well below the chronic effects levels for neutral lipophilic compounds when they are not actively metabolised into more toxic

metabolites (McCarty, 1991; Van Wezel *et al.*, 1995). A universal extrapolation from acute to chronic body residue level, assuming equal sensitivity for different species, may not be valid, however. As was mentioned above, exposure to a weathered harbour dredged sediment did not affect the fertilisation success of *A. marina*. The same sediment, however, induced significant effects in several reproductive endpoints, including fertilisation success, for *M. balthica* and the suspension feeding bivalve *Cerastoderma edule* (Timmermans *et al.*, 1996). Furthermore, significant mortality in the bivalves *M. edulis* and *Spisula subtruncata*, exposed to the BaP spiked sediments, was observed during a severe frost period in the winter of 1996/1997, indicating an interaction between natural stress and contaminant stress (Foekema *et al.*, 1998; Kaag *et al.*, 1998). This effect was most pronounced in *S. subtruncata*, a species that is very sensitive to cold. In *M. edulis*, which is not very sensitive to frost (Seed & Suchanek, 1992), mortality was lower. Mortality in *M. edulis* probably was only observed because the frost period occurred very early that winter, as no significant mortality was observed during a frost period which occurred during midwinter 1995/1996. Nevertheless, significant effects of contaminants on the endurance of *M. edulis* to stresses they naturally experience have been observed (Veldhuizen-Tsoerkan, 1991). Although *A. marina* is only tolerant to frost to a limited extent (Sommer *et al.*, 1996) and in that respect is intermediate between *M. edulis* and *S. subtruncata*, it did not show BaP related mortality, although the body residue levels of BaP in *A. marina* were orders of magnitude higher than in both bivalves. Obviously, *A. marina* is less sensitive than bivalves, with regard to the endpoints considered. However, other endpoints may be more sensitive. As is discussed in Chapter 6, the fact that the accumulation of BaP increased with increasing levels of BaP spiked to the sediments, might indicate that as yet unidentified effects of BaP, resulting in increased exposure, were present. Furthermore, the feeding rate of *A. marina* seems to be as sensitive to sediment quality as amphipod survival (Thain *et al.*, 1996).

The IVF assay is open to improvement. Fertilisation success shows a considerable variation between individual females, which makes it difficult to discriminate between test populations (Chapter 4, 5 and 6). A highly variable fertilisation success has also been observed in the field, but this is mainly caused by the fact that sperm concentrations in the field are very variable and generally suboptimal (Williams *et al.*, 1997). In the IVF assays, sperm is extracted from the body cavity of males in order to make a sperm suspension for the fertilisation of naturally spawned eggs. This sperm has not been subjected to the final maturation step inducing the activation and subsequent spawning of free sperm (Pacey & Bentley, 1992b). Although free and active sperm may be present in the coelom (Bentley, 1985), the concentrations may vary unpredictably, causing the observed variation in the fertilisation success. As sperm extracted from the body cavity may be activated *in vitro* by adding the maturation hormone 8,11,14-eicosatrienoic acid (Pacey & Bentley, 1992b), it is possible to use quantified, active sperm suspensions for the IVF assays. This would also enable the assessment of sperm quality in relation to sediment contamination. Assessing the sperm quality might be important (Chapter 3). Exposure of adult sea stars (*Asterias rubens)* to PCBs mainly affected oocyte development in females (Den Besten *et al.*, 1990), whereas BaP and pesticides were found to affect the quality of sea urchin sperm in direct aquatic exposures (Hose & Puffer, 1983; Nelson, 1990). The eggs of *A. marina* can only be spawned after they have entered the metaphase of the first meiosis. Eggs that are not able to enter this stage cannot be spawned and will be resorbed during winter (Howie, 1961b). The effects of contaminants on the development of the eggs until the mature stage that can be spawned may thus go unobserved, as the affected eggs are

retained in the body cavity. No assessment has, however, been made of the presence of eggs in the body cavity of spent females during the IVF assays.
It might be useful to incorporate more advanced larval stages into the IVF procedure. Organic contaminants may be immobilised in the egg lipid until metamorphosis occurs, as was found for the ragworm, *Neanthes arenaceodentata* (Rossi & Anderson, 1977). The early larval development of the sea urchin, *Paracentrotus lividus*, appeared to be more sensitive than fertilisation in early life stage testing (Pinto *et al.*, 1995).

7.4. Concluding remarks

The newly developed spiking procedure, enabling the preparation of large amounts of test sediments, seems to be a suitable alternative to conventional whole-sediment spiking procedures. Using a more decomposed organic carrier, instead of macroalgae, might improve the suitability of the technique for compounds less lipophilic than BaP and would probably also reduce the maturation period needed before experimentation.

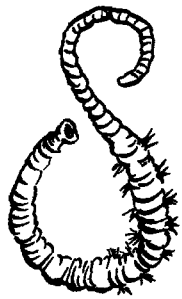
The importance of direct uptake of contaminants from ingested sediment has clearly been demonstrated. The most important mechanisms governing the uptake of contaminants associated with ingested material, digestion and solubilisation, will result in additional uptake relative to the uptake from pore water alone, because these digestive processes affect the partitioning of the chemicals and, thus, the equilibrium between the compartments involved (e.g. ingested pore water, food organic carbon and body lipids). Whether uptake from ingested food is indeed additive, or rather should be seen as an alternative route, probably depends on contaminants as well as organism characteristics. Landrum (1989) estimated that the accumulation of BaP by a freshwater amphipod (*Diporeia sp.*) could be fully explained by uptake from ingested sediment particles, whereas for other, less lipophilic compounds, uptake from pore water also played a role. Due to the large amounts of water pumped through the feeding funnel, direct uptake from the pore water probably does not play a significant role in the uptake of contaminants by the lugworm, *A. marina*.

With respect to the endpoints studied, *A. marina* is obviously less sensitive to contaminants, at least PAHs, than bivalves. The resolution of the IVF assay is seriously hampered by the variability in fertilisation success, caused by the use of immature sperm. Refinement of the procedure and the incorporation of more advanced larval stages should result in a more sensitive assay. The overall sensitivity needs to be tested against a wide range of contaminants characteristic of field sediments.

Due to its intimate relationship with the sediment and its relative insensitivity to contamination, *A. marina* is a suitable organism with which to assess the bioavailability of sediment bound contaminants. With regard to the use of *A. marina* in environmental monitoring programmes, more research is needed in order to elucidate the influence of naturally fluctuating conditions on body residue levels (Chapter 4). Comparisons of body residue levels in *A. marina* and in *M. edulis* caged near the sampling site may give some insight into the relative distribution of contaminants between water and sediment.
Since *A. marina* is not always found in relevant sediments and cannot easily be transplanted in cages, as is applied to *M. edulis* in active biological monitoring (ABM), an inverse ABM may

be applied. *A. marina* may be exposed to relevant sediments in mesocosms situated on the shore. This has the advantage that the sediments may be simultaneously subjected to a battery of sediment tests, incorporating different species and endpoints. The observed effects may then not only be correlated to bulk sediment chemistry, but also to the bioavailable fraction.

Chapter



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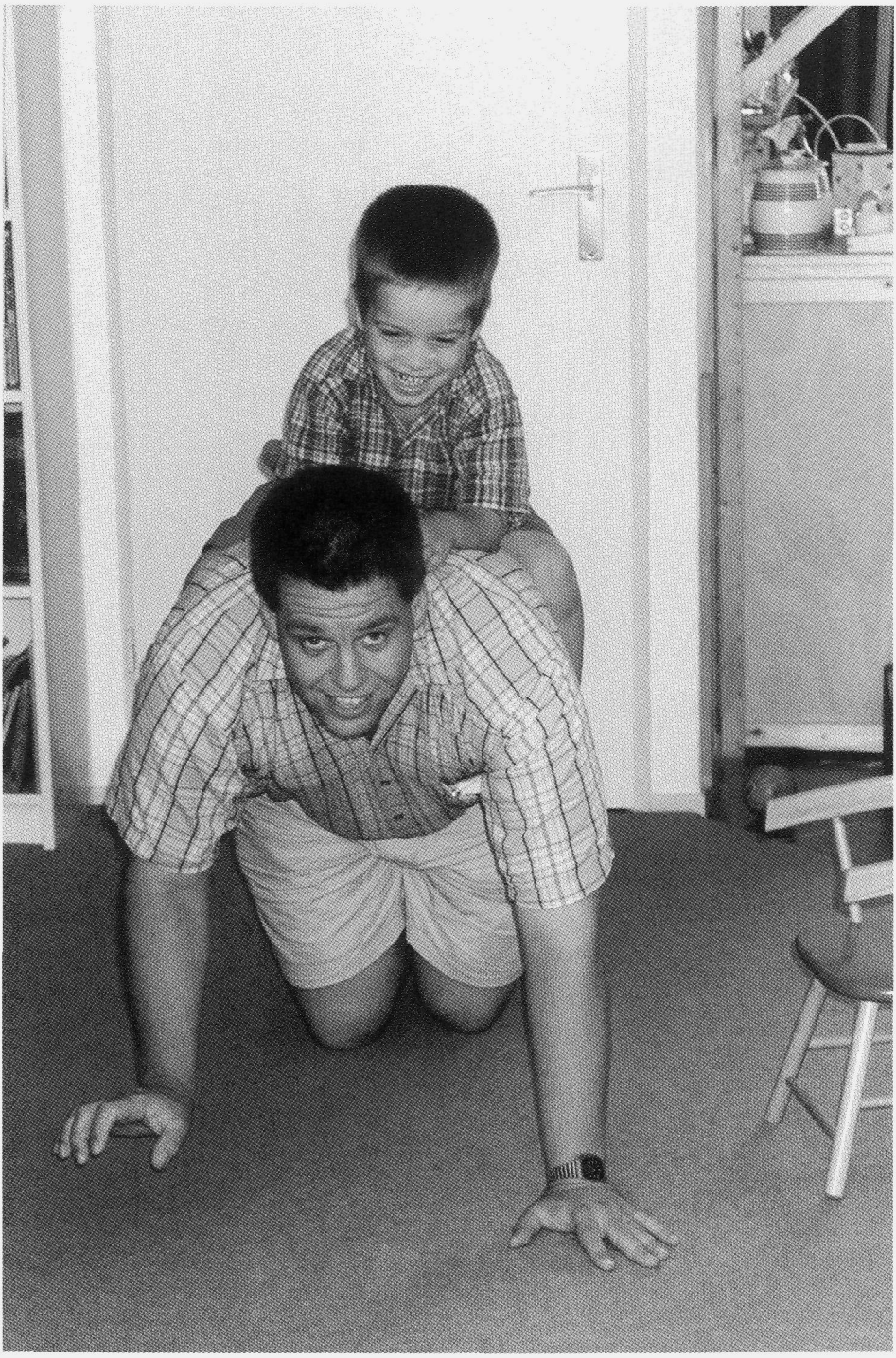

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