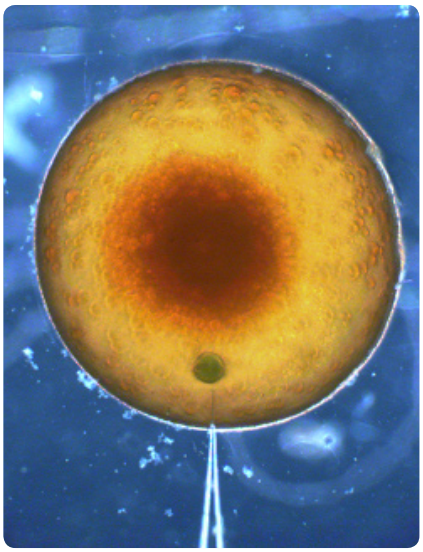
Riskbedömning av förorenade sediment

- ekotoxikologiska metoder som underlag för beslut

RAPPORT 5596 • SEPTEMBER 2006



Kunskapsprogrammet

HÅLLBAR SANERING

Riskbedömning av förorenade sediment – ekotoxikologiska metoder som underlag för beslut om hållbar sanering

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ISBN 91-620-5596-8.pdf
ISSN 0282-7298

Tryck: Elektronisk publikation

Omslagsfoton: © Gun Åkerman, Stockholms universitet (ägg från regnbåge)
© Roger Huononen, Yolda Enviromental Consulting (Sedimentpropp)

Förord

Ett av riksdagens miljömål är Giftfri miljö, och i detta mål ingår att efterbehandla och sanera förorenade områden. Brist på kunskap om risker med förorenade områden och hur de bör hanteras har identifierats som hinder för effektivt saneringsarbete. Naturvårdsverket har därför initierat kunskapsprogrammet Hållbar Sanering.

Den här rapporten redovisar projektet ”Riskbedömning av förorenade sediment” som har genomförts inom Hållbar Sanering. Projektet omfattar en kunskapssammanställning av metodik för kvantitativ riskbedömning av förorenade sediment och en erfarenhetsåterföring från två norska forskningsprojekt. Den innehåller även ett förslag till ett nytt testsystem för karaktärisering av förorenade sediment.

Projektgruppen var sammansatt av personal från WSP Environmental, Institutionen för tillämpad miljövetenskap (ITM) vid Stockholms universitet, och Norsk Institutt for Vannforskning (NIVA). På WSP Environmental fungerade Marie Arnér och Andrew Petsonk som bollplank/granskare medan Johan Persson var uppdragsledare och biträdande handledare åt Niklas Ricklund (ITM). John Sternbeck på WSP ansvarade för den slutliga redaktionella bearbetningen. På ITM var Magnus Breitholtz och Henrik Sundberg huvudansvariga. Magnus handledde examensarbetaren Niklas Ricklund samt doktoranderna Jenny Karlsson och Ulrika Dahl. Laborativt arbete med extraktioner utfördes av Kerstin Grunder, och nanoinjektioner av Gun Åkerman. Britta Eklund deltog i arbetet med slutrapporten och handledde på laboratoriet. På NIVA skrev Kristoffer Næs och Torgeir Bakke varsitt PM. Kontaktperson för Hållbar Sanering har varit Niklas Johansson på Naturvårdsverket.

Naturvårdsverket har inte tagit ställning till innehållet i rapporten. Författarna svarar själva för innehåll, slutsatser och eventuella rekommendationer.

Stockholm i oktober 2006

Innehåll

1 Sammanfattning

Projektet RAFS var huvudsakligen inriktat på ekotoxikologiska metoder och effekter relaterade till förorenade sediment. Vår utgångspunkt var att beslut som ska ligga till grund för sanering av områden med komplex föroreningsblandning bör baseras på ekotoxikologiska tester utförda på extrakt från det aktuella området. I föreliggande slutrapport sammanfattas 1/ en aktuell kunskapssammanställning, 2/ erfarenhetsåterförningar från två norska projekt samt 3/ förslag till ett nytt testsystem samt referensdata för karaktärisering av förorenade sediment. Resultaten från projektet förväntas underlätta bedömning av ekotoxikologiska effekter inför åtgärdsbeslut i pågående och framtida saneringsprojekt.

Genom en enkätundersökning konstaterades att svenska beslutsfattare (länsstyrelser) hittills mer sällan använt ekotoxikologiska test för bedömning av sedimentkvalite. Beslutsfattarna motiverar det med att det är svårt att tolka resultaten. Kunskaps-sammanställningen visar att dagens ekotoxiko-logiska riskbedömningar ofta baseras på jämförelser med riktvärden för enskilda föroreningar, och bedömningen av samverkanseffekter beaktas inte. Föreningen ”Society of Environmental Toxicology and Environmental Chemistry” (SETAC) har en väl utarbetad metodik för riskbedömning av föroreningar i sediment. Det saknas dock interntaionellt godkännande för sådan riskbedömning. EU har före-slagit ett flertal ekotoxikologiska test för föroreningar i sediment och dessa bör kunna användas mer inom svensk riskbedömning.

Förorenade sediment är en av de stora miljöfrågorna i Norge idag. Historiskt sett har punktkällor till den marina miljön skapat lokalt starkt förorenade sediment vilka nu representerar en potentiell källa för påverkan under kommande tiotals år, trots genomförda utsläppsminskningar. De norska myndigheterna har därför lanserat omfattande planer för sanerings- eller efterbehandlingsåtgärder i hamn- och fjordsediment. Sådana åtgärder är kostsamma och kräver ett besluts-underlag som ger kostnadseffektiva förslag. I Norge finns därför ett färdigt riskbedömningssystem för föroreningar i sedimenten och det kallas RAS. Systemet kommer att prövas intensivt under 2005–2006. Kontakter med norska nyttjare av systemet bör vara av stort intresse för svenska beslutsfattare.

I de starkt dioxinförorenade norska Grenlandsfjordarna har effektbiomarkörer studerats i omgångar sedan 80-talet. Trots att dioxinföroreningen har påvisats ge effekter på bland annat blåmussla och torsk så har den kunskapen inte använts som beslutsunderlag för sanerings- eller efterbehandlingsåtgärder eftersom norsk praxis kräver bevis för effekter på populationsnivån. Grunden för denna praxis är att man befarar att åtgärder kan genomföras som ändå inte riktas mot effektbiomarkörens sanna agens.

Inom RAFS-projektet genomfördes även en experimentell studie där sedimentens toxicitet från förorenade områden jämfördes med kontrollområden. Den potentiella toxiciteten av olika sediment som kan vara föremål för saneringsaktiviteter jämfördes genom att exponera och undersöka hoppkräftan Nitocra spinipes samt embryon och larver från regnbåge (Oncorhynchus mykiss). Tanken var att en integrerad tolkning av resultaten från båda biotesterna ska ligga till grund för beslut

rörande sanering av sediment. Representanter från två viktiga ekologiska organisationsnivåer – fisk och kräftdjur – inkluderades för att erhålla en god ekotoxikologisk grund.

I det kombinerade testsystemet med hoppkräfta och regnbåge så gav den komplexa blandningen av föroreningar i sedimentextrakten skador vid 70–160 gånger lägre halt än vad som observerades av en enskild miljöförening. Vidare utvecklades en ny exponeringsmetod för föroreningar med låg vattenlöslighet. Det gäller användandet av kiselgel som ”bärare” av opolära extrakt och föroreningar vid ekotoxtestning för vattenlevande organismer.

Det kombinerade testsystemet med kräftdjur och regnbåge, som provats och delvis utvecklats inom RAFS-projektet, ger information om sedimentens potentiella toxicitet med avseende på organiska föroreningar och det ger ett bra underlag för beslut om prioritering av saneringsåtgärder mellan olika förorenade sediment. Metodiken kan därmed i ett tidigt stadium av saneringsprocessen ersätta de dyrare och mer svårtolkade analyserna av kända substanser, som främst ligger till grund för prioriteringsbeslut idag. Dessutom möjliggör de förenklade testsystemen med extrakt en minskning av antalet felkällor som är kopplade till de olika sedimentens specifika sammansättning och komplexitet. Med hjälp av referensdata som redovisas i RAFS-projektet så kan nya områden testas och jämföras med starkt industriellt påverkade sediment (Frierfjorden, Örserumsviken), stadsmiljöpåverkat sediment (Riddarfjärden) och bakgrundssediment (Björkskär och Slingsviken). Resultaten från RAFS-projektet redovisas i denna slutrapport.

2 Summary

The RAFS-project was mainly focused on ecotoxicological test methods and effects related to polluted sediments. We claim that prioritizations of remedial activities of polluted sediments should, in early phases, be based on ecotoxicological data derived from tests using sediment extracts. In the current report we summarize 1) a literature review on Sediment Quality Assessment (SQA), 2) experiences from two Norwegian remedial projects and 3) a new test approach and reference data to be used in future assessments of polluted sediments.

Via an inquiry that was sent to a number of Swedish decision-makers (i.e. Länsstyrelser) it was concluded that ecotoxicological test methods are seldom or never used in Swedish SQAs. This was mainly motivated by the difficulties that these decision-makers claim are associated with the interpretation of the data derived from such tests. Our literature review also showed that today's ecotoxicological assessment of polluted sediments is mainly based on comparisons of background levels of known pollutants with literature toxicity data on single substances. Synergistic effects of pollutants in the sediments are as a consequence seldom considered. The Society of Environmental Toxicology and Chemistry (SETAC) has developed a useful methodology for SQA but this methodology has not been internationally harmonized. Within the European Union (EU) a number of ecotoxicological test methods for investigations of sediment pollution have been proposed and established over the years and these methods could be used more frequently in Swedish SQAs.

Sediment pollution is a major environmental issue in Norway today. Historically, a number of point sources released pollutants into the aquatic ecosystem, which have ultimately ended up in sediments. Although emissions from these point sources have been drastically reduced, there is a concern that pollutants present in the sediments may be available for organisms in the aquatic environment. Responsible Norwegian authorities have as a consequence introduced a number of remedial activities of sediments in polluted harbour and fjord areas. Such activities are costly and must therefore be based on decision-relevant information, giving a sound combination of ecological relevance and cost effectiveness. Norway has therefore introduced a new assessment system for polluted sediments, which is called RAS. This system will be tested extensively during 2005 and 2006. Contacts between Norwegian and Swedish decision-makers could be of great interest for adequate Swedish SQAs in the future.

In the Grenland fjords of Norway, which are heavily polluted with dioxins, a number of biomarkers of effects have been studied since the 1980's. Despite the fact that dioxin pollution has been linked to adverse effects on blue mussels and fish (cod), this knowledge has not been used as decision-relevant information in remedial activities since Norwegian praxis demands evidence of population-level effects. The reason for this is that there is a risk that measures may taken that in reality will not be directed to the true causative agent behind the effects observed.

Within the RAFS-project we also developed an ecotoxicological test approach to rank potential toxicity between different locations by investigating several exposure routes and adverse effects on different biological organisation levels. Early life-stages of two ecologically relevant tests species, i.e. the copepod *Nitocra spinipes* and rainbow trout (*Oncorhynchus mykiss*), were exposed to organic sediment extracts from both polluted and reference sites. Our results clearly show that this new approach preferably can be used as first screening step in the prioritising process of remedial activities. By using biological effects in terms of potential toxicity, numerous disadvantages that accompany mere chemical analyses may be avoided. The three potentially most toxic locations (Örserumsviken, Frierfjorden and Riddarfjärden) contains, at least partly, chemicals that act through similar toxicological pathways and are most likely structurally similar. Finally, the modified *N. spinipes* test system using silica gel as carrier was demonstrated being a sensitive screening tool of hydrophobic toxicants. Overall, the crustacean and fish results were consistent. Our results clearly show that sediment extracts from the locations that we expected to be most polluted, *i.e.* former industrial locations, also caused the most significant effects on development and mortality in early life-stages of both crustaceans and fish. Similarly, the sediment extracts from reference or low-polluted locations were not very toxic in either of the test organisms. When solely PAH concentrations in the sediments were compared, however, the most potentially toxic sediment was not the worst polluted, underlining the importance of including biological effects for reliable risk assessments.

3 Frågeställning

Naturvårdsverket, länsstyrelser, kommuner och konsulter ställs regelbundet inför uppgiften att fatta beslut om huruvida förorenade mark- och vattenområden ska saneras. Besluten om åtgärder baseras på en sammanvägning av miljö- och hälsorisker samt tekniska och ekonomiska aspekter. En begränsning i beslutsunderlaget är de betydande kunskapsluckor som finns avseende ekotoxikologiska effekter. Många frågor är otillräckligt utredda, till exempel: Vilka olika tester finns? Vilka olika effektmått eller indikationer på effekter är lämpliga i svenska förhållanden? Kan effekter av komplexa blandningar av föroreningar förutsägas?

Tiotusentals kemikalier är för närvarande i användning inom Europeiska unionen men endast ett fåtal (<100 st) har blivit tillförlitligt riskbedömda med avseende på effekter i människa och miljö (ECB, 2002). När kemikalier når recipienter kan de börja interagera, vilket kan medföra antagonistiska, additativa eller synergistiska effekter i de organismer som lever där. Det medför att enbart analys av föroreningshalter ger ett osäkert underlag för bedömning av ekotoxikologiska effekter. Eftersom kunskapen är begränsad redan för enskilda kemikaliers risker så är det uppenbart att kunskapen om deras samverkanseffekter är än mindre. I de flesta miljöundersökningar ställs man dock inför problemet att den matris som ska studeras innehåller en komplex blandning av organiska och oorganiska substanser.

När det gäller lakvatten och komplexa industriella avloppsvatten finns idag riktlinjer för hur de ska testas med avseende på potentiella effekter i miljön. Kontrollen sker vanligtvis genom att en integrerad kemisk (föroreningshalter) och biologisk karakterisering (toxiska effekter på ett antal standardtestorganismer) utförs med regelbundna intervall (NFS, 2001). Även om tanken bakom dessa undersökningar ofta är god så brister inte sällan resultatet på grund av att ”fel” testvariabler och testorganismer används. Exempelvis är det inte ovanligt att korttidstester på letala effekter i fisk, kräftdjur och alger ligger till grund för beslut som ska skydda naturliga populationer. För framförallt persistenta organiska föreningar är användandet av ett sådant testbatteri tveksamt eftersom subletala effekter (utveckling och fortplantning) efter en längre tids exponering är ett större miljöproblem.

Vidare påverkas testresultaten av föroreningarnas biotillgänglighet. Teoretiska bedömningar av biotillgängligheten i såväl vatten (Burkhard, 2000) som jord och sediment har i allmänhet en mycket stor osäkerhet – eller kräver åtminstone mycket detaljerad information om sorptionsförhållanden/isotermer (Cornelissen et al., 2005) – och tester där föroreningarna exponeras via den aktuella miljömatrisen ger också osäkerheter. Testning av extrakt från den förorenade matrisen (sediment, jord) minskar dessa osäkerheter och ger istället ett mått på potentiell toxicitet.

Föreliggande rapport är huvudsakligen inriktad på ekotoxikologiska metoder och effekter relaterade till förorenade sediment. Vår utgångspunkt är att beslut som ska ligga till grund för sanering av områden med komplex föroreningsblandning, bör baseras på ekotoxikologiska tester utförda på extrakt från det aktuella området. De huvudsakliga frågeställningarna är:

- Vilka olika tester finns?
- Vilka olika effektmått eller indikationer på effekter är lämpliga i svenska förhållanden?
- Kan effekter av komplexa blandningar av föroreningar förutsägas?

Genom projektet erhålls förutom en aktuell kunskaps- och erfarenhetssammanställning även förslag till ett nytt testsystem samt referensdata för karaktärisering av förorenade sediment. Resultaten från projektet förväntas underlätta bedömning av ekotoxikologiska effekter inför åtgärdsbeslut i pågående och framtida saneringsprojekt.

Figur 1. Bottensediment fungerar som livsmiljö för många djur och växter i basen av akvatiska och marina näringskedjor. Bilden visar bland annat död mans hand, stjöstjärnor och sjöpungar. Foto: Mikael Eriksson.

4 Metoder och genomförande

Projektet omfattade tre huvudmoment: (I) kunskapssammanställning (II) erfarenhetsuppföljning, och (III) experiment med två kompletterande ekotoxtestsystem.

4.1 Kunskapssammanställning

Det är av stort intresse för en beslutsfattare att ha tillgång till en sammanställning och utvärdering av metodik för kvantitativ riskbedömning av förorenade sediment. Ricklund (bilaga 1) genomförde kunskapssammanställningen i form av ett examensarbete vid Stockholms universitet, och han skrev på engelska för att underlätta internationell diskussion av innehållet. Examensarbetet omfattade även en laborativ del med Nitocra-testet och Niklas handleddes då av flera anställda på ITM. Utöver Niklas examensarbete så utgör föreliggande slutrapport en sammanställning av kunskap.

4.2 Erfarenhetsuppföljning

En erfarenhetsuppföljning gjordes av två norska forskningsprojekt där metoder för riskbedömning studerats. Dessa redovisningar (bilaga 2–3) syftade till att ge en överblick av det nuvarande kunskapsläget samtidigt som de ger möjlighet att se framåt och planera det fortsatta arbetet. Exempelvis via förslag om vilka utbildningar som bör anordnas inom området.

4.3 Projektet Veileder

Det ena projektet, som finansierats av norska statens forurensiningstilsyn (SFT), heter ”Veileder for risikobedømning av forurensede sedimenter”. Det har genomförts av NIVA i samarbete med Norsk geoteknisk institutt (NGI) och slutrapporterades under våren 2005. Projektet ”Veileder---” syftade till att producera ett dokument med allmän vägvisning för riskbedömning av förorenade sediment, och vi ansåg att det var mycket relevant att få en redovisning av detta.

4.4 Projektet DIG

Det andra projektet heter ”Dioxiner i Grenlandsfjordene” (DIG) och omfattade flera underprojekt som alla syftade till att ge underlag för en riskbedömning som slutligen skulle ligga till grund för beslut om eventuella saneringsåtgärder. Bland annat genomfördes platsspecifik ekotoxtestning i syfte att kvantifiera effekter av miljögifter i fjordsystemet på utvalda arter och livsstadier. Detta är intressant för att påvisa aktuell erfarenhet från arbete med ekotoxtestning i syfte att underlätta riskbedömning. Berörd personal från NIVA skrev ett kort PM om nyttan med ekotoxtestresultaten för riskbedömningen inom deras projekt.

Figur 2. Frierfjorden i Grenlandsfjordarna är en av Norges mest förorenade recipienter. Sedimenten är kraftigt förorenade av bland annat dioxiner från en numera nedlagd magnesiumfabrik (fotot). Foto: Johan Persson.

4.5 Försök med kompletterande testsystem för ekotoxdata

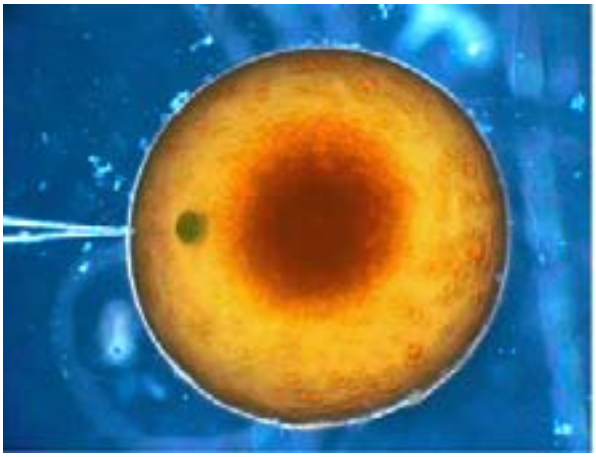
Den potentiella toxiciteten av olika sediment som kan vara föremål för saneringsaktiviteter jämfördes genom att exponera och undersöka hoppkräftan Nitocra spinipes, och komplettera undersökningen med experiment på embryon och larver från regnbåge (Oncorhynchus mykiss). Tanken var att en integrerad tolkning av resultaten från båda biotesterna ska ligga till grund för beslut rörande sanering av sediment. Representanter från två viktiga ekologiska organisationsnivåer – fisk och kräftdjur – är därmed inkluderade för att erhålla en god ekotoxikologisk grund.

Vanligtvis inkluderas även alger i detta testbatteri men för persistenta organiska föroreningar är fisk och kräftdjur ofta betydligt mer känsliga än alger. Sedimentens toxicitet från förorenade områden jämfördes med kontrollområden, varefter en relativ toxicitet erhölls. Sediment är lätta att provta och innehåller ofta höga halter av organiska miljögifter, vilket borgade för ett känsligt instrument för att upptäcka potentiell toxicitet.

Bottensediment från Örserumsviken, Slingviken, Frierfjorden, Riddarfjärden och Stockholms yttre skärgård användes. Den toxiska potentialen av Örserumsvikens sediment var av särskilt intresse eftersom det tidigare är sanerat (Örserum, 2004). Slingsviken (15 km söder om Örserumsviken) har låg föroreningsgrad och är omgiven av jordbrukslandskap och fungerade som referens till Örserumsviken. Frierfjorden är en av Europas mest dioxinkontaminerade områden och god kunskap finns om den kemiska sammansättningen av andra miljögifter såsom polyklorerade naftalener (PCN), polyklorerade bifenyler (PCB) och polycykliska aromatiska kolväten (PAH). Stockholms ytterskärgård har visserligen ett oljepåslag från fartyg, men representerar i övrigt en relativt opåverkad miljö till skillnad från den kraftigare belastade Riddarfjärden.

Isolering av exponeringslösningar erhölls genom att föroreningar från sedimenten extraherades med Soxhletextraktor (Ishaq et al., 1999) varpå det organiska extraktet delades i två delar. Den ena delen laddades på kiselgel som låg i botten av experimentbehållaren där kräftdjuren hölls. Den andra delen av extraktet löses i

triolein (naturlig förekommande fettsyra i fiskägg) varpå hexan dunstades bort och extrakten injicerades i nybefruktade regnbågsägg (figur 3).



Figur 3. Nanoinjektion av organiskt sedimentextrakt i nyberfruktat ägg från regnbåge. Foto: Henrik Sundberg.

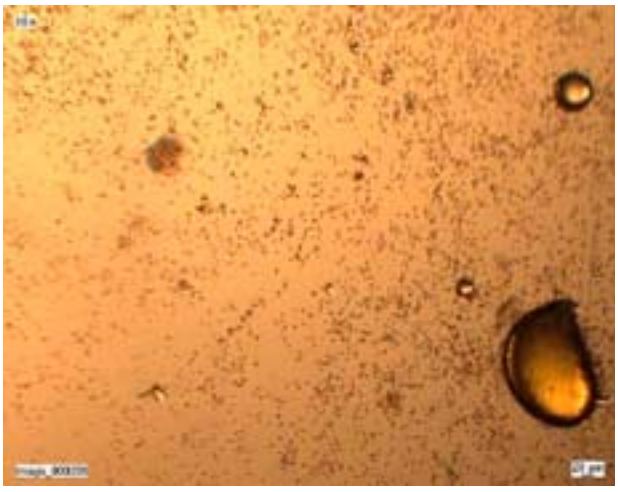
Kräftdjuret *Nitocra spinipes* (figur 4) är vanligt förekommande i brackvatten och marina miljöer, är liten (vuxen ~0.5–0.8 mm), har en sexuell fortplantning och snabb livscykel (generationstid 15–20 dagar i 20 ºC) samt är enkel att odla på lab. Arten har därför i snart 30 år använts i ekotoxikologiska undersökningar för att studera letala och subletala effekter av enskilda substanser, komplexa avloppsvatten samt lakvatten från avfallsanläggningar (Öhman et al. 2000; Breitholtz et al. 2003a; RVF 2003).

Figur 4. Kräftdjuret *Nitocra spinipes* är vanligt förekommande i brackvatten och marina miljöer, och odlas sedan 70-talet som försöksdjur på lab. Foto: Niklas Ricklund.

En organisms välmående är vanligen korrelerat till den individuella tillväxthastigheten, som kan mätas såväl i fält som på lab med olika former av biokemiska markörer, till exempel enzymaktivitet eller RNA-baserade index (Runge och Roff

2000). I kräftdjur har det tidigare visat sig vara framgångsrikt med bulkanalys av nukleinsyror (det vill säga RNA och DNA) för att estimera individuell tillväxt (Gorokhova och Kyle 2002; Gorokhova 2003) samt toxiska effekter (McKee och Knowles 1986). Dessa metoder kan också förutsäga populationseffekter i ett tidigt skede av en lång exponering (till exempel Breitholtz et al. 2003b), vilket avsevärt minskar tidsåtgången och därmed kostnaden för att ta fram relevanta ekotoxikologiska data. Den logiska grunden för att mäta RNA och DNA på individnivå baseras på att mängden RNA är nära sammankopplat med proteinsyntesen; det vill säga att dess koncentration i aktiva vävnader vanligtvis varierar med en organisms resurser som kan användas för tillväxt. DNA-mängden i kroppens celler är däremot relativt konstanta för ett specifikt utvecklingsstadium, vilket medför att den kan användas som ett index över antalet celler. Båda nukleinsyrorna är således involverade i proteinsyntesen och den cellökning som krävs för tillväxt. På så sätt kan RNA:DNA kvoten anses vara ett index över proteinsynteskapaciteten per cell (Alberts et al. 1983).

Nitocra spinipes exponerades för fem olika organiska sedimentextrakt i varierade doser (totalt 4 per sediment samt åtskiljda av en faktor 5) laddade på kiselgelen (figur 5). Försöken startades med 10–15 äggbärande honor (F_0 generation) per replikat (totalt 4 per behandling). Då varje hona generellt sett ger upphov till cirka 15 ungar per kull och kan föda åtminstone 5–6 på varandra följande kullar, kommer det i varje replikat efter cirka två veckors tid att finnas ett stort urval av varierande livsstadier. Detta erbjuder ett gott statistiskt underlag för att slumpmässigt sampla 2–3 juveniler (F_1-generation) per replikat för vidare nukeinsyraanalyser (RNA:DNA) (Gorokhova och Kyle 2002; Gorokhova 2003). Totalproteinhalter analyserades enligt Jones et al. (1996). Just de juvenila stadierna hos *Nitocra spinipes* har visat sig vara speciellt känsliga för organiska miljöföroreningar i tidigare studier (till exempel Breitholtz et al. 2003a)



Figur 5. Organiskt sedimentextrakt bildar droppar (bilden) i vatten och omöjliggör ekotoxikologiska tester. En ny metod för att undvika problemet utvecklades på ITM. Extrakten indunstades på kiselgel som fick fungera som föroreningskälla i testsystemet. Foto: Niklas Ricklund.

Sammanfattningsvis förväntades RNA:DNA-kvoterna ge ett känsligt och generellt mått på toxiska effekter på såväl individuell nivå som på populationsnivå hos *Nitocra spinipes*. Då effekter på populationsnivå ofta anses vara nödvändiga att ta fram för att utföra ekologiskt sunda miljöriskbedömningar och andra ekotoxikologiska undersökningar (OECD 1998; Forbes et al., 2001), förväntades även RNA:DNA-kvoterna erbjuda en vetenskapligt tillförlitlig men samtidigt kostnadseffektiv metodik för framtagande av just effekter på populationsnivå.

Regnbåge är en salmonid med välkänd biologi rörande enzymer, nervsystem hormonsystem *et cetera* och har därför använts som modellart i många ekotoxikologiska undersökningar. I testsystemet exponerades nybefruktade regnbågsägg genom att de framtagna organiska extrakten injicerades med nanoinjektionsmetoden (Åkerman och Balk, 1995). Denna metod härmar maternal exponering – en av de viktigaste exponeringsvägarna av lipofila föreningar hos fisk sker från moder till avkomma (Niimi, 1983) – och medför att utvecklade embryon och larver är kroniskt exponerade för kemikalierna i extraktet eftersom de livnär sig på den näring som finns i gulan. Som biologiskt system har tidiga livsstadier i salmonider visat sig vara känsligare än andra vertebratsystem för lipofila substanser (Walker och Peterson 1992). Eftersom en känd dos injicerades i varje enskilt ägg så underlättas jämförelsen av toxisk potential mellan olika områden. Trots att extrakten injiceras i äggen är kemikalierna som ger toxisk effekt biotillgängliga eftersom den toxiska effekten sker i embryots celler, det vill säga att de har passerat cellmembran. Metoden har använts i flertalet andra undersökningar (till exempel Ishaq et al., 1999) samt för att finna de potentiellt mest giftiga kemikalierna i ”Projekt Örserumsviken” (Sundberg et al. 2003), och användes delvis för beslutsunderlag inför sanering av Örserumsvikens bottensediment (Axelman et al., 1998).

Fem sedimentextrakt om tre doser (separerade av en faktor 5) injicerades i nybefruktade regnbågsägg (36 ägg i varje exponeringsgrupp). Förutom sedimentextrakt används tre olika kontrollexponeringar: i) 5 olika doser av den kända carcinogenen benzo(a)pyren, ii) ägg endast injicerade med triolein samt iii) oinjicerade ägg. Embryonen fick sedan utvecklas och mortalitetsfrekvensen dokumenterades. Missbildningar dokumenterades när regnbågslarverna kläckts. Efter ytterligare en månad när larverna förbrukat ca 2/3 av gulan avslutades försöket genom att avliva alla larver. Genom att jämföra specifika missbildningar erhölls även information om andra typer av toxiska effekter än de symptom vi observerar, till exempel ödem: en effekt av störd cirkulation; blödningar: störd leverfunktion; misssbildningar på skelett: en effekt av störd cirkulation. Om mortaliteten var lägre under embryonalutvecklingen (innan kläckning) än under gulesäckslarvstadiet (efter kläckning) tolkades det som exponering av kemikalier som blivit ”toxikologiskt aktiverade” vid metabolism.

5 Resultat och diskussion

5.1 Kunskapssammanställning

Genom en enkätundersökning konstaterade Ricklund (bilaga 1) att svenska beslutsfattare (länsstyrelser) ofta använder exponeringsanalyser, men hittills mer sällan har använt ekotoxikologiska effekttester för bedömning av sedimentkvalité. Beslutsfattarna motiverar det med att det är svårt att tolka resultaten.

Efter en litteraturgenomgång sammanfattar Ricklund (bilaga 1) att hittillsvarande sedimentkvalitetsbedömningar (SQA, ”sediment quality assessment”) huvudsakligen baseras på kemisk analys som jämförs med riktvärden för enskilda föroreningar (SQG, ”sediment quality guidelines”). Utvecklingsbehovet för kostnadseffektiva, pålitliga, ekologiskt relevanta och känsliga ekotoxikologiska tester förefaller att vara stort, och ligger också i linje med EU:s strategi för miljöriskbedömning (ERA, ”environmental risk assessment”; EU, 2003).

Ricklund (bilaga 1) diskuterar vidare att riktvärden för sediment kan baseras på såväl halter av enskilda föroreningar, som på ekotoxikologiska effekter av komplexa blandningar, och USA, Kanada, Australien och Nya Zeeland har mer utvecklade metoder för användning av ekotoxikologiska effektmått för SQG än de som används inom EU.

Vidare skriver Ricklund (bilaga 1) att föreningen SETAC (”Society of Environmental Toxicology and Environmental Chemistry”) har utarbetat förslag för hur sedimentkvalitetsbedömning bör genomföras. SETAC förespråkar att SQG används som ett av flera kemiska och biologiska verktyg, och den samlade bedömningen från dessa ska ge riktlinjer för bevisföring (LOE, ”lines of evidence”) angående sedimentets kvalitet. Dessa riktlinjer för bevisföring bör i första hand vara:

- Sammansättning och geografisk utbredning av föroreningen
- Förväntad eller acceptabel artdiversitet eller artabundans av bentisk biota i frånvaro av föroreningen
- Biotillgänglighet, bioackumulerbarhet, och potential för såväl kroniska som akuta effekter av föroreningen på akvatisk biota
- Sedimentets och föroreningens beständighet (”fate and transport”)
- Risk för att akvatisk biota och likande resurser förorenas.

Riktlinjerna för bevisföring vägs samman till en bedömning (WOE, ”weight of evidence”) som blir specifik för ett förorenat sedimentområde. Den sammanvägda bedömningen blir därmed ett verktyg för beslutsfattarna i frågan om prioritering mellan åtgärder i olika områden.

Ricklund (bilaga 1) redovisar vidare att det för närvarande finns ett flertal ekotoxtester för sediment och att EU (2003) föreskriver tester med märlkräftor (amfipoder), havsborstmaskar (polycheater), tagghudingar (echinodermater), slemmaskar (nematoder) och även med mikrokosmer där flera arter studeras samtidigt. Testen är utformade som kroniska, subkroniska eller akuta med testvariabler som till exempel överlevnad, tillväxt, reproduktion och samhällssammansättning. Det

finns även flera testbatterier som utprövats i forskningsprojekt från de Stora sjöarna i Nordamerika. Inom OECD, vars testprogram EU numera anammat, finns dessutom även ett antal akuta, subkroniska och kroniska testmetoder (t.ex. OECD Test Guidelines No 218 and 219) med mygglarver (*Chironomus tentans* och *C. riparius*) som helt eller delvis exponeras via spikade sediment och som därmed även kan vara relevanta för testning av förorenade naturliga sediment. Förutom ovan nämnda testvariabler har det visat sig att morfologiska förändringar i form av deformerade mundelar hos dessa mygglarver kan vara känsliga för miljögifter som finns i sediment.

En ny bok om riskbedömning av förorenade sediment har i år getts ut av SETAC (Wenning et al., 2005). Boken sammanfattar ”the Pellston Workshop on the use of sediment quality guidelines and related tools for the assessment of contaminated sediments” som hölls 18–22 augusti 2002 i Fairmont, Montana, USA. Deltagarna indelades i fem arbetsgrupper som fick jobba med ett av dessa fem ämnen:

1. Sammanställning av vetenskapligt underlag för olika SQG-metoder.
2. Utvärdera SQG för deras möjlighet att upptäcka effekter eller icke-effekter av sedimentföroreningar i laboratorietester och bentiska samhällsbedömningar.
3. Uppskatta betydelsen av andra tillgängliga verktyg för utvärdering av sedimentförorening.
4. Utforska vilken roll SQG och liknande kemiska och biologiska bedömningsverktyg har för beslutsfattare.
5. Uppskatta nyttan av SQG och liknande verktyg för utvärdering av sediment i akvatiska miljöer.

Boken ger en synopsis för varje diskussionsämne, samt innehåller ett flertal fackartiklar som ytterligare beskriver frågorna.
Förutom de projekt angående riskbedömning av förorenade sediment som diskuterats i föreliggande slutrapport så pågår förstås flera andra runtom i världen. I till exempel Venediglagunen (figur 6) muddras kanaler och farleder regelbundet och ett riskbedömningsprotokoll har använts sedan 1993. Därvid begränsas användningen av muddermassorna beroende på deras fysiska och kemiska sammansättning. Protokollet identifierar olika klasser av massor beroende på föroreningshalter (http://www.salve.it).

Figur 6. I italienska Venediglagunen muddras kanaler och farlder regelbundet och ett riskbedömningsprojekt finns för vidare användning av massorna. Foto: Andrew Petsonk.

5.2 Erfarenhetsuppföljning

5.2.1 Projektet Veileder

Bakke et al. (bilaga 2) redogör att de norska riktlinjerna för riskbedömning av förorenade sediment nu finns beskrivna som bedömningssystemet ”RAS” (”Risk assessment of contaminated sediments”) vilket har utvecklats specifikt för myndigheter och andra problemägare som handhar saneringsplaner för förorenade fjord- och hamnsediment. RAS är tänkt att fungera som ett verktyg för att välja ut förorenade sedimentområden för förbättringsåtgärder. Behovet av riktlinjer uppstod mot bakgrund av att det idag finns restriktioner för konsumtion av fisk/skaldjur i 31 norska fjordar och hamnområden. Flertalet restriktioner gäller fisk och blåmusslor och grundas på att de innehåller höga halter av PCB och PAH. Eftersom primärkällorna anses ha minskat drastiskt när renare industriteknik infördes på 1990-talet, så är det största problemet idag sedimentens bidrag som sekundärkällor.

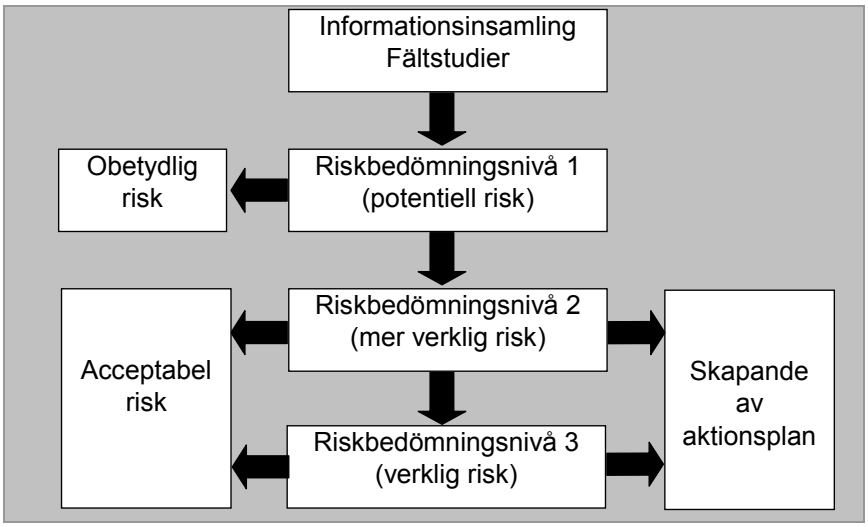
Det norska riskbedömningssystemet baseras på liknande system från Nederländerna, EU, Kanada och USA, men även riskmodeller för norska oljeborrningsprojekt har fungerat som förebild.

RAS-systemet omfattar riskbedömning av föroreningens påverkan på organismer i sedimenten och på den omgivande miljön som inkluderar människan. Systemet är uppbyggt i tre nivåer (figur 7) där steget från en nivå till en högre karakteriseras av ökande komplexitet i bedömningen, mer hänsyn till lokala förhållanden och mindre konservativa bedömningar.

På riskbedömningsnivå 1 identifieras sedimentområden som utgör en obetydlig risk för miljön, och detta görs med en minimerad arbetsinsats. Sedimentens potentiella risk baseras på uppmätta koncentrationer av ett urval föroreningar, samt generella toxicitetstest. Koncentrationerna och toxicitetsdatan utvärderas mot på

förhand givna miljökvalitetsstandarder (”environmental quality standards”, EQS). Toxicitetsdatans syfte är att täcka in kombinationseffekter samt effekter av föroreningar som inte analyseras kemiskt. Konceptet med PEC / PNEC (”predicted environmental concentration / predicted no effect concentration”) används för att utvärdera koncentrationerna och toxicitetsdatan. En kvot som överstiger 1 tolkas som en indikation på risk. Som ett krav på minsta dataunderlag för riskbedömningsnivå 1 anges att för ett sedimentområde med en area på 50 ha eller mindre så ska minst 5 sedimentlokaler provtas. Större eller topografiskt mer komplexa områden kräver gradvis större dataunderlag. För att ett område ska bedömas som riskfritt så krävs att alla individuella prover uppvisar koncentrationer och toxicitet som understiger miljökvalitetsstandarden.

Eftersom miljökvalitetsstandarden som används på riskbedömningsnivå 1 vanligen är mycket konservativ så kan det innebära att många sedimentområden klassas som riskfulla – fler än vad tid och ekonomiska resurser räcker till att åtgärda. På riskbedömningsnivå 2 genomförs därför en prioritering mellan olika områden, och den baseras på en rankningsskala som anger hur mycket miljökvalitetsstandarden totalt överskrids. Rankningsskalan baseras på ett index för potentiell risk (PRI, ”potential risk index”) och det anges för varje enskilt prov som summan av alla kvoter av uppmätta koncentrationer (eller toxicitet) och miljökvalitetsstandarden, se bilaga 2. För att PRI ska kunna användas så kräver RAS-systemet att samma variabler mätts på de sedimentområden som ska jämföras.



Figur 7. Det norska riskbedömningssystemet för förorenade sediment (RAS), efter Bakke et al.

Ytterligare moment inom riskbedömningsnivå 2 är att uppskatta i vilken omfattning föroreningar frigörs och transporteras till andra områden, risk för människor, samt risk för effekter på organismer, populationer och ekosystem. Eftersom dessa moment vanligen motsvarar ambitionsnivån i lokala och regionala miljöplaner så anser Bakke et al. (bilaga 2) att RAS-systemet kan användas till att uppskatta sedimentens bidrag till den totala miljörisken.

Föroreningsfluxen (F, mg m^{-2} dag^{-1}) längs olika exponeringsvägar/transportvägar från sedimentet till den omgivande miljön (diffusion, biodiffusion, erosion, och näringskedjetransport) och till människan (konsumtion av marin föda, sedimentintag, kallsupar med mera), samt förväntade koncentrationer i målorganismer (fisk och skaldjur) och i målbiotoper (vattenkolumnen) beräknas med hjälp av generella ekvationer och parametrar. Olika exponeringsvägar antas för olika typer av områden, till exempel rekreationsområden. Om den beräknade föroreningstransporten bedöms vara orimlig så rekommenderas tester för biotillgänglighet och bekrivningar om sådana tillhandahålls.

Den totala fluxen från sedimenten till vattenkolumnen (F_{tot}) ger därmed underlag för en uppskattning av en föroreningshalt i vattenkolumnen (C_{sv}, µg/l) och den jämförs med en miljökvalitetsstandard för vatten (HC_5, µg/l), på samma vis som för sedimenten på riskbedömningsnivå 1 (kvoten ska vara under 1). Den totala fluxen genom näringskedjor till människan (F_{bio}, mg m^{-2} dag^{-1}) ger i sin tur underlag för en bedömning av dagligt intag av föroreningar som härstammar från sedimenten (dos, mg kg^{-1} dag^{-1}). Denna dos jämförs med maximalt tolerabel livstidsintag (MTR, mg kg^{-1} dag^{-1}) och i RAS-systemet görs antagandet att 10 % av människoföda kommer från havet. Alltså accepteras att dosen får vara upp till 0,1MTR. Sedimentområden som underskrider miljökvalitetsstandarden i riskbedömningsnivå 2 bedöms därmed utgöra en acceptabel risk. De områden som inte friklassas på riskbedömningsnivå 2 bedöms vidare i riskbedömningsnivå 3, men kan också resultera i skapandet av en aktionsplan för begränsing av risken.

På riskbedömningsnivå 3 görs ytterligare förfiningar i antaganden som förefaller att vara allt för konservativa. Syftet med riskbedömningsnivå 3 är att verifiera slutsatser från riskbedömningsnivå 2, eller att göra dessa mer trovärdiga genom att tillämpa platsspecifika parametervärden i fluxberäkningarna. RAS-systemet anger inga specifikationer för hur riskbedömningsnivå 3 ska utföras eftersom den måste skräddarsys för varje enskilt fall. Möjliga moment föreslås likväl kunna omfatta till exempel mätningar av föroreningsfluxer med hjälp av sedimentfällor, bentiska kammare, mätning av platsspecifika partikel–vatten-fördelningskoefficienter, mätning av föroreningshalter i nyckelorganismer, platsspecifik numerisk modellering av ”fate and transport” och effekter av sedimentens föroreningar, identifiering av strukturen för den bentiska faunan, biodiversitet och känslighet.

Bakke et al. (bilaga 2) redovisar slutligen ett exempel på hur RAS-systemet tillämpats för riskbedömning av benso(a)pyren, PCB-153 och kvicksilver i sediment i norska Bispeviken i Oslo hamn. Kvicksilver utgjorde en acceptabel risk utifrån riskbedömningsnivå 2, medan benso(a)pyren och PCB-153 med samma bedömning var en risk både för miljö och människa.

RAS-systemet finns sedan mars 2005 offentliggjort via Statens Forurensningstilsyns hemsida (www.sft.no; Breedveld et al., 2005a–b; Systad et al., 2004) och ska testas intensivt under 2005–2006. Utvecklingen och hittillsvarande tillämpningar av RAS-systemet har påvisat flera frågor som bör besvaras bättre och de är:

- Mätning av koncentrationen i porvatten förefaller att vara en mer pålitlig metod än att uppskatta densamma från mätningar av koncentrationen i partiklar och omräkning via fördelningskoefficeienter för partikel–vatten (K_d).
- Toxicitetstesten som förespråkas för närvarande (bilaga 2) bör utvärderas för deras tillämplighet eftersom de hittills förefaller att mäta andra egenskaper än enbart sedimentets föroreningskoncentration.
- Biomagnifiering och effekter av komplexa blandningar av flera föroreningar är otillräckligt bedömt.
- Flera miljökvalitetsstandarder är provisoriska och saknas till exempel för enskilda PCB-föreningar. Det ger en obalans i bedömningen av PCB relativt PAH för vilka det finns miljökvalitetsstandarder.
- Tributyltenn (TBT) i hamnområden förefaller att ligga extremt långt över de effektnivåer som rapporteras i litteraturen. Det gör att TBT helt dominerar riskbedömningens resultat. Samma gäller för benzo(a)pyren.

5.2.2 Projektet DIG

Næs et al. (bilaga 3) redogör för att förorenade sediment är en av de stora miljöfrågorna i Norge idag. Historiskt sett har punktkällor till den marina miljön skapat lokalt starkt förorenade sediment som efter att betydande utsläppsreduktioner genomförts nu representerar en potentiell källa för påverkan som kan fortgå under tiotals år. De norska myndigheterna har därför lanserat omfattande planer för saneringsåtgärder eller efterbehandlingsåtgärder i hamn- och fjordsediment. Sådana åtgärder är kostsamma och kräver ett beslutsunderlag som ger kostnadseffektiva förslag. Grenlandsfjordarna ligger vid Porsgrunn cirka 10 mil sydväst om Oslo och har mottagit stora mängder dioxiner från Norsk Hydros magnesiumfabrik på Herøya (figur 2) som var i drift 1950–2002. Föroreningen har medfört restriktioner och kostråd för hantering och konsumtion av havsmat. Utsläppen minskades avsevärt de sista 25 åren och man trodde att kostråden skulle kunna hävas vid år 2000, men det har inte skett. Koncentrationen av dioxin har minskat i miljön, men långsammare än förväntat.

Mot bakgrund av detta finansierade Norges Forskningsråd och Norsk Hydro forskningsprojektet DIG. Syftet var att skapa kunskap som ger förståelse för kemisk och biologisk transport och effekter i Grenlandsfjordarna, men även att få fram kunskaper om generella miljöprocesser som kan användas för att förstå situationen i andra fjordsystem. Förutom grundforskning skulle kunskapen också kunna brukas som underlag för beslut om åtgärdsbehov av sanering eller efterbehandling. En slutrapport för DIG-projektet finns (Næs et al., 2004) och ett flertal delrapporter och forskningsartiklar har publicerats.

Angående biologiska effekter av dioxiner i Grenlandsfjordarna så kunde man kring 1986 när utsläppen fortfarande var relativt höga, konstatera att det bara kunde påvisas effekter i blåmussla. I mitten av 90-talet inleddes en begränsad övervakning av fisk och blåmussla. Effekter på mjukbottenorganismer kunde dittils främst tillskrivas organisk belastning, begränsat vattenutbyte och syrebrist. Övervakningsprogrammet kom att visa att både torsk och blåmussla var klart påverkade.

Angående blåmussla fastslogs att effekterna var orsakade både av saltförhållanden och dioxinbelastningen, men för torsk var dioxinbelastningen den största orsaken.

Inför DIG-projektets start fanns det alltså data som tydde på att fisken var påverkad av dioxin. Målet med projektet blev därför att fastslå om effekterna fortfarande kunde påvisas, om de var årstidsberoende, och om även andra fiskarter var påverkade – främst lax (*Salmo trutta*) och skrubba (*Platichtys flesus*). DIG-projektet medgav även en utökad möjlighet att koppla effekterna till föroreningshalter i organismerna, samt att använda fler nya testmetoder.

Biomarkörerna som studerades mättes i fisklever och innefattade aktivitet av avgiftningsenzymerna cytokrom P4501A (CYP1A), glutation S-transferas (GST), UDP-glukuronyltransferas (UDP-GT) och glutationreduktas (GR). Dessutom mättes äggviteämnet vittellogenin i blodet hos hanfisk för att undersöka östrogena effekter. Eftersom fisklarver anses vara känsliga för dioxiner så gjordes även försök med utsättning av befruktade sillägg. För slutvärderingen av mätresultaten noterades också allmänna faktorer som ålder, storlek, kön, årstid och kondition. Resultaten påvisade signifikanta årstidsvariationer för flertalet biomarkörer och även skillnader mellan den inre mest förorenade fjorden (Frierfjorden) och en yttre något mindre förorenad fjord (Eidangerfjorden). Angående silläggens utveckling kunde dock ingen skillnad påvisas mellan Frierfjorden och en referenslokal (Flødeviken), och båda testgrupperna utvecklades normalt.

Vid en jämförelse med data från andra norska kustlokaler så kunde man dock konstatera att torsken i Grenlandsfjordardne hade förhöjd aktivitet av avgiftningsenzymerna.

Svaga tecken på östrogenpåverkan hos torsk fastslogs, men den kunde inte knytas till dioxinföroreningen. Næs et al. (bilaga 3) konstaterar att det därför finns ett forskningsbehov för vidare undersökningar av dioxinpåverkan av hormonreglering i fisk.

Vidare tyder långa tidsserier på att torskbeståndet minskade i Grenlandsfjordarna på 60- och 70-talet, men på grund av databrist så kan inte heller det kopplas till dioxinbelastningen.

Næs et al. (bilaga 3) avslutar med att konstatera att biomarkörer ger information om individuell hälsa hos fisk och andra organismer. Informationen är ofta specifik nog att knytas till miljögiftsbelastning, och det gjordes delvis i Grenlandsfjordarna. Emellertid är den stora frågan om dessa effekter betraktas som så allvarliga att man ska rekommendera eller genomföra saneringss- eller efterbehandlingsåtgärder. I den frågan har det i Norge varit praxis att kräva bevis för påverkan även på populationsnivån. Biomarkörresultaten från Grenlandsfjordarna har hittills inte använts som beslutsunderlag för förvaltning. Även om myndigheter önskat göra det så har problemägarna invänt att mätningarna inte är tillräckligt ämnesspecifika, och därmed riskerar att leda till åtgärder som är kopplade till andra/fel föroreningar i utsläppet.

WSP noterar att utvecklandet av det datorbaserade verktyget SEDFLEX (SFT, 2005) för kostnadseffektiva beslutsunderlag beträffande föroreningshalter i sediment för närvarande är fokus i ett fortsättningsprojekt av DIG-projektet. Verktyget

är tänkt att användas på riskbedömningsnivå 3 i RAS-systemet (figur 7), och det bör vara av intresse även för svenska beslutsfattare.

5.3 Experiment med kompletterande testsystem för ekotoxdata

Breitholtz et al. (bilaga 4) sammanfattar att svenska sedimentbedömningar vanligen baseras på riktvärden för enskilda föroreningar, och menar att eftersom det mycket sällan har påvisats samband mellan analyserade föroreningshalter och potentiell toxicitet så finns det ett stort behov av ekologiskt relevanta, pålitliga och känsliga ekotoxikologiska tester som förbättrar beslutsunderlaget för prioritering av saneringsåtgärder mellan olika områden.

Testen med hoppkräfta och regnbåge fungerade bra och kunde påvisa skillnader i potentiell toxicitet av sedimentextrakt från de fem undersökta områdena. Den sammanvägda bedömningen visade att extrakt från Örserumsviken och Frierfjorden var mest potentiellt toxiska, följt av de från Riddarfjärden, Slingsviken och Björkskär. Det var ett förväntat resultat med utgångspunkt från att Örserumsviken och Frierfjorden är industriellt påverkade områden, medan Slingsviken och Björkskär borde vara relativt opåverkade. De industriellt påverkade sedimenten kunde alltså påvisas att vara mer toxiska än Riddarfjärdssedimentet som bör avspegla belastningen från storstadsmiljön och Mälaren. Med kännedom om enbart uppmätta föroreningshalter så hade den sammanvägda bedömningen blivit långt mer komplex eftersom det är flera olika ämnen att beakta, och dessutom mer osäker eftersom riktvärden för flera ämnen inte är så tillförlitliga (bilaga 4).

De två ekotoxtesten gav i stort sett samstämmiga resultat, men hoppkräftan indikerade att extraktet från Örserumsviken var mer toxiskt än det från Frierfjorden, medan regnbågen visade det omvända. Regnbågstestet påvisade lika toxicitet för Slingsviken och Björkskär, medan hoppkräftstestet rankade Slingsviken som mer toxisk.

Vidare orsakade sedimentextraktet från Frierfjorden en förhöjd frekvens av blödningar bland exponerade regnbågslarver. Breitholtz et al (bilaga 4) konstaterar att det är i linje med att exponering för dioxinföroreningar i andra studier gett liknande skador på fisk.

Mätningen av RNA i kräftdjuren kunde inte påvisa förändrad tillväxthastighet efter exponering av extrakt från Freierfjorden och Örserumsviken, och övriga stationer testades därför inte.

En mycket intressant observation gällande kvantifiering av samverkanseffekter är att Breitholz et al (bilaga 4) påvisade den av Sundberg (2005) först dokumenterade skadan ”assymetrisk gulesäck” i de regnbågslarver som exponerades för sedimentextrakt från Frierfjorden, Örserumsviken och Riddarfjärden, men inte i övriga stationers extrakt. Samma frekvens av assymetrisk gulesäck observerades även bland de larver som blivit exponerade för 5 mg benzo(a)pyren per kg vått ägg (positiv kontroll). De doser vid vilka skadan uppstod i testen med extrakt från provstationerna var 60 g sediment per kg vått ägg, vilket med de tidigare uppmätta halterna av PAH i dessa sediment motsvarade 31–72 µg bens(a)pyren per kg vått ägg.

Alltså uppstod skadan vid en halt av bens(a)pyren som var 70–160 gånger lägre än vad som observerades för det enskilda ämnet, och det tolkade Breitholtz et al som ett starkt tecken på att samverkanseffekter med andra ämnen sker.

Ett ytterligare mycket intressant ”spin off resultat” från utvecklandet av det kombinerade ekotoxtestsystemet med hoppkräfta och regnbåge var att Breitholtz et al (bilaga 4) tog fram en ny exponeringsmetod för föroreningar med låg vattenlöslighet. Det gäller användandet av kiselgel som ”carrier” av opolära extrakt och föroreningar vid ekotoxtestning för vattenlevande organismer. Metoden liknar den inom fysikalkemin använda generatorkolonnen som möjliggör (påskyndar) beredningen av en homogen vattenlösning av svårlösliga kemikalier genom att öka arean för kontakt med vattnet (t.ex. Hawker & Connell, 1988; Shiu et al., 1988), men har troligen aldrig tidigare nyttjats på detta vis för ekotoxtestning.

Breitholz et al (bilaga 4) påpekar också att metoden med hoppkräfta och regnbåge kan ge högre biotillgänglighet av föroreningarna jämfört med förhållandet i naturen. De vill dock understryka att syftet med testsystemet var att erhålla kunskap om potentiell toxicitet och att jämföra den mellan olika områden. Dessutom är sedimentextrakten framtagna på lika sätt vilket borgar för hög reproducerbarhet och hög jämförbarhet.

6 Allmänna rekommendationer

Ekotoxikologiska tester används i relativt liten omfattning i nuvarande svenska riskbedömningar för föroreningar i sediment. Ekotoxikologisk riskbedömning baseras ofta på jämförelser med riktvärden för enskilda föroreningar, och bedömningen av samverkanseffekter beaktas inte. SETAC har en väl utarbetad metodik för riskbedömning av föroreningar i sediment (bilaga 1; Wenning et al., 2005). Det saknas dock internationellt godkännande för sådan riskbedömning. EU (2003) har föreslagit ett flertal ekotoxikologiska test för föroreningar i sediment och dessa bör kunna användas mer inom svensk riskbedömning (bilaga 1 & 4).

I Norge finns sedan våren 2005 ett riskbedömningssystem för föroreningar i sedimenten och det kallas RAS. Systemet kommer att prövas intensivt under 2005–2006 (bilaga 2). Kontakter med norska nyttjare av systemet bör vara av stort intresse för svenska beslutsfattare.

I de starkt dioxinförorenade norska Grenlandsfjordarna har effektbiomarkörer studerats i omgångar sedan 80-talet. Trots att dioxinföroreningen har påvisats ge effekter på bland annat blåmussla och torsk så har den kunskapen inte använts som beslutsunderlag för saneringss- eller efterbehandlingsåtgärder eftersom norsk praxis kräver bevis för effekter på populationsnivån. Grunden för denna praxis är att man befarar att åtgärder då kan genomföras som ändå inte riktas mot effektens sanna agens.

Det kombinerade testsystemet med kräftdjur och regnbåge, som provats och delvis utvecklats inom RAFS-projektet, ger information om sedimentens potentiella toxicitet med avseende på organiska föroreningar och det ger ett bra underlag för beslut om prioritering av saneringsåtgärder mellan olika förorenade sediment (se även nedan). Med hjälp av referensdatan som redovisas i bilaga 4 så kan nya områden testas och jämföras med starkt industriellt påverkade sediment (Frierfjorden, Örserumsviken), stadsmiljöpåverkat sediment (Riddarfjärden) och bakgrundssediment (Björkskär och Slingsviken).

Kunskapsbehovet för ekotoxikologiska tester förefaller att vara stort i Sverige. Några termer som dykt upp inom RAFS-projektet och som kan vara bra att minnas sammanfattas i tabell 1.

Tabell 1. Ordlista för riskbedömning av förorenade sediment.

Engelska	Engelsk akronym	Svenska
sediment quality assessment	SQA	sedimentkvalitetsbedömning
sediment quality guideline	SQG	riktvärde som kan avse en enskild förorening eller ekotoxikologiskt effektmått för en komplex blandning
environmental risk assessment	ERA	miljöriskbedömning
chemcial- and biological lines of evidence	LOE	kemiska och biologiska bevisföringslinjer
environmental quality standard	EQS	miljökvalitetsstandard
predicted environmental concentration / predicted no effect concentration	PEC / PNEC	predicerad koncentration i miljön relativt predicerad koncentration vid vilken ingen effekt förväntas
potential risk index	PRI	index för potentiell risk

7 Specifika rekommendationer för rankingmetodik

RAFS-projektet rekommenderar att den framtagna rankingmetodiken för att bedöma potentiell giftighet av sediment, som är förorenade med företrädesvis organiska föreningar, främst ska användas vid mer storskaliga prioriteringsbeslut, där det är av stor vikt för berörda myndigheter att identifiera det eller de sediment som har den största inneboende farligheten för miljön. Eftersom metodiken baseras på giftighetsundersökningar av organiska extrakt från sedimenten är det viktigt att belysa att resultaten från de enskilda ekotoxikologiska fisk- och kräftdjurstesterna i de flesta fall överskattar biotillgängligheten av de organiska föreningarna i sedimenten. Denna överskattning görs dock i såväl förorenade sediment som referenssediment och innebär vidare att *försiktighetsprincipen*, som är ett fundament i EUs nya kemikalielagstiftning REACH, är inbyggd i metodiken. På så sätt medför användandet av metodiken att möjliga risker med föroreningar i sediment inte underskattas.

Som ett rankinginstrument ska den föreslagna metodiken komma in i ett tidigt stadium av saneringsprocessen. Finns det klara indikationer på att det eller de sediment som ska genomgå en prioriteringsprocess (för att fastställa typ av sanering eller om sanering över huvud taget behövs) till största delen innehåller organiska föreningar bör rankingmetodiken ersätta kemiska analyser av bakgrundshalter av kända organiska substanser. Finns det däremot indikationer på att även oorganiska komponenter i sedimenten kan bidra till den totala giftigheten rekommenderar RAFS-projektet att giftighetsrankingen bör kompletteras med kemiska analyser av t.ex. metaller i sedimenten. Sådana kemiska analysdata bör användas för jämförelser med befintliga toxicitetsdata av enskilda metaller eller metallföreningar. I möjligaste mån skall dessa toxicitetsdata baseras på tester med de i rapporten föreslagna arterna. Detta för att erhålla en mer komplett, representativ och relevant riskbild. Förslagsvis bör rankingtabellen som anges i bilaga 4 i dessa fall utökas och inkludera även en oorganisk komponent, som beskriver relativa riskkvoter baserade på jämförelser mellan effekter och exponering. Om det finns tydliga indikationer på att de organiska föroreningarna utgör den största risken för miljön bör rankingen viktas så att de oorganiska komponenterna inte får alltför stor betydelse i den slutgiltiga prioriteringen.

RAFS-projektet vill vidare förtydliga att även om det aktuella projektet har fokuserat på den akvatiska miljön och tester med regnbåge och hoppkräftan *Nitocra spinipes*, är det fullt möjligt att använda andra arter. En noggrann validering av metoderna med sådana alternativa arter rekommenderas dock innan en definitiv undersökning görs. Avslutningsvis menar projektgruppen också att det är fullt möjligt att testa förorenad mark med den föreslagna rankingmetodiken. För att bedöma den potentiella giftigheten av förorenad mark bör dock terrestra organismer användas eftersom akvatiska och terrestra organismer skiljer sig åt i form av exempelvis livsstrategier, ekologiska anpassningar och födointag.

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Bilaga 1. Ekotoxikologiska test-metoder för sediment

Förliggande bilaga genomfördes som ett examensarbete vid ITM, Stockholms Universitet, under 2005. Författare var Niklas Ricklund och handledare var Magnus Breitholtz och Johan Persson. Rapporten har även publicerats som ett examensarbete vid denna instutition.

A new Silica-based Ecotoxicity Test with the Harpacticoid Copepod *Nitocra spinipes* for Extracts from Natural Sediments with application to Sediment Quality Assessment (SQA)

Contents

Abstract

Present Sediment Quality Assessment (SQA) mainly relies on chemical analysis and Sediment Quality Guidelines (SQGs) as assessment tools. To ensure data quality of SQGs, to improve *in situ* measurements of ecotoxicity, and in the end, to ensure quality of SQAs, the development of cost-effective, reliable, ecologically relevant and sensitive ecotoxicity tests is important, and also in line with the perspective of the European Commission's Environmental Risk Assessment (ERA) strategy. Present work focus on the development of a sublethal ecotoxicity test with high bioavailability conditions, examining potential toxicity on the ecologically relevant harpacticoid copepod *Nitocra spinipes* exposed to extracts from polluted sediments, and also to give an introduction to present SQA in which SQGs together with chemical analysis and ecotoxicity tests are the most important tools. In the experimental part gravid female *N. spinipes* fed with the red micro algae *Rhodomonas salina,* were exposed to silica gel spiked with the hexane-soluble fraction of extracts (Soxhlet extraction) of sediments from Örserumsviken. On day 16, the number of copepodites and adults were counted, and RNA content for copepodite stage three were examined in each concentration. The RNA content reflects the level of metabolic activity and has been used as an index of both nutritional status and growth rates of an organism. A dose-response relationship was observed for the population structure, but no significant toxic effect (which we have seen earlier in similar tests) could be detected from the RNA content analysis. Earlier experiments, in which Larval Development Rate (LDR) was examined, suggested an effect in population structure but this endpoint was expected to be less sensitive than the RNA content. It remains unclear why the RNA content failed to indicate toxic effects from the sediment in the present experiment, but some explanations were suggested. The RNA content is not commonly used in the context of ERA and SQA, but has a potential to become a very potent endpoint. More tests with *N. spinipes* and RNA content in combination with other endpoints and test conditions may, however, be needed before a test concept obliging all demands can be proposed for standardization. It was concluded that the silica gel appeared to be a useful carrier of the sediment extracts in association with the micro algae, performing a reasonable high bioavailability of pollutants. Further, a simple endpoint (in respect to performance, but ecologically very relevant) as population structure of *N spinipes* was demonstrated to be considerably useful for screening sublethal toxicity of hydrophobic pollutants.

OBJECTIVES

The objective of this work was to develop a sublethal ecotoxicity test using *Nitocra spinipes* exposed to extract from polluted sediments (experimental part) and to give an introduction to present SQA (part I), in which SQGs with chemical analysis (part II) and ecotoxicity tests (part III), are the most important tools. Conclusions are summarized in part V.

PART I

Introduction to present SQA

Present SQA is mainly derived from the London Convention 1972 (LC72), which proposed a methodology for limiting environmental impacts of dumping and spreading of polluted sediment (Stuer-Lauridsen *et al*. 2001). Seventy-eight countries signed the LC72, including the majority of the European nations. Consequently, the framework of this convention is reflected, to a variable extent, in all of these countries sediment assessment work.

The LC72 has later (1996-1997) been complemented and made more stringent. The LC72 assessment methodology is basically simple. It proceeds with physical and chemical characterizations of sediments, followed by biological (i.e. ecotoxicity) tests, from which biological effects are predicted. In addition to this, governments should establish an (a) "Action list", which is numerical limit concentrations for pollutants and (b) an "Impact hypothesis", which is an exhaustive report of environmental consequences associated with the pollution. From this information, decisions concerning monitoring programs and management are made. The 1972's "Action list" is similar to present SQGs, which by definition are "numerical limits (i.e. concentrations of substances) or narrative statements, recommended supporting and maintaining designated uses of the aquatic environment" (Smith and MacDonald 1999). Alternatively, "numerical chemical concentrations intended to be protective of biological resources, or predictive of adverse affects to those resources, or both" (Wenning and Ingersoll 2002). In scientific literature, the term "threshold values" also often is used.

SQG has become an important and widely used tool and several established methodologies for SQA are built up around SQG (e.g. Stuer-Lauridsen *et al*. 2001, Wenning and Ingersoll 2002). This is easiest explained by the fact that comparing measured field concentrations of pollutants to a SQG represents the simplest form of SQA, which generally enhances fast management decisions and lowers costs. In spite of this, there are concerns about the methods used to derive SQGs, and to solely rely on SQGs in SQA (O'Connor *et al* 1998, Stuer-Lauridsen *et al*. 2001, Wenning and Ingersoll 2002, Bilaga 1). Unfortunately, this is often the case, but to solely rely on SQGs may lead to wrong conclusions of the risks associated with the pollution situation. The underlying supposition in these cases is that SQGs can be used instead of *in situ* measurements of toxicity, but this may not be the case (Wenning and Ingersoll, 2002). SQGs most often must be complemented by site-specific ecotoxicological information. In addition, to be able to make sound management decisions, volume considerations of polluted project material must be taken into account (Hansson and Rudén 2004). In present SQA there are typically no regulatory distinction made in the decision tree between a situation involving a few hundred cubic meters and a few hundred *millions* cubic meters of project material (Chapman *et al* 2002).

The most widely practiced method to establish SQGs is the use of background concentrations. Concentrations are measured at reference sites and compared to polluted sites. Degree of pollution and safety margins for communities are estimated. However, this strategy alone does not provide information for safe interpretations of limit concentrations. Instead, ecotoxicity testing is strongly recommended (European Commission 2003, Smith and MacDonald 1999, Wenning and Ingersoll 2002). Ecotoxicity tests can be used both to derive SQGs and as a tool for in situ measurements of sediment toxicity. The Nordic countries Denmark, Sweden and Norway mainly rely on the use of background concentrations instead of ecotoxicity tests (Stuer-Lauridsen *et al*. 2001) to derive SQGs. In Denmark and Sweden ecotoxicity tests have been used sporadically, i.e. in larger restoration projects and during, *in situ* measurements of toxicity (e.g. Engvall, M *et al* 1996, Pedersen *et al* 2001, Sundberg *et al* 2003). In the present study, from a feedback-form (bilaga 1) concerning usage of methods for ERA among secondary decision-makers in Sweden, it became clear that ecotoxicity tests are not so often used. A common opinion among seems to be that ecotoxicity tests are not very necessary in ERA and results from ecotoxicity tests are difficult to interpret. Out of thousands of national environmental investigations over the years, the 16 responding counties (out of 21asked) could refer to about 40 occasions where ecotoxicity tests have been used. Of these 40 occasions, Microtox™ is the most frequently utilized test. Sublethal endpoints with ecologically relevant species have never or rarely been investigated. On the contrary, the Netherlands may be one of the few exceptions from this strategy within the European Union (EU) (Stuer-Lauridsen *et al*. 2001).

Compared to the EU, Canada, Australia, New Zealand and USA have come further and use SQGs based on standard ecotoxicity tests (Stuer-Lauridsen *et al*. 2001). The Canadian SQG is derived from an iterative process, where the idea is that new ecotoxicity test results together with existing data are continuously incorporated and the SQG is constantly improved (Smith and MacDonald 1999). In this context, it is worth mentioning the Great Lakes project (US EPA 1998) in North America. The project has been progressed since 1978 and was initiated by the United States Environmental Protection Agency (USEPA) and Environment Canada in consultation with other federal departments and agencies. The Great Lakes Project is a huge environmental binational project concerning assessment and restoration of a heavily polluted lake region, in which the United State's and the Canada's perspectives (illustrated in for instance: MacDonald and Ingersoll 2002) of SQA have been allowed to develop. The primary goal in the Great Lakes Project is virtual elimination of persistent toxic substances from the Great Lake Basin. That is, substances resulting from human activity and particularly those that are bioaccumulating. The goal is achieved through a variety of programs and actions, but mainly through pollution prevention, in line with the *Agenda 21: A Global Plan for the 21st century* (adopted at the United Nations Conference on Environment and Development). The Great Lakes Project has generated immense amounts of data and the effectiveness of the ecotoxicity tests used in the Great Lakes Project have been accurately evaluated (e.g. Burton and Ingersoll 1996).

General methodology for SQA

Current SQGs and the methods used to derive SQGs are generally appropriate to make some management decisions. Although, according to the Society for Toxicology and Environmental Chemistry (SETAC), well familiarized with questions at issue associated with SQA, SQGs should be seen as one of several optional tools. These tools should be used together in a common methodology to develop chemical- and biological Lines of Evidence (LOE), for polluted sediments. In principle, several LOE are needed to properly evaluate polluted sediments. These LOE are in the first place:

- Nature and extent of pollution
- Expected or acceptable diversity and abundance of benthic biota in the absence of contamination
- Bioavailability, bioaccumulation and effects of contamination (the potential for chronic as well as acute effects) on aquatic organisms
- Stability of sediments and pollutants (fate and transport)
- Risk of contamination to aquatic biota and associated resources

Available tools to obtain this information are for instance:

- SQGs (which involves chemical analysis of sediment and sediment ecotoxicity tests)
- Sediment ecotoxicity tests
- Resident exposed communities (not necessarily benthic)
- Bioaccumulation tests
- Biomarkers and/or histopathology

In addition to this there are also non-biological tools for geological-, hydrological- and chemical characterizations (see PART II) of sediment.

According to SETAC, these LOE should be weighed to build up a Weight of Evidence (WOE) for a polluted site. This should point out the direction for decision-makers and form the basis for management decisions. SQA methodologies should include three basic characteristics:

- Multiple LOE
- Multiple tiers
- An iteration process

These characteristics do not necessary form a new concept, but have its support in several other organizations' guidance documents (European Commission 2003, Smith and MacDonald 1999), with great influence on the subject. To incorporate LOE into a WOE, SETAC proposes the following methodology:

1) Define measurement and assessment endpoints
2) Select appropriate and multiple LOE
3) Select and apply assessment tools within the chosen LOE in multiple tiers
4) Analyze collected information and create a WOE

5) Identify data gaps and risks (iterative process)

Conclusions from this methodology may enhance selecting management options, which involve:

6) Listing of management alternatives
7) Comparing risks associated with alternatives
8) Comparing costs of alternatives
9) Apportioning the sediment at a site among the selected alternatives

Sediment quality and SQG as an assessment tool

Sediment quality has not always been considered as an issue. Sediments have in ERA more or less been considered as a secure reservoir for harmful pollutants, but this has later shown to be a delusion, founded on the lack of knowledge of ongoing processes (see PART III) in sediments. The importance of sediment quality can be illustrated as follows:

Sediment quality is important because it influence the health of aquatic organisms, which may be exposed to toxic and bioaccumulative substances through their interactions with sediments. Sediments also influence the environmental fate of many toxic and bioaccumulative substances in aquatic ecosystems. Many substances form associations with particulate matter and are eventually incorporated into sediments (Allan 1986). Consequently, sediments may also act as long-term sources of these substances to the aquatic environment (Larsson 1985, Salomons *et al*. 1987, Loring and Rantala 1992). Therefore the use of SQGs for evaluating the toxicological significance of sediment-associated substances has become an important part of protection and management of freshwater, estuarine, and marine ecosystems (Smith and MacDonald 1999).

The numerical limit concentrations defining the SQG should be based on the Predicted No Effect Concentration (PNEC) of substances, which results from effect measurements with ecotoxicity tests. The PNEC is a central parameter of the general concept of Environmental Risk Assessment (ERA), which is according to the European Commission (2003) completed in four steps:

a) **Hazard identification** - indicates the adverse effect that a substance has the potential to cause.
b) **Dose-response assessment** - estimates the relationship between the level of exposure to the substance and the incidence and severity of an effect on the organism. The assessment activities of a) and b) are the result from ecotoxicity testing and the final outcome of these two is the PNEC.
c) **Exposure assessment** - brings about the Predicted Environmental Concentration (PEC), which is the concentration of the substance in different environmental compartments (aquatic, terrestrial, atmosphere etc.)
d) **Risk characterization** - the PEC and PNEC are used to calculate PEC/PNEC ratios for environmental compartments. If the PEC/PNEC ratio is higher than one there is a concern that there may be a risk for organisms in that specific

compartment. In case the number and quality of the ecotoxicity data is low, safety factors (10, 100 or 1000) are multiplied to the PEC/PNEC ratio and hence the risk of the substance of interest is increased.

The PNEC should represent the lower limit of the range of substance concentrations that are usually not or never associated with adverse biological effects. If the PNEC is set too low it will be associated with a number of false positives. That is, a number of sites will be addressed as "toxic", while they are not. If the PNEC is set too high it will conversely be associated with a number of false negatives. Improvement of methods (e.g. ecotoxicity tests) for determination of PNECs will lead towards determination of actual concentrations, NECs, which will limit the frequency of false positives and negatives. Among the factors (see PART III) that causes overlap between *effect* and *no effect* data include (Wenning and Ingersoll 2002):

- Other substances that cause effect
- Differing bioavailability
- Differences in responses among organisms and errors in measurement of concentrations or responses

PNEC can in the SQG preferably be derived from a recommended minimum data set (Table 1), a so-called test battery, which is the minimum data that a numerical limit should be based on (Smith and MacDonald 1999, Wenning and Ingersoll 2002, Burton and Ingersoll 1996). The minimum data set ensures data quality of SQGs and can at the same time be used as guidance during *in situ* measurements of toxicity in sediment.

Table 1. Minimum data set from the Protocol for derivation of Canadian sediment quality guidelines for the protection of aquatic life (Smith and MacDonald 1999).

Minimum Data Set Requirements for Freshwater Sediment Quality Guidelines.
At least four studies are required on two or more sediment-resident invertebrate species that occur in North American waters. These must include at least one benthic crustacean species and one benthic arthropod species (other than a crustacean).
At least two of these studies must be partial or full life-cycle tests that consider ecologically relevant endpoints (e.g., growth, reproduction, and developmental effects).

Depending if the data is derived from sediment toxicity tests, or other medium (i.e. water), SQGs can be:

1) Mechanistically
2) Empirically

Mechanistically based SQGs are developed and tested using PNEC for aquatic organisms, almost exclusively according to the Equilibrium Partition (EqP) theory

(see 3.1.), when data for sediment dwelling organisms are missing (Wenning and Ingersoll 2002). The EqP theory has received criticism due to wrongly estimated exposure concentrations (Fredriksson *et al* 2003, Persson 2003). Empirically based SQGs are developed using large databases with matching measures of sediment chemistry and toxicity with field collected samples. Several algorithms are used to define specific concentrations associated with particular levels of effect or no effect (ERLs, ERMs, TELs, PELs, AETs).

A problem associated with the mechanistically SQG, which is based on casualty, is that it may underestimate adverse biological effects of a mixture of substances. Conversely, the empirically SQG has a tendency of incorrectly attribute adverse biological effects by a mixture (whole sediment) to a single substance. However, the use of mixture models improves the predictability of a SQG, which is an advantageous property.

PART II

Exposure analysis of sediment

Exposure analysis is implemented through chemical analysis and gives information of the concentrations of substances in sediments or other media. However, known concentrations of substances in sediments are not equivalent with the concentrations sediment-dwelling organisms are truly exposed to, which is due to factors that influence bioavailability (see 3.1.). Thus, one should have in mind that exposure analysis does not give any answers to which effects these measured concentrations may cause.

Exposure analysis of sediments is preceded by an investigation of the object, its environmental pollution history and possible pollution sources (Naturvårdverket, rapport 5254). The exposure analysis often generates enough information to point out priority pollutants. Relevant questions in exposure analysis are:

- What substances should be measured?
- Where and how should samples be taken?
- What to do with samples?

What substances should be measured?

There are certain groups of substances that are frequently recognized as environmental pollutants. According to structural properties of these substances, they can be divided into five groups, which are presented in Table 2.

Table 2. Common environmental pollutants.

Group of substances	Common environmental pollutants within group of substances
Metals	almost all metals
chlorinated organic compounds	PCBs (polychlorinated biphenyls), polychlorinated dibenzodioxins, polychlorinated dibenzofurans, chlorophenols
Aromatic compounds	PAH (polycyclic aromatic hydrocarbons), phenols, cinolines, pyridines
inorganic salts	cyanides, fluorides
other organic substances	flame-retardants, nonyl-phenols, mineral oils, tin-organic compounds

A group of pollutants are often associated with a particular type of industrial activity, pollution situation etc. This kind of knowledge is today widespread and commercial companies (e.g. Analytica, SWECO) offers batteries of analytic tests suitable for common pollution situations.

Where and how should samples be taken?

LOCALIZATION OF THE POLLUTION

It is often convenient to use some kind of method to localize pollutants, which can be concentrated to a small area, a hot spot or scattered at a larger. For this purpose, several tools are available (Table 3).

Table 3. Examples of techniques for localizing pollutants.

Type of method	**Comment**
Geophysical field measurements	To measure physical properties (electricity, magnetism and radioactivity) in the ground and disturbance of these from pollutants.
-georadar	Detects differences in ground composition and metal objects like a barrel or a pipeline.
-resistivity measurements	Is used for detection of ground layers.
-CPT (Cone Penetration Testing)	Measures the resistance from a drill cone penetrating the ground. Detects for example areas where digging has been done.
Chemical field measurements	Gives an idea of pollutant concentrations in different mediums.
-XRF (X-Ray Fluoroescens)	A simple and fast but not very precise technique for measuring of metals and As.
-PID, FID (Photo- /Flame Ionization Detector)	For detection of volatile organic compounds.
-infrared spectrophotometry	Also useful for detection of volatile organic compounds.
-Coulometric methods	Semiquantative measurements of different substances in water-solution.
-FOCS (fiber optical chemical sensors)	Sensitive for one substance or a group of substances.
-immunoassay	Designed enzymes sensitive for one substance or a group of substances.
Other	
-Passive Accumulation Sampler (SPMD, PISCES etc).	A Passive Accumulating Sampler (PASs) is a non-biological object capable of accumulating substances without the supply of power.
-Data simulating programs	Programs based on fugacity models designed for calculating PEC values.

COLLECTING SAMPLES

There are several methods used to choose sample spots when a polluted area has been localized (Table 4). In principal, it is appropriate to collect samples in such a way that statistic analysis is favored (i.e. large number of samples collected randomly). Reference samples are often taken to reveal background concentrations from an unpolluted area nearby the polluted site. When samples are taken in the actual sediment, this is done down to a depth where sediment is free from pollutants, which varies with sediment- and pollutant-type. For this purpose, a sediment corer, an Ekman collector or a bottom striker is preferably used.

Table 4. Examples of strategies for sample-collection.

Strategy	Achievement	Comment
Aimed sample-collection	**The polluted spots are known and samples are taken at the site.**	**There might be spots which are unknown**
Systematic sample-collection	**Samples are taken after suitable geometric patterns.**	**Large risk of systematic errors.**
Randomized sample-collection	**Samples are taken from random spots.**	**There is a possibility that areas are missed.**
		Data suitable for statistic calculations.
Systematic, randomized sample-collection	**Samples are taken from random spots in a geometric pattern.**	**The strategy eliminates most of the possible errors from systematic and randomized strategy.**
Stratified sample-collection	**The polluted area is divided into smaller areas of which samples are taken systematically or randomly.**	**The strategy is suitable for areas with differing pollution-pattern.**

What to do with samples?

STORAGE AND HANDLING OF SAMPLES

Depending on type of pollutant and sediment there are different methods (e.g. Environment Canada 1994, ASTM 2002) for handling and storage of samples. In general samples are kept cold, but not below zero and in non-reactive beakers. Sediment-samples containing substances sensitive to pH-changes, or easily oxidized, should not be in contact with air.

SAMPLE ANALYSIS

Considering a polluted sediment-sample, for example from a strongly trafficked area in a city. The extremely complex mixture of organic derivates and metals in different speciation makes it in practice impossible to completely characterize the sediment. Common analytical methods in SQA are compiled in Table 5. Considering the commonly occurring pollutants described in Table 4 (see 2.1.), metals are preferably detected with ICP-AES/-QMS/-SFMS, AFS, chlorinated organic substances may be detected with GC-ECD, aromatic substances with HPLC or GC-MS, which also is appropriate for other organic substances.

Table. 5. Compilation of standard analytic methods.

Method	Comment
HPLC, HPTLC	High Performance Liquid Chromatography and High Performance Thin Layer Chromatography, primarily suited for separation of substances that are not volatile, that are thermally unstable or possess some kind of reactive or electrically charged functional groups. PAH- and creosote screening can be carried out with this method.
ICP-AES	At high temperature (10,000ºC), most elements emit light of characteristic wavelengths, which can be measured and used to determine the concentration. The sample being analyzed is introduced into the plasma as a fine droplet aerosol. Light from the different elements is separated into different wavelengths by means of a grating and is captured by light-sensitive detectors.
ICP- QMS	ICP-QMS uses plasma of the same type as in ICP-AES, but here it is used to convert elements to ions, which then are separated by mass in a mass spectrometer. Detection limits are lower than in ICP-AES; certain elements can be detected at the ng/L level in aqueous solutions.
ICP-SFMS	Allows analysis of sample types that cannot be handled by conventional ICP-MS (seawater and biological samples). Can separate particles with smaller differences in mass “high resolution” ICP-MS.
GC-MS	In Gas Chromatography the sample (1-5 µl) is vaporized in an injector and carried by a gas stream through a column where the compounds are separated. They are then registered as separate peaks by means of a detector. Substances eluted from the column of the gas chromatograph are ionized and mass spectrometry provides detection of the substances and gives information of its molecular structure, by means of the fragmentation pattern in the mass spectrum. Modified GC-analysis is standard tool when screening for environmental pollutants.
GC-ECD	An ECD (Electron Capture Detector) has high sensitivity and selectivity for halogenated compounds, e g chlorinated pesticides and PCB is also sensitive to organic peroxides and nitro compounds. A disadvantage is that the linear range is small.
GC-MSD	An MSD (Mass Selective Detector) enhances the analysis of PAH that can be problematic because of the tendency of these compounds to adhere to the surfaces of the analytical system, particularly ones with high molecular weights. Typical PAH analyses, therefore, show decreasing response and sensitivity with increasing molecular weight
Micro coulometric titration	Sample is combusted in oxygen and amount organic halogenides is determined. The method is a screening-method applicable as EOX, AOX or POX.

PART III

Effect analysis of sediment

The gained information from the exposure analysis is central in SQA, but since it neither gives information about bioavailability nor toxicity and there are no reliable SQGs available, it needs to be complemented with effect analysis. In a situation when the concentrations of pollutants in sediment are known, the most important question remains unanswered:

- Can ecotoxicological effects be expected?

A substance of a specific concentration apparent in one system (lake, geographical region etc) can potentially have a major impact on the surrounding ecosystem, while the same concentration of the same substance may have no effect in another system. Consequently, this makes a SQG for one region possibly useless to another. The primary underlying reason to this problem is the variation of sediment constitution among aquatic systems (Smith and MacDonald 1999, Wenning and Ingersoll 2002, European Commission 2003).

It should also be mentioned that commonly there is a natural variation in sensitiveness to pollutants among individuals/populations of same species in different areas (due to genetic factors or stress factors as starvation, diseases, predation etc). However, individual variation and presumably variation in sensitivity is smaller when using laboratory-cultured animals, which limits this source of error.

Bioavailability of hydrophobic substances in sediments

Bioavailability is a medical term defined as: proportion of a drug or foreign substance absorbed in the gastro-intestinal tract of an organism (Timbrell 2000). The term has later been adapted to the ecotoxicology, but in its new context the term has at the same time become somewhat more complex and difficult to handle, due to the more complex system studied. The environment contains a lot of organisms, innumerous exposure pathways, substances in mixes and physical-chemical conditions, compared to the controlled uptake in the gastro-intestinal tract of an organism. Considering bioavailability in the ecotoxicological context, in some situations, it may invite to confusion that a substance by definition not is considered taken up when it is present in the gut of an organism. For instance, this may be the case while examining uptake of pollutants in very small organisms.

In order to enhance understanding and estimation of ecotoxicological bioavailability, bioaccumulation factors (BAFs), bioconcentration factors (BCFs) and biomagnification factors (BMFs) have become increasingly important (Mackay and Fraser 2000). The BAF is defined as $C_{X\ organism}/C_{X\ water}$, and tells us about the uptake and accumulation of substances in organisms during field conditions (BAF>1 gives accumulation). The BAF considers all exposure pathways, e.g. dietary absorption, transport over respiratory surfaces and dermal exposure**.** The BCF is also defined

as $C_{X\ organism}/C_{X\ water}$ but only involves uptake of the freely dissolved (in water) fraction of a substance via respiratory surfaces and/or the skin, usually under laboratory conditions. The freely dissolved fraction is commonly referred to as the bioavailable fraction. The BMF is defined as $C_{X\ organism}/C_{X\ food}$ and is a special case of bioaccumulation: the concentration of a pollutant in an organism exceeds the concentration in the diet, due to dietary absorption. In the gastro-intestinal tract the bioavailability of a substance generally increases enormously, enhancing dietary absorption. Worth clarify, is that substances that bioaccumulate does not necessarily biomagnify. Persistent hydrophobic substances that also are subject of long-range transport (for instance PCBs, DDTs and PBDEs) are the most eager to accumulate in organisms (Mackay and Fraser 2000).

The sorbed fraction of a hydrophobic substance in sediment is by definition (Mackay and Fraser 2000) excluded from the bioavailable fraction. Therefore, the understanding of sorption processes is very relevant for the understanding of bioavailability. Sorption of hydrophobic substances to sediments (and also soils) strongly depends on the amount of organic material present (Karichoff *et al* 1979, Karichoff 1981). Previously, it has been proposed that the sorbed fraction is linearly dependent of the fraction (f_{oc}) of organic material and that sorption is implemented through partitioning (Karichoff *et al* 1979, Karichoff 1981). For example, $C_{sediment}=K_{partition} * C_{water}$ (the plot generates a partition *isotherm*) and equilibrium is assumed in system. It is referred to as the Equilibrium Partitioning theory (EqP theory). From this point of view partitioning between organic substances and sediments can be treated in similar manner to that between an organic solvent phase and water (Chiou *et al* 1983). Later, this standpoint have become somewhat adjusted, because studies have shown that geosorbents often exhibit non-linear sorption behavior (Luthy *et al* 1997). That is, $C_{sediment}=K_{partition} * C_{water}^{n}$, where n reflects to the linearity of the sorption process. Equilibrium expressions for sorption may be invalid due to slow kinetics in many natural systems (Pignatello and Xing 1996). The underlying reasons to this are that pollutants are sequestred, which encompasses diffusion limitations, adsorption and partitioning. Sequestration can for instance be observed as trends of hysteresis, extractabilities and decreasing desorption rates (slow fraction) of lipophilic substances (Luthy *et al* 1997). These are incompatible with a simple phase partitioning process, and may reflect [1]heterogeneity effects and non-equilibrium conditions.

Different types of organic matter have been recognized to contribute differently to the linearity of the overall sorption process. In “soft or rubbery” materials absorption is dominant while in “hard or glassy” absorption (diffusion limited) or adsorption may occur. Thus, differing linearity also is a consequence of the sequestration processes and can be illustrated with some examples. For instance, absorption into natural amorphous organic matter should probably always show linear behavior, while that to condensed organic matter may exhibit some combination of linear and non-linear behavior. Adsorption may generate linear or non-linear isotherms depending on surface properties. Adsorption to non-polar heterogeneous

[1] Several mechanisms working simultaneously at the sorption-process.

organic surfaces is presumably non-linear, but adsorption to hydrophilic mineral surfaces may not be, because coverage is small and energy differences among sorption sites are small (competitive adsorption may occur). However, there is no reason to believe that only one sorption mechanism dominates in any particular case. In natural systems it is likely that more than one process contributes to the rate-limited sorption behavior (Luthy *et al* 1997).

Sorption processes are most often non-specific and the thermodynamic driving force for these processes is primarily hydrophobic expulsion. Thus, changes in polarity and aromatic carbon content in sediment may cause large effects on the sorption processes. Because sorption is promoted with increasing hydrophobicity (of sorbent or sorbate) gives that only certain cases, will be sensitive to isomeric or steric arrangements of the sorbates. For instance, sorbate molecules that are planar and/or are provided with a large area may be favored in adsorption. These properties allows more dispersion forces between sorbent and sorbate. Desorption of large molecules can also be thermodynamically unfavorable due to steric hindrance (i.e. "ink bottled pores"). The remaining slow fraction limits the overall degradation-rate of the substance by microbes, because of reduced bioavailability. It has been concluded that microbes far more readily degrade substances from the water phase than the sorbed phase (Pignatello and Xing 1996). The slow fraction also complicates extraction with non-water miscible solvents (i.e. hexane). Extraction of aged sediment samples is superior with hot water miscible solvents (for example toluene). To take into account in SQA, is that simultaneously to the decreasing degradation rate of an aged sample, its toxicity also is decreasing. That is, an aged sample in sediments often is less toxic than a freshly added (Pignatello and Xing 1996).

Except from amount and type of organic matter, particle size of sediments may affect sorption process (Luthy *et al* 1997). This is especially true for "hard and glassy" inorganic materials (i.e. quartz) where adsorption is more important and competitive adsorption may occur. In spite of this, in many systems the dependence of particle size is absent (Pignatello and Xing 1996). This may be due to non-competitive situations, i.e. the concentration of the substance is low. It may also be due to that porosity exists in an outer shell of particles that is of similar thickness among size fractions. Besides this, adsorption often occurs in the pores of the material, and pore size (number of pores/area) may not always be related to particle size.

The described processes give information of how environmental conditions can affect bioavailability and toxicity of pollutants. Consequently, in SQA these processes must be taken into account, to be able to create relevant WOE.

Ecotoxicity tests for SQA

Ecotoxicity testing gives information about potential effects of pollutants in sediments. It can be used for either *in situ* measurements of toxicity, as a necessary complement to SQGs, or to derive SQGs. With this in mind, it should be clear that no fully accepted international, standardized test methods for whole sediment are currently available. Most of the existing whole sediment tests measure acute toxicity; only a few measure long-term, sublethal endpoints. Only the latter tests are

considered applicable to SQA, because of the long-term exposure of sediment dwelling organisms to sediment bound substances that occur under field conditions (European Commission 2003).

An inventory of tests with marine organisms for the evaluation of dredged material and sediments was compiled by the Federal Environment Agency of Germany, UBA (Herbst and Nendza, 2000), and in addition a detailed review paper on aquatic ecotoxicity tests including marine sediment test methods was prepared by OECD (1998a). With support from this gathered information the European Commission (2003) has presented a list of recommended tests (Table 6) for SQA.

Table 6. Ecotoxicity tests for sediments recommended by the European Commission (2003).

Test Organism	Acute or ChronicTest	Duration	Endpoints	Reference	Comments
Amphipods					
Corophium sp. (C. Volutator or C. Arenarium)	Chronic	28d	Survival, growth and reproduction	ASTM (1993), Environment Canada (Burton 1992) (OECD, 1998a recommended)	Degrader. Organisms can be field collected. Cultivation causes intermediate to high expenses. Organism does not like coarse sediment. Low concern with regard to animal welfare. Ecologically important organisms. Relevance for exposed ecosystems high. SOP available with field-collected organisms. Ringtested.
Leptocheirus plumulosus	Chronic	28d	Survival, growth and reproduction	ASTM (1993) Environment Canada (Burton, 1992), USEPA (1996)	Degrader. Grain size has significant effect on survival, growth and reproduction. Low concern with regard to animal welfare. Ecologically important organisms. Relevance for exposed ecosystems very high. SOP available with field collected organisms. Ringtested.
Polychaetes					
Nereis/Neanthes sp. Naeanthes arenaceodentata-kan cultivated	Subacute/chronic	12-28d	Survival – suvival/growth	ASTM (1994)	Degrader. Distributed widely throughout the world. Can be cultivated on the laboratory. Low concern with regard to animal welfare. Relevance for exposed ecosystems very high. SOP available, equipment and test species commercially available. Ringtested.
Arenicola marina	Chronic	28d	Survival	ASTM (1994) (OECD, 1998a recommended)	Degrader, wide tolerance of sediment grain size. Organism is found extensively over the OSPAR and Helsinki conventions area; cultivation is difficult. Low concern with regard to animal welfare. Relevance for exposed ecosystems very high. SOP available, equipment and test species commercially available. Ringtested.
Arenicola marina	Subacute	10	Casting rate	Thain and Bifield (2001)	See above row. Changes in feeding rate have consequences for sediment communities. SOP available, equipment and test species commercially available. OSPAR ringtested.
Echinodermes					
Echinocardium cordatum	Acute/subchronic	14d	Survival	Stonkhorst, in press. (OECD, 1998a recommended)	Degrader. SOP available with field collected organisms. Ringtested.
Microcosm					
Nematodes	Chronic	60d	Community structure	(Austen and Somerfield, 1997)	

Besides the recommendations of the European Commission, ecotoxicity tests using *Hyalella azteca* and *Chironomus tentans* are very well described by USEPA (2000) and evaluated by Burton and Ingersoll (1996). Referring to the latter article from the Great Lakes project no less than 24 different organisms were tested for 97 endpoints at 4 sites (altogether 7600 data points), and evaluated in respect to sensitivity, discrimination and redundancy. Conclusions from this work generated three optional test batteries (Table 7), optimal for the Great Lakes. However, there is some degree of confidence that recommended organisms also probably would be sensitive or discriminatory at other sites. It should be noted that the recommended tests in this study also have been recommended in earlier North American studies. (Burton and Ingersoll 1996) The most sensitive endpoint was the avoidance/preference behavior of *Diporeia*, which is not a sublethal endpoint and consequently not qualifies for the European Commissions testing criteria for sediments.

Table 7. Optimal sediment test batteries for the Great Lakes Basin derived from Principal Component Analysis (Burton and Ingersoll 1996).

	Test Organism	Duration	**Endpoints**	**Reference**
Option 1	Hyalella azteca	14d	Survival, length, sexual maturation	USEPA 1994a
	and *Ceridaphnia dubia*	7d	Survival, reproduction	ASTM 1995
	or *Chironomus riparius*	14d	Survival, length	USEPA 1994a
	or *Daphnia magna*	7d	Survival, reproduction	USEPA 1994a
	or *Pimephales promelas*	7d	Larval survival and weight	ASTM 1995
	or *Diporeia*	5d	Avoidance/ preference	ASTM 1995, Burton *et al* 1989
	or *Hexagenia bilineata*	10d	Survival, molting frequency	ASTM 1995
Option 2	C. dubia	(see above)	(see above)	(see above)
	or *C. riparius*			
	and *Diporeia*			
	or *H. bilineata*			
Option 3	D. magna			
	and *P. promelas*			
	and *Diporeia*			
	or *H. bilineata*			

Ecotoxicity test constituents

Ecotoxicity tests should be *cost-effective, reliable, ecologically relevant and sensitive*. A *cost-effective* test can be performed in big scales, which gives a good statistical basis. It should be able to perform not just in welfare countries but also in development countries where pollution of the environment often is severe. Cost-effectiveness generally decreases with more advanced techniques and testing time. *Reliability* derives from reliable results and the quality, which international standardization programs assure. The *ecological relevance* of the test is due to the extent to which the test result can be applicable to the actual ecosystem. The *sensitiveness* must be high to receive an early indicator of the pollution situation, for

protection of the most sensitive species. To meet these demands there are primarily three parameters in the ecotoxicity test that can be modulated (Breitholtz 2002):

(a) organism
(b) medium
(c) endpoint

ORGANISM

There are several sediment-dwelling organisms proposed to be appropriate for sediment ecotoxicity testing (see 3.2.). The choice of organism is likely to affect all demands of an ecotoxicity test. In general, a test-organism should preferably exist at an investigated site, for not being considered ecologically irrelevant. A test-organism should also be easy to keep in the laboratory and to culture. It should be easily collected in the field most of the year and should be easily and successfully maintained in the laboratory for a period of at least twice the testing period (acclimatization period + test duration) (OECD 1998). The suitability of a test organism for laboratory experiments depends a lot on the knowledge about the organism: its habits, physiology and sensitiveness for different pollutants, which can be extremely diverse.

The crustacean harpacticoid copepod *Nitocra spinipes* (Breitholtz 2002), which was used in the experimental part of this work, among other crustaceans (Chandler and Green 2001), have previously shown to be useful in ecotoxicity testing. Crustaceans are the second largest subphylum after insects among the invertebrates, which account for 95% of all known species on earth. The crustaceans are also abundant in all kinds of waters and serve as food for many economically valuable fish species. *N. spinipes* together with all other crustacean harpacticoid copepods comprise more than 3000 species. They are mostly free-living benthic organisms and are usually the second most abundant group of animals (after nematodes) in marine benthic communities. (Breitholtz 2002) Although the endocrine functions of *N. spinipes* are not fully understood, its physiology (Abraham and Gopalan 1975) is well described and it practices sexual reproduction.

MEDIUM

As with natural sediments, the medium in an ecotoxicity test has the potential to affect both test organisms and test pollutants, and consequently the outcome of the test result. Therefore it is important to be aware of and to control all molecular mechanisms involving the medium in the ecotoxicity test. It is also important that test concentrations of the substance or effluent remain constant during the whole test period. Concentrations of hydrophobic substances often decline over time in chronic tests, in such a way that early test periods yield disproportionately higher exposure concentrations than later periods. This may be a consequence of slow fractions (see 3.1). Thus, those life-stages used when initiating a test often are exposed to the highest concentrations. However, this problem can be overcome by initiating test with different organism life-stages (Chandler and Green 2001).

Present ecotoxicity testing of sediments focus primarily on the Spiked Sediment Toxicant Test Approach (SSTT) (Smith and MacDonald 1999). Briefly, a reference/baseline-sediment is spiked with known concentrations of chemicals, either alone or in combination. Usage of reference/baseline-sediments allows an evaluation of which (i.e. "all") substances that may cause toxicity but is not uncontroversial (Chapman *et al* 2002). Comparing actual sites to reference/baseline conditions can generate unrealistic restoration goals.

ENDPOINTS

Owing to differences in sensitivity to substances among test organisms, it is recommended to focus on more than one endpoint (OECD 1998). Physiological and biochemical responses of individual organisms are often relatively sensitive and reliable, but in general they are difficult to interpret in an ecological context. Subjective endpoints, like behavioral effects, and endpoints at population/community level (population structure) gains in ecological relevance but are often less sensitive, demands more time and increases costs (full life cycle tests with many individuals). According to OECD (1998) toxicity endpoints may be compiled in the following five groups:

1) Endpoints at population or community level:
- population survival and growth
- age structure
- fecundity
- species composition and community tolerance

2) Endpoints related to individuals or groups of organisms of similar age:
- survival/lethality (or immobility)
- growth and survival of specific life stages
- reproduction and survival or early life stages
- avoidance/behavioral effects
- gross deformities and morphological effects

3) Endpoints related to specific toxicity mechanisms of substances (e.g. genotoxicity)

4) Physiological endpoints (e.g. effects on metabolic processes, inhibition of respiration)

5) Biochemical endpoints (induction or inhibition of enzymatic activity, etc.)

Ecotoxicity tests may include several endpoints. Life cycle tests include some or all of the endpoints listed above, while short-term acute toxicity studies mainly focus on survival, which is ecologically very relevant but insensitive endpoint.

EXPERIMENTAL PART

Toxicity test on Nitocra Spinipes

Introduction

Sediment samples in general comprise groups of substances (PCBs, PAHs etc) extraordinary complicated to test for ecotoxicological effects, which may be due to its non water-soluble properties (Breitholtz 2002). These substances have also often shown to be a major reason for environmental impacts (e.g. Sundberg *et al* 2003, Lithner *et al* 2003, USEPA 1998, Naturvårdsverket rapport 4165), and as mentioned, currently there are no fully accepted international standardized tests available for whole sediment (European Commission 2003). Therefore it is important to develop ecotoxicity tests for sediments.

When making management decisions in SQAs it has to be stated whether the goal is low-/non toxic sediments or sediments free from pollutants. Toxicity is strongly influenced of bioavailability (see 3.1) and conditions for bioavailability may change in natural systems. Therefore, it is reasonable to propose a test where *potential* toxicity of sediments is estimated, during enhanced or high bioavailability conditions (Breitholtz, personal communication).

In our test concept, the ecologically very relevant test organism *N. spinipes* (see 3.3.1.) is exposed to extracts from sediments through all exposure paths (that is, dietary absorption, transport over respiratory surfaces and dermal exposure) in a system "free" from elements that may limit bioavailability, i.e. organic material that is not food. The extraction of sediments allows increased concentration of pollutants and elevated sensibility of the test. Also, natural sediments are excluded to limit the possible effects of unknown physical-chemical-processes. Consequently, the test is appropriate to compare potential toxicity between different sites.

The simplicity of the test system favors cost-effectiveness and reliability. Besides this, several endpoints can be studied from the test system. Except from the traditional population structure analysis, which comprises endpoints as counting of adults and larvae, we included analysis of the RNA content of the copepods. RNA content in organisms is coupled to physiological stress, e.g. stress proteins, or heat shock proteins (Hsp) (Mayer and Bukau 1998). Bulk analysis of nucleic acid (*i.e.* RNA and DNA) has been successfully used in copepods and daphnids to assess individual growth (Gorokhova and Kyle 2002; Gorokhova 2003). These indices were also found as a promising tool to predict population-level responses at an early stage (Breitholtz *et al.* 2003).

The experimental part of this work features the development of a new cost-effective, reliable, ecologically relevant and sensitive ecotoxicity-test, for estimating toxicity of sediment. Part four thus includes:

a) Collection of sediment samples
b) Extraction of sediment samples.

c) Four successive ecotoxicity tests (acute tests I-II, 6 day LDR tests I-II) in which the test concept was tried out, with improvements in each series.
d) Two tests (I-II) of the new 16-day sub lethal ecotoxicity test.

Materials and methods

COLLECTION OF SEDIMENT SAMPLES

Twenty surface bottom sediment samples (top 3 cm) were randomly collected on 21st of October 2004 from the middle part of Riddarfjärden at a water depth of 15-21 m using a Kajak-type gravity corer from boat.

EXTRACTION OF SEDIMENT SAMPLES

(The staff at the ITM Laboratory for Aquatic Ecotoxicology performed all procedures.) The sediment samples (see 4.2.1.) were pooled and homogenized at 10ºC in a glass beaker with a stainless steel spoon and kept in precleaned polypropylene jars with lid prior to extraction. The organic compounds were extracted wet for 24 h in toluene using a Soxhlet apparatus connected with a Dean-Stark trap for water removal.

ACUTE TESTS I-II

In the first experiment five sets of four 20ml beakers were added with 3mg commercial salmon feed (Astra-Ewos, Södertälje, Sweden). Of the five sets of test-beakers, four (except control) were prepared with n-hexane (Merck, PA, LiChrosolv), and three (accept control and solvent control) were prepared with n-hexane solved sediment extracts from Örserumsviken (Sundberg *et al* 2003), which were already prepared and ready to use. N-hexane was transferred with a pipette equipped with a glass syringe. Losses of solvent due to vaporizing were instantly controlled and compensated for on scales. The n-hexane solved sediment extract was stirred out in the salmon feed in different amounts, corresponding to actual sediment masses (salmon feed and sediment in relation; 1:1, 1:10, 1:100 by dry weight). The beakers were left standing in a ventilated chamber until the n-hexane was considered to be completely vaporized (approximately 2 h). Each beaker was filled to 10ml with GF/C-filtrated seawater and five adult *N. spinipes* were added. Mortality was studied after 96 h.

The second experiment was performed as the first, except for the preparation of the spiked salmon feed, which this time were milled into smaller pieces and mixed with the extract in the absence of a much larger amount n-hexane (5ml n-hexane/g fish-food), to enhance homogeneity. The amounts of salmon feed to sediment (10:1, 1:1, 1:10) were this time prepared from the same solution of n-hexane solved extracts.

6-DAY LDR TESTS I-II

The third experiment was performed as the second (with the same prepared salmon feed), with the differences that eight nauplii were used instead of five adults per

beaker and five beakers per set were used instead of four. The sublethal endpoint Larval Development Rate (LDR), from nauplii to copepod, was studied after six days, instead of mortality. (LDR = number of $copepods_{DAY\ 6}$ / number of $nauplii_{START}$.) Evaporation losses were compensated for with distilled water.

The fourth experiment was performed as the third, but with five sets of four beakers with five nauplii in each beaker. The spiked food was changed from salmon feed to micro algae, *Rhodomonas salina*, which is a natural food source for the *N. spinipes*. This time, all sediment extracts was solved in a common, large volume of n-hexane (100ml). Volumes of the solved sediment extract, derived from 1.25mg, 10mg and 100mg of natural sediment, were transferred to test-beakers. This was done quickly and cork was put on after each transfer, to minimize evaporation losses. The test beakers were left in a ventilated chamber for about 24 h, to vaporize all n-hexane (although, no signs of n-hexane can be traced with microscope or by scent after approximately 2 h). Finally micro algae (50ul of $5*10^7$/cells/ml, i.e. 0,141 mg dw), copepods and water were added to each beaker.

16-DAY SUBLETHAL TESTS I-II

The first of two sublethal test attempts were performed with five sets of ten test beakers. Artificial baseline sediment of pure silica gel (Merck, silica gel 60, Ø= 0,063-0,200mm, 400mg/beaker) was used as a carrier (i.e. sorbent, see 3.1.) of the sediment extracts. The silica gel of three beakers was spiked with sediment extract derived from 1.25mg, 10mg and 100mg sediment, respectively (following the handling procedure of sediment extract described in 4.2.4. in the fourth experiment). To each beaker a single dose (50ul of $5*10^7$/cells/ml, i.e. 0,141 mg dw) of red algae was added together with 10ml GF/C filtrated seawater and four ovigerous females. Evaporation losses were compensated for by adding distilled water. The test was finalized on day 10 due to abnormally high lethality in controls (see discussion). No endpoints were measured.

The second sublethal test attempt was performed as the first accept from that food (micro algae, i.e. *R. Salina*) was added three times/week (0,141 mg dw, 7 times) and seven sets of ten replicate test beakers were used, with 200mg silica gel and extracts derived from 0.02mg, 0.1mg, 0.5mg, 2.5mg and 12.5mg. The test was finalized on day 16.

Microplate fluorometric high-range assay with the RiboGreen was performed to quantify RNA in individual copepods after extraction with N-laurylsarcosine followed by RNase digestion as described in detail elsewhere (Gorokhova and Kyle 2002). Measured RNA concentrations were expressed as $\mu g\ ind^{-1}$. Five randomly selected copepods, in copepodite stage three (CIII), from each concentration were identified and collected, with all instruments washed in Rnase Erase (Q Biogen, Invitro, Sweden AB. Cat. Number 2440-204). Identification of copepods was performed with photographic software programme Leica IM50, Image Manager, on a microscope slide. CIIIs were preserved in RNA *later* (Sigma-Aldrich CO, USA. Lot 073K10485). After CIIIs were removed, Lugol's solution was added to all test beakers for the population structure analysis. Copepod individuals in each test beaker were identified as adults or copepods and counted the following day.

After about two weeks, CIIIs were put in vials containing 1.5 ml extraction buffer (1% v/w sacrosyl in TE buffer [Tris EDTA Buffer, Q Biogen, Invitro, Sweden. Cat. Number TE1X1000]). Cells were opened by sonication in ultra sonic bath filled with ice, two times one minute. Vials were then carefully shaken at room temperature on a multiple vial head for one and a half hour, diluted 1:4 with extraction Buffer and shaken for additional 15 minutes. Fluorescence measurements were performed according to Gorokhova & Kyle 2002 using a microplate fluorometer (FLUOstar OPTIMA) and a black, solid, flat bottom microplate. Working solutions for RNA was diluted in StB in concentrations ranging 0.01, 0.06; 0.12 $\mu g \cdot ml^{-1}$. Working solutions for DNA was diluted in StB in concentrations ranging 0.01; 0.05; 0.08 $\mu g \cdot ml^{-1}$. Plate included extracted CIII samples (two replicates of each extracted CIII sample), blanks, RNA and DNA standards (three replicates). Samples and standards were treated with RiboGreen (Ribogreen^{TM} RNA Quantitation Kit, Molecular Probes Göteborgs Termometerfabrik, Sweden Cat nr R11490) and the plate was scanned. After first scanning, Rnase was added to all samples as well as to DNA standards, and plate was incubated for 30 minutes at 37°C before a second scanning. The amount of RNA was calculated according to RNA standard curve, using the difference between scan #1 and #2. DNA concentrations were calculated according to the DNA standard curve derived from scan #2. Fluorescence measurements were performed using fluorometer FLUOstar Optima (BMG Labtechnologies, microplate reader, filters: 485 nm for excitation and 520 nm for emission) and black solid flat-bottom microplates (COMBO; Labsystems, cat. # 9502067). The plate was scanned with 0.2 sec well measurement time, 10 measurements per well.

STATISTICAL ANALYSIS

The results were statistically analyzed with One-way Anova, Post Hoc tests (Dunnett's 2-sided T-test and Tamhane) and regression analysis.

Results

ACUTE TESTS I-II

No effect (mortality) was observed.

6 DAY LDR TEST I

No effect on mortality or LDR was observed at the doses (extracts derived from 0,3, 3 and 30 mg sediment/(8 nauplii*10ml water*3mg salmon feed), respectively) (Figure 1). There were large variations within the groups and high mortality in the solvent control (SC). Using linear regression a negative dose-response relationship can be suggested in LDR (R^2=0,9712).

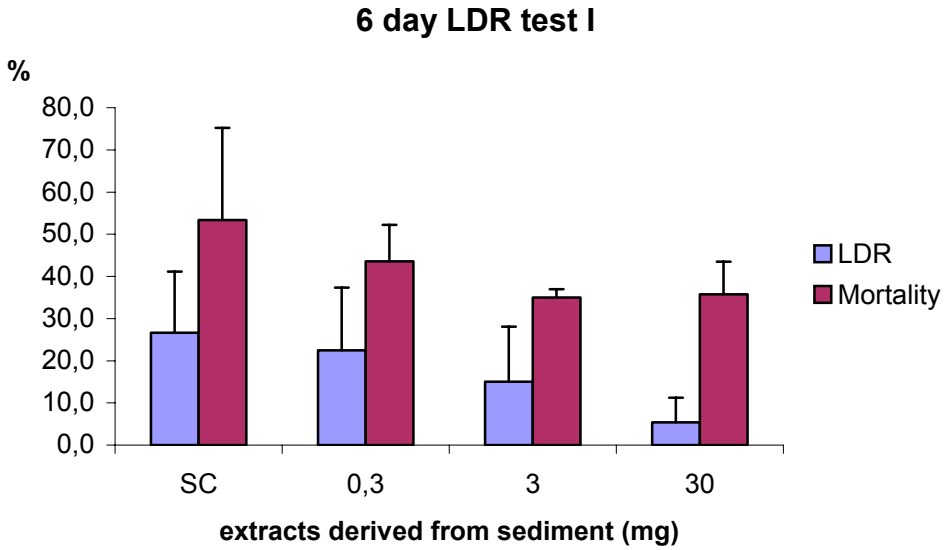

Figure 1. Larval Development Rate and mortality of N. spinipes exposed to extracts derived of sediments from Örserumsviken. Error bars indicate 95% confidence interval of means.

6 DAY LDR TEST II

A significant effect in LDR was observed in the highest dose (extracts derived from 100mg sediment/(5 nauplii*10ml water*0,141mg dw micro algae)) and a negative exponential dose-response relationship could be suggested with regression analysis (R^2=0,9911). Mortality was not significantly increased in any of the treatment compared to the control. (Figure 2).

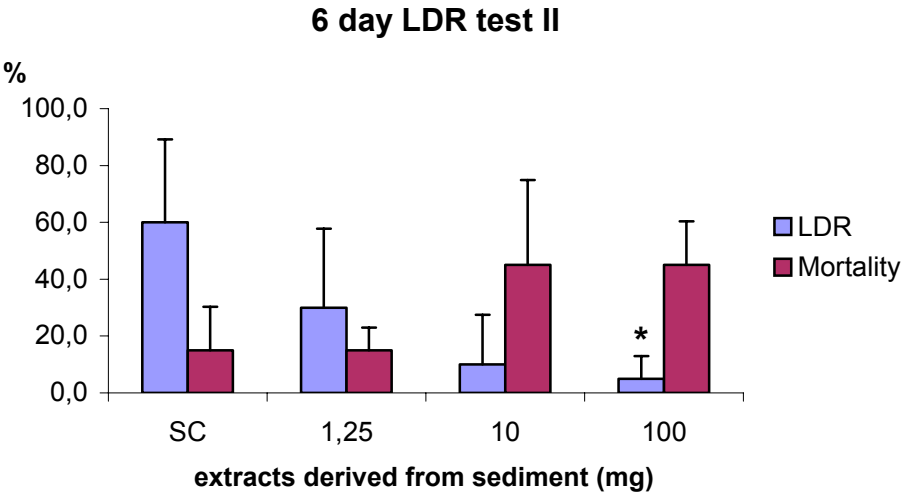

Figure 2. Larval Development Rate and mortality of N. spinipes exposed to extracts derived of sediment from Örserumsviken. Error bars indicate confidence 95% interval of means. Asterisks denote significant differences from control (p<0,05).

16 DAY SUBLETHAL TEST I

The test was interrupted at day 10, due to high mortality in the control.

16 DAY SUBLETHAL TEST II

A significant effect was observed in number of adults in the highest dose (extracts derived from 12,5 mg sediment/(10ml water*5 ovigerous females*200mg silica gel*(7*0,141)mg dw micro algae)) (Figure 3). No effect in amount RNA was observed.

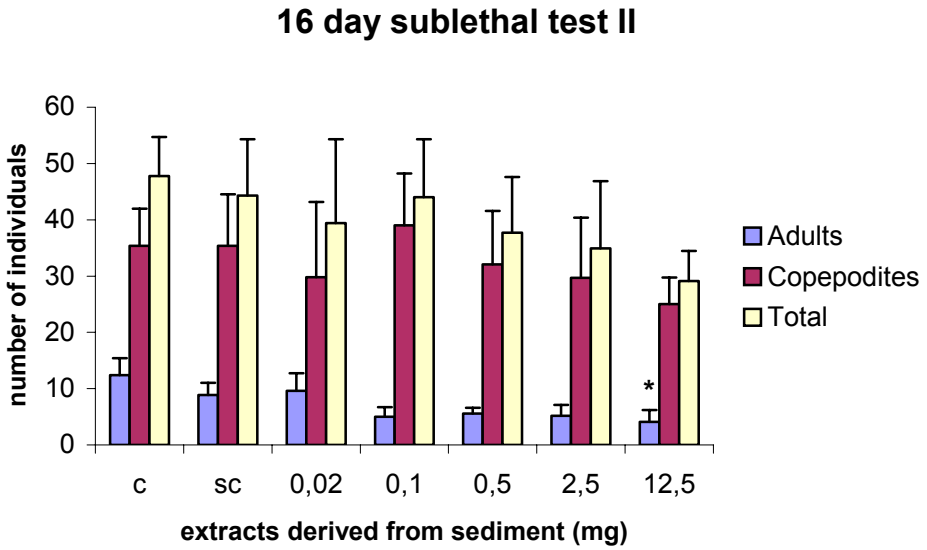

Figure 3. Number of adults and copepodites of N. spinipes after 16-day exposure to extracts derived of sediment from Örserumsviken. Error bars indicate 95% confidence interval of means. Asterisks denote significant differences from control ($p<0,05$).

Discussion

16-DAY SUB LETHAL TESTS I-II

In the second sublethal test a significant effect was observed in number of adults in the highest dose, but no effect was observed in number of copepods or total number of individuals. The reasons for the absent effect may be due to:

1) Low toxicity in the chosen dose interval (most probably).
2) Detoxification in test system due to added micro algae.

The effect observed among the adults was observed in the same dose range as in the 6-day LDR test II. This suggests that the observed effect in the LDR test may be due to toxicity rather than stickiness of the extract (see 4.4.2.), since no sticky droplets were present in the 16-day sub lethal test (because of the present silica gel).

No significant effect from the RNA-analysis was observed. I suggest five possible explanations:

1) Exposure of pollutants often decrease through time in single-dose chronic sublethal tests (Chandler and Green 2001), leading to significantly lower concentrations of pollutants in the subsequent test-period. Survivors on day 16 might have suffered less stress from the extracts than those exposed in the beginning of the experiment. However, such a scenario may be more probable if using more volatile pollutants, or if the sorbent is of such nature (i.e. other than the silica) that a slow fraction of the pollutants is promoted (see 3.1.).
2) Detoxification of the system due to the additions of organic material, i.e. micro algae.
3) The poorly understood evertebrate detoxification-system of N. spinipes.
4) The response mechanisms of N. spinipes to hexane-extractable substances from sediment may be of other character than coupled to RNA levels.
5) The response mechanisms at RNA level of N. spinipes to the hexane extractable fraction may need more time than 16 days to be initiated. It is known from bioconcentration tests that lipophilic substances generally need about 28 days for reaching equilibrium with test organisms of same size as N. spinipes (Ingersoll, Brunson and Dwyer 1998).

It would be interesting to investigate which other endpoints that could be useful and possibly measured parallel to the current ones in our test.

In the first sub lethal test, the high mortality in controls was with great certainty due to deficiency in food (i.e. micro algae) in the test system. To confirm this assumption survivors from controls were fed for one more week after finalizing the test, which quickly led to an awakening among the copepods and some females even generated new nauplii.

LDR ENDPOINT

To construct a comfortable method to prepare the beakers with the hydrophobic extracts was a lot more difficult than we first expected. Firstly, the extract was extremely unwilling to absorb to the salmon feed (Acute tests I-II and 6-day LDR test I). Secondly, the hydrophobic extract was difficult to spread out as a plane film on the bottom of the beaker, which the micro algae were meant to absorb extract from (6-day LDR test II). The dry extract immediately formed droplets when water was added. The droplets seemed sticky and at some occasions they acted as fly-catchers on the nauplii, which were hindered in their feeding in the higher doses. This, of course, may lead to misinterpretations of results. One can be tempted to believe that slow growth rate in higher concentrations are due to toxicity of the extract, while it is rather the stickiness of the extract. However, the results from the 16-day sub lethal test II indicated that this suspicion was incorrect (see 4.4.1.), which suggests that the LDR is a useful and considerably sensitive endpoint in our test concept.

ACUTE TOXICITY TESTS

The absent mortality in the two acute toxicity tests may be caused by too low concentrations of the extracts. However, some of the test organisms instantly were in immediate contact with the sediment extracts, visible to the very eye as small yellowish droplets (which some of the copepods even attached to). This may demonstrate an insensibility and weakness of acute tests as assessment tool to reveal ecotoxicological effects.

Before the experiments were started, the extracts solved in n-hexane, were believed to be absorbed to the salmon feed, which would profit exposure through digestion. After the two first experiments this seemed not to be the case, the extract rather attached to the surrounding glass and the major exposure path for the sediment extract became dermal instead of digestive. This problem was larger when using adults. Adults are opportunists capable of ignoring food for several days if they do not find it suitable (Breitholtz, personal communication), which could be the case with the spiked salmon feed. Another problem is that adults are good swimmers and can avoid the bottom of the beakers if they found this environment stressful, which also is much probable in the presence of the extracts. These could be the reasons for the absent of mortality in the first two experiments. However, these problems could theoretically be overcome if using nauplii instead of adults. The opportunistic characteristics of the nauplii are not as developed as of the adults, the nauplii must feed. Besides this, they do not swim and are therefore forced to stay in close contact with the extracts. The choice of nauplii is from this point of view more practical when not examining full life-cycle endpoints.

FOOD SOURCE FOR N. SPINIPES

The salmon feed used in the acute tests has by convention and lack of alternatives frequently been used in experiments with *N. spinipes* since the early 70´s. The components of this food is not known due to the policy of the producers, but it has recently been discovered that one of its components is an endocrine disrupter, which among other already known factors, like the lack of ecological relevance and the suspected low uptake of the extracts, makes it doubtable for ecotoxicity tests (Magnus Breitholtz, personal communication). Another difficulty with the commercial salmon feed is that it was excellent to hide in for the copepods, which was a major problem when counting them by light microscopy. These reasons, which came up during the first experiments, made it interesting to change the food source to a more natural food source for *N. spinipes*, i.e. the micro algae *R. salina*. The micro algae made it easier to observe and count the copepods and the test also became more ecologically relevant. The only problem the micro algae may have caused was the *increasing* detoxification of the system. Also, in longer tests (>1 week) the unconsumed micro algae starts to degenerate, which may initiate unknown physical-chemical processes in the test system.

SILICA GEL AND EXTRACTS - EXPOSURE OF POLLUTANTS

Presumably, the sorption of hydrophobic substances in our test system (as in natural sediments) primarily was controlled by present organic material. But in the first four experiments (see 4.2.3 and 4.2.4.) the doses of the extracts probably exceeded the amount that immediately could undergo sorption to the present food (extracts formed large droplets visible to the eye, see 4.4.2.), delaying equilibrium. In contrast, in the following two experiments (see 4.2.5.), the silica gel facilitated an even exposure of the extracts by contributing with a large sorption area, spreading out the extracts. This may have enhanced substances to faster reach equilibrium, or at least come closer to equilibrium. The extracts were expected to adsorb to the silica gel weekly, i.e. through hydrophobic repulsion and dispersion forces, following a linear sorption isotherm (Luthy *et al* 1997) (see 3.1.).

A mass balance calculation (Equation 1) of the final test system suggested that when equilibrium was reached, the hydrophobic example substances (i.e. PCB-180, PCB-52 and 2378-TCDD) exclusively (100%) was associated to organic carbon, i.e. 84% to Dissolved Organic Carbon (DOC) and 16% to Particulate Organic Carbon (POC). For the example substances, I used a median $K_{Si\text{-}Water}$=4,61 (l/kg) for *ortho*-substituted PCBs from Bucheli and Gustafsson (2003). To some degree, this result supports the introducing discussion (see 4.1.) about the exposure situation in the test system. It is reasonable to propose that a large fraction of the pollutants was exposed to the organisms through the micro algae, which contributed to the DOC and POC. In spite of this, there were uncertainties about some of the input values in the mass balance calculation, e.g. the amount- and sorbent properties of the organic carbon represented by the micro algae. Also, the calculation supposed equilibrium in test system, which probably never was reached during the test period. This may for instance be supported from the visible yellow film of extracts, which remained in the silica gel when this was present, and the large visible droplets when no silica gel was present. However, it is possible that pollutants similar to the example substances were not included in this yellow film/droplets and thus perhaps were in equilibrium.

$$m\_tot = m\_water + m\_silica + m\_DOC + m\_POC + m\_N.\ spinipes + m\_air$$

Equation 1. Total mass of a substance in the test system where *m_water, m_silica* etc are the mass of an example substance in each phase.

If assuming equilibrium, the results from the mass balance calculation may indicate that the main exposure pathway for the pollutants was *not* implemented through bioconcentration from the water phase, but rather food ingestion of micro algae. Because, of an arbitrary amount (1 mol) of each example substance in the test system, the calculation predicted 0% (~10^{-5} mol) of the example substances present in the water phase and organisms, but 100% (~1 mol) in the organic matter (DOC and POC), which includes the micro algae.

For the system, the calculation suggested that a substance with a low volatility will be present in the water phase (by a few percent), if its $K_{OW}<500$, approximately. This is far below the K_{OW} of the example substances (e.g. PCB-52, $K_{OW}=6{,}7*10^5$), which explains why the calculation suggested that "no" example substances were present in the water phase.

Consequently, with the information from the mass balance calculation and the experimental results (see 4.3.) I suggest that:

- The most probable main exposure pathway of the pollutants was through the food ingestion of the micro algae. With time, it is possible that the amount of POC and DOC, which not is food (e.g. moulting products, faeces etc), became larger, causing a detoxification of the system.

- An unknown amount of the exposure was implemented through diffusive mechanisms in the water phase, which the model could not predict due to the likely non-equilibrium conditions in the system. These conditions may have caused a release of pollutants from the silica gel to the water phase.

Also, it should be mentioned that natural sediments often are rich of silica (i.e. quartz). Thus, with the silica gel present, the test system gained in ecological relevance since it resembled a somewhat more natural habitat for the copepods.

SOLVENTS

A spectrum of acetone solvable hydrophobic substances remained in the sediment after the hexane-soluble fraction was removed. Of course, when only the latter was included in our test, this may lead to a false estimation of toxicity of the sediment samples.

N-hexane vaporizes quickly when aerated, which obstructed volume measurements. Usage of an ordinary pipette when handling hexane-solved extracts was not an option, because the plastic part of the syringe would most probably partly solve in the n-hexane, and there was also a risk that substances in the extract would associate to the plastic. For reliable measures of amount extracts, it was practicable to use a glass injection syringe on a regular pipette and to instantly control and compensate for vaporization on scales.

RIDDARFJÄRDEN

The sediment of Riddarfjärden is a biotope strongly affected by the heavy trafficked area of central Stockholm. Upstream are Västerbron and E4 situated and downstream is Slussen. The water in Riddarfjärden flows quickly and the seabed is a transport-bottom, constantly changing due to the transport of material (Sundberg, personal communication). This consequently lowers the environmental pressure from pollutants, which can be expected to be very high. Some of the samples near the München-brewery and small boat harbor seemed extremely polluted (black, oily). No nematodes or any other organisms were observed in any samples, which is noticeable. The pipe-sample taker was not effective when sediment contains large material, like gravel. Unfortunately, because of lack of time these sediment

samples could not be used in this work, the ecotoxicological results will be accounted for in a future ITM report.

APPROPRIATENESS OF TEST CONCEPT FOR SQA

Referring to the introduction (see 4.1.) of the experimental part and considering the results (see 4.3.), after some refinements of the methodic, I cannot see it inappropriate to recommend our test concept for SQA. The test facilitates prioritization between polluted sites when making management decisions and can be used with other tools to create relevant LOE (see 1.1.). In spite of this, the test may not appropriate for establishing SQGs. Presumably, it would generate a large number of false positives (see 1.2.) due to the high bioavailability conditions in our test system compared to natural sediments. In other words, the high bioavailability conditions in our test system can be said both are the strength and the weakness of the test concept.

Experimental Conclusions

Our test concept may be useful for SQA. Simple endpoints (in respect to performance, but still ecologically relevant) as analysis of mortality, LDR and population structure of *N spinipes* were demonstrated to be considerably sensitive for screening sublethal toxicity of hydrophobic pollutants in sediments. More tests with *N. spinipes* and RNA content in combination with other endpoints, i.e. length measurements (Ghorokova, personal communication) and test conditions may be needed before a test concept can be proposed for standardization. The micro algae (*R. Salina*) worked well in the test system and the silica gel appeared to be a useful carrier (i.e. reference/baseline sediment) of the sediment extracts, performing a reasonable high bioavailability of pollutants. To completely understand the exposure mechanisms of the pollutants in the test system, the mass balance calculation (see 4.4.5.) would need some refinements.

PART V

Summarizing Conclusions

- There is an international requirement in present SQA for (standardized) cost-effective, reliable, ecologically relevant and sensitive ecotoxicity tests to improve effect analysis.
- The test concept developed in this work may be useful in SQA.
- Accurate methodologies for SQA are available and applied in Canada and the U.S.
- The great experience in Canada and the U.S. of SQA should less experienced countries (for instance Sweden and the other Nordic countries) take benefit of, to improve the quality of their SQA.
- Present exposure analysis in SQA is considerably efficient.

Acknowledgements

I would like to thank Dr. Magnus Breitholtz (ITM), Dr. Johan Persson (WSP), the group for biotesting at ITM and all other friendly and helpful people I have met at ITM. My time at ITM has been great and I have learned a lot!

Renare Mark enkätsvar, januari 2005

16 av de 21 länen svarade på utskickad enkät. 10 av 16 län har i enstaka (totalt ca 40) undersökningar använt sig av biotester. Jämtlands (Z) och Jönköpings (F) län har vid flest tillfällen (8 vardera) använt sig av biotester. Stockholms (AB) län har 6-10 gånger utfört biotester i annan (okänd) regi. Microtox är det mest använda testet (E, Y, Z, F, BD, K), därefter fiskfysiologiska undersökningar (G, Z, O, AC). Andra biotester som använts är tester på vattenmossa (M, Z), bottenfauna (Z, O), alg (F), mussla (E).

Samtliga svarande län har mycket ofta genomfört metallanalyser i sina undersökningar (i huvudsak tungmetaller). 5 län (G, AB, S, M, Y) anger ”ca alla undersökningar”. ICP-AES omnämndes (BD, S) som analysmetod. Undersökningar enligt SPIMFAB har utförts regelbundet (>10 tillfällen) i samtliga svarande län utom U (2 tillfällen). Organiska summaparametrar har använts vid ett fåtal tillfällen (EOX ca 4 tillfällen, EGOM ca 3 tillfällen, EOCL 1 tillfälle, m.fl.) i 9 län (T, X, Y, Z, G, BD, F, AB, K). Mer specifika analysmetoder (okända, ev. GC/MS) har använts istället (AC, Z).

3 län (T, X, S) anser att biotester ej varit nödvändiga i de undersökningar som utförts. 2 län (O, BD) menar att MIFO manualens vägledning är bristande. 2 län (F, Z) påpekar att jämförvärden för tester saknas. Vidare omnämns MIFO rapporten inte vara etablerad i branschen (M, K) och experter har varit skeptiska till rapportens test metoder (Z). Testerna i MIFO är ekonomiskt omotiverade och ingen gör fullständiga fas II undersökningar (Z). MIFO rapporten är inte anpassad till analys labbens utbud (E). Den oenighet som råder emellan olika aktörer (konsulter, analyslab, myndigheter) gällande rekommendationer om testmetoder skapar osäkerhet hos länsstyrelser (K). En uppdatering av MIFO rapporten är nödvändig (Z) och skulle göra den mer användbar (Y). Vilken typ av uppdatering länen Y och Z avser anges inte. 1 län (T) framhåller att MIFO rapporten saknar brister.

Slutsatser

Den exponeringsanalys av miljögifter som utförs i länsstyrelsers regi bör anses hålla en förhållandevis hög nivå. Länsstyrelsernas behov av kompetens och utrustning inom detta område tillgodoses av etablerade analyslab. Detta ställs i kontrast till avsaknaden av effektanalys inom länsstyrelsers undersökningar, vilket i stor utsträckning negativt kan påverka kvalitet och tillförlitlighet inom miljöriskbedömning. Alltså: det finns ett behov av standardiserade batterier av biotester med tydliga instruktioner för tolkning av test resultat.

Det finns ett missnöje riktat mot den grund MIFO rapporten erbjuder för miljöriskbedömning. Detta missnöje understryker brister i rapportens utformning. Samtidigt effektiviseras miljöriskbedömning om konsekvent metodik samt standardiserade tester för exponerings- och effekt analys tillämpas. Exempel på detta finns i USA och Kanada. Ett dokument av liknande karaktär som MIFO bör därför finnas till hands, vilket aktörer inom miljöriskbedömning kan luta sig tillbaka på.

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Bilaga 2. Det norska systemet för riskbedömning av sediment

Risk assessment of contaminated sediments – development of a Norwegian Risk Assessment System (RAS)

PM prepared by Torgeir Bakke and Aud Helland, NIVA, in cooperation with Gijs Breedweld, NGI, Espen Eek, NGI, Torsten Källqvist, NIVA, Amy Oen, NGI and Anne Kibsgaard, NGI.

Contents

Introduction

The Norwegian guidelines for risk assessment of polluted sediments have been developed to assist authorities and other problem owners dealing with remedial action plans for polluted fjord and harbour sediments. The need for guidelines arose from the fact that Norway today has restrictions on consumption of fish and/or shellfish in 31 fjord and harbour areas. Most of the restrictions are on fish and blue mussels and are due to high levels of PAH and PCB. Since the primary sources of pollution were drastically reduced during the 1990ies by cleaner industrial technologies, the major concern today is the bottom sediments and their contribution of pollutants to the surrounding marine environment.

The Norwegian risk assessment system is based on similar systems from the Netherlands (Verbruggen et al. 2001), the European TGD (European Chemicals Bureau), Canada (British Columbia) and the USEPA. Use of elements from the Norwegian offshore risk models for drill cuttings and produced water (Johnsen et al. 2000, Smit et al. 2005) has also been considered.

The risk assessment system (RAS) will be presented on the internet (www.sft.no) in the course of 2005.

Risk assessment principles

The RAS is intended to be applied as a tool to select polluted sediment areas for mitigating action. The RAS covers the risk that such sediments may have a negative impact on resident organisms or that they cause negative effects on the surrounding environment including human health.

The RAS follows a tiered approach with 3 levels of assessment as illustrated in figure 1. The step from one tier to the next is typically characterized by

- increased complexity in the assessment,
- stronger reflection of local conditions,
- less conservatism in estimates and in factors and coefficients used.

Description of the risk process

Tier 1 – Potential risk

The aim of the Tier 1 RAS is to identify with a minimum of effort the sediment areas that represent an insignificant environmental risk. This is done by assessing their 'potential' risk on basis of measured concentrations of selected contaminants and general toxicity tests evaluated against a set of environmental quality standards (EQS). For the concentrations of contaminants this is equivalent to a PEC/PNEC approach. Toxicity tests on pore water and organic extract of the sediments are included to cover combined effects of all contaminants present as well as compounds not included in the chemical analysis.

The EQS values applied (some shown in Table 2) have mostly been derived from literature values on environmental risk (Verbruggen et al. 2001) and risk to

human health (Baars et al. 2001). Where these values differ for a specific compound the lowest concentration has been selected as the EQS.

The Tier 1 RAS requires that a minimum set of sediment samples are subjected to the chemical and toxicological analyses proposed. For a sediment area of 50 000 m^2 or less a minimum of 5 sediment locations shall be sampled. Larger or topographically more complex areas require gradually larger number of samples. The required minimum set of compounds to be analyzed is shown in Table 2. Compliance according to the RAS requires that all compounds from all individual samples show concentration and toxicity below the EQS.

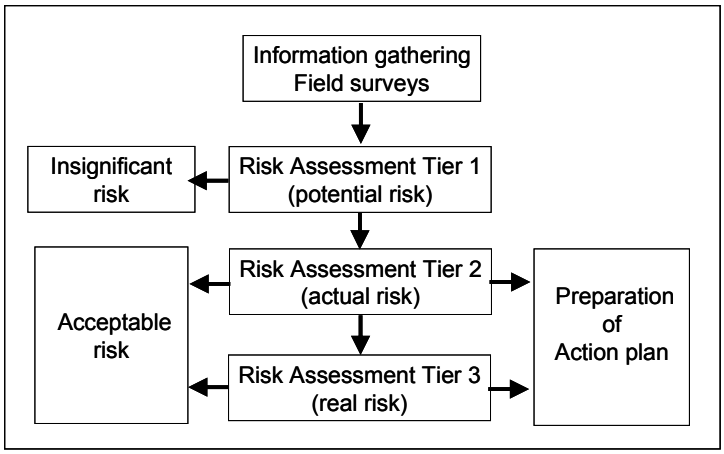

Figure 1. Outline of the Norwegian Risk Assessment System for contaminated sediments

Table 1. Minimum range of parameters to be analyzed in sediments subjected to the RAS.

Parameter group	Parameter
Physical characterization	Water content, content of silt and clay (fraction < 2µm and < 63µm),
Heavy metals	Hg, Cd, Pb, Cu
Non-chlorinated organic compounds	PAH_{16} THC (total hydrocarbons)
Chloro-organic compounds	PCB_{7}
Other compounds	TOC, TBT
Toxicity tests	Skeletonema (pore water and extract) DR-CALUX/EROD (extract)

Tier 2 – Actual Risk

Sediment areas that fail to comply with the Tier 1 EQS are normally subjected to Tier 2. Due to the conservative nature of the EQS values in Tier 1 the situation may arise that more sites will have to be treated in Tier 2 than time and money allow. Hence one may want to prioritize the most polluted sites. A ranking system for this is proposed in the RAS, based on the degree of exceedance of the Tier 1 EQS. The ranking system produces a Potential Risk Index (PRI) for each sample as the sum of all measured/EQS ratios for the parameters analyzed in that sample, and an

overall PRI for the sediment area as the mean of all sample PRIs. A prerequisite for using the PRI to rank sites is that the PRI is calculated on the same set of compounds at all sites.

In Tier 2 the 'actual' risk is assessed along 3 lines:

- risk for mobilization and transport of contaminants to other areas
- risk to human health
- risk of effects on organisms, populations and ecosystems.

These 3 lines also correspond to the most typical levels of ambition expressed in the environmental goals underlying local and regional environmental management plans. In this context one may envisage the RAS as a mean to assess the contribution from the sediments to the total risk that the environmental goals will not be achieved.

The Tier 2 assessment is based on estimates of fluxes of contaminants along several likely transport routes from the sediments towards surrounding ecosystems and humans (diffusion, biodiffusion, erosion, and food chain transport), and of likely concentrations in target species (e.g. fish and shellfish) and biotopes (e.g. the water column). A series of flux calculation formulas are given and general parameter and coefficient values for these formulas are proposed, in case site specific information is lacking. For human health risk assessment the RAS provides contaminant flux estimates along transport routes resulting in exposure via seafood consumption, sediment ingestion, swallowing of seawater with resuspended sediments, etc. The latter should be used at sites intended for recreational use. Tier 2 also requires selected whole sediment toxicity tests to be performed. If the theoretical food chain transport estimates are deemed unreliable, a test on bioavailability of the sediment bound contaminants is recommended and described.

The results from Tier 2 for the risk of effects on the ecosystem and on human health are assessed for compliance against the same acceptance values as are used to develop the EQS for Tier 1. Since common EQS values for risk of dispersion to other areas have not been developed, the RAS suggests how alternative acceptance criteria for dispersion may be adopted by the authorities or the problem owner.

Sediment areas that comply with the relevant EQS and other criteria are deemed to pose an acceptable environmental risk and no remedial action is required. Non-compliance will normally trigger a process of remedial action planning, alternatively a Tier 3 risk assessment.

Tier 3 – Real risk

If non-compliance in Tier 2 is suspected to be caused by an over-conservative or in other ways unreliable assessment, a Tier 3 risk assessment may be performed before a decision on action planning is made. The purpose of Tier 3 is to verify the Tier 2 results or to improve their reliability by application of site specific coefficients and parameter values. The RAS does not give any advice or specifications for Tier 3, since Tier 3 has to be tailored to each specific situation. Possible elements in a Tier 3 risk assessment could be e.g.:

- Direct measurements of fluxes of contaminants from the sediments (e.g. sediment traps, benthic landers)
- Determination of site specific equilibrium coefficients for the compounds in question
- Determination of contaminant body burden in key organisms
- Site specific numerical modeling of the fate and effects of the sediment contaminants
- Benthic fauna structure, biodiversity, sensitivity.

An example of application

As part of the RAS development project the system was used to assess the environmental risk from the harbor areas of Oslo, Drammen and Tromsø. The results from the assessment of a smaller part of the Oslo Harbour named Bispevika are briefly presented here to illustrate the outputs from the RAS, as well as some weaknesses detected in the present version of the RAS.

Tier 1 Risk assessment of Bispevika, Oslo Harbour

Sediment samples were taken specifically for this trial from 5 selected subareas in Bispevika. The samples were taken by use of a 0,1 m^2 van Veen grab and the upper 0-5 cm was subsampled for the analyses. Exceedance of the EQS is given Table 2, which clearly shows that none of the samples comply with all EQS. Non-compliance was seen for several of the metals and for all the organic contaminants analyzed, and in the toxicity tests. Hence the sediments in Bispevika represent an unacceptable potential environmental risk, and a risk assessment Tier 2 should be performed.

The PRI value for the five samples varied from 519 to 756, giving an overall PRI for Bispevika of 651. The exercise also showed that the main contribution to the PRI is from two PAH compounds: Benzo(a)pyrene and Indeno(1,2,3-cd) pyrene. Furthermore the heavy metals contribute very little to the PRI compared to the organic contaminants. It is also a weakness that EQS values have only been developed for sumPCB_7 and not for the individual congeners such as for the PAHs.

Table 2. Level of EQS exceedance (= sediment value/EQS) in samples from Bispevika July 2004. Exceedance above 1 are shaded. EQS values are in mg/kg dry sediment unless otherwise stated.

Parameter\Site	R6	P7	Q8	R9	O10	Tier 1 EQS
Arsen	0,3	0,3	0,2	0,3	0,3	60
Lead	1,1	1,5	1,2	1,1	1,3	175
Cadmium	0,1	0,1	0,1	0,1	0,1	30
Copper	4,5	4,6	4,6	4,2	5,1	70
Chromium	0,2	0,2	0,2	0,2	0,2	500
Mercury	2,5	3,0	3,1	3,1	3,0	1
Nickel	0,9	1,0	0,9	1,0	1,0	40
Zink	0,8	0,9	0,8	0,8	1,0	700
Naphtalene	33	50	55	39	50	0,02
Fenantrene	2,0	3,8	3,0	2,4	3,8	0,4
Antracene	60	104	84	71	120	0,007
Fluorantene	6,7	12	10	7,2	17	0,18
Benzo(a)antracene	10	17	16	11	18	0,06
Chrysene	1,0	1,8	1,6	1,1	2,1	0,82
Benzo(k)fluoranthene	15	32	33	22	40	0,06
Benzo(a)pyrene	143	243	229	171	257	0,007
Indeno(1,2,3-cd) pyren	147	167	183	118	152	0,006
Benzo(ghi)perylene	11	15	15	11	15	0,08
PCB-7	4,8	9,9	6,5	8,0	7,2	0,01
Mineral oil (THC)	22	30	26	30	34	50
TBT	54	54	31	12	13	0,035
Porewater % growth inhibition algae	1,74	1,96	1,72	1,98	2	50 %
Organic extract test in algae. EC50(mg/l)	3,0	3,3	2,9	2,2	1,6	>2000 mg/l
Risk index PRI	**525**	**756**	**709**	**519**	**745**	

Tier 2 Risk assessment of Bispevika

This risk assessment resulted in a series of flux estimates, averaged over the 5 samples, for each of the contaminants. Fluxes via diffusion, erosion and food chain transport are estimated (as examples the results for mercury, benzo(a)pyrene, and sumPCB_7 given in Table 3).

The example calculations in Table 3a show that the actual risk of effects on organisms in the water column is acceptable for mercury and PCB153 (exceedance < 1), but not for benzo(a)pyrene. Taking also Tier 1 results into account, this shows that mercury and PCB153 (probably, since sumPCB_7 by far exceeds the EQS) poses an unacceptable risk of effects for organisms in the sediment at Bispevika, but not for organisms in the water column. Benzo(a)pyrene shows an unacceptable risk of ecological effects in both compartments. The same considerations have to be made for all contaminants analyzed.

Table 3b shows that the risk for human health from contaminants in the seafood from Bispevika exceeds the acceptable limits strongly for benzo(a)pyrene and PCB153, but not for mercury.

Table 3. Calculations from the Tier 2 risk assessment for 3 contaminants from Bispevika (chosen as examples).a

Compound	F_{diff} mg/m^2/yr	F_{eros} mg/m^2/yr	F_{tot} mg/m^2/yr	C_{sv} µg/l	HC_5 µg/l	Exceedance of HC_5
Mercury	0,036	19	19	0,015	0,11	0,14
Benz(a)pyr	0,18	9,2	9,3	0,0078	0,005	1,55
PCB153	0,0013	0,081	0,0082	0,0001	0,0002	0,33

b

Compound	F_{bio} mg/m^2/yr	Dosis mg/kg/d	10%MTR mg/kg/d	Exceedance of 10%MTR
Mercury	1,7E-4	5,5E-7	1,0E-5	0,054
Benz(a)pyr	7,5E-1	2,3E-3	1,0E-4	46,2
PCB153	5,5E-3	1,8E-5	1,0E-6	18,1

F_{diff}: Flux of contaminants from biodiffusion
F_{eros}: Flux of contaminants from erosion (primary propeller effects)
F_{tot}: Total flux of contaminants from the sediments to the water column
C_{sv}: Contaminant concentration in the Bispevika water column due to F_{tot}
HC_5: EQS water concentration effects limits
F_{bio}: Flux of contaminants through the food chain
Dosis: estimated lifetime daily intake of contaminant from the sediments via seafood consumption
10%MTR: Maximum acceptable lifetime dosis in man from seafood alone (assuming that 10 % of the overall contaminant intake will come from seafood) (cf Baars et al. 2001).

In this exercise direct measurements of bioaccumulation of organic contaminants from the Bispevika sediments were also made, according to the procedure recommended by the RAS. This generated site specific values for the biota-to-sediment accumulation factor (BSAF). When substituting these BSAFs for the BSAFs value based on the standard Kd and BCF equilibrium coefficients into the RAS formulas, and repeating the calculations in Table 3b, it appeared that PCB153, but not benzo(a)pyrene, still exceeded the 10%MTR acceptance level given in Table 3b. Hence the site specific calculations were less conservative than the general, showing that the RAS worked as intended in this example.

Tier 2 also comprises a whole-sediment toxicity test on the survival of lugworms (*Arenicola marina*) exposed for 10 days directly to the sediments. The acceptance mortality has been set at 20 % (a common limit for such long term survival tests). The results showed mortalities in the range 47 to 93 % for the 5 sediment samples. Hence, all samples violated the acceptance limit, which is in accordance with the toxicity tests from Tier 1.

The unanimous conclusion from the Tier 2 assessment was that the Bispevika sediments poses an unacceptable actual risk both to human health, organisms and populations in the sediment itself, and organisms in the surrounding marine environment. The next step would then normally be to prepare an action plan for the sediments.

Future developments of the Norwegian RAS

The RAS is publicly available at the SFT homepage by the 1 March 2005. The period 2005 and 2006 is considered a fairly intense testing period, in particular since the Norwegian regional action plans for contaminated sediments are to be finished by the end of 2005. The RAS is already used and will be more widely used in this connection.

Development and application of the present version of the RAS have shown several area of potential improvement. The ones which have been brought forward to the authorities for consideration at present are

- The proposed EQS and the calculations performed in Tier 2 are highly dependent on the sediment-water partitioning coefficients (Kd) used. The measurements of bioavailability of contaminants from the three trial sites have shown that the proposed Kd values for the PCBs are reasonably in harmony with the measured values (proposed are higher by a factor of 1 – 10), whereas the proposed and measured Kd's for the PAHs may differ by more than 3 orders of magnitude, and difference varies strongly from one site to another. One should therefore consider whether pore water rather than sediment concentrations should be applied in the RAS.
- The toxicity tests proposed should be evaluated for suitability when more results are generated, since they also appear to reflect other properties of the sediments than contaminant content.
- The issues of biomagnification and of combined effects from several contaminants are not covered sufficiently by the RAS.
- Some of the EQS values proposed for Tier 1 are provisional, and there is a lack of EQS for individual PCB congeners. The latter results in an unbalanced emphasize between PAHs and PCBs in the RAS.
- Harbour sediment TBT concentrations may be extremely high compared to effects levels seen in the literature. This may cause TBT to govern the results of the risk assessment completely. The dominating role of TBT and to some extent benzo(a)pyrene should be assessed further to see if this is justified.

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Bilaga 3. Användning av biomarkörer i Norge

Risikovurdering av forurensede sedimenter (RAFS): Norske erfaringer fra biomarkøresponser i fisk fra dioksin-forurensede Frier- og Eidangerfjord, S-Norge

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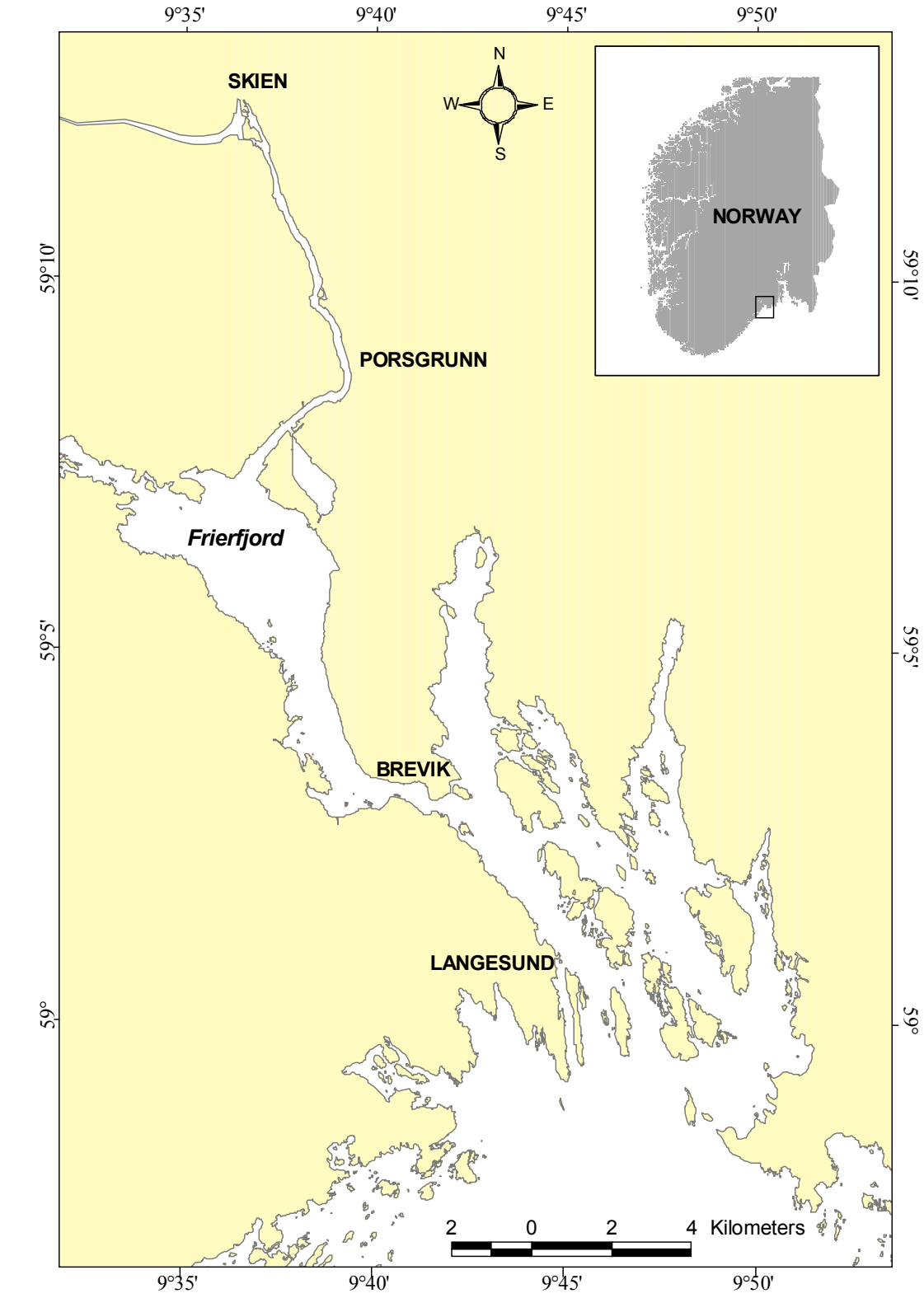
Contents

Bakgrunn

Betydningen av sedimenter forurenset med ulike typer miljøgifter er et av de store miljøspørsmålene i Norge i dag. Historisk har store primærutslipp fra punktkilder til det marine miljø medført lokalt, sterkt forurensede sedimenter. Etter at primærutslippene i Norge nå er betydelig redusert, representerer sedimentene en potensiell kilde til påvirkning som kan strekke seg over flere 10-år. Myndighetene har derfor lansert omfattende planer for opprydding i havne- og fjordsedimenter. En slik opprydding er kostbar. Det er derfor viktig at beslutningsgrunnlaget er kunnskapsbasert slik at tiltak kan gjennomføres mest mulig kostnadseffektivt.

Grenlandsfjordene har mottatt store tilførsler av dioksiner fra Norsk Hydros magnesiumfabrikk på Herøya (1951-2002). Forurensningen har ført til restriksjoner og råd knyttet til omsetning og inntak av sjømat fra området. I de siste tjuefem årene frem til nedleggelsen av bedriften er det blitt gjennomført store utslippsreduserende tiltak, og konsentrasjonene i miljøet i Grenlandsfjordene har avtatt betydelig. I 1990 hadde man forhåpninger om at restriksjonene på fangst og konsum av sjømat kunne oppheves ved årtusenskiftet. Dette har imidlertid ikke skjedd.

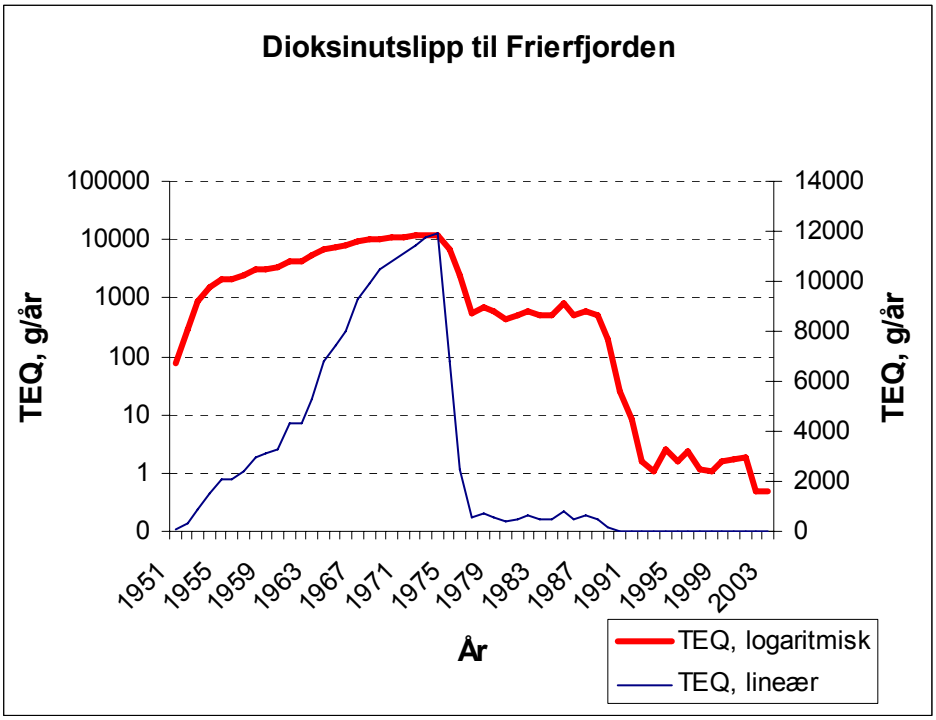
Påvirkningen fra forurensede sedimenter på vannmasser og organismer er komplisert og krever inngående prosesskunnskap. For å forstå situasjonen i Grenlandsfjordene og samtidig erverve kunnskap som også er relevant for andre miljøgiftbelastede fjorder, var det behov for grunnleggende forskning. Med denne bakgrunnen har Norges Forskningsråd og Norsk Hydro i samarbeid finansiert forskningsprosjektet ”Dioksiner i Grenlandsfjordene – *DIG*” (Næs et al. 2004) hvor hovedmålet har vært å gi en helhetlig forståelse av kjemisk og biologisk flyt og effekter av dioksiner i Grenlandsfjordene, Figur 1. Foruten å utføre grunnleggende forskning skulle kunnskapen og resultatene kunne brukes som grunnlag for beslutning om tiltak. Dette PMet oppsummerer effekter på villfisk fra området. Ytterligere dokumentasjon finnes i Hylland et al. (2004).



Figur 1. Oversiktskart over Grenlandsfjordene

Tilførsler av dioksin til Grenlandsfjordene

I 1951 startet Norsk Hydro produksjon av magnesium på Herøya. I den prosessen ble dioksiner og også andre klororganiske forbindelser dannet som biprodukter ved klorering av magnesiumoksid for å gi vann-fri magnesiumklorid. Det førte til betydelige utslipp til Frierfjorden, Figur 2.



Figur 2. Utslipp av dioksiner beregnet som 2,3,7,8-TCDD-toksisitetsekvivalenter til Frierfjorden fra magnesiumfabrikken på Herøya. Tall fra før 1987 er estimert ut fra relasjon til verdier av andre klorerte hydrokarboner. Kilde: Trond Gulbrandsen, Norsk Hydro Forskningssenteret. Dataene er presentert både i en logaritmisk og lineær skala. Den lineære skalaen viser tydeligere den store utslippsreduksjonen rundt 1980.

Effekter på villfisk i Grenlandsfjordenene

Tidligere undersøkelser

Eventuelle effekter av dioksinene i fjordsystemet har tidligere blitt vurdert i forskjellige sammenhenger. Et bredt spekter av metoder ble benyttet i en stor internasjonal workshop allerede i 1986 (Bayne et al. 1988). Til tross for at tilførslene på det tidspunktet var høye, var det bare noen av metodene som viste at det faktisk var helseeffekter hos fisk og blåskjell (*Mytilus edulis*) under workshop'en i 1986 (Widdows and Johnson 1988). Det eksisterer lange dataserier for tilstanden for samfunn på bløtbunn og hardbunn i fjordene og det ble gjennomført en begrenset overvåking av eventuelle helseeffekter hos fisk og blåskjell midt på 1990-tallet (Hylland et al. 1997). Effekter på bløtbunn har generelt kunnet tilskrives organisk

belastning, begrenset vannutskiftning og oksygenmangel. Det har foreløpig ikke vært gjort en gjennomgang av eventuelle dioksin-relaterte effekter på hardbunnssamfunnet i fjorden. Overvåkingsprogrammet i 1996 viste at det var åpenbar påvirkning på både blåskjell og torsk (*Gadus morhua*) i fjordområdet. Blåskjellene fra Frierfjorden hadde mindre ressurser igjen til reproduksjon og vekst enn blåskjell lenger ut i fjorden, sannsynligvis på grunn av dioksinbelastningen. Effektene kunne imidlertid i noen grad også tilskrives forskjeller i hydrografi (salinitet) i de undersøkte områdene. Effektene på torsk som ble observert i 1996 kunne imidlertid entydig tilskrives dioksin-påvirkning. Før gjennomføringen av DIG-prosjektet fantes det altså data som tydet på at det kunne være påvirkning av fisk i området. Målet med DIG-prosjektet var å avklare om slike effekter vedvarer i fjordområdet, om de også gjelder andre fiskearter enn torsk (sjøørret (*Salmo trutta*)og skrubbe (*Platichtys flesus*)) og om effektene er sesongavhengige. Det ble også mulig å relatere eventuelle effekter til konsentrasjoner i organismene i en større grad i dette nye prosjektet og det ble benyttet et bredere spekter av metoder enn i det tidligere prosjektet.

Biologiske effekter av dioksiner

Biologiske effekter av miljøgifter kan identifiseres og overvåkes ved bruk av metoder som spenner fra påvirkning av bunndyrsamfunnet til endringer i nivåer av proteiner eller enzymer (biomarkører). Mens den økologiske relevansen er størst for metoder som vurderer effekter på samfunn, er metodene i den andre enden av skalaen (biomarkører) bedre egnet til å identifisere og kvantifisere miljøgiftspesifikke effekter. Biomarkører benyttes til å vurdere eventuelle effekter av miljøgifter på helsen til enkeltindivider av fisk eller virvelløse dyr. Det er imidlertid ikke mulig i dag å forutsi effekter på populasjoner med utgangspunkt i biomarkørresponser. Det meste av metabolismen i virveldyr (som fisk) skjer i leveren. De fleste av biomarkørene blir derfor målt i lever.

Hos alle organismer er det enzymer som katalyserer nedbrytningen av fettløselige, organiske stoffer. Dette kan være både naturlige stoffer, som steroide hormoner, eller fremmedstoffer, som polysykliske aromatiske hydrokarboner (PAH), polyklorerte bifenyler (PCB) eller dioksiner. Slik nedbrytning foregår oftest i to trinn, som kalles fase-I og fase-II. **Fase-I** aktivitet utgjøres hovedsakelig av et enzym som hos fisk kalles **cytokrom P4501A** (forkortes ofte **CYP1A**). Aktiviteten til dette enzymet i fisk og pattedyr blir vanligvis målt med et substrat som heter 7-etoksyresorufin og responsen kalles EROD. Det er også mulig å måle konsentrasjonen av enzymet direkte. Dioksiner er blant de stoffene som sterkest påvirker disse enzymene – eksponering for dioksiner fører til en økning i mengde enzym og aktivitet. Andre miljøgifter kan også ha samme effekt (PAHer, plane PCB) eller hemme enzymet (tributyltenn, TBT). **Fase-II** enzymer katalyserer en sammenkobling (konjugering) av fremmedstoffer med vannløslige komponenter slik at de kan skilles ut, hovedsakelig gjennom galle. Disse enzymene vil endre metabolitter fra fase-I reaksjonene, men også virke direkte på noen stoffgrupper (som i utgangspunktet er mer vannløslige). Det er flere grupper av fase-II enzymer. Hos fisk dekkes fase-II aktivitet av enzymfamiliene **glutation S-transferase (GST)**,

UDP-glukuronyl transferaser (UDP-GT) og sulfotransferaser. Disse enzymene vil også påvirkes av miljøgifter som dioksiner, men krever oftest høyere eksponeringsnivåer enn fase-I før de øker i mengde og aktivitet. I dette prosjektet ble GST målt. Belastning med ulike miljøgifter vil kunne føre til økte mengder frie radikaler i celler, såkalt oksidativ stress. Alle celler har et batteri av mekanismer til forsvar mot slike effekter. Et sentralt enzym er **glutation reduktase (GR)** som "resirkulerer" glutation som er oksidert (noe som skjer ved oksidativ stress). Det forventes at det blir mer av dette enzymet ved økt belastning med frie radikaler i cellene. Også andre miljøbetingelser vil imidlertid kunne forårsake oksidativ stress, blant annet generell stress og lave oksygennivåer.

Hos hunnfisk vil østrogener frigis fra gonadene og gi økt syntese av forstadier til eggeplommeprotein og eggeskallsproteiner i leveren. Disse proteinene transporteres så med blodet til gonadene der de bygges inn i rogn. Hos de fleste fiskearter vil det være svært lave konsentrasjoner av eggeplommeprotein (**vitellogenin**) i blodet til hannfisk, men også hos hannfisk kan syntesen av proteinet "skrus på" av østrogener. Ved å måle vitellogenin i hannfisk kan en derfor identifisere og kvantifisere tilstedeværelsen av miljøøstrogener. Dioksiner har anti-østrogeneffekt hos pattedyr, men lite er kjent om eventuelle effekter på fisk. Tidligere undersøkelser i Grenlandsfjordene har gitt resultater som tyder på østrogeneffekter.

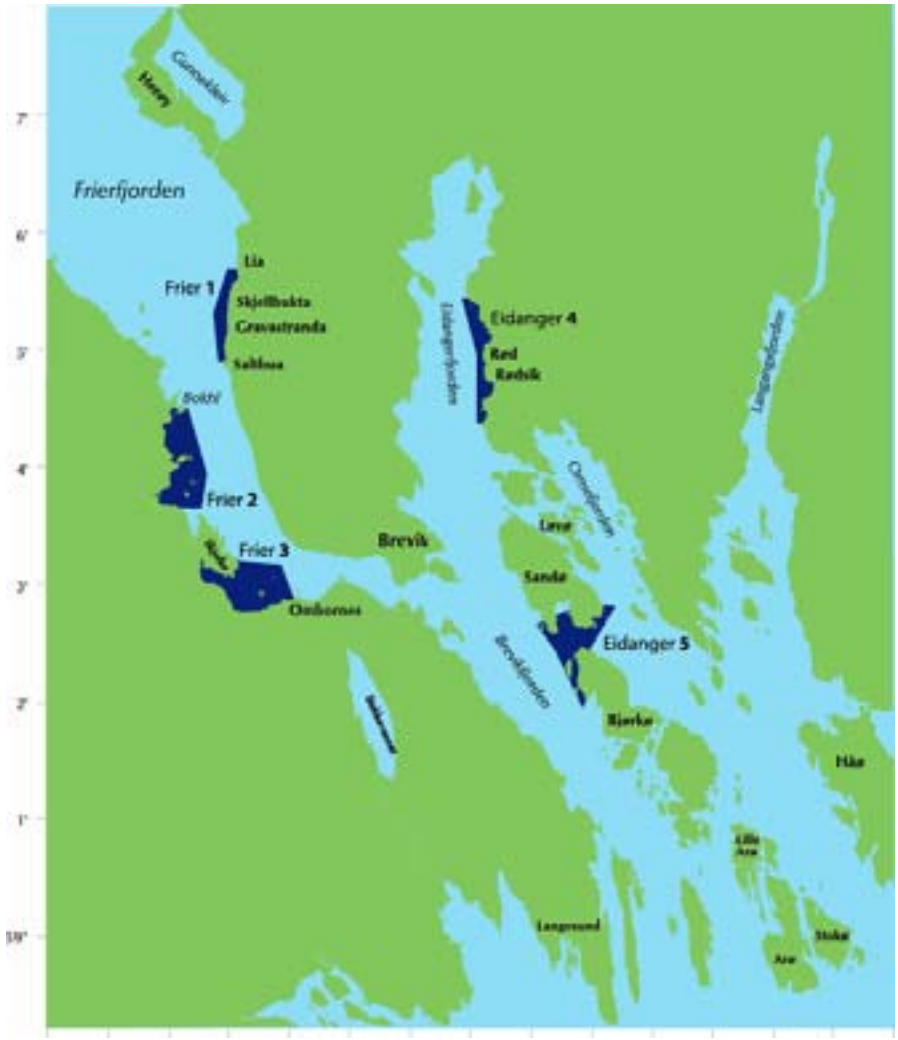
Det er kjent at fiskelarver er spesielt følsomme for dioksiner. I dette prosjektet ble det satt ut befruktede silde-egg for å undersøke eventuelle slike effekter.

I en vurdering av helsen til fisk eller andre organismer er det avgjørende å ha kontroll på faktorer som kan påvirke biomarkør-responser i tillegg til miljøgiftbelastning. Slike faktorer er **alder, størrelse**, **kjønn**, **årstid** og **kondisjon**.

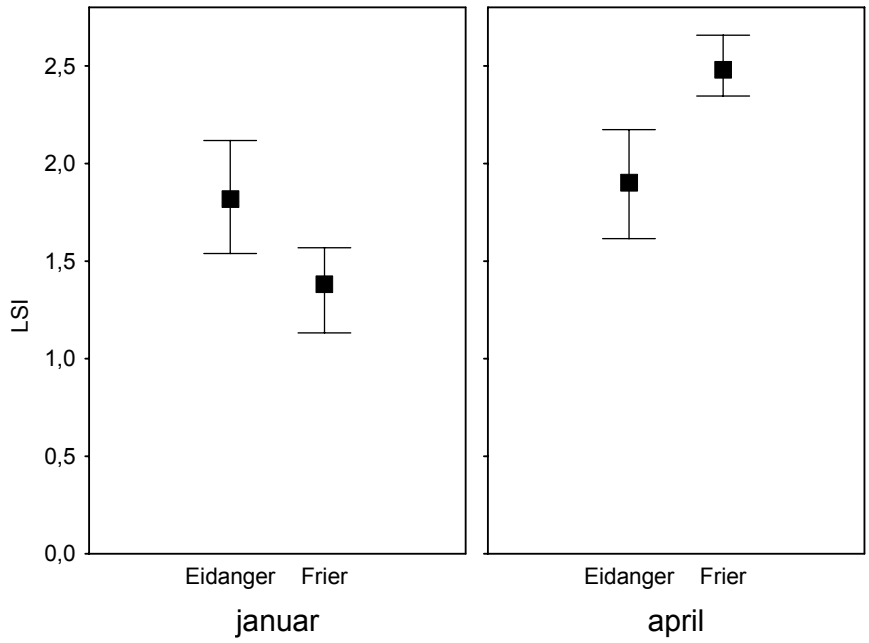
Påvirkning på villfisk fra Grenlandsfjordene

GENERELL STATUS FOR FISKEN

Fisk ble samlet fra to fjordområder, **Figur 3**. Dette ble gjort fordi det var forventet at næringsnettene ville være sammenlignbare og at den viktigste forskjellen på fisk i de to områdene ville være eksponering for dioksin. Resultatene viser at det bare var en faktor 2-3 i forskjell på nivåer av dioksiner i fisk innsamlet i Frierfjorden (høyest) og i Eidangerfjorden. Gjennomgående syntes fisk fra de to fjordområdene å ha like betingelser gjennom hele året. Unntaket var småtorsk, som tilsynelatende hadde dårligere betingelser i Frierfjorden om vinteren, men der de raskt kunne "spise seg opp" på våren, **Figur 4**. Kondisjon ble her vurdert som forholdet mellom levervekt og totalvekt. Siden torsk i stor grad lagrer overskuddsnæring i leveren, kan dette forholdet gi en pekepinn om næringsforholdene.



Figur 3. Områder for innsamling av villfisk

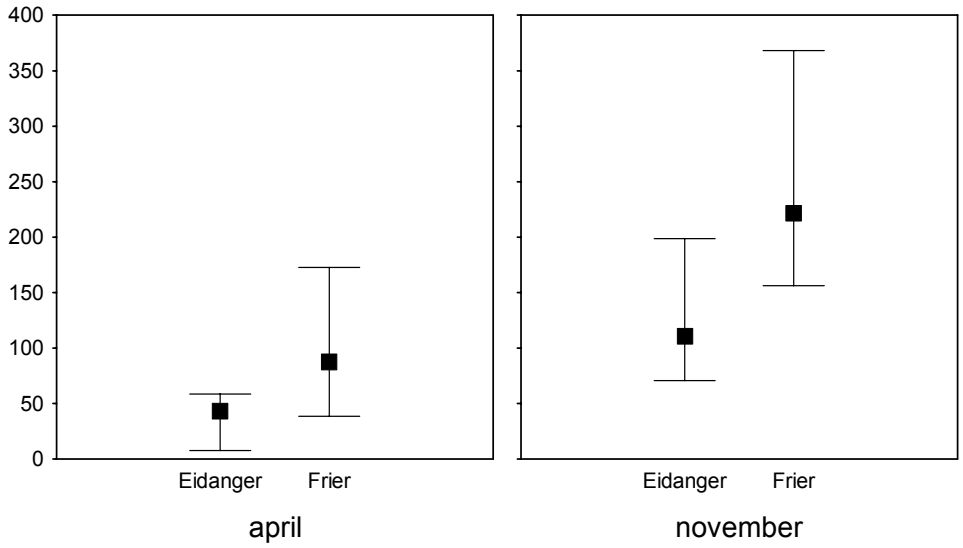


Figur 4. Lever-somatisk indeks (forholdet mellom lever- og kroppsvekt) hos småtorsk innsamlet i januar og i april i de to fjord-områdene. Figuren viser median og kvartiler (25/75-persentiler).

Det er ikke usannsynlig at langgrunne områder i Frierfjorden er spesielt gunstige områder for fødeinntak på våren for småtorsk, noe som vil kunne forklare denne forskjellen. Slike forskjeller vil også være mest tydelige for småfisk (det ble bare innsamlet voksne sjøørret og skrubbe). Til tross for dette tydet resultatene fra analysene av trofisk nivå på et kortere næringsnett i Eidangerfjorden enn i Frierfjorden. Dette vil kunne ha konsekvenser for akkumulering av dioksiner, men bare indirekte på effektene.

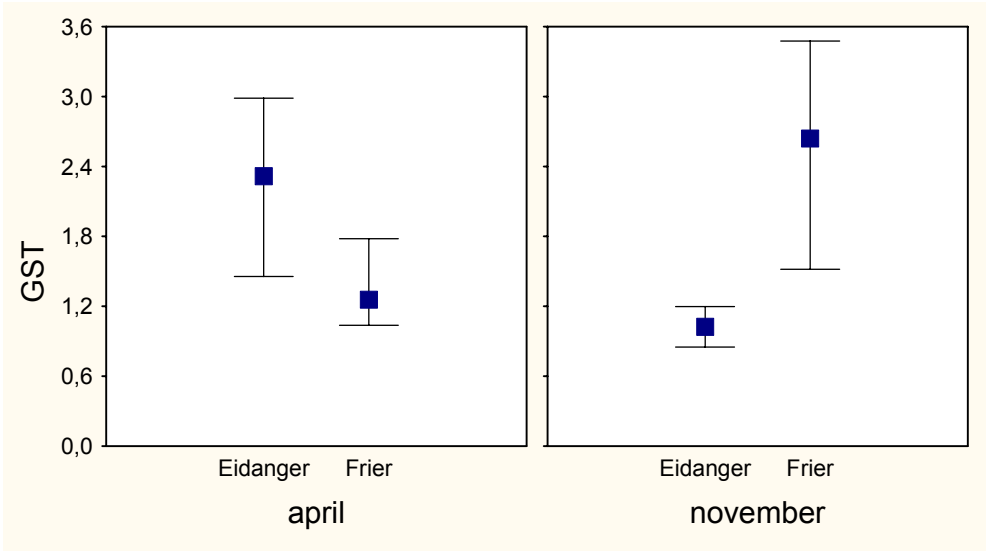
PÅVIRKNING AV AVGIFTNINGSSYSTEMENE FOR ORGANISKE MILJØGIFTER

Hos alle tre artene og til alle årstider var det gjennomgående høyere aktivitet og konsentrasjon av cytokrom P4501A i fisk fra Frierfjorden sammenlignet med fisk fra Eidangerfjorden (se **Figur 5** for resultatene fra torsk). Resultatene viser også en forventet årsvariasjon i aktiviteten av enzymet. En dobling av dioksinkonsentrasjonen i fisk fra Frierfjorden sammenlignet med fisk fra Eidangerfjorden er altså tilstrekkelig til at det gir en signifikant økning i aktiviteten av dette enzymsystemet, til tross for at det allerede er høye aktiviteter. Alle resultater tilsier at fiskene i de to områdene er relativt stasjonære og det synes altså ikke å være en tilvenning hos fiskene til dioksineksponeringen.



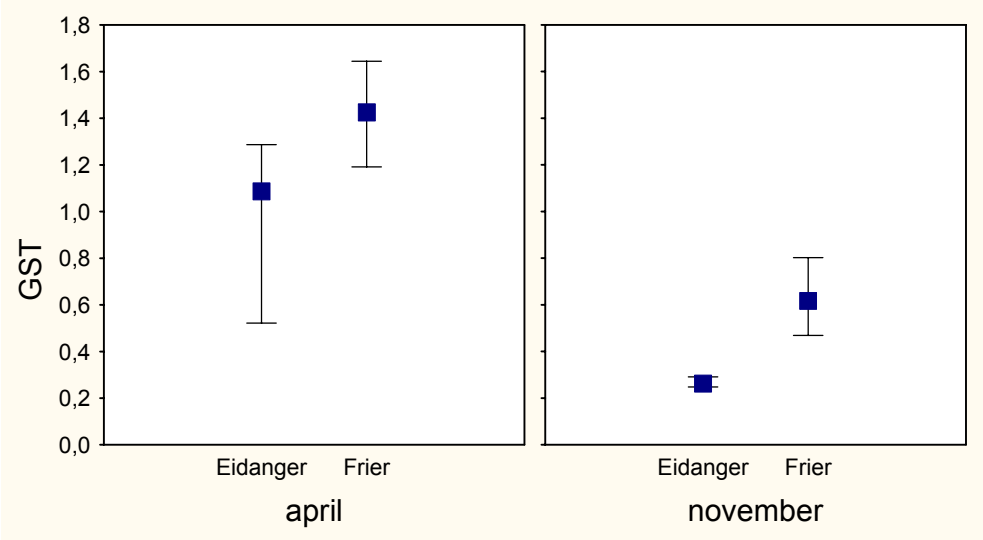
Figur 5. Cytokrom P4501A (pmol/min/mg protein) aktivitet i torsk innsamlet i april og november. Figuren viser median og kvartiler (25/75-persentiler).

Resultatene for glutation *S*-transferase illustrerer hvordan det kan være vanskelig å tolke biomarkør-responser med begrensede kunnskaper om naturlige nivåer og hvorfor det kan være viktig å ha kunnskap om sesongvariasjon. For torsk er det åpenbart ulike responser hos fisk i Frierfjorden i forhold til fisk i Eidanger både vår og høst, men det er bare for torsk innsamlet i november det er en forventet økning hos Frierfjord-fisk, **Figur 6**. Det er mest sannsynlig at det motsatte resultatet som ble sett for torsk i april, er knyttet til fysiologiske endringer hos fisken like etter gyting, men det er begrensede kunnskaper om dette. Det er imidlertid klart at det er forskjeller i fase-II responser mellom torsk i de to fjordområdene, trolig knyttet til den ulike dioksineksponeringen.



Figur 6. Glutation *S*-transferase aktivitet (µmol/min/mg protein) i torsk (begge kjønn) innsamlet i april og november. Figuren viser median og kvartiler (25/75-persentiler).

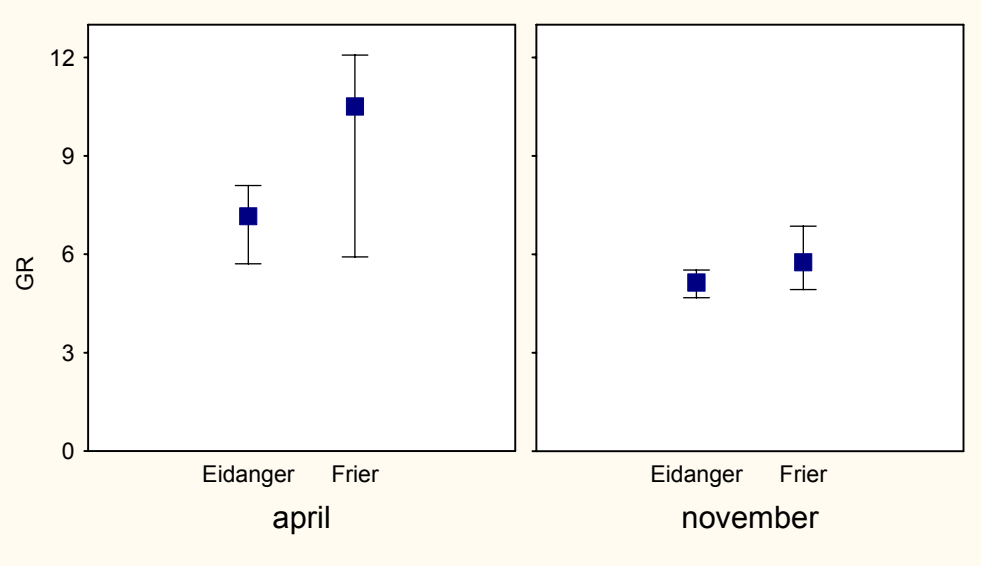
Resultatene for GST viser også en annen egenskap ved følsomme metoder slik som biomarkører, nemlig at det kan være artsforskjeller. For sjøørret var det forhøyd aktivitet av GST i Frierfjordfisk både vår og høst, **Figur 7**. Sjøørret gyter på høsten og det er tydelig lavere nivåer av fase-II enzymet da, men forskjellen mellom områdene beholdes. Resultatene for skrubbe viser nødvendigheten av kunnskap om spesifikke arter; hos skrubbe var det ingen åpenbare forskjeller mellom fjordene på noe tidspunkt (ikke vist).



Figur 7. Glutation *S*-transferase aktivitet (µmol/min/mg protein) i sjøørret (begge kjønn) innsamlet i april og november. Figuren viser median og kvartiler (25/75-persentiler).

EFFEKTER PÅ ANTIOKSIDANT-FORSVARET

Forsvaret mot frie radikaler er mer artsspesifikt enn fase-I og fase-II responser, blant annet fordi det er mange ulike enzymer og anti-oksidanter involvert og ulike arter har generelt variasjoner i strategi for beskyttelse mot skader av frie radikaler. Fri radikaldannelse kan skade proteiner, membraner og DNA i cellene og kan blant annet føre til unormal cellevekst (kreft). Resultatene som ble funnet i dette prosjektet viser som forventet at det er store artsforskjeller. Det var egentlig bare for sjøørret at det var en tydelig økning hos Frierfjordfisk sammenlignet med fisk fra Eidangerfjorden, **Figur 8**. Som for GST er det sannsynlig innvirkning av de fysiologiske endringene knyttet til gyting i november. Høy metabolsk aktivitet i leveren knyttet til produksjon av kjønnsprodukter vil også påvirke andre biokjemiske prosesser.



Figur 8. Glutation reduktase aktivitet (nmol/min/mg protein) i sjøørret (begge kjønn) innsamlet i april og november. Figuren viser median og kvartiler (25/75-persentiler).

ØSTROGEN-EFFEKTER

Som nevnt i innledningen, har noen tidligere resultater pekt på at det kan være en østrogen påvirkning på torsk i Grenlandsfjordene (Hylland og Braaten 1996). Resultatene som ble funnet i dette prosjektet støtter i noen grad opp under de tidligere funnene, men det er ikke dramatiske effekter. Hanntorsk fra Frierfjorden synes å ha noe forhøyde konsentrasjoner av vitellogenin gjennom året sammenlignet med torsk fra Eidanger. Like før gyting øker konsentrasjonen av vitellogenin i blodet til Eidangertorsk til samme nivå som hos torsk i Frierfjorden. Det er usannsynlig at dioksiner er østrogenagonister (at de binder til reseptoren for østrogen). To mulige mekanismer er at dioksiner påvirker enten omsetning av testosteron til østradiol (øker mengden av den cytokrom P450 som katalyserer den omdannelsen) eller at dioksin påvirker den hormonelle ”feedback” for østradiol og derved øker syntesen av hormonet som regulerer østradiolsyntesen i gonadene.

Frekvens av feilutvikling hos sildelarver

I mai 2001 ble det registrert moden sild (*Clupea harengus*) i Frierfjorden, og det ble derfor besluttet å forsøke å gjennomføre et befruktningsforsøk for å sammenlikne kvalitet til plommesekklarver klekket fra egg inkubert i Frierfjorden og et kontrollområde uten dioksinforurensing (Bergstad og Knutsen 2004b).

”Rennende” sild ble fanget på sildegarn 24/5-2001 på vestsiden av Frierfjorden ved Sildeberget. To hunner og én hann ble strøket, og eggene festet til plastplater før de ble befruktet med melke fra hannen. Den 25/5 ble halvdelen av eggene fraktet til Forskningssatsjonen Flødevigen og plassert i klekkesylinder forsynt med vann fra 75 m dyp. Resten av eggene ble plassert på bøye på 6-7 m dyp nær fagststedet for silda. Den 5/6-2001 ble eggene fra Frierfjorden hentet og fraktet til Flødevigen hvor de ble plassert i klekkesylinder under samme miljøbetingelser som de øvrige.

Klekkingen var for begge grupper påbegynt 11/6-2001. Den 13/6 ble det tatt ut prøver av plommesekklarver for vurdering av kvalitet. Kvalitetsvurderingen ble foretatt under lupe mens larvene var levende. Begge grupper egg synes å klekke tilfredstillende og ganske synkront. Sammenlikningen som er gjennomført, indikerer at det ikke var forskjellig frekvens av feilutvikling for larvene fra eggruppene inkubert i Frierfjorden og Flødevigen. Feilutviklingsfrekvensen var ikke unormalt høy for noen av gruppene.

Fisk fra Grenlandsfjordene i forhold til andre områder

Undersøkelser av biomarkørresponser i andre fjordområder har bidratt til at en i dag, for enkelte fiskearter og noen biomarkører, har en tilstrekkelig kunnskapsbase til å kunne avgjøre om miljøgiftbelastningen gir effekter på fiskens helse.

Torsk er den arten som i hovedsak benyttes i det landsomfattende årlige overvåkingsprogrammet JAMP (Green et al. 2002). Siden 1998 har det vært gjennomført enkelte biomarkøranalyser innen dette programmet, deriblant cytokrom P4501A aktivitet (EROD). Videre har det vært gjennomført noen grunnleggende studier om årsvariasjon i cytokrom P4501A hos skrubbe ved Universitetet i Oslo (Hylland et al. 1998). Det eksisterer ikke samme bakgrunnsinformasjon for sjøørret.

Torsk i både Frierfjorden og Eidangerfjorden har høyere fase-I aktivitet enn torsk innsamlet i uforurensede områder langs kysten og langt høyere enn torsk fra Barentshavet. Tilsvarende er fase-I aktiviteten i skrubbe fra begge områdene høyere enn det som er funnet i andre studier. Dioksinnivåene i Grenlandsfjordene fører altså til økt fase-I metabolisme hos fisk og det er en dose-avhengig respons hos noen arter (torsk, sjøørret). Forhøyd aktivitet av fase-I enzymer vil gi endret omsetning også av kroppens egne stoffer slik som hormoner, men det er ikke tilstrekkelig kunnskap til å kunne forutsi hvilke effekter dette kan ha på vekst, utvikling, reproduksjon eller overlevelse hos fisken. Det var forskjeller mellom fjordområdene også for den delen av fase-II systemet som ble målt. De naturlige nivåene av total GST-aktivitet (fase-II) i lever hos fisk er høye og det skal derfor en betydelig miljøgiftbelastning til for å påvirke dette systemet. Resultatene for fase-II viste igjen at det er viktig å ha en gjennomtenkt prøvetaking for effekt-studier, blant annet å unngå perioder når organismer er reproduktivt aktive, sammenligne arter og skille på størrelse og kjønn.

Det var svake tegn til endringer i østrogeneffekter hos torsk, men det er foreløpig ikke mulig å knytte dette til dioksineksponering. Det er behov for eksperimentelle forsøk for å avklare eventuelle effekter av dioksin på hormonregulering hos fisk.

Det er i praksis ikke mulig å ekstrapolere med sikkerhet fra biomarkørresponser til effekter på populasjoner. Data fra strandnotinnsamling gjennomført gjennom mange titalls av år av Havforskningsinstituttet (Flødevigen) tyder på at rekrutteringen av torskefisk ble redusert i Grenlandsfjordene på 60- og 70-tallet. Det er imidlertid ikke data som tilsier at dette skyldes dioksinbelastningen i fjordområdet.

Resultatene fra prosjektet viste også at det er åpenbare forskjeller mellom ulike fiskearter i deres respons til miljøgifter. Det er en generell oppfatning at laksefisk er generelt mer følsomme enn andre grupper (og gir sterkere respons), men resultatene fra dette prosjektet tyder også på at torsk er anvendelig. Skrubbe er kanskje den europeiske, marine arten det har vært gjort mest forskning på og nye molekylære verktøy utvikles også for denne arten. Også tidligere erfaringer har imidlertid vist at blant annet fase-I responser hos skrubbe er vanskelige å tolke. Det er behov for et omfattende bakgrunnsmateriale for denne arten før den kan brukes effektivt i effektovervåking. Det er et begrenset bakgrunnsmateriale for sjøørret, men omfattende kunnskap om andre laksefisk, særlig regnbueørret. Siden en ikke uten videre kan anta at laksefisk er like er det også behov for mer kunnskap om forventede responser i denne arten, men resultatene i dette prosjektet tyder på at også sjøørret er en følsom art og en egnet indikatororganisme.

Bruk av biomarkørdata som grunnlag for tiltak

Biomarkører gir informasjon om individuell helse for fisk (eller andre organismer). Informasjonen er ofte spesifikk nok til at den kan knyttes til miljøgiftbelastning. Dette er også til dels tilfelle i Grenlandsfjordene. Spørsmålet er imidlertid om disse effektene sees på som så bekymringsfulle at en ønsker å pålegge/gjøre tiltak. I den sammenheng har det ofte vært tradisjon for å kreve at påvirkningen også skal kunne påvises på populasjonnivå.

Hittil har ikke resultatene fra biomarkøranalysene Grenlandsfjordene blitt brukt direkte som grunnlag for forvaltning selv om det nok har vært et ønske fra myndighetene å gjøre dette, eller i allefall inkludere resultatene i beslutningsgrunnlaget. Fra problemeiers side hevdes det ofte at målingene ikke er stoffspesifikke nok slik at man kan risikere å gjøre tiltak, som ikke direkte kan knyttes til kjente komponenter i utslippet.

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Bilaga 4. En ny metod för att mäta toxiska effekter från sediment-extrakt

A novel approach to rank potential toxicity of organic sediment extracts in juvenile harpacticoid copepods (*Nitocra spinipes*) and rainbow trout (*Oncorhynchus mykiss*)

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Abstract

In polluted areas harmful chemicals are present as complex mixtures. It has been demonstrated a poor relationship between analyzed pollutants and their contribution to potential toxicity. Interactive toxicological effects, which occur during exposure of complex mixtures, can therefore not be estimated from mere chemical analyses. Still, in Sweden current Sediment Quality Assessment mainly rely on the use of background concentrations to derive Sediment Quality Guidelines; whereas ecotoxicological information is seldom used. As a consequence, there is an urgent need to develop ecologically relevant, reliable and sensitive ecotoxicological tests and methodologies, to improve prioritisation of remedial activities between different locations.

The objective of the present project was therefore to develop an ecotoxicological test approach to rank potential toxicity between different locations by investigating several exposure routes and adverse effects on different biological organisation levels. In sediments, hydrophobic pollutants are generally of major concern, but many ecotoxicity tests used today are aquatic test systems; hydrophobic chemicals are therefore difficult to investigate. Using whole sediment in these systems is also difficult since *e.g.* water content, types of organic carbon, redox-potential and pH, which influence the toxicity, differ between sediments. In our approach, we have therefore used organic sediment extracts and exposed early life-stages of both the copepod *Nitocra spinipes* and rainbow trout (*Oncorhynchus mykiss*). The *N. spinipes* system was adapted for exposure of hydrophobic compounds. The main test variables were mean larval development rate (LDR) and mortality. Rainbow trout were used to investigate biological adverse effects on the individual and the cell level in an intact vertebrate. In the rainbow trout system we investigated abnormalities and mortalities in early developmental stages.

To test the developed approach, five different sediments were collected: Örserumsviken, Frierfjorden and Riddarfjärden as polluted locations as well as Slingsviken and Björkskär as reference locations.

Overall, the crustacean and fish results were consistent. Our results clearly show that sediment extracts from the locations that we expected to be most polluted, *i.e.* former industrial locations, also caused the most significant effects on development and mortality in early life-stages of both crustaceans and fish. Similarly, the sediment extracts from reference or low-polluted locations were not very toxic in either of the test organisms. When solely PAH concentrations in the sediments were compared, however, the most potentially toxic sediment was not the worst polluted, underlining the importance of including biological effects for reliable risk assessments.

The results from the present project show that the developed approach may then be used for priority setting of environmental remedial activities of polluted sites. We have also described a reliable semi-quantitative tool based on potential toxicity of hydrophobic compounds that can be used as first screening step in the prioritising process of remedial activities. By using biological effects in terms of potential toxicity, numerous disadvantages that accompany mere chemical analyses

may be avoided. The three potentially most toxic locations (Örserumsviken, Frierfjorden and Riddarfjärden) contains, at least partly, chemicals that act through similar toxicological pathways and are most likely structurally similar. Finally, the modified *N. spinipes* test system using silica gel as carrier was demonstrated being a sensitive screening tool of hydrophobic toxicants.

Sediment Quality Assessment

Current knowledge

Present *Sediment Quality Assessment* (SQA) is mainly derived from the London Convention 1972 (LC72), which proposed a methodology for limiting environmental impacts of dumping and spreading of polluted sediment (Stuer-Lauridsen and Birkved, 2001). Seventy-eight countries signed the LC72, including the majority of the European nations. Consequently, the framework of this convention is, to a variable extent, reflected in all of these countries' sediment assessment work.

The LC72 has later (1996-1997) been complemented and also made more stringent. The LC72 assessment methodology is basically simple. It proceeds with physical and chemical characterizations of sediments, followed by biological (*i.e.* ecotoxicity) tests, from which biological effects are predicted. In addition to this, governments should establish an:

- *Action list*, which is numerical limit concentrations for pollutants;
- *Impact hypothesis*, which is an exhaustive report of environmental consequences associated with the pollution.

From this information, decisions concerning monitoring programs and management are made. The 1972's "Action list" is similar to present *Sediment Quality Guidelines* (SQGs), which by definition are "numerical limits (*i.e.* concentrations of substances) or narrative statements, recommended supporting and maintaining designated uses of the aquatic environment" (Smith and MacDonald, 1999). Alternatively, "numerical chemical concentrations intended to be protective of biological resources, or predictive of adverse affects to those resources, or both" (Wenning and Ingersoll, 2002). In scientific literature, the term "threshold values" is often also used.

SQG has become an important and widely used tool and several established methodologies for SQA are built up around SQG (*e.g.* (Stuer-Lauridsen and Birkved, 2001; Wenning and Ingersoll, 2002). For instance, the simplest form of a SQA may be represented by comparisions of measured field concentrations of pollutants to a SQG. This generally enhances fast management decisions and lowers costs. In spite of this, there are concerns about the methods used to derive SQGs, and to solely rely on SQGs in SQA (O'Connor *et al.*, 1998); (Stuer-Lauridsen and Birkved, 2001; Wenning and Ingersoll, 2002). The latter scenario is unfortunately often the case. However, to solely rely on SQGs may lead to wrong conclusions of the risks associated with the pollution situation. The underlying supposition in these cases is that SQGs can be used instead of *in situ* measurements of toxicity, but this may not be the case (Wenning and Ingersoll, 2002). SQGs should be

complemented by site-specific ecotoxicological information. In addition, to be able to make sound management decisions, volume considerations of polluted project material must be taken into account (Hansson and Rudén, 2005). In present SQA there are typically no regulatory distinction made in the decision tree between a situation involving a few hundred cubic meters and a few hundred *millions* cubic meters of project material (Chapman *et al.*, 2002).

The most widely practiced method to establish SQGs is the use of *background concentrations*. Concentrations are measured at reference sites and compared to polluted sites. Degree of pollution and safety margins for communities are estimated. However, this strategy alone does not provide information for safe interpretations of limit concentrations. Instead, *ecotoxicity testing* is strongly recommended (European Commission, 2003; Smith and MacDonald, 1999; Wenning and Ingersoll, 2002). Ecotoxicity tests can be used both to derive SQGs and as a tool for *in situ* measurements of sediment toxicity. The Nordic countries Denmark, Sweden and Norway mainly rely on the use of background concentrations instead of ecotoxicity tests (Stuer-Lauridsen and Birkved, 2001) to derive SQGs. In Denmark and Sweden ecotoxicity tests have been used sporadically, i.e. in larger restoration projects and during *in situ* measurements of toxicity (*e.g.* (Engwall *et al.*, 1996; Pedersen *et al.*, 2001; Sundberg, 2005). From a feedback-form concerning usage of methods for ERA among secondary decision-makers in Sweden, it became clear that ecotoxicity tests are not so often used. A common opinion among these secondary decision-makers seems to be that ecotoxicity tests are not necessary in SQA and that results from ecotoxicity tests are difficult to interpret. Out of thousands of national environmental investigations over the years, the 16 responding counties (out of 21 asked) could refer to about 40 occasions when ecotoxicity tests have been used. Of these 40 occasions, Microtox™ is the most frequently utilized test. Sublethal endpoints with ecologically relevant species have never or rarely been investigated. With few exceptions (*e.g.* the Netherlands) this scenario seems to be the situation within the European Union (EU) (Stuer-Lauridsen and Birkved, 2001).

In comparison, Canada, Australia, New Zealand and USA, use SQGs based on standard ecotoxicity tests (Stuer-Lauridsen and Birkved, 2001). The Canadian SQG is derived from an iterative process, where the idea is that new ecotoxicity test results together with existing data are continuously incorporated and the SQG is constantly improved (Smith and MacDonald, 1999). In this context, it is worth mentioning the Great Lakes project (US EPA, 1998) in North America. The project has been progressed since 1978 and was initiated by the United States Environmental Protection Agency (US EPA) and Environment Canada in consultation with other federal departments and agencies. The Great Lakes Project is a huge environmental binational project concerning assessment and restoration of a heavily polluted lake region, in which the United State's and the Canada's perspectives (illustrated in for instance: (MacDonald and Ingersoll, 2002) of SQA have been allowed to develop. The primary goal in the Great Lakes Project is virtual elimination of persistent toxic substances from the Great Lake Basin. That is, substances resulting from human activity and particularly those that are bioaccumulating. The

goal is achieved through a variety of programs and actions, but mainly through pollution prevention, in line with the *Agenda 21: A Global Plan for the 21st century* (adopted at the United Nations Conference on Environment and Development). The Great Lakes Project has generated immense amounts of data and the effectiveness of the ecotoxicity tests used in the Great Lakes Project have been accurately evaluated (*e.g.* (Burton, Jr. *et al.*, 1996).

Available methodologies

Current SQGs and the methods used to derive SQGs are in general sufficient to make some management decisions. According to the Society for Toxicology and Environmental Chemistry (SETAC), which is an organization well familiarized with questions at issue associated with SQA, SQGs should however be seen as one of several optional tools. These tools should be used together in a common methodology to develop chemical- and biological *Lines of Evidence* (LOE), for polluted sediments. In principle, several LOE are needed to properly evaluate polluted sediments. These LOE are in the first place:

- Nature and extent of pollution
- Expected or acceptable diversity and abundance of benthic biota in the absence of contamination
- Bioavailability, bioaccumulation and effects of contamination (the potential for chronic as well as acute effects) on aquatic organisms
- Stability of sediments and pollutants (fate and transport)
- Risk of contamination to aquatic biota and associated resources

Available tools to obtain this information are for instance:

- SQGs (which involves chemical analysis of sediment and sediment ecotoxicity tests)
- Sediment ecotoxicity tests alone
- Resident exposed communities (not necessarily benthic)
- Bioaccumulation tests
- Biomarkers and/or histopathology

In addition to this there are also non-biological tools for geological-, hydrological- and chemical characterizations of sediments.

According to SETAC, these LOE should be weighed to build up a Weight of Evidence (WOE) for a polluted site. This should point out the direction for decision-makers and form the basis for management decisions. SQA methodologies should include three basic characteristics:

- Multiple LOE
- Multiple (testing) tiers
- An iteration process

These characteristics do not necessary form a new concept, but have its support in several other organizations' guidance documents (European Commission, 2003; Smith and MacDonald, 1999), with great influence on the subject. To incorporate LOE into a WOE, SETAC proposes the following methodology:

- Define measurement and assessment endpoints
- Select appropriate and multiple LOE
- Select and apply assessment tools within the chosen LOE in multiple tiers
- Analyze collected information and create a WOE
- Identify data gaps and risks (iterative process)

Conclusions from this methodology may enhance selecting management options, which involve:

- Listing of management alternatives
- Comparing risks associated with alternatives
- Comparing costs of alternatives
- Apportioning the sediment at a site among the selected alternatives

Sediment quality and SQG as an assessment tool

Sediment quality has not always been considered as an issue. Sediments have in Environmental Risk Assessment (ERA) more or less been considered as a secure reservoir for harmful pollutants, but this has later shown to be a delusion, founded on the lack of knowledge of ongoing processes (see below) in sediments.

Sediment quality is important because it influence the health of aquatic organisms, which may be exposed to toxic and bioaccumulative substances through their interactions with sediments. Sediments also influence the environmental fate of many toxic and bioaccumulative substances in aquatic ecosystems. Many substances attach to particulate matter and are eventually incorporated into sediments (Allan, 1986). Consequently, sediments may also act as long-term sources of these substances to the water column (Larsson, 1985; Loring and Rantala, 1992; Salomons *et al.*, 1987). Therefore the use of SQGs for evaluating the toxicological significance of sediment-associated substances has become an important part of protection and management of freshwater, estuarine, and marine ecosystems (Smith and MacDonald, 1999).

The numerical limit concentrations defining the SQG should be based on the Predicted No Effect Concentration (PNEC) of substances, which results from effect measurements with ecotoxicity tests. The PNEC is a central parameter of the general concept of ERA, which is according to the European Commission (2003) completed in four steps:

- *Hazard identification* - indicates the adverse effect that a substance has the potential to cause.
- *Dose-response assessment* - estimates the relationship between the level of exposure to the substance and the incidence and severity of an effect on the

organism. The assessment activities are the result from ecotoxicity testing and the final outcome (also called *effect assessment*) is the PNEC.

- *Exposure assessment* - brings about the Predicted Environmental Concentration (PEC), which is the concentration of the substance in different environmental compartments (aquatic, terrestrial, atmosphere *etc.*)
- *Risk characterization* - the PEC and PNEC are used to calculate PEC/PNEC ratios for environmental compartments. If the PEC/PNEC ratio is higher than one there is a concern that there may be a risk for organisms in that specific compartment. In case the number and quality of the ecotoxicity data is low, safety factors (10, 100 or 1000) are multiplied to the PEC/PNEC ratio and hence the risk of the substance of interest is increased.

The PNEC should represent the lower limit of the range of substance concentrations that are usually not or never associated with adverse biological effects. If the PNEC is set too low it will be associated with a number of false positives. That is, a number of sites will be addressed as "toxic", although they are not. If the PNEC is set too high it will conversely be associated with a number of false negatives. Improvement of methods (e.g. ecotoxicity tests) for determination of PNECs will lead towards determination of actual concentrations, NECs, which will limit the frequency of false positives and negatives. Among the factors (see below) that causes overlap between *effect* and *no effect* data include (Wenning and Ingersoll, 2002):

- Other substances that cause effect
- Differing bioavailability
- Differences in responses among organisms and errors in measurement of concentrations or responses

PNEC can in the SQG preferably be derived from a recommended minimum data set (Table 1), a so-called test battery, which is the minimum data that a numerical limit should be based on (Burton, Jr. *et al.*, 1996; Smith and MacDonald, 1999; Wenning and Ingersoll, 2002). The minimum data set ensures data quality of SQGs and can at the same time be used as guidance during *in situ* measurements of toxicity in sediment.

Table 1. Minimum data set from the *Protocol for derivation of Canadian sediment quality guidelines for the protection of aquatic life* (Smith and MacDonald, 1999).

Minimum Data Set Requirements for Freshwater Sediment Quality Guidelines.
At least four studies are required on two or more sediment-resident invertebrate species that occur in North American waters. These must include at least one benthic crustacean species and one benthic arthropod species (other than a crustacean).
At least two of these studies must be partial or full life-cycle tests that consider ecologically relevant endpoints (e.g., growth, reproduction, and developmental effects).

Depending on if the data is derived from sediment toxicity tests, or other medium (*i.e.* water), SQGs can be:

- Mechanistically
- Empirically

Mechanistically based SQGs are developed and tested using PNEC for aquatic organisms, almost exclusively according to the Equilibrium Partition (EqP) theory (see below), when data for sediment dwelling organisms are missing (Wenning and Ingersoll, 2002). The EqP theory has received criticism due to wrongly estimated exposure concentrations (Fredriksson *et al.*, 2003; Persson, 2003). Empirically based SQGs are developed using large databases with matching measures of sediment chemistry and toxicity data related to field collected samples. Several algorithms are used to define specific concentrations associated with particular levels of effect or no effect.

A problem associated with the mechanistic SQG, which is based on casualty, is that it may underestimate adverse biological effects of a mixture of substances. Conversely, the empiric SQG has a tendency of incorrectly attribute adverse biological effects by a mixture (whole sediment) to a single substance. However, the use of mixture models improves the predictability of a SQG, which is an advantageous property.

EXPOSURE ASSESSMENT OF SEDIMENTS

Exposure assessment is implemented through chemical analysis and gives information of the concentrations of substances in sediments or other media. However, known concentrations of substances in sediments are not equivalent with the concentrations sediment-dwelling organisms are truly exposed to, which is due to factors that influence bioavailability (see below). Thus, one should have in mind that exposure analysis does not give any answers to which effects these measured concentrations may cause. Exposure assessment of sediments is preceded by an investigation of the object, its environmental pollution history and possible pollution sources (Naturvårdverket, rapport 5254). The exposure analysis may generate enough information to point out priority pollutants, but since sediments are always composed of complex mixtures of chemicals, presently unknown toxic chemicals

will not be found. However, it is only via the effect assessment that it is possible to distinguish between harmful sediments (*i.e.* that are toxic) and those that do not pose a threat to natural populations.

The main objective with the present project was to find a reliable methodology to semi-quantify, or rank, potential toxicity of sediments, via sediment extracts. For a thorough compilation on the *exposure assessment* we therefore refer to other literature (*e.g.* Mackay and Fraser, 2000). Nevertheless, since both biological and chemical processes in sediments will have a major impact on the effect assessment, we will give a short introduction to some aspects of bioavailability of hydrophobic substances in sediments.

Bioavailability is a medical term defined as: proportion of a drug or foreign substance absorbed in the gastro-intestinal tract of an organism (Timbrell, 2000). The term is also used within the field of ecotoxicology, but in its new context the term has at the same time become somewhat more complex and difficult to handle, due to the more complex system studied. The environment is comprised of a vast number of species, innumerous exposure pathways, substances in mixes and diverse physical-chemical conditions, compared to the controlled uptake in the gastro-intestinal tract of an organism. Considering bioavailability in the ecotoxicological context, in some situations, it may invite to confusion that a substance by definition is not considered taken up when it is present in the gut of an organism. For instance, this may be the case when examining uptake of pollutants in very small organisms.

In order to enhance understanding and estimation of ecotoxicological bioavailability, bioaccumulation factors (BAFs), bioconcentration factors (BCFs) and biomagnification factors (BMFs) have become increasingly used (Mackay and Fraser, 2000). The BAF is defined as $C_{X\ organism}/C_{X\ water}$, and tells us about the uptake and accumulation of substances in organisms during field conditions. The BAF considers all exposure pathways, e.g. dietary absorption, transport over respiratory surfaces and dermal exposure**.** The BCF is also defined as $C_{X\ organism}/C_{X\ water}$ but only involves uptake of the freely dissolved (in water) fraction of a substance via respiratory surfaces and/or the skin, usually under laboratory conditions. The freely dissolved fraction is, especially among chemists and modellers, commonly referred to as the bioavailable fraction but this statement is often strongly argued among ecotoxicologists. Even though uptake through the water often may be the main exposure route for aquatic organisms, ingestion of particle-adsorbed (hydrophobic) substances is also important (Breitholtz and Wollenberger, 2003; Fliedner, 1997). For deposit-feeding invertebrates living in sediments, ingestion of particle-adsorbed substances may in fact be the major route of exposure. (Forbes *et al.*, 1998) have, for instance, used experimental data for fluoranthene and feeding selectivity in the deposit-feeding polychaete *Capitella* in a reaction-diffusion model to calculate the importance of uptake from contaminated sediments versus pore-water. Their results showed that the uptake was 20 to 30 times greater from the sediment than from the pore-water. The BMF is defined as $C_{X\ organism}/C_{X\ food}$ and is a special case of bioaccumulation: the concentration of a pollutant in an organism exceeds the concentration in the diet because in the gastro-intestinal tract the

bioavailability of a substance generally increases enormously, enhancing dietary absorption. Worth clarify, is that substances that bioaccumulate does not necessarily biomagnify. Persistent hydrophobic substances that also are subject of long-range transport (for instance PCBs, DDTs and PBDEs) are the most eager to accumulate in organisms (Mackay and Fraser, 2000).

The sorbed fraction of a hydrophobic substance in sediment is by definition (Mackay and Fraser, 2000) normally excluded from the bioavailable fraction. Even though this is not true in reality, the understanding of sorption processes is still very relevant for the understanding of bioavailability. Sorption of hydrophobic substances to sediments (and also soils) strongly depends on the amount of organic material present (Karickhoff *et al.*, 1979; Karickhoff, 1981). Previously, it has been proposed that the sorbed fraction is linearly dependent of the fraction (f_{oc}) of organic material and that sorption is implemented through partitioning (Karickhoff *et al.*, 1979; Karickhoff, 1981). For example, $C_{sediment} = K_{partition} \times C_{water}$ (the plot generates a partition *isotherm*) and equilibrium is assumed in system (EqP). From this point of view partitioning between organic substances and sediments can be treated in similar manner to that between an organic solvent phase and water (Chiou *et al.*, 1983). Later, this standpoint have become somewhat adjusted, because studies have shown that geosorbents often exhibit non-linear sorption (Luthy *et al.*, 1997). That is, $C_{sediment} = K_{partition} \times C_{water}^{n}$, where n reflects to the linearity of the sorption process.

The described processes give information of how environmental conditions can affect bioavailability and toxicity of pollutants. Consequently, in SQA these processes must be taken into account, to be able to create relevant WOE.

EFFECT ASSESSMENT OF SEDIMENTS

The information gained from the exposure assessment is central in SQA, but since it neither gives information about bioavailability nor toxicity and there are no reliable SQGs available, it needs to be complemented with effect assessment. In a situation when the concentrations of pollutants in sediment are known, the most important question remains unanswered:

- Can ecotoxicological effects be expected?

A substance of a specific concentration apparent in one system (lake, geographical region *etc*.) can potentially have a major impact on the surrounding ecosystem, while the same concentration of the same substance may have no effect in another system. Consequently, this makes a SQG for one region possibly useless to another. The primary underlying reason for this problem is the variation of sediment constitution among aquatic systems (European Commission, 2003; Smith and MacDonald, 1999; Wenning and Ingersoll, 2002).

Ecotoxicity testing traditionally gives information about potential effects of pollutants in sediments. It can be used for either *in situ* measurements of toxicity, as a

necessary complement to SQGs, or to derive SQGs. With this in mind, it should be clear that no fully accepted international, standardized test methods for whole sediment are currently available. Most of the existing whole sediment tests measure acute toxicity; only a few measure long-term, sublethal endpoints. Only the latter tests are considered applicable to SQA, because it is the long-term exposure of sediment dwelling organisms to sediment bound substances that occur under field conditions (European Commission, 2003).

Individual ecotoxicological tests can be described in terms of their (i) cost, (ii) ecological relevance (validity), and (iii) reliability (reproducibility), and (iv) sensitivity (predictive power). There is often a trade-off between these properties: First, low-cost testing often has a lower relevance to risk assessment than more expensive testing, due to short-term exposures and low complexity of the model. Second, there is a conflict between cost-containment and reliability, since measures to ensure reliability include increased experimental systems and improved measurement apparatus and procedures. Thirdly, there is also often a trade-off between a test's relevance and its reliability. Increasing the complexity of test models often implies a decrease in reliability. This is due to the large number of parameters included in the model, which will increase the rate of random errors. Finally, increasing sensitivity in a model includes both using a sensitive species and endpoint, but can also include increased sample size, which usually implies increased costs. If we had a (sufficiently large number of) test(s) that fulfilled the criteria of low cost, high ecological relevance, and high reliability, testing and risk assessment would be easy. In reality every test is a trade off between these aspects, and the combination of characteristics of the test more or less optimized.

In designing an ecotoxicological test, the trade-off between (i) cost, (ii) ecological relevance (validity), and (iii) reliability (reproducibility), and (iv) sensitivity (predictive power), often involve modulation of the following characteristics (Environment Canada, 1995):

- organism
- medium
- endpoint

There are several sediment-dwelling organisms proposed to be appropriate for sediment ecotoxicity testing (Table 2). The choice of organism is likely to affect all demands of an ecotoxicity test. In general, a test-organism should preferably exist at an investigated site, for not being considered ecologically irrelevant. A test-organism should also be easy to keep in the laboratory and to culture. It should be easily collected in the field most of the year and should be easily and successfully maintained in the laboratory for a period of at least twice the testing period (acclimatization period + test duration) (OECD, 1998). The suitability of a test organism for laboratory experiments depends a lot on the knowledge about the organism: its habits, physiology and sensitiveness to different pollutants, which can be extremely diverse (Forbes and Calow, 2002; Roex *et al.*, 2000); reviewed in (Breitholtz *et al.*, 2001; Breitholtz, 2002).

Owing to differences in sensitivity to substances among test organisms, it is recommended to focus on more than one endpoint (OECD, 1998). Physiological and biochemical responses of individual organisms are often relatively sensitive and reliable, but in general they are difficult to interpret in an ecological context. Subjective endpoints, like behavioral effects, and endpoints at population/community level (population structure) gains in ecological relevance but are often less sensitive, demands more time and increases costs (full life cycle tests with many individuals).

As with natural sediments, the medium in an ecotoxicity test has the potential to affect both test organisms and test pollutants, and consequently the outcome of the test result. Therefore it is important to be aware of and to control all molecular mechanisms involving the medium in the ecotoxicity test. It is also important that test concentrations of the substance or effluent remain constant during the whole test period. Concentrations of hydrophobic substances often decline over time in chronic tests, in such a way that early test periods yield disproportionately higher exposure concentrations than later periods. This may be a consequence of slowly desorbing fractions. Thus, those life-stages used when initiating a test often are exposed to the highest concentrations. However, this problem can be overcome by initiating test with different organism life-stages (Chandler and Green,).

Present ecotoxicity testing of sediments focus primarily on the Spiked Sediment Toxicant Test Approach (SSTT) (Smith and MacDonald, 1999). Briefly, a reference/baseline-sediment is spiked with known concentrations of chemicals, either alone or in combination. Usage of reference/baseline-sediments allows an evaluation of which (*i.e.* "all") substances that may cause toxicity but is not uncontroversial (Chapman *et al.*, 2002). Comparing actual sites to reference/baseline conditions can generate unrealistic restoration goals.

An inventory of tests with marine organisms for the evaluation of dredged material and sediments was compiled by the Federal Environment Agency of Germany, UBA (Herbst and Nendza, 2000), and in addition a detailed review paper on aquatic ecotoxicity tests including marine sediment test methods was prepared by OECD (1998). With support from this gathered information the European Commission (2003) has presented a list of recommended tests (Table 2) for SQA.

Table 2. Ecotoxicity tests for sediments recommended by the (European Commission, 2003).

Test Organism	Acute or Chronic Test	Duration	Endpoints
Amphipods			
Corophium sp. (C. Volutator or C. Arenarium)	Chronic	28d	Survival, growth and reproduction
Leptocheirus plumulosus	Chronic	28d	Survival, growth and reproduction
Polychaetes			
Nereis/Neanthes sp. Naeanthes arenaceoden-tata-kan cultivated	Subacute/chronic	12-28d	Survival – suvival/growth
Arenicola marina	Chronic	28d	Survival
Arenicola marina	Subacute	10	Casting rate
Echinodermes			
Echinocardium cordatum	Acute/subchronic	14d	Survival
Microcosm			
Nematodes	Chronic	60d	Community structure

Besides the recommendations of the European Commission, ecotoxicity tests using *Hyalella azteca* and *Chironomus tentans* are described by (US EPA, 2000) and evaluated by (Burton, Jr. *et al.*, 1996). Referring to the latter article from the Great Lakes project no less than 24 different organisms were tested for 97 endpoints at 4 sites (altogether 7600 data points), and evaluated in respect to sensitivity, discrimination and redundancy. Conclusions from this work generated three optional test batteries (Table 3), optimal for the Great Lakes. However, there is some degree of confidence that recommended organisms also probably would be sensitive or discriminatory at other sites. It should be noted that the recommended tests in this study also have been recommended in earlier North American studies (Burton, Jr. *et al.*, 1996). The most sensitive endpoint was the avoidance/preference behavior of *Diporeia*, which is not a sublethal endpoint and consequently not qualifies for the European Commissions testing criteria for sediments.

Table 3. Optimal sediment test batteries for the Great Lakes Basin derived from Principal Component Analysis (Burton, Jr. *et al.*, 1996).

	Test Organism	Duration	Endpoints
Option 1	*Hyalella azteca*	14d	Survival, length, sexual maturation
	and *Ceridaphnia dubia*	7d	Survival, reproduction
	or *Chironomus riparius*	14d	Survival, length
	or *Daphnia magna*	7d	Survival, reproduction
	or *Pimephales promelas*	7d	Larval survival and weight
	or *Diporeia*	5d	Avoidance/ preference
	or *Hexagenia bilineata*	10d	Survival, molting frequency
Option 2	*C. dubia*	(see above)	(see above)
	or *C. riparius*		
	and *Diporeia*		
	or *H. bilineata*		
Option 3	*D. magna*		
	and P. promelas		
	and Diporeia		
	or H. bilineata		

It should also be mentioned that commonly there is a natural variation in sensitiveness to pollutants among individuals/populations of same species in different areas (due to genetic factors or stress factors as starvation, diseases, predation *etc.*). However, individual variation and presumably variation in sensitivity is smaller when using laboratory-cultured animals, which limits this source of error.

Introduction to experimental part

For prioritising remedial activities between different locations reliable, ecologically relevant and sensitive tools are needed. In polluted areas harmful chemicals are generally always present as complex mixtures. Toxicological interactive effects, which occur during exposure of complex mixtures, cannot be estimated from mere chemical analyses. Furthermore, several field studies have demonstrated poor relationships between analyzed pollutants and their contribution to potential toxicity (Brack, 2003; Sundberg, 2005), which reveals our limited knowledge concerning which compounds are responsible for toxic effects in field situations. Exposure assessments (chemical analyses) will therefore only give limited information on the environmental hazard at a certain location. In addition, interspecies differences regarding sensitivity to pollutants have been demonstrated in number of studies (Breitholtz, 2002; Tanguay *et al.*, 2003). Another important factor that complicates the extrapolation from chemical analysis to ecotoxicological effects are that different developmental stages are not equally sensitive to environmental stress. Early life-stages for instance, are generally more sensitive than juvenile and adult stages.

In sediments, hydrophobic pollutants are generally of major concern, but many ecotoxicity tests used today (see section 1.3.2.2) are aquatic test systems; hydrophobic chemicals are therefore difficult to investigate. Using whole sediment in these systems is also difficult since *e.g.* water content, redox-potential and pH, which influence the toxicity, differ between sediments. The physico-chemical properties of the sediment itself may also have a significant impact of the bioavailability of the substances in the sediment (see section 1.3.1). None the tests described in Tables 2 and 3 includes vertebrates, by use of intact vertebrates – with all its biological complexity – a broader toxicological evaluation may be facilitated.

The objective of the present project was therefore to develop an ecotoxicological test approach that rank potential toxicity between different locations by investigating several exposure routes and adverse effects on different biological organisation levels. This approach may then be used for priority setting of environmental remedial activities of polluted sites.

We have investigated two trophy levels, three exposure routes (diffusive uptake, food uptake and maternal transfer), several developmental stages and several toxic mechanisms by exposing different early life-stages of both the copepod *Nitocra spinipes* and rainbow trout (*Oncorhynchus mykiss*) to organic sediment extracts. The *N. spinipes* system was adapted for exposure of hydrophobic compounds. The main test variables were mean larval development rate (LDR) and mortality. In order to assess the individual growth rate, a microplate based fluorometric high-range assay was also used to measure individual RNA content. Rainbow trout were used to investigate biological adverse effects on the individual and the cell level in an intact vertebrate. In the rainbow trout system we investigated abnormalities and mortalities in early developmental stages.

Material and Methods

Chemicals

Toluene (*p.a.* grade), *n*-hexane (LiChrosolv®), silica gel (60 puriss), anhydrous sodium sulphate (*p.a.* grade), copper (*p.a.* grade fine powder <63 µm) were obtained from Merck (Darmstadt, Germany). Tris EDTA Buffer (TE buffer, Cat. Number TE1X1000) and Rnase Erase was bought from Q Biogen (Invitro, Sweden AB. Cat. Number 2440-204). N-laurysarcosine, (sarcosyl, CAS No 97-78-9), Triolein (T-7140, 99%) and RNAlater® was purchased from Sigma-Aldrich (St. Lois, MO, USA). RiboGreen Ribogreen™ RNA Quantitation Kit, Molecular Probes (RiboGreen, Cat nr R11490) was obtained from Göteborgs Termometerfabrik, Sweden. Working solutions for RNA were diluted in StB in concentrations ranging 0.14; 0.43; 0.86 µg × mL^{-1}. Distilled Water (DNAase RNAase free. Cat nr 1097-015) was obtained from GIBCO BRL, Inviro Sweden AB. Extraction Buffer (ExB) was prepared by mixing sarcosyl (1%, v/w) in TE Buffer. Standard Buffer (StB) was prepared by mixing sacrosyl (0.2%, v/w) in TE Buffer.

Sediment sampling

Bottom sediment samples were collected using a Kajak type gravity corer. Table 4 describes the collected sediment samples from the five locations and literature data of some hydrophobic pollutants from each location. The sediments from Slingsviken and Björkskär served as background sediments with relatively low pollutant burdens. The sediment from Örserumsviken served as our positive reference since this sediment recently has been remediated. The sediment from Örserumsviken contains high levels of polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and mercury (Hg), which mainly originates from a former pulp mill industry located at the inner bay. The pollutant burden in the sediment of Riddarfjärden (central part of Stockholm) originates most likely mainly from traffic and other anthropogenic activities. Frierfjorden, which is highly contaminated by PAHs and PCDD/Fs owing to discharges form different production plants at the inner fjord (Persson *et al.*, 2002), was included for investigating the relative ranking of potential toxicity compared with Örserumsviken.

Table 4. Description of collected sediment samples and literature data of some pollutants in each location.

	Örserumsviken	Frierfjorden	Riddarfjärden	Björkskär	Slingsviken
Position[a]	N57 44, E16 40	N59 07, E9 65	N59 30 E18 02	N59 22, E19 08	N57 38, E16 30
Water depth (m)	1–2	56	15–21	78	3–4
Sediment depth (cm)	0–10	0–15	0–3	0–5	0–10
Pooled samples (n)	10	1	20	4	7
ΣPAH[b] (µg/kg dw)	8700 [c]	4900[d]	11000[e]	530[f]	2100[c]
ΣPCB[g] (µg/kg dw)	7800[h]	29	100[e]	2.2[f]	n.a.[i]
ΣPCDD/F[j] (µg/kg dw)	n.a.	7000	n.a.	n.a.	n.a.

[a] Coordinates in World Geodectic System 1984 (WGS-84). [b] Sum concentration of the following compounds: phenanthrene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, triphenylene, benzo[*b*+*k*+*j*]fluoranthene, Benzo[a]pyrene, indeno[1,2,3-*cd*]pyrene and benzo[*ghi*]pyrene. [c] From (Sundberg *et al.*, 2005a). [d] From (Nœs, 1999). [e] From (Sternbeck *et al.*, 2003). [f] From (Hansson.T *et al.*, 2005). [g] Sum concentration of the following PCB congeners (IUPAC no): 28, 31, 52, 118, 123, 132, 153, 138, 180, 193. [h] From (Sundberg *et al.*, 2005b). [i] Not analysed. [j] Sum concentration of 24 PCDD/Fs congeners, data obtained from (Persson *et al.*, 2005).

Organic extraction – preparation of exposure solutions

Pooled wet sediment samples were extracted for 24–50 hours in toluene using a Soxhlet apparatus coupled to a Dean-Stark trap for water removal (Lamparski and Nestrick, 1980). Dry weights were gravimetrically determined after extraction. Extracts were cleaned on a deactivated (10% H_2O) silica gel column (I.D. 1 cm)

with *n*-hexane (10 mL of *n*-hexane/g silica gel) as eluent. Elemental sulphur was removed by the addition of a small amount of elemental copper, ultrasonification-bath (4 ×15 min) and overnight incubation at room temperature. Total extracts were obtained after a final clean-up with a silica gel column topped with 1 cm of sodium sulphate and with *n*-hexane as eluent.

Nitocra spinipes experiments

SUBCHRONIC TEST

The *Nitocra spinipes* used in the present project were collected from the ITM stock culture, which origins from one female isolated from a sediment sample over 25 years ago (Bengtsson, 1978).

In the subchronic tests with *N. spinipes* two main test variables were observed, *i.e.* larval development ratio (LDR), which gives a picture of the mean developmental rate among a large group of animals, and mortality. The naupliar (larval) and copepodite (juvenile) stages are morphologically distinct in copepods, therefore, the transition from the last naupliar to the first copepodite stage is easily observed. Larval development ratio is normally recorded after 5–7 days, when about 50% of the control animals have reached a copepodite stage, and is expressed as the ratio of copepodites to the total number of animals at the beginning of the test (Breitholtz and Bengtsson, 2001; Breitholtz and Wollenberger, 2003; Breitholtz *et al.*, 2003).

In addition to the mentioned test variables measured, we also wanted to analyze nucleic acid levels in individual copepods as a measure of individual growth rate. Identification of biochemical changes, such as RNA contents, which are related to metabolism (Dahlhoff 2004), can be used to determine if an organism has been exposed to a stress, including contaminants (Yang *et al*. 2002). The rationale is based on the fact that the RNA content of tissues or whole organisms consists primarily of ribosomal RNA (rRNA). Consequently, concentration of rRNA, at any given time is directly related to the protein synthesis of a cell (Elser *et al.* 2000). The quantity of RNA is directly linked to the growth of the individual (Saiz *et al*. 1998). For example, in small metazoans, which have high metabolic rates of biosynthesis, a high portion of RNA is required (Brown *et al*. 2004). DNA exists in a quasi-constant quantity in a somatic cell and therefore might be used as an index of the number of cells (Buckley *et al.* 1999). Based on this, it is becoming increasingly common to assess growth rates in a variety of aquatic animals by RNA:DNA ratio (fish: Buckley *et al.*, 1999; lobsters: Rosa and Nunes 2003; krill: Cullen *et al.* 2003, copepods: Saiz *et al.* 1998, Campbell *et al.* 2001, Gorokhova 2003; daphniids: Vrede *et al*. 2002; cirripeds: Desai and Anil 2002; corals: Meesters *et al.* 2002). In toxicological tests, these indices were also found to be useful in nematodes (Ibiam and Grant 2005) and algae (Yang *et al.* 2002). However, in microcrustaceans, such as daphniids, copepods and decapod larvae, RNA content alone may be a more sensitive endpoint than the RNA:DNA ratio due to the growth- and ontogeny-related fluctuations in DNA content (Gorokhova and Kyle 2002, Rosa and Nunes 2003, Gorokhova 2003).

For practical reasons, i.e. to be able to both record the larval development ratio and analyze nucleic acid levels, two tests were performed in parallel for each sediment extract. In order to disperse the extract uniformly and prevent droplets on the bottom of the test beakers, 100 mg (to cover the bottom) silica gel was used as carrier of the sediment extract. The test beakers containing silica gel with spiked extract were prepared the day before the start of the test to avoid residues from the solvent. In both tests, solvent control and extracts corresponding to 1.5 mg, 4.5 mg, 13.5 mg and 40.5 mg whole sediment were used.

Prior to start of each LDR test, about 300 gravid females were evenly isolated in six beakers, containing 100 mL natural seawater (salinity ~6.5 ‰) and a suspension of the red micro alga *Rhodomonas salina* (500 µL of 5×10^7 cells/mL, *i.e.* 1.2 mg dw). Nauplii released within 24 hours were randomly transferred (8 nauplii per replicate) to 20 mL test beakers containing artificial baseline sediments and 5 mL natural seawater (salinity ~6.5 ‰). Ten replicates per treatment (including control) were used in all tests. The copepods were fed with a suspension of *R. salina* three times a week (each time 25 µL of 5×10^7 cells/mL, *i.e.* 0.06 mg dw). Evaporation losses were compensated for with distilled water each time the copepods were fed. The nucleic acid test, on the other hand, started with ovigerous females (4 per replicate) and lasted for about 14 days. Five replicates per treatment (including control) were used in all tests. Otherwise the procedures were the same as for the larval development ratio test. When nauplii released by these ovigerous females reached copepodite stage III (CIII; the most suitable stage for this type of analysis, which is reached after 10-12 days among control larvae) (Gorokhova and Kyle, 2002), they were randomly selected for further RNA analysis (see below). Owing to lack of effects using this biomarker to measure individual growth in the two most toxic sediment extracts (*i.e.* Örserumsviken and Frierfjorden; see below), we did not analyse the other three sediment extracts.

Although, in the present project, we do not report acute toxicity data from the sediment extracts, a test system was developed for the purpose range finding. The test system (*i.e.* beakers, silica gel, handling of extracts *etc.*) is the same as for the subchronic test above, although food is not added. Otherwise, the procedures from the standardized acute toxicity test (SIS, 1991) are followed. In the present project, this test was only used to find a suitable dose range using the sediment extract that was believed to be most toxic (*i.e.* Örserumsviken). To be able to compare (rank) the potential toxicity, this dose range was used in the subchronic toxicity testing with all the other sediment extracts.

NUCLEIC ACID ANALYSIS

Microplate fluorometric high-range assay with the RiboGreen was performed to quantify RNA in individual copepods after extraction with N-laurylsarcosine followed by RNase digestion as described in detail elsewhere (Gorokhova and Kyle 2002). Measured RNA concentrations were expressed as $\mu g \times ind^{-1}$. Five randomly selected CIII individuals from each treatment were identified and collected using instruments washed in Rnase Erase. Identification of CIII individuals was

performed with the photographic software programme Leica IM50, Image Manager, on a microscope slide. The CIII individuals were preserved in RNA later.

After 14 days exposure (see above), CIII individuals were transferred to vials containing 1.5 mL extraction buffer (1% v/w sacrosyl in TE buffer [Tris EDTA Buffer, Q Biogen, Invitro, Sweden. Cat. Number TE1X1000]). Cells were opened by sonication in ultra sonic bath filled with ice, two times one minute. Vials were then carefully shaken at room temperature on a multiple vial head for one and a half hour, diluted 1:4 with extraction Buffer and shaken for additional 15 minutes. Fluorescence measurements were performed according to Gorokhova and Kyle (2002) using a microplate fluorometer (FLUOstar OPTIMA) and a black, solid, flat bottom microplate. Working solutions for RNA was diluted in StB to concentrations of 0.01, 0.06, 0.12 µg/mL. Working solutions for DNA was diluted in StB to concentrations of 0.01, 0.05, 0.08 µg /mL. Plate included extracted CIII samples (two replicates of each extracted CIII sample), blanks, RNA and DNA standards (three replicates). Samples and standards were treated with RiboGreen and the plate was scanned. After first scanning, Rnase was added to all samples as well as to DNA standards, and plate was incubated for 30 minutes at 37 °C before a second scanning. The amount of RNA was calculated according to an RNA standard curve, using the difference between scan #1 and #2. DNA concentrations were calculated according to the DNA standard curve derived from scan #2. Fluorescence measurements were performed using fluorometer FLUOstar Optima (BMG Labtechnologies, microplate reader, filters: 485 nm for excitation and 520 nm for emission) and black solid flat-bottom microplates (COMBO; Labsystems, cat. # 9502067). The plate was scanned with 0.2 sec well measurement time, 10 measurements per well.

Rainbow trout experiment

To increase the homogeneity of the biological material, only one family pair was used. Eggs and seminal fluid from rainbow trout were collected and shipped to our laboratory from Vilstena fiskodling (Fjärdhundra, Sweden) on the day of fertilisation. Artificial fertilisation and water swelling were performed at 8.2 ˚C, where upon eggs were placed in cup-shaped depressions in 1% agarose gel cast in square petri dishes (Falcon, Becton Dickinson Labware, Franklin Lake, NJ, USA) with maximum 36 eggs/dish (Åkerman and Balk, 1995).

The exposure solutions and benzo[*a*]pyrene (positive control) were dissolved in the carrier substance, triolein. Two additional controls were used: carrier controls (eggs exposed solely to triolein) and uninjected controls. The day after fertilisation, rainbow trout eggs were exposed to the solutions using the nanoinjection technique (Walker *et al.*, 1996; Åkerman and Balk, 1995). Briefly, triolein-dissolved exposure solutions were transferred, using a vacuum suction pump, into aluminium silicate capillaries (Sutter Instrument CO., Novato, CA, USA) with sharp elliptical tips. Exposures were performed under a stereo-microscope (Leica MZ8, Leica Microscopy and Scientific Instruments Group, Heerburg, Switzerland) with the aid of a one-dimensional hydraulic manipulator (Narishige, Tokyo, Japan) and a Pico-injector (Medical Syst. Corp., Greenvale, NY, USA). After the penetration of the

chorion and the vitelline membrane, the solutions were injected into the yolk of the egg. Before injection, each capillary was individually calibrated by adjusting the nitrogen gas pressure and the time of injection so that the diameter of the triolein droplet corresponded to the desired dose (less than 1‰ [v/v] of the egg volume).

Control and exposure groups were kept in darkness in identical individual flow-through systems (36 individuals/system) with an average temperature of 8.8 °C (7.8–9.5 °C), containing 2 L of Stockholm municipal drinking water filtered in three consecutive steps (nominal pore size: 50 µm – active carbon – 10 µm) and aerated. Mortality was recorded every second day during the experiment. Abnormalities among newly hatched larvae were investigated under the stereomicroscope. The experiment was terminated 28 days post-hatch when surviving larvae were euthanised.

Statistics

In the *N. spinipes* experiments statistical differences in mortality and larval development ratio between exposure and control groups were determined using one-way ANOVA, followed by Dunett's two-sided post hoc test. Homogeneity of variances was tested using the Levene test. In the rainbow trout experiment statistical differences in mortalities and abnormalities between exposure and control groups were determined using Fisher's exact test. As α-level for statistically significant differences between groups a *p*-value of less than 0.05 was used.

Results

Nitocra spinipes experiments

Mean larval development and mortality of early life stages after 6 days exposure

LDR and mortality after 6 days exposure in *N. spinipes* exposed to the five sediment extracts are displayed in Figures 1 (A-E). The sediment extract from Örserumsviken (Fig. 1A) was the only extract that caused significant differences in morality, compared to the control ($p<0.01$). At the doses corresponding to 13.5 mg and 40.5 mg sediment, the percentage mortality was 33.8 **%** and 65.1 %, respectively**,** compared to 3.5 **%** in the control. The sediment extract from Örserumsviken also caused the most significant decrease in LDR compared to the control ($p<0.001$). In fact, the lowest observed effect concentration (i.e. LOEC) was also the lowest dose tested. Hence, we cannot exclude the risk that even lower doses could have caused effects as well. The sediment extract from Frierfjorden (Fig. 1B) also caused significant effects on LDR. At the doses corresponding to 13.5 and 40.5 mg sediment, the decrease was significant from the control ($p<0.001$). Similar effects were also observed at the dose corresponding to 40.5 mg sediment in the tests with the other three sediment extracts (Figs. 1C-E). Interestingly, at the dose corresponding to 4.5 mg sediment from Slingsviken (Fig. 1E), there was an increase in LDR compared to the control ($p<0.05$), indicating that something in the sediment had a positive effect on mean development rate among the larvae.

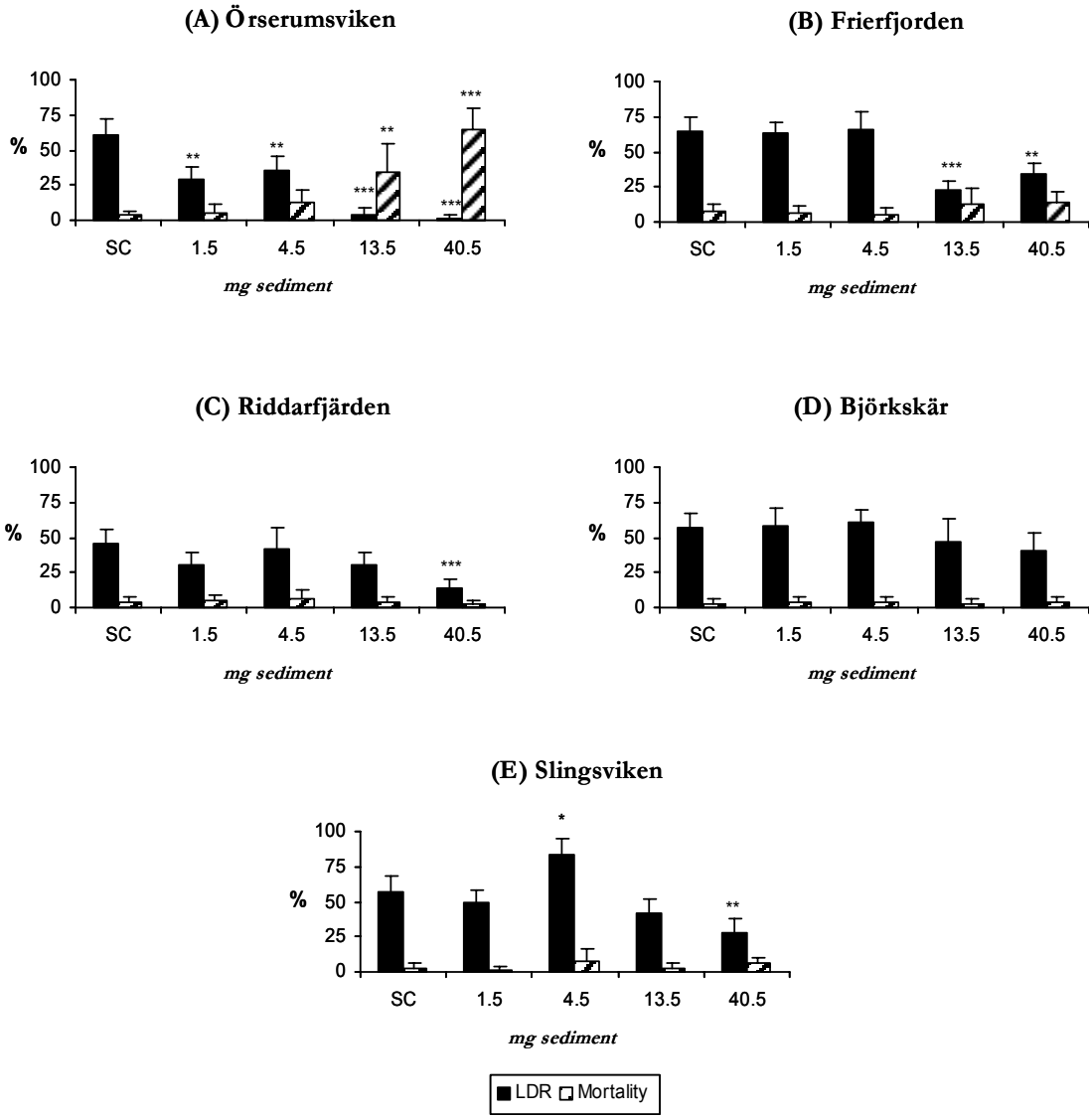

Figure 1. Larval development ratio (LDR, black bars) and mortality (hatched bars) in *N. spinipes* exposed to sediment extracts from five locations (A-E; for positions see Table 4) loaded on silica gel for 6 days. Error bars indicate 95% confidence intervals of means. Asterisks denote significant differences from the solvent control (SC) (**p*<0.05, ***p*<0.01, ****p*<0.001).

Nucleic acid levels

The results from the nucleic acid analysis on copepodite stage III animals collected from the tests with extracts from Örserumsviken and Frierfjorden is presented in Table 5. None of the extracts however caused any significant effects on individual growth rate as measured by individual RNA content. Since these two sediment extracts didn't cause any effect on RNA content, we did not test the extracts from the three less toxic sediments.

Table 5. Average levels of RNA (µg) in *N. spinipes* copepodite stage III individuals (n=4) collected from the tests using extracts from Örserumsviken and Frierfjorden. 95% confidence intervals of means are presented in brackets.

Treatment *(corresponding to mg sediment)*	Örserumsviken *µg RNA/ind*	**Frierfjorden** *µg RNA/ind*
Solvent control	0.045 (±0.016)	0.038 (±0.009)
1.5 mg	0.054 (±0.008)	0.037 (±0.009)
4.5 mg	0.040 (±0.008)	0.036 (±0.011)
13.5 mg	0.041 (±0.012)	0.032 (±0.013)
40.5 mg	0.037 (±0.018)	0.034 (±0.009)

Rainbow trout experiment

Mortality

Mortalities at the embryonal and larval stages are displayed in Figures 2A–F. No significant differences were observed between carrier and uninjected controls, statistical differences between exposed larvae were therefore compared against carrier controls. Except for the highest dose of benzo[*a*]pyrene, which caused a significant increase of embryonal mortality (Fig. 2F), the sediment extract from Frierfjorden (Fig. 2B) was the only exposure causing significant increases of embryonal and larval mortalities. The highest dose of the extract from Riddarfjärden (Fig. 2C), however, caused a non-significant (p=0.11) 38% increase of embryonal mortality.

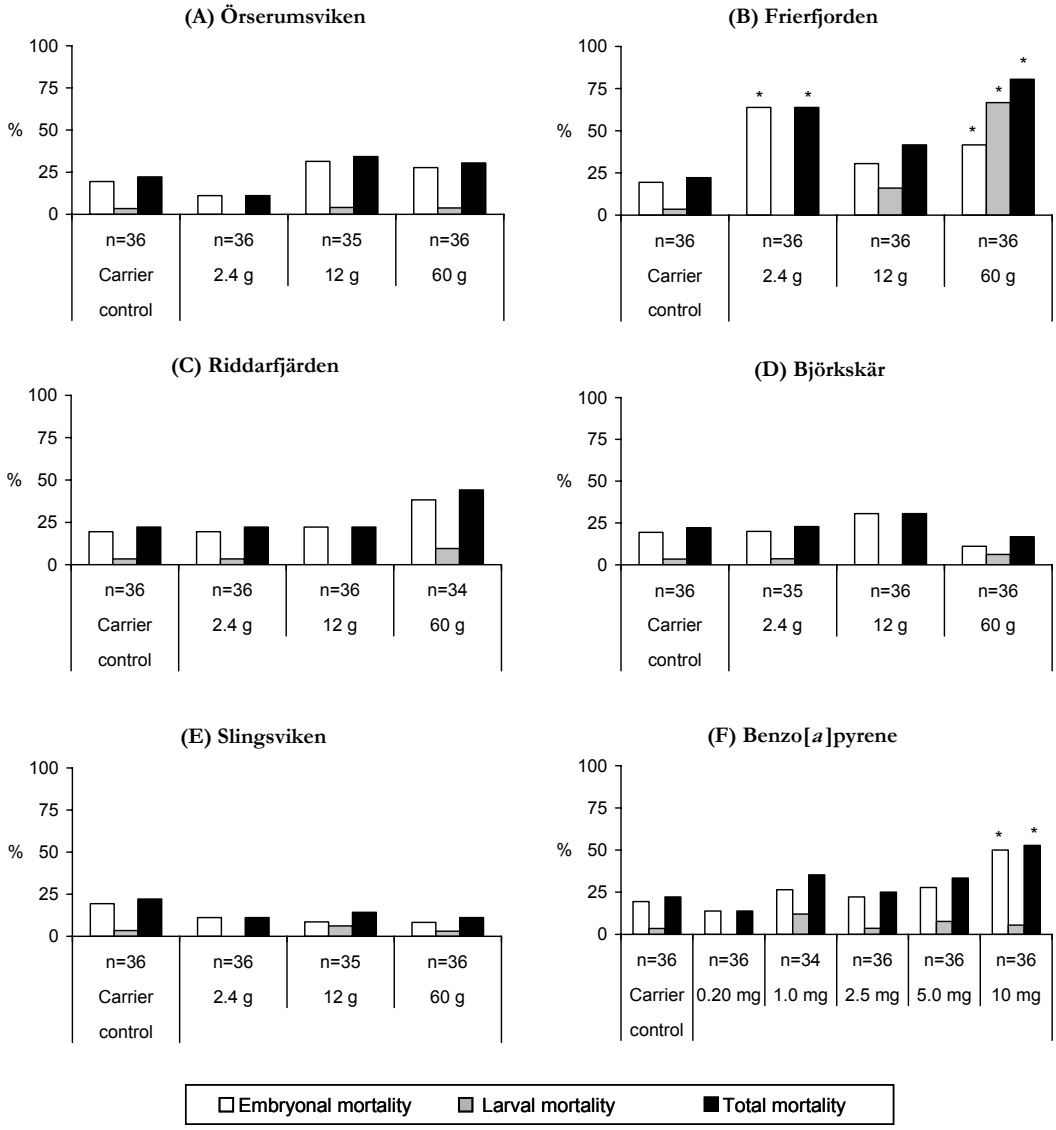

Figure 2. Embryonal (white bars), larval (grey bars) and total mortality (black bars) among rainbow trout exposed as newly fertilized eggs to sediment extracts from five locations (A-E; for positions see Table 4) using the nanoinjection technique. Triolein injected eggs were used as carrier controls and graded doses of (F) benzo[*a*]pyrene were used as positive controls. n depicts number of injected eggs and doses are given as dry weight/kg wet eggs. *Significantly (p<0.05) different from carrier control.

Abnormalities

Newly hatched larvae suffered from several types of abnormalities, including edema in the yolk sac and pericardium, hemorrhages in the yolk sac, head, trunk and tail region, craniofacial deformities, scoliosis and asymmetry of the medio-lateral axis of the yolk sac (asymmetric yolk sac, first described in (Sundberg *et al.*, 2005a). For brevity, frequencies of newly hatched larvae suffering from hemorrhages, asymmetric yolk sac and frequencies of abnormal larvae are displayed in Figures 3A–F. The two control groups – carrier controls and uninjected controls – did not statistically differ in number of abnormalities; exposure groups were therefore compared with carrier controls for statistical analyses. Except for the sediment

extracts from Björkskär (Fig. 3D) and Slingsviken (Fig. 3E), evident dose-response effects were observed in the different sediment extracts. Hemorrhages were significantly increased in larvae exposed to the highest doses of benzo[*a*]pyrene (Fig. 3F) and of the sediment extracts from Örserumsviken (Fig. 3A) and Frierfjorden (Fig. 3B). Larvae with asymmetric yolk sac were found in three doses of benzo[*a*]pyrene (Fig. 3F, 1.0, 2.5 and 5.0 mg/kg egg) and the highest doses of the sediment extract from Riddarfjärden (Fig. 3C), Örserumsviken (Fig. 3A) and Frierfjorden (Fig. 3B). The highest doses of the sediment extract from Örserumsviken and Frierfjorden were the only exposures causing significantly increased frequencies of abnormal larvae.

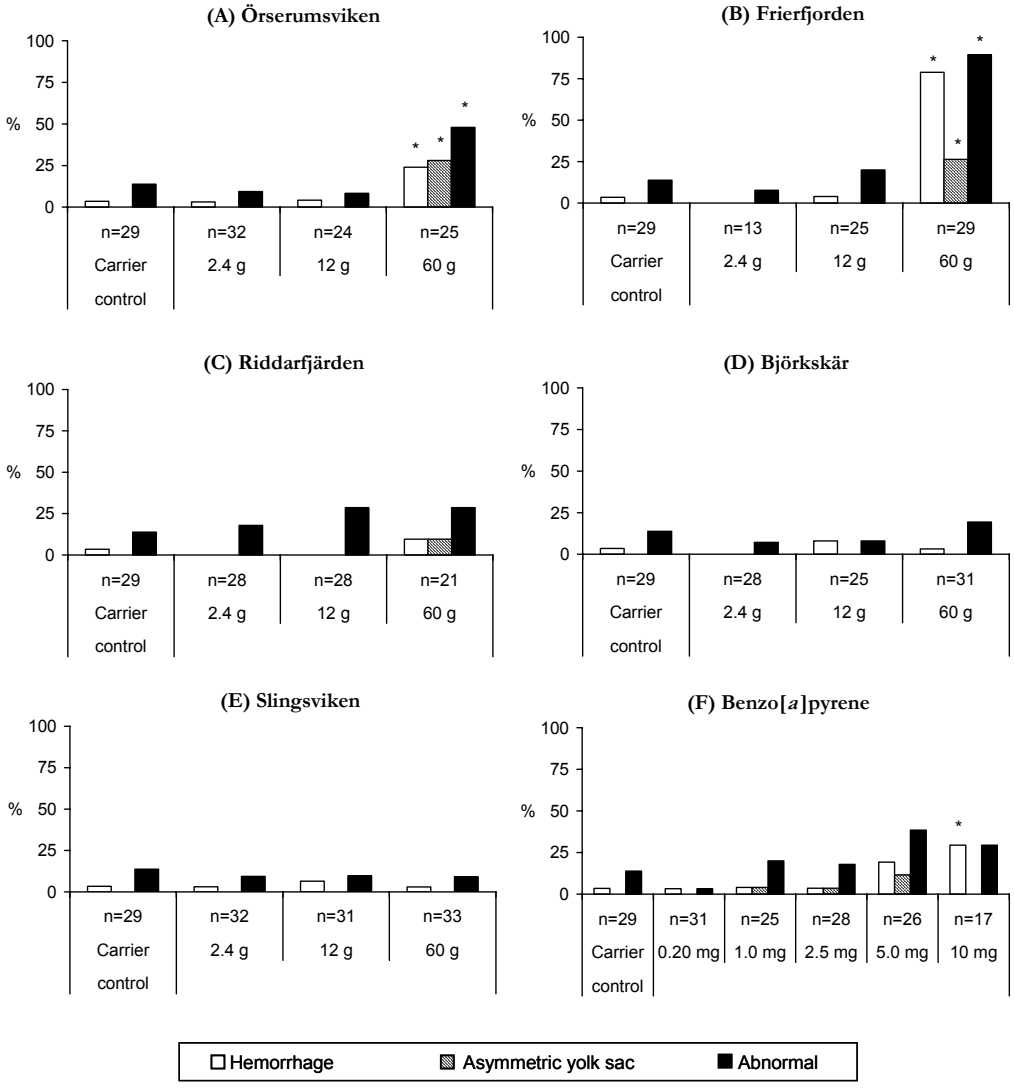

Figure 3. Frequencies of hemorrhage (white bars), asymmetric yolk sac (hatched bars) and frequencies of any kind of abnormality (black bars) were recorded in newly hatched rainbow trout larvae exposed as newly fertilized eggs to sediment extracts from five locations (A-E; for positions see Table 4) using the nanoinjection technique. Triolein injected eggs were used as carrier controls and graded doses of (F) benzo[*a*]pyrene were used as positive controls. n depict number of investigated larvae and doses are given as dry weight/kg wet eggs. *Significantly (p<0.05) different from carrier control.

Discussion

In the present project we have developed a semi-quantitative approach to rank potential toxicities of organic chemicals in bottom sediments from different locations. Overall, the crustacean and fish results were consistent but owing to species differences and different test variables used, some minor differences were still observed, which will be discussed below (see section 5.3). Our results clearly show that sediment extracts from the locations that we expected to be most polluted, *i.e.* former industrial locations, also caused the most significant effects on development and mortality in early life stages of both crustaceans and fish. Similarly, the sediment extracts from reference or low-polluted locations were not very toxic on either of the test organisms. When solely PAH concentrations in the sediments were compared, however, the most potentially toxic sediment was not the worst polluted, underlining the importance of including biological effects for reliable risk assessments.

In the present project we have also shown that it is possible to test organic sediment extracts in aquatic test systems by using silica gel as a carrier to diminish the risk of droplets (via a drastically increased surface area) and thereby also increase the bioavailability of the organic substances in the sediment extracts (see section 5.1). Although this method may overestimate the risk of sediment toxicity, we are confident that it may serve as a useful screening tool to rank potential toxicity of sediment extracts. In fact, this finding may also have positive implications for regular ecotoxicity testing of organic substances in environmental risk assessment, especially if toxic effects can be related to levels of substances tested in the test organisms (*i.e.* the body burden). Regardless of somewhat enhanced bioavailability, this aquatic test system could then serve as a quantitative assessment tool. Consequently, since we investigate potential toxicity in this aquatic test system it is suitable as a semi-quantitative assessment tool.

As a final outcome of this work we have developed a simple ranking system, which takes into account both crustacean and fish toxicity of sediment extracts, and which can be used in the prioritising process of remedial activities of sediments (see section 5.4.).

Nitocra spinipes experiment

To construct a practical test method using hydrophobic extracts (i.e. hexane) was far more difficult than we first expected. Even though not presented in this report, numerous of tests were conducted before reaching the final test method, which was used to obtain the reported results. First, the extract was extremely unwilling to absorb to the micro algae (i.e. the food for the copepods). Second, the hydrophobic extract was difficult to disperse as a plane film on the bottom of the test beakers, where the micro algae were initially meant to absorb the extract from. The dry extract immediately formed droplets when water was added. The droplets seemed sticky and at some occasions they acted as flycatchers on the nauplii, which were

hindered in their feeding at the higher doses. This, of course, lead to misinterpretations of results obtained.

Presumably, the sorption of organic substances in our test system (as in natural sediments) was controlled by organic material. In our initial pilot experiments we did not use the silica gel, which probably caused the extracts to exceed the amount that could immediately undergo sorption to the present food and instead formed visible droplets (Ø ~2–3 mm). As a result, the hydrophobic chemicals were less bioavailable owing to the thermodynamic behaviour of hydrophobic chemicals, *i.e.* they prefer being adhered to each other in droplets rather than being dissolved in the water-phase. In contrast, the silica gel facilitated an even exposure of the extracts by contributing with a large sorption area, dispersing the extracts. Consequently, this modification of the test system likely enhanced better uptake of the organic chemicals and also advocates reliable repetition of the experiment due to a more homogenous exposure.

Silica as artificial sediments has been use in a few other ecotoxicity tests with invertebrates. For instance, (McDonald and Haynes,) concluded, from a 20-day test with the worm *Neanthes arenaceodentata*, that the mean individual dry weight was almost identical for worms held in artificial silica sediments and field-collected sediments. Quartz sand has also been used as control sediment in a Sediment Contact test with *Bacillus cereus* (Heise and Ahlf, 2005). However, to the best of our knowledge the use of silica gel as a carrier of organic extracts has not been reported earlier.

According to these findings, we conclude the test facilitates prioritisation between polluted sites when making management decisions (*e.g.* priority setting) and can be used with other tools to create relevant LOE. In spite of this, the test may not be appropriate for establishing SQGs in units of mg/kg dw sediment. Presumably, it would generate a large number of false positives owing to the high bioavailability conditions in our test system compared to natural sediments. On the other hand, natural sediments are often rich of silica (*i.e.* quartz) and the natural habitat of *N. spinipes* is sandy bottoms, suggesting an increase in ecological relevance.

Rainbow trout experiment

Nanoinjection, the exposure technique used in the rainbow trout experiment mimics maternal transfer, which is an important exposure route for hydrophobic xenobiotics in fish (Walker *et al.*, 1996). Nanoinjection have been used in a number of studies for investigating potential toxicity of hydrophobic chemicals (Ishaq *et al.*, 1999; Sundberg *et al.*, 2005a; Sundberg *et al.*, 2005b). Even though the exposure solutions are introduced into the yolk, the chemicals must pass a number of biological membranes before the observed effects can occur. Hence, the extracted chemicals are bioavailable and thus pose a potential risk *in situ*.

Since we investigated five different endpoints (embryonal and larval mortality, hemorrhages, asymmetric yolk sac and abnormal larvae) each representing different toxicopathic responses that can be induced by specific or several types of compounds or genetic reasons and since the extracts are complex mixtures containing

innumerable chemicals and the toxic actions are only known for a few of those chemicals, we can only speculate in which chemicals are the etiological agents. Based on our findings, however, some general remarks can be made. The extract from Frierfjorden caused a significant increase of hemorrhagic larvae, which is accordance with high levels of PCDD/Fs that are known to be strong inducers of this toxicopathic response in rainbow trout larvae (Tanguay *et al.*, 2003). Sediment extracts from three locations (Örserumsviken, Frierfjorden and Riddarfjärden) caused asymmetric yolk sac among larvae, indicating that these locations contain chemicals that act through similar mode of action. Since asymmetric yolk sac is not found among control larvae this endpoint is regarded as the most sensitive indication of xenobiotic exposure. In addition, based on our findings in previous studies (Sundberg *et al.*, 2005a) these locations do most likely contain potentially toxic chemicals that are structurally similar, *i.e.* different kind of polycyclic aromatic compounds. Benzo[a]pyrene cannot by itself cause the asymmetric yolk since 5 mg/kg egg is needed to induce similar frequencies of larvae suffering from this abnormality (Fig. 3F), while the levels in those three extracts corresponds to doses of 31–72 µg benzo[*a*]pyrene/kg egg.

Interspecies differences of toxic response

In the *N. spinipes* test system the sediment extract from Örserumsviken was the most toxic followed by the extract from Frierfjorden, while in the rainbow trout test system the extract from Frierfjorden was the most toxic. Different exposure routes and different toxicological pathways most likely explain this discrepancy.

In the *N. spinipes* test system toxic chemicals enter the animals mainly via the gastrointestinal tract or via diffusion through the skin (see section 5.1). In the rainbow trout test system the chemicals enter the animals via diffusion through biological membranes (see section 5.2) from the yolk into the developing embryo, contributing to differences in administered dose between the two systems. Many of the toxic effects observed in rainbow trout are governed by metabolic activation. The rainbow trout have a more developed and complex biological system, including metabolising and detoxification enzymes, resulting in different toxicopathic responses between the two species.

Ranking

In the *N. spinipes* experiment two main endpoints investigated were larval development status and mortality. The potentially most lethal sediment was Örserumsviken. In fact, this was the only sediment extract that caused a significant effect on mortality. Effects on larval development were observed for all sediment extracts, except for Björkskär, which is a background location. The other background location, Slingsviken, contained substances that both caused an increase and decrease in mean LDR. The most significant effects on larval development were observed in the extracts from the sediment from Örserumsviken, followed by Frierfjorden. The sediment from Riddarfjärden caused a strong inhibition in the highest treatment.

Consequently, the overall ranking for the different sediments are: 1 Örserumsviken, 2 Frierfjorden, 3 Riddarfjärden, 4 Slingsviken and 5 Björkskär (Table 6).

In the rainbow trout experiment the potentially most lethal sediment was Frierfjorden followed by Riddarfjärden. The sediment from Frierfjorden caused also the most developmental abnormalities followed by Örserumsviken. While the sediment from Riddarfjärden also induced developmental abnormalities its potential toxicity was less than that from Örserumsviken. The two background locations (Slingsviken and Björkskär) did not show any effects on mortality or developmental abnormalities for the doses used here. Consequently, the overall ranking for the different sediments are: 1 Frierfjorden, 2 Örserumsviken, 3 Riddarfjärden, 4 Slingsviken and Björkskär (Table 6).

The combined ranking of the five sediments' potential toxicity in juvenile *N. spinipes* and rainbow trout is compiled in Table 6. This ranking indicates that the sediments from Örserumsviken and Frierfjorden have the highest potential toxicity. The sediment from Riddarfjärden has an intermediate potential toxicity whereas the combined ranking of the two reference locations, Slingsviken and Björkskär, indicate low potential toxicity. However, Riddarfjärden has a relatively high water flow out to the Baltic Sea of approximately $10^6 \times$ m3/day (www.stockholmvatten.se), while Örserumsviken, which served as our positive reference sediment, has a water flow out of the bay that is 200 times less (Fanger *et al.*, 2003). Therefore, the total load of potentially toxic substances passing by the City Hall of Stockholm is most likely higher than those dredged from Örserumsviken.

If we solely had used the literature data of PAH concentrations from the five stations, Riddarfjärden would be considered more polluted than Örserumsviken and Frierfjorden, while including other pollutants such as PCBs and PCDD/Fs the interpretation would be even more complex. An important consideration is that the current knowledge of which compounds that actually are the most potentially toxic is severely limited. In Örserumsviken for instance, other polycyclic aromatic compounds than commonly analysed PCBs and PAHs contributed to the major part of potential toxicity (Sundberg, 2005). Similar findings have also been demonstrated in other locations (Brack, 2003). By investigating potential toxicity as a first screening step in the process of prioritising remedial activities, we could avoid these theoretical implications.

Table 6. Semi-quantitative ranking of the different organic sediment extracts based on their potential toxicities investigated by the *N. spinipes* and the rainbow trout experiments. A high score in the combined ranking column indicates a low potential toxicity.

Location	*N. spinipes*	*Rainbow trout*	*Total points*	*Combined ranking*
Örserumsviken	1	2	3	**1**
Frierfjorden	2	1	3	**1**
Riddarfjärden	3	3	6	**2**
Slingsviken	4	4	8	**3**
Björkskär	5	4	9	**4**

Conclusions

We have described a reliable semi-quantitative tool based on potential toxicity of hydrophobic compounds that can be used as first screening step in the prioritising process of remedial activities.

By using biological effects in terms of potential toxicity, numerous disadvantages that accompany chemical analyses may be avoided.

The three potentially most toxic locations (Örserumsviken, Frierfjorden and Riddarfjärden) contains, at least partly, chemicals that act thorough similar toxicological pathways and are most likely structurally similar.

The modified *N. spinipes* test system using silica gel as carrier was demonstrated being a sensitive screening tool of toxicants with low water solubility.

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Riskbedömning av förorenade sediment

- ekotoxikologiska metoder som underlag för beslut

RAPPORT 5596

NATURVÅRDSVERKET
ISBN 91-620-5596-8
ISSN 0282-7298

Den här rapporten redovisar en sammanställning som omfattar kvantitativ riskbedömning av förorenade sediment. Den presenterar även erfarenheter från två norska projekt. Det första, RAS (Risk Assessment System), är ett nytt riskbedömningssystem för förorenade sediment. I det andra, DIG (dioksiner i Grenlandsfjordene), har effekterna av dioxiner på olika organismer i norska fjordar undersökts.

I rapporten presenteras även ett nytt testsystem för att karaktärisera förorenade sediment. Med detta testsystem kan effekter av komplexa blandningar av föroreningar påvisas vid betydligt lägre halter än för varje enskilt ämne för sig.

Naturvårdsverket har inte tagit ställning till innehållet i den här rapporten. Författarna svarar själva för innehåll, slutsatser och eventuella rekommendationer.

Kunskapsprogrammet Hållbar Sanering samlar in, bygger upp och sprider kunskap om förorenade mark- och vattenområden. Genom Hållbar Sanering kan myndigheter, forskare och företag söka bidrag för utredningar, seminarier och utvecklingsprojekt som täcker kunskapsluckor på kort och lång sikt. Hållbar Sanering styrs av en programkommitté som består av representanter från Banverket, Göteborgs stad, KTH, Linköpings Universitet, Länsstyrelsen i Kalmar, Naturvårdsverket, Norges Teknisk- Naturvetenskaplige Universitet; SGI, SLU, Sydkraft SAKAB och Umeå Universitet.

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