

Environmental effects of oil and gas exploration on the benthic fauna of the Norwegian Continental Shelf

- *An analysis using the OLF-database*

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LIST OF PAPERS

This thesis is based on the following papers:

- I. Leung, K.M.Y., Bjørgesæter, A., Gray, J.S., Li, W.K., Lui, G.C.S., Yuan, W. & Lam, P.K.S., 2005. Deriving sediment quality guidelines from field-based species sensitivity distributions. *Environmental Science & Technology* 39 (14), 5148-5156.
- II. Bjørgesæter, A., Leung, K.M.Y. & Kwok, K.W.H., 2008. Deriving sediment quality guidelines (SQGs) on the Norwegian Continental Shelf from field based species sensitivity distributions – a quantile regression approach. *Environmental Pollution*. Manuscript.
- III. Kwok, K.W.H., Bjørgesæter, A., Leung, K.M.Y., Lui, G.C.S., Gray, J.S., Shin, P.K.S. & Lam, P.K.S., 2008. Deriving site-specific sediment quality guidelines for Hong Kong marine environments using field-based species sensitivity distributions. *Environmental Toxicology and Chemistry* 27 (1), 226-234.
- IV. Bjørgesæter, A., Kwok, K.W.H., Leung, K.M.Y., Gray, J.S. & Lam, P.K.S., 2008. Can we protect 95% of species in natural marine benthic communities including rare species by setting sediment quality guidelines at hazardous concentration of 5% (HC5)? *Environmental Science & Technology*. Submitted.
- V. Bjørgesæter, A. & Gray, J.S., 2008. Setting sediment quality guidelines: A simple yet effective method. *Marine Pollution Bulletin* 57 (6-12), 221-235.
- VI. Uglund, K.I., Bjørgesæter, A., Bakke, T. & Fredheim, B., 2008. Assessment of environmental stress with a biological index based on opportunistic species. *Journal of Experimental Marine Biology and Ecology* 366, 169 – 174.

ABSTRACT

The main objective of these studies was to utilize the large amount of co-occurring fauna and chemical data collected in different contamination zones around offshore installations to derive ecologically relevant protection levels for marine benthic communities on the Norwegian Continental Shelf (NCS). The data used are collected in regular monitoring surveys in the vicinity of oil- and gas installations at the NCS and stored in the OLF-database (OLF = The Norwegian Oil Industry Association). The methods developed in this thesis try to integrate chemical and biological criteria to provide better and more ecologically relevant guidance in environmental management and risk assessment.

In the first study we developed a novel approach to derive field-based species sensitivity distributions (f-SSDs) and field-data-derived sediment quality guidelines (SQGs) based on concurrent data of sediment chemistry and the abundance of common benthic species (I). The f-SSDs may be used as benchmarks for probabilistic risk assessment and the SQGs can be used as site specific guidelines or integrated into existing SQGs. A 50% decrease in abundance of 5% (HC5, the 5% Hazardous Concentration) of the taxa was used as a measure of effect. The effect concentrations of sensitive benthic species were derived by ordinary least square regression (OLS). To try to strengthen the link between ecological theories and our response model and to reduce the effect of confounding factors, the ecological concept of limiting factors (Liebig's law of the minimum) was incorporated in constructing f-SSDs, by using quantile regression methods to quantify the limiting response functions along contamination gradients (II). The practicalities of the f-SSDs approach in other polluted areas and on a smaller scale were investigated by deriving f-SSD and site specific SQGs for the marine environment of Hong Kong (III). In this study, the data screening criteria of acceptable minimum resolution on the y-axis (abundance) was also tested. Our result suggested that it is possible to halve or more than halve the maximum abundance from the original criteria of 100 individuals per species, in the Hong Kong environment (dataset).

Rare species constitute a major part of the species richness in marine benthic ecosystems (25% - 60%, in these studies depending on scales and definitions). Without knowing the sensitivity of these rare species towards chemicals, it is impossible to know whether a predefined protection level (e.g. HC5) can be protective to 95% of

species in biological assemblages. We utilized the f-SSD approach to investigate whether field-data derived SQGs for common benthic species are likely to be protective also for rare species; using both real field data and computer simulated species distributions (V). Our results suggest that rare benthic species, as a group, are relatively tolerant towards the contamination levels found around offshore installations on the NCS. Inclusion of our estimates of rare species sensitivity had little effect on both the shape of the original f-SSD and the SQGs estimates for all examined chemicals, except barium, suggesting that the majority of rare species are also protected in our previously estimated SQGs for the NCS.

All SQGs derived from the f-SSDs approach (I, II, III, IV) were in general more conservative than the existing guidelines derived from laboratory test data; suggesting that benthic fauna on the NCS are affected at lower concentrations than used in international guidelines presently in operation. In some cases our highest estimated effective concentrations for sensitive species were lower than the suggested threshold levels in use, e.g. for PAHs (polycyclic aromatic hydrocarbons) from HK waters. Interpreting the currently used SQGs in countries worldwide to the potentially affected fraction of species in our f-SSDs translates to a protection level that would on average protect around 80% of the investigated taxa (II, III). The reason for an observed effect at lower contaminant concentration levels in these studies may be due to: (1) differences between biological and environmental conditions in the field and laboratory and (2) differences between guidelines derived for single contaminants and for a mixture of contaminants.

Experience shows that multivariate analyses techniques (e.g. Nonmetric MultiDimensional Scaling, NMDS) are well suited to detect changes in species composition in benthic biological communities. Multivariate analyses techniques were therefore used to test the results from the SSDs studies, and to test the hypothesis that setting SQGs at 4-times background concentrations will give sufficient protection for the benthic fauna on the NCS (V). Slightly disturbed fauna assemblages were identified in 121 contamination gradients, incorporating more than 2,000 species from different habitats and geographic regions on the NCS. The contamination levels in sediment samples having disturbed fauna were compared with the levels in control samples, and if statistically significantly different, they were used to estimate the effect level for structural disturbance of the benthic communities. Our results from this study supported the f-SSD-analysis in that benthic fauna on the NCS are affected at lower levels than

existing SQGs, but also demonstrated the need for more site-specific SQGs. The protection levels derived using the f-SSDs approach (II) was too low (i.e. overprotective) in habitats with naturally occurring high metal concentrations and too high (i.e. underprotective) in habitats with natural occurring low metal concentrations. Our hypothesis that setting SQGs at 4-times background concentrations in various habitats will give sufficient protection for the benthic fauna on the NCS may be a useful rule of thumb for metals, as the overall background concentrations eliciting effect was 3.6 times. In the same gradients, the total hydrocarbon levels eliciting effect ranged, on average from 26 mg/kg to 99 mg/kg.

The increase in abundance of known opportunistic species at increasing contamination levels provides strong evidence of a pollution induced disturbance in the benthic communities. We utilized this to develop a simple biotic index (BIOSTRESS) based on the relative abundance of only five well-known opportunistic polychaetes species that are common in polluted sediments (VI). Its performance to detect changes in community structure was comparable to NMDS in sediment samples with relatively high hydrocarbon levels (> 50 mg/kg).

The results from these studies emphasize the need for better integration of ecology within the field of ecotoxicology. It highlight the importance of establishing field monitoring programs and appropriate database for chemical and biotic data so that we better can better assess the actual risk and effect of contamination on natural ecosystems. Our results provided favourable evidence in support of the use of the f-SSD approach and multivariate analysis to derive ecologically relevant SQGs with a view to protecting the natural biological assemblage.

1 INTRODUCTION

Marine soft sediments comprise one of the largest and oldest habitats in the world (Gray, 2002b). The organisms that live on and in marine sediments, termed *benthos* (derived from the Greek word meaning “depths of the sea”) include a wide range of animals that play an important role in ecosystem processes such as nutrient cycling, contaminant metabolism, dispersion, burial and secondary production (Snelgrove, 1998). On a global scale, marine sediments are the quantitatively most important sites for carbon burial (Wollast, 1991). The benthos are usually divided into different organism size groups (macrofauna, meiofauna and microfauna) residing in (infauna) and on (epifauna) sediments. The macrofauna (defined in my studies as those retained on a 1 mm sieve) often dominates the species richness and the biomass in marine sediments (Snelgrove, 1998; Snelgrove, 1999). Uglund et al. (2003) estimated that the macrofaunal species richness on the Norwegian Continental Shelf (NCS) was 5,152; with Annelida (polychaetes), Mollusca, Arthropoda (crustaceans) and Echinodermata as the most abundant and species rich phyla’s. Species rich fauna of other phyla is also registered on the NCS and as many as 20 of the 29 known non-symbiont animal phyla on Earth were represented in the data material used in the present studies.

1.1 Offshore industry and environmental monitoring

The petroleum offshore industry has been active on the Norwegian Continental Shelf (NCS) for more than 40 years (Figure 1). At present, 226 fixed oil and gas producing facilities are situated on the NCS. The number of wells per offshore installation varies widely and can reach up to several hundred (e.g. a total of 169 development and exploration wells have been drilled between 1982 and 2007 at the Valhall field). In total, about 3,614 exploration and development wells have been drilled on the NCS since the entry of the first exploration well on Balder in the North Sea, October 1966 (NPD fact pages; www.npd.no/engelsk/cwi/pbl/en/index.htm).

An oil/gas well undergoes different stages during its lifespan; starting with drilling and completion to production until it is finally abandoned. All stages are accompanied by undesirable discharge of liquids and solids to the sea. In contrast to freshwater and terrestrial ecosystems, contaminants discharged to the open sea will most often disperse rapidly and spread over large distances from the pollution sources. Sediments are the ultimate resting place of the many organic and inorganic contaminants that enter the sea. Pollutants may attach to particles and sink to the sea bed where the risk of harmful exposure may be significant for several years after discharge (Olsgard and Gray, 1995; UKOOA, 1999). The pollution-induced impact on marine sediments from the offshore petroleum activity on the NCS is primarily from drilling operations. In

2004, drilling chemicals constituted 82% of the total chemical discharge from the oil industry (The Norwegian Oil Industry Association, 2004). Most of these chemicals (> 90%) are so-called green chemicals that are regarded to pose little or no environmental risk to marine organisms. Their chemical status (green, yellow, red and black) is partly founded on the ongoing process of testing acute toxicity of single chemicals (OSPARCOM-tests). The Norwegian offshore benthos monitoring programme investigate the state of the natural environment around all offshore installations within a region every third year (Figure 1). It is regarded as one of the exemplary marine biological monitoring programmes in the world. The long term support of this programme and the cooperation between oil companies and management agencies is unique (Gray et al., 1999). The data are stored in the OLF-database, a database



Figure 1. Map of the Norwegian Continental Shelf, including Svalbard with regional boundaries and oil and gas fields. The regions that are regularly monitored are: Region I - Ekofisk, Region II - Sleipner, Region III - Oseberg, Region IV - Tampen (Statfjord), Region VI - Haltenbanken (Trøndelag), and Region IX Southern Barents Sea (Finnmark). Source: www.sft.no.

maintained by the operators at the NCS. Today the dataset constitutes more than 2,000 marine sediment dwelling macroinvertebrate taxa distributed on around 2 millions individuals. The large amount of biological and chemical data that have been collected and stored in this database provides a good basis for testing and describing empirical pattern of natural population and community structure in relation to pollution (I – VI).

1.1.1 *Pollutant sources (drilling discharges)*

The drilling operations produce two main types of waste (Figure 2); cuttings produced by the action of the drilling bit and used drilling mud. Drilling mud is a mixture of clay (bentonite), drilling weight material (barite, or ilmenite), organic polymers, salts and other chemicals suspended in a liquid. The weighting agents comprise up to 90% of the mud. It contains heavy metals as impurities and is together with clay the main source of heavy metals in the drilling discharges (Neff, 2005; Frost et al., 2006). Three types of drilling mud (liquid) are used on the NCS: oil- (OBM), synthetic- (SBM) and water-based (WBM) mud. Historically OBM and oil contaminated cuttings have been the main source of hydrocarbons finding its way down to the bottom sediments. However, due to the large impact on the benthic fauna around offshore installations the dumping of OBM drill cuttings were not allowed and from 1. January 1993 the OBM and its cuttings had to be brought to land for treatment or re-injected in suitable formations. Today, therefore, the input of oil is clearly reduced and the main sources of hydrocarbons are from production oil getting into the drilling mud, added chemicals, completion fluid, cutting ingredients, and oil from the geological formations being drilled (Frost et al., 2006).

During active drilling, drill cuttings are discharged continuously to the sea floor while drill mud is reprocessed and recycled and discharged intermittently, usually with a larger batch (200 m³) at the end of the drilling operation (Breuer et al., 2004). The fate of chemicals in the water column and the deposition of the drill cuttings and mud on the sea floor will depend on local oceanographic conditions (e.g. prevailing current, water depth), type (e.g. mud, particle size, attachment, agglomeration and hydrophobic properties) and the amount of drilling wastes (Khondaker, 2000; Rye, 2002). In some areas large cutting piles (2,000 – 240,000 m³) are found underneath the installations (Jensen, 2004). These accumulations may act as a secondary pollution source to aquatic and benthic organisms (Breuer et al., 1999). See also UKOOA web-site:

www.oilandgas.org.uk, for studies on cutting piles. The deposition on the sea floor may be re-distributed (e.g. waves, currents, bioturbation), and hydrocarbons and metals undergoes chemical and physical changes (speciation) and biological degradation, accumulation, migration, e.g. through bacterial activity and assimilation into the gut of benthic fauna.

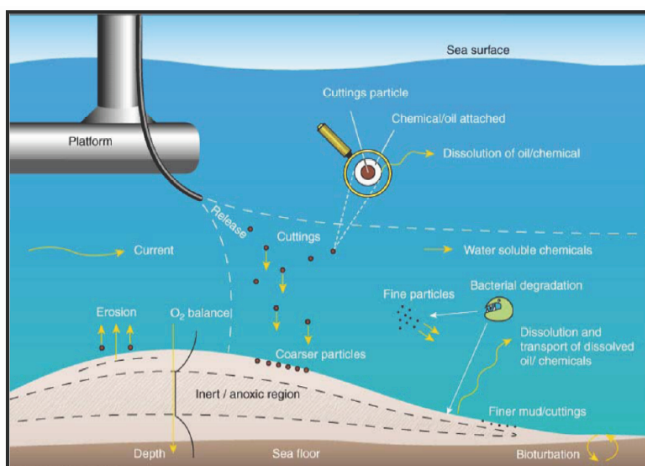


Figure 2. An illustration of some of the processes influencing the nature of contamination gradients around the offshore installations that are investigated in the studies presented in this thesis. The particle size of drill cuttings is in the range of clay (< 2 μm) to coarse gravel (> 3 cm) and the barite particles range from 0.7 μm to 50 μm . Source: (Rye et al., 2006).

1.1.2 Pollutants in drilling discharges

The selection of substances investigated in this thesis is based on the following criteria (cf. Frost et al., 2006): **(1)** relative concentration in drilling mud and cuttings, **(2)** potential for bioavailability and **(3)** toxicity or potential for other non-toxic disturbance (burial, change in grain size and oxygen depletion) to marine organisms **(4)** regularly monitored on the NCS.

The metals of most concern in drilling discharges are (Neff et al., 1987, 2000; Neff, 2005): Barium (Ba), Copper (Cu), Cadmium (Cd), Chromium (Cr), Mercury (Hg), Lead (Pb) and Zinc (Zn). Some of the metals are necessary for normal development in animals (Cu, Cr and Zn) while the others have no known biological function (Ba, Cd, Hg and Pb) (Kapustka et al., 2004). Reused drilling mud may contain concentrations of Ba more 100,000 times background concentrations and metals such as

lead (Pb) and zinc (Zn) at more than 100 times natural background concentration (Neff, 2005). Total discharges of metals on the NCS as impurities in barite or ilmenite per year is in the order of (source: <http://www.sft.no/> - yearly reports of discharged oil and chemicals from oil platforms): Cu (3,000-5,000 kg), Cd (50 – 600 kg), Cr (700-1,500 kg), Hg (20 -700 kg), Pb (2,600 - 24,000 kg) and Zn (2,000 – 3,600 kg). All, except Ba (Neff, 1987) are regarded as acute or chronic toxic and pose a threat to resident benthic fauna (Luoma and Carter, 1991; Bryan and Langston, 1992). For example, Cu used in antifouling paints on boats and Hg may be of special concern as it is susceptible to biomagnification in the form of methylmercury (CH₃Hg) in aquatic food webs (cf. Gray, 2002a).

Oil-hydrocarbons contains traces of several thousand different hydrocarbons. It can be divided into five main groups (Patin, 1999): **(1)** aliphatic saturated hydrocarbons **(2)** aliphatic unsaturated noncyclic hydrocarbons, **(3)** saturated cyclic and polycyclic compounds, **(4)** aromatic unsaturated cyclic compounds, and **(5)** unresolved mixtures. Total PAHs (polycyclic aromatic hydrocarbons) and other aromatic compounds, NPD (naphthalenes, phenanthrenes and dibenzothiophenes), and decalines (decahydronaphthalene) are considered the major contributor to the toxicity (e.g. mutagenic properties) of hydrocarbons in sediments (e.g. Patin, 1999). Aliphatic hydrocarbons (petroleum THC) above decane are insoluble which means that they have low bioavailability and probably make a minor contribution to the toxicity to marine organism in drilling discharges (Frost et al., 2006). At high concentrations they may cause damage to benthic ecosystems by physical/chemical alteration of sediments (e.g. smothering and organic enrichment leading to oxygen depletion). The lighter fraction of aliphatic hydrocarbons (up to octane) is volatile and rarely accumulates to potential toxic concentrations in sediments. As oil is not a single homogenous product it is difficult in laboratory studies to mimic the exposure of organisms to oil and oil products in the field (Chapman et al., 1990; Swartz, 1999 - cf. the “mixture paradox” on page 29). It is generally neglected in ecotoxicological research that also marine sediments consist of a mixture of pollutants (i.e. a non-homogenous product), and this is one of the outstanding questions that is addressed in the field based species sensitivity distributions (f-SSDs) studies (**I**, **II**, **III**). Experience shows that a number of key species dominate the feedback structure of the benthic community in sediments at high organic load (e.g. Kingston et al., 1987; Kingston, 1992, see also Figure 3 and associated text). This observation was used to develop a simple biotic univariate index

(BIOSTRESS) that quantifies the degree of disturbance around installations based on the relative abundance of only five well-known opportunistic polychaetes species (VI). Intriguingly, this apparently active control of a few species follows Liebig's Law in a more modern form (cf. Berryman, 1993; Berryman, 2003), which is the ecological theoretical basis, though in a more traditionally view, for the f-SSDs approach (II, III, IV).

Barium will mostly be present as insoluble BaSO₄ in the sediment (because of the high concentrations of sulphate (SO₄) in marine environment). Ba is discharged in large quantities however and may have a mechanical impact on benthic organisms (cf. Schaanning et al., 2008). Barite contain however heavy metal as impurities making it difficult to separate toxic and mechanistic effects. This again stresses the importance that it is the combined effects that are of interest when predicting effects in the field. It has been demonstrated that barite particles can damage gills of both suspension and deposit feeding bivalves; the sea scallop *Placopecten magellanicus*, common cockle *Cerastoderma edule* and the mud-dwelling and deposit feeding *Macoma balthica* (Cranford et al., 1999; Barlow and Kingston, 2001). In a study of the colonization of an estuarine community, Tagatz and Tobia (1978) showed that fewer individuals and species colonized sand covered by barite compared to controls. Despite the fact that BaSO₄ is insoluble, Ba has been shown to accumulate in benthic organisms living in sediments around offshore installations (Sadiq and Zaidi, 1990; Sadiq et al., 1996) as well as in controlled drilling mud experiments (Ruus et al., 2005). Ruus et al. (2005) calculated a BioAccumulation Ratio (BAR; i.e. adjusted for accumulation in control sediments) from 66–342 for the omnivorous ragworm *Nereis diversicolor* and from 25–75 for the scavenger and predatory dog whelk, *Hinia reticulata*. They found the high BARs interesting from a toxicological point of view since Ba appears to interact with calcium-sensitive processes in cells. This experiment demonstrates the importance of resolving all pathways of bioaccumulation to avoid underestimating full exposure (Luoma, 1996), and that even a minute fraction of the total sediment pollutants assumes considerable importance in estimating bioavailability since pollutants accumulate in sediments up to several orders of magnitude above the levels found in water (Bryan and Langston, 1992).

1.1.3 Bioavailability of the pollutants

Bioavailability describes the portion of a contaminant that can be taken up by the organism from its environment and food and is subsequently transported, distributed and metabolized by the organism (Kördel et al., 1997). A chemical accumulates in an organism when the rate of uptake exceeds the rate of elimination. For a chemical to bioaccumulate, it must be bioavailable. Thus for a chemical to become a hazard it has to be bioavailable. According to Jenne and Luoma (1977), the availability of trace elements to organisms may be influenced by at least four general factors: (1) the physiological and ecological characteristics of the organism, (2) the forms of trace elements in ingested solids, (3) the forms of dissolved trace elements in sediment, and (4) the chemical and physical characteristics of water (cf. Ruus et al., 2005). To these four general factors one may add exposure time of fauna to pollutants which is important as chronic toxicity must involve all sensitive portions of an organism life-history.

Although there is an understanding of many of these general factors individually (Jenne and Luoma, 1977; Chapman et al., 1998b), at present it is only the SEM:AVS (SEM = simultaneously extracted metals, AVS = acid-volatile sulfide) ratio and the $F_{oc} \cdot K_{oc}$ (F_{oc} = weight fraction of organic carbon in sediment, K_{oc} = partition coefficient organic carbon-water) which provides the best mechanistic based procedure for the prediction of metals and organic non-ionic substance sediment toxicity (Di Toro et al., 1991; Chapman et al., 1998b). Due to AVS and organic carbon strong binding affinity they affect the pollutant concentration in the pore water and consequently, if organic carbon or AVS is in excess, the metals or organic substances are not likely bioavailable. An example of how partitioning of pollutants between sediment and water phase are used to derive sediment quality guidelines within the EU-Water Frame Directive, Norwegian coastal waters (including fjords and harbors) and on the NCS is given in Chapter 1.2.1.

The partitioning method is based on that free metal ions (M^{2+}) are regarded as the most bioavailable species, and mechanisms that increase the binding affinity of metals tend to reduce metal mobility, bioavailability and toxicity (Chapman et al., 1998b). Important metal binding phases in sediments includes: acid-volatile sulfide (AVS), particulate organic carbon (POC), iron oxyhydroxides (FeOOH), and manganese oxyhydroxides (MnOOH). Their binding affinities will however depend the

redox potential (Eh) of the sediments, e.g. AVS will only be valid in anaerobic conditions, POC is valid for anaerobic and aerobic sediments, and FeOOH and MnOOH is valid for aerobic sediment. Overlaying and pore water chemistry (e.g. pH, redox potential and metalcomplexing ligands) may further change the binding affinity of metals and may either reduce or increase metal bioavailability and toxicity. For example, a small increase in redox potential may enhance the release of metals from sediments to porewaters and hence increase the bioavailability of metals. So it is already complex before the introduction of the species into the environment. A change in redox potential may be caused by biological activity (bioturbation, feeding behavior etc.) but also by abiotic disturbance of sediments (storms, underwater currents etc.). Thus different species do not only experience different exposure to chemicals based on their biological traits, but also affect their (and others) potential exposure of chemicals.

There has been some controversy about the role of dietary pollutant uptake (cf. Luoma, 1996) but it has become clear that suspension feeders, and not at least deposit feeders can accumulate a substantial proportion (> 98%) of their body burden from ingestion of organic and inorganic bound pollutants (Selck et al., 1998; Wang et al., 1999; Timmermann and Andersen, 2003; Selck and Forbes, 2004). A preliminary analysis of the feeding category to the 400 most common species on the NCS yielded the following rank (giving each species a score from 0 – 3 for each category): surface deposit feeders (355), subsurface deposit feeders (223), omnivore/carnivore (224), suspension feeders (169), large detritus/sand-lickers and commensal (39) (Fleddum et al., in prep.). Combined with other biological traits (activity pattern, body form, size etc.) one may get hundreds of different combinations, emphasizing the importance of using species with different modes of living in ecotoxicological tests (cf. Ruus et al., 2005).

Using a sequential extraction procedure of cuttings material (both OBM and WBM) deposits in the North Sea, Westerlund et al. (2001) showed that the metals are found in fractions loosely bound to particles and thus have a potential to accumulate in benthic organisms. Westerlund et al. (2002) showed that Cu and Zn were more available from barite based formulations than from natural sediments, while other metals were less available (Cd, Cr and Pb). The drilling mud study of Ruus et al. (2005) referred to above demonstrated in addition to Ba, additional accumulation of Cd, Cu, Hg, Pb, Zn and PAHs from five different drilling mud formulations (four with barite as weighing agent and one with ilmenite).

The large number of physical, chemical and biological processes may interfere with bioavailability in marine sediments. The metal gradients in these studies are quantified by nitric acid (HNO₃) digestion according to Norwegian Standard [NS] 4770 on the whole (< 500 µm) grainsize fraction. It is a partial digestion method and has been used for the last 15 years as an operational definition of the bioavailable fraction of metals in sediment (Luoma, 1989; Di Toro et al., 1990). It is aimed to only target the most labile mineral phases (Fe and Mn oxides, sulphides, carbonates and organics) and those metals sorbed outside of grains or within porewater (Campbell et al., 1998). This is the fraction that have potential to exert an influence on biota as it is unlikely that lattice bound metals are bioavailable (Luoma, 1989; e.g. Scouller et al., 2006; but see Bettiol et al., 2008). Although digestion with a weak acid may improve the correlations of metal concentrations with bioavailability, there is no single universal extractant procedure that can predict bioavailability of contaminants in sediments (Luoma, 1989; Chapman et al., 1998b; Chapman et al., 2003). When performing a risk assessment (e.g. comparing chemical criteria derived from laboratory bioassay and environmental concentration), it is important that both the chemical concentration and the chemical criteria are based on similar levels of bioavailability. Quantile regression methods offer a method to reduce the effect of some of these factors (II), but due to the large natural temporal and spatial variations in environmental conditions, it is difficult to define generic and fixed SQGs (V).

1.1.4 *The toxicity of mixtures*

Contaminates occur in sediments as a complex mixture of covarying substances and their derivatives. Thus, any observed ecological effects are the result of the cumulative toxicological actions of multiple pollutants. Potential ecological impacts of simultaneous multiple pollutants are not well understood, especially for low doses. In polluted sediments, contaminants may act synergistically (e.g. fertilizer and pesticides), additive (e.g. two similar type of pain killers) or antagonistically (e.g. snake-antidotes) to cause greater, equal or less effect on an organism than would be expected by the toxicity of each contaminant separately. An example of the significance of considering the effect of mixtures and chemical interactions on overall toxicity is the synergistic toxicity effects of mercury and other heavy metals (Al, Pb, Zn, Cd) in amalgams (Shubert et al., 1978): Injecting rats with LD-1 dose (i.e. the dose that is shown to kill 1

rat out of 100) of mercury and lead resulted in 100% death, and not the 1% – 2% that would be expected if the combined effect of Hg and Pb was strictly additive. Similar synergetic effects have been demonstrated in marine organisms; e.g. the combined effect of Cd, Cu, and Zn on zebra mussel *Dreissena polymorpha* (Kraak et al., 1994) and the Australian ghost shrimp *Callinassa australiensis* (Ahsanullah et al., 1981). In predicting effects in the field it is the combined effects that are of interest. Regression and multivariate analysis together with field data may offer a good method to evaluate the combined risk of multiple chemical exposures to multi-species systems (Thrush et al., 2008, **I, II, III and V**).

1.2 Effects of anthropogenic disturbances in natural ecosystems

Toxic effects of metals occur first when the capacity of an organism to regulate the internal concentration is lost, i.e. when the rate of uptake exceeds the rates of physiological or biochemical detoxification and excretion (Rainbow, 1996). It is the physiological characteristic of a species and previously exposure to contamination levels (adaptation and acclimation) that determine if it will be adversely affected by the pollutants or not. Experience shows that oil related contamination is an important factor in structuring benthic communities around offshore installations (Davies et al., 1984; Kingston et al., 1987; Bakke et al., 1990; Gray et al., 1990; Kingston, 1992; Olsgard and Gray, 1995; Carroll et al., 2000; Renaud et al., 2008). In a comprehensive study of the effects of offshore industry activities on benthic communities on the NCS, Olsgard and Gray (1995) showed that the effects of contaminants (i.e. polluted areas) closely followed the patterns of contamination and concluded that the observed faunal effects were primarily related to concentrations gradients of THC, barium, strontium, zinc, copper, cadmium and lead. The initial effects correlated best with barium and THC, whereas in later years the effects were best correlated with heavy metals; but with both temporal and local variations.

Figure 3, left panel, shows a typical sampling design and placement of stations around an offshore installation. The layout of stations is often in a cross-shaped pattern with a higher concentration of stations closest to the platform and along the main current direction. This design has proven well suited to discovering gradients in both chemical contamination and faunal community composition with increasing distance

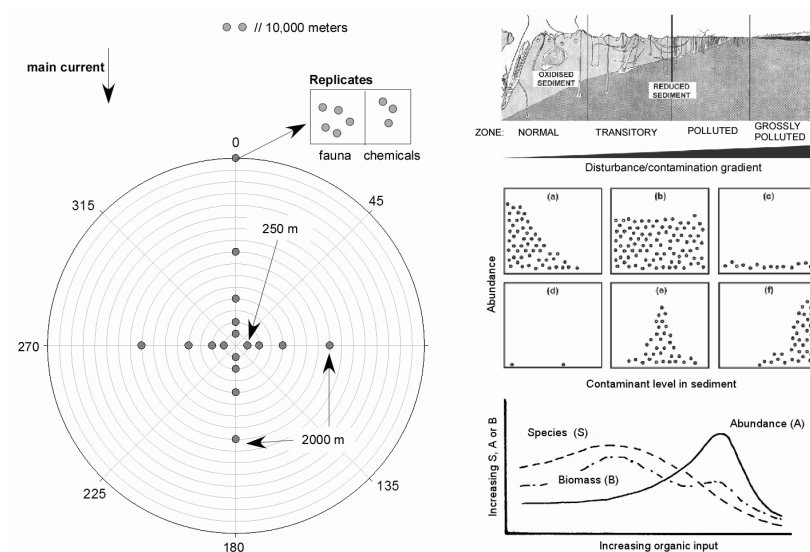


Figure 3. Illustrations of typical sampling design (left panel) making it possible to assess the effect of drilling discharge: (1) Disturbance and contaminant gradient after Pearson and Rosenberg, 1978 (upper right panel), (3) various possibilities of abundance of benthic organisms occurring along the gradient of contaminant (middle right panel): (a) sensitive species, (b, c) two indifferent species with different occurrences, (d) a rare species, (e, f) one opportunistic species and one highly opportunistic species, and (3) Species Abundance Biomass (SAB) distribution (lower right panel).

from installations. The offshore monitoring surveys have shown that under various types of impact sources the “equilibrium” community is disturbed and some species decrease in abundance while others become more abundant (Figure 3, right panels). The replacement of sensitive species by tolerant species is a common response in many types of contaminated systems (Blanck et al., 1988), and is one of the most consistent indicators of organic and inorganic pollution (Kapustka et al., 2004). The theoretical ecological basis is that of life history strategies *r*, *k* and *T* (Pianka, 1970), and the succession in relation to organic enrichment and pollution of the marine environment (Pearson and Rosenberg, 1978), operating within general hypothesis of species diversity (Huston, 1979), intermediate hypothesis (Connell, 1978) and Lotka Volterra models (Lotka and James, 1925; Volterra, 1926; Volterra, 1928). When the organic matter concentration in the sediment increases the boundary between the toxic and anoxic layers called the Redox Discontinuity Boundary (RPD) moves towards the surface (Figure 3, upper right panel). In the normal and transitory zone individuals with low tolerance may be eliminated either by a direct toxic effect or as a result of reduced

competitive ability, facilitation or reduced avoidance of predators (Gray et al., 1979). In the transitory and polluted zone, deep burrowing species do not survive and many sensitive species disappear or decrease in abundance (Figure 3, middle right panel). Effects in these zones (contamination load) on characteristic and important bioturbating species such as the brittle star *Amphiura filiformis* may have indirect effect on the total benthic respiration and thus distribution of other species (cf. Figure 9). The process continues so that the only survivors are a few polychaetes and finally the RPD layers meets the surface and the only species surviving are a few species of bacteria and nematode worms (this may occur in the cutting piles and only one survey in the database include samples from this zone). The monitoring sampling design allowed response of benthic species populations to be assessed along a “mixture dose-response” gradient in field conditions in an uncontrolled way analogous to the doses-response experiments conducted in laboratory bioassay tests (Adams, 2003). The relationship between the abundance of a particular species and the concentration of a specific chemical in the sediment can be plotted, and any sensitive species is readily revealed (Figure 3, lower right panel; **I, II, III**).

In more crude terms as the organic content increases (Figure 3, lower right panel) at first some new species come into the systems as there are more food resources (increase in species richness. S), then the biomass (B) increases. Before the collapse of the system there was large abundance (A) of a few tolerant polychaete species such as *Chaetozone* and *Capitella* (**VI**). The dominant species are often those with a flexible life-history strategy (r-selection), including; direct development, high reproduction (early maturation and larvae throughout the year) and the ability to undergo short-term adaptation (genetic tolerance) or acclimation (non-genetic tolerance) to new environmental condition (Gray et al., 1979). Gray et al. (2002) suggested that the major effect at this stage is from hypoxia rather from organic enrichment per se, and since *Capitella* have a requirement for hydrogen sulphides as a settlement clue it may be “attracted” to hypoxic areas (cf. Gray et al., 2002).

1.2.1 *Methods to assess impact concentrations of chemicals*

Although it is often believed that protection levels based upon laboratory data are conservative and represent “worst-case scenarios” (Chapman, 1995; Chapman et al., 1998a; Chapman, 1999), the lack of response of a process to contaminate in the

laboratory does not necessary mean that responses will be not detectable in nature. It is for example noteworthy that the effect of DDT on birds' egg shell thinning and TBT gender-bender effects on snails were first detected in field studies and not in the laboratory (cf. Luoma, 1996).

The greatest source of uncertainties in deriving assessment end points (e.g. a PNEC; the concentration below which organisms in the area of interest are unlikely to be adversely affected) is the extrapolation of laboratory bioassay results to the natural environment. The requirement to both culture and maintain test species in the laboratory restricts the selection of possible test species and the species used are often not very representative for the large spectrum of species, with varying degree of sensitivity that

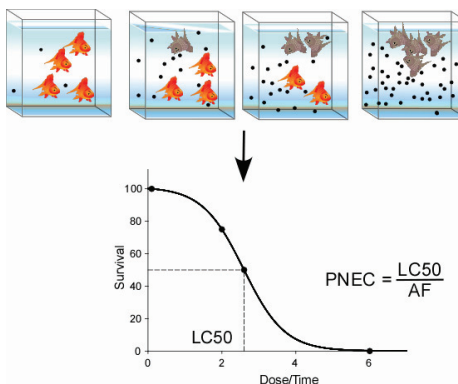


Figure 4. Dose-response relationship based on laboratory toxicity test (bioassay), here fish. LC50 is the lethal concentration to 50% of the individuals but any level of biological organization (e.g. molecular, cellular, organ and organism), effect level (e.g. NOEC, the highest concentrations with no observed effect and LOEC, the lowest concentration with observed effect) and life history variable (growth, reproduction, swimming speed etc.) can be used to estimate an effect concentration.

may occur in natural ecosystems. This exercise of extrapolation therefore involves many often untested assumptions. The outcome is often that the PNECs may be either overprotective or underprotective, depending upon the biological and environmental conditions that apply at each natural site (Kapustka et al., 2004; Crane et al., 2007). This severely limits the usefulness of the chemical criteria in Ecotoxicological Risk Assessment (ERA). The two main approaches used to extrapolate lab data to field data to obtain an estimate of the field Predicted No Effect Concentration (PNEC) is (1) using an assessment factor approach or, when sufficient data are available, (2) from statistical extrapolation.

The assessment factor approach is based on the myth that protecting the most sensitive species will protect the whole ecosystem (Cairns, 1986; Chapman et al., 1998a). A PNEC is derived by dividing the lowest measured endpoint (e.g. NOEC) by an assessment factor that addresses the following uncertainties: (1) inter- and

intraspecies variation, (2) acute-to-chronic extrapolation, and (3) laboratory-to-field extrapolation (Figure 4). Each of the above uncertainties can be addressed separately (Chapman et al., 1998a), or as suggested in the EU-Technical Guidance Document (EC, 2003) by using standard assessment factors, ranging from 10 to 10,000 depending on choice of test organisms, endpoint measured and for how long they have been tested. Even nowadays, these types of experiments are still producing results that have very little relevance for the ecological effects that may occur in the natural environment. Within the Water Framework Directive (the most important piece of water legislation in Europe for many years), the assessment factor approach is used to estimate Environmental Quality Standards (EQS) for marine sediments for lead (Pb) and cadmium (Cd) (EU-RAR). EQS for Pb and Cd is based on laboratory bioassay with lowest No Observable Effect Concentrations (NOECs) for the sewage worm, *Tubifex tubifex* (534 mg/kg) and for the red bloodworm (midge), *Chironomus salinarus* (115 mg/kg), respectively. This is obvious not the most sensitive species in marine sediments and if the PNEC will give an adequate protection level of the benthic community will depend on the assessment factor used.

Experience shows that different species differ in their sensitivity towards a single chemical. This may be due to differences in life history, physiology, morphology and behavior. The species sensitivity distributions (SSDs) approach is a statistical description of the variation among a set of species in toxicity of a certain compound or mixture (Figure 5). SSDs consider variation between species and not

within species and do not attempt to explain why species differ in sensitivity. It is a probabilistic approach in contrast to the deterministic procedure of assessment factors. When plotted as a cumulative distribution function (CDF) it may be used in a “forward” way ($x \rightarrow y$) and in an “inverse” ($y \rightarrow x$) way. Forward use yields information about the proportion of the community likely to be affected, or the probability that an individual

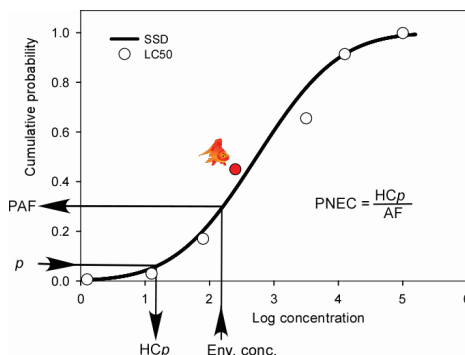


Figure 5. Species sensitivity distribution combining results from various single species bioassay. Forward use ($x \rightarrow y$) in risk assessment and inverse use ($y \rightarrow x$) in EQC is indicated. After (Posthuma et al., 2001).

species will be affected (i.e. a risk assessment), while inverse use yields a measure of environmental quality criteria at a certain cutoff value (e.g. a PNEC or HCp). The probability distribution models that are commonly used by different regions to construct SSDs are the normal distributions, the triangular, the logistic and the Burr II distribution (Wheeler et al., 2002). There is no biological or toxicological motivation to choose one model over another, and an alternative that is utilized in this thesis (**I, II, III, IV**), is to use bootstrapping which does not make any assumptions of a specific distribution (Newman et al., 2000; Wheeler et al., 2002). The use of SSDs in ecological risk assessment procedures environmental quality criteria (EQC) has increased in the last decade because they can introduce greater statistical confidence into risk assessment processes when compared to traditional assessment factor approaches. Today it is used worldwide and so far the SSD method is the only significant basis to predict toxic effects from bioassay on natural ecosystems with multiple species. The SSDs is however no better than the choice of test organism and the endpoints, and since ecologically relevant toxicological data from long-term studies is mostly lacking for marine sediment-dwelling organisms, assessment factors must also be applied on the HC_p-values.

The SSDs method is incorporated in the EU-Water Framework Directive (EC, 2003; Lepper, 2005), in the new official sediment quality guidelines for Norwegian inshore regions (Bakke et al., 2007; Källqvist et al., 2007) and the ERMS-program (Environmental Risk Management System) with the aim to estimate risk from drilling discharge on

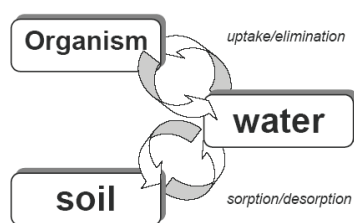


Figure 6. An illustration of the process underlying the equilibrium partitioning concept. Source (Jager, 2003).

benthic communities on the NCS (Frost et al., 2006). As sediment bioassay data are missing (Frost et al., 2006), the PNEC is derived using an equilibrium partitioning (EqP) approach (Figure 6). The EqP-approach aims to predict the concentration of a contaminant that will dissolve in the interstitial pore water (the partitioning coefficient, K_p based on the SEM:AVS ratio for specific metals and the $F_{oc} \cdot K_{oc}$ product for specific organic compounds) and to use this coefficient to adjust PNECs derived from aquatic toxicity tests (e.g. Figure 5). The above programme incorporated slightly modified versions of the Dutch EqP-model (Crommentuijn et al., 2000). The Dutch model

derives an acute value (Maximum Permissible Concentrations, MPC) and a chronic value (Negligible Concentration, NG) using this formulae: $MPC_{sed} = C_{b_{sed}} + (MPA_{water} * K_{p_{sed}})$, where MPA is a PNEC estimated from aquatic toxicity test results and C_b is the background concentrations in sediments. The negligible concentration (NG) is estimated as MPC shown above, but by dividing MPA_{water} with a factor of 100 ($MPA_{water}/100 =$ the negligible addition or NA). The MPC and NC correspond to threshold levels which should never be exceeded by any single chemical and the NC should not be exceeded by any single chemical over longer periods. A great advantage using the EqP-approach is that it utilizes existing toxicology data and considers the question of bioavailability. It is however only valid in aerobic conditions and does not consider all bioavailable pathways (e.g. food), mixture effect and it is based on sensitivity of aquatic organism tested in laboratory. This may be adjusted using assessment factors.

Using this method or slightly modified versions yields for Zn the following chronic threshold levels: the Netherlands 145 mg/kg (NG), Norwegian coastal water 360 mg/kg (upper Limit Class II) and for NCS 21.16 mg/kg ($PNEC_{sed}$). All three Zn levels are intended to be the no observed chronic effects over long exposure times. Although it is some differences in use of E_p (e.g. ERMS use the partitioning coefficient between Barite and water), assumed organic carbon in sediments (e.g. the Netherlands assume a 10% organic content in marine sediments while the Norwegian SQGs assume 1%), the large observed differences is due to different interpretation of assessment factors. For example, the Dutch interpretations, suggests that all values would, in practically terms, be equal to the used background concentrations (within 5 mg/kg). Thus although scientifically sound, one may argue that the EqP-approach in practical (ecological) terms does not differ in this example from the assessment approach (divide the lowest LC50, NOEC etc. by a number). The problems with current laboratory approaches for deriving PNECs can potentially be, at least in part overcome through the proposed f-SSD approach (I, II, III).

1.2.2 *Methods to assess impact on biological populations and communities*

A number of biological criteria have been developed in order to assess whether biological assemblages are disturbed or not disturbed. Species richness, various modifications of Rényi entropy (e.g. the Shannon-Wiener index, the Simpson index)

and species abundance distributions (SADs) are easily measured and intermediate in complexity. SADs was used by Gray (1979; 1981) to detect initially disturbed fauna exposed to various pollution sources such as organic waste, oil and toxic substances by showing that under slight disturbance the community is not in equilibrium, giving a departure of the lognormal distributional pattern. A variety of elaborations have been developed based on this basic idea; such as k-dominance plots (Lambhead et al., 1983) and abundance/biomass comparison (ABC) plots (Warwick, 1986; Magurran, 2004). Gradient analysis techniques (or ordinations) are commonly used to detect pollution-induced changes in benthic communities (e.g. Gray et al., 1990; Clarke, 1993; Olgard and Gray, 1995; Currie and Isaacs, 2005), and are regarded as the most sensitive techniques for distinguish site groupings of disturbed benthic assemblages related to oil activity (Olgard and Gray, 1995). Ordinations may be divided into two main approaches; eigenanalysis-methods (e.g. PCA = Principal Components Analysis and CA = Correspondence Analysis) which is based on statistical species response models (linear or unimodal) to latent gradients, and distance-based methods (e.g. NMDS = Nonmetric Multidimensional Scaling) which is based on a distance matrix or a similarity matrix (e.g. Euclidian or Bray Curtis measures) (Goodall, 1954; Bray and Curtis, 1957; Kruskal, 1964; Hill, 1973; Clarke, 1993). The statistical techniques used to place sediment samples (and species) along the latent gradient is somewhat intricate; eigenanalysis is the solution of linear algebra (for small matrixes) or an iteration process of a series of recurrent regression and calibrations (for large matrixes), while distance based methods are purely geometrical (moving samples around to one find the best solution). The final outcome is simple however, and may be presented in a 2-dimensional ordination diagram for further interpretation (linking the pattern to species in the case of distance based methods, and to environmental variables) and generating hypothesis. Regression analysis and direct gradient analysis (constrained ordination) may be used to predict and test *a priori* hypothesis about species abundance and species response functions (mode, tolerance and modal abundance) to a single or a set of environmental variables (Ter Braak, 1987; Austin, 2007). The latter technique is a multivariate parallel to regression, treating several species and several predictor variables simultaneously (e.g. CCA = Canonical Correspondence Analysis). A relative recent method, CAP (Canonical Analysis of Principal Coordinates) provides a constrained ordination of the data on the basis of any measure of dissimilarity, a constrained version of NMDS (Anderson and Willis, 2003). Constrained ordination

methods may also be used to extract the best environmental variables in a stepwise forward selection of variables (e.g. CA/CCA; NMDS/CAP).

The above biological criteria have a great potential for use in ecotoxicology, as they reflect the natural assemblage (population and communities) and environmental conditions (see Clarke, 1999; Sparks et al., 1999 for a discussion of traditionally multivariate methods potential use in ecotoxicology). The priority has however been to develop biological criteria and best tools to identify biological effects from contaminants and not to provide chemical guidelines (see Anderson et al., 2006 for one noticeable exception). For example among the 10 parameters (e.g. the Shannon Wiener index, the Evenness index, SADs, top ten indicator species, Bray-Curtis similarity matrix, and subjective classification) required by the Norwegian Authorities for assessing the status of the benthic communities, none are intended to provide chemical guidelines (see Trannum et al., 2006 for a review of the different methods used in the environmental monitoring on the NCS). The lack of methods to derive chemical guidelines from field based biological criteria is possibly because population response to stresses are complex and the cause of e.g. population change can be difficult to differentiate. It is however possible to use regression analysis together with SSDs to (1) estimate single species sensitivity based on population abundance and (2) model species sensitivity distributions to estimate community level response to pollutants so that relevant biological criteria can be integrated with chemical criteria **(I, II, III)**.

2 Discussion

Single species toxicity testing may be of limited value in marine sediment habitats (Gray, 1999; Lam and Gray, 2001). Marine sediment faunal diversity is considerably higher than in lakes and rivers (for which bioassay was intended) and a large number of the benthic species are found at very low densities and occurrences, and will therefore never be tested (Gray et al., 2005, **IV**). We

simply do not have the scientific capacity of ecology to extrapolate bioassay data to predict effect on populations and ecosystem (see Power and Adams, 1997 special section for perspectives of the scientific community). It is of importance to protect biodiversity (structure) and complex biological interactions (functions) such as bioturbation, secondary production, contaminant metabolism and nutrient cycling. A fundamental question is: “*what should or could be measured to indicate response of ecosystems (structure or function) when they are exposed to chemical stressors*” (Lam and Gray, 2001). There is today no consensus on which structural (e.g. changes in abundance, species richness and community composition) or functional (e.g. bioturbation, bioirrigation, nutrient cycling, energy flow) effects are the most appropriate endpoints in ecotoxicological studies. Structural parameters are usually measured as surrogates of function under the assumptions that ecosystem functioning is less sensitive because of functional redundancy. In this context, protecting structure may be considered as a conservative approach. Biological responses to pollutants have been demonstrated on all levels of biological organizations; molecular, cell, organ, individual, population, community and ecosystem. The ecological relevance of a study increases with increasing complexity but it may be more difficult to identify clear cause-effect relationship (Figure 7). Regression analysis of population abundance is a convenient measure of population level response to pollutants, and multivariate analyses are suitable to study changes in community structure (**I, II, III, V**).

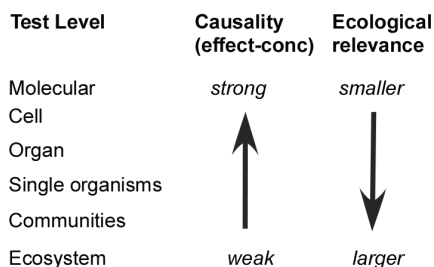


Figure 7. Relationship between test level (complexity of study) and casual relationship and ecological relevance. After (Caquet et al., 2000).

Another key question can be raised: “*where should one try to measure the effect*”. Interactions between organisms and their environment are fundamental in ecology but not in ecotoxicology. Ecological systems are intrinsically complex; possible biological effects caused by contaminants at one biological level are likely to cascade through the entire system as natural assemblages of organisms are affected; reflecting the importance of biological interactions (e.g. population density effects, facilitation, competition and predator-prey relationship) and biological attributes (e.g., susceptibility of different life stages and life cycle variability, life-history strategies, tolerance, trophic level, rareness). All these biological interactions are affected by dynamic geochemical factors and interactions of their environment. Indeed, pollutants often interact with natural stressors such as low oxygen levels and reduced food availability and may thus be imperative for already stressed organisms (cf. Thrush et al., 2008). This complexity and the lack of stringent laws in ecology has led to the beliefs by non-ecologist that ecotoxicology cannot profit from ecological experiments and observations (see Van Straalen, 2003): Egler stated (1970): “Nature is not only more complex than we think. It is more complex than we can think”. Colyvan and Ginzburg (2003) point out however that it is a mistaken belief that ecology is a too complex science to have laws and that this is partly due to unrealistic expectations of what a law is; - laws describe idealized situations, have many exceptions and need not be explanatory or predictive (e.g. Kepler’s laws of planetary motion). Experience suggests that only some of the many biotic and abiotic interactions are strong and important (Berryman, 1993; Berryman, 2003), and e.g. the basic principle of ordinations of reducing dimensionality is only possible because the species respond to the same few underlying complex-gradients and because the number of strong coenoclines therefore is low (R. H. Økland 2006 – Univ. of Oslo gradient analysis course compendium). A more concrete argument is that field observation data cannot infer causality since it is based on correlation. This is a crucial point, as causality cannot be unambiguously established for any observation studies (Clarke, 1999; Adams, 2003). It is in this relation important to distinguish between a possible strong mechanistic causal relationship between a pollutant and an observed effect in the laboratory and the casual relationship between this observation and the actual effect in natural systems. The first is off course impossible to determine from field observation data, since the actual observed effect on e.g. a population may be due to the combined effect of cumulative and synergetic effects of several stressors. At the Norwegian Continental Shelf causality in the weaker sense has however been

established between stressors and the benthic fauna, shown by studying data from large-scale replicated sampling across a range of oil and gas fields for more than 40 years of field monitoring surveys. It should not be very controversial to claim that the leap of imagination required to visualize causality between a significant result in, say the worm *Tubifex tubifex* and Pb in a standard bioassay, is an order of magnitude greater than that for a convincing demonstration of change in marine benthic populations or species assemblages (cf. Clarke, 1999). As argued by Clarke (1999), if there is a trade off among simplicity, sensitivity and realism (as it is since we are lacking relevant ecological toxicity data for marine benthic species) in methods chosen to assess environmental impact, realism surely deserves more emphasis than it receives in current practice (see also Lam and Gray, 2001).

To this end traditional field sampling and monitoring across pollution gradients may be a good complementary approach to assess impact of toxicants on benthic marine life (Lam and Gray, 2001; Thrush et al., 2008). Field surveys describe patterns that emerge from the effects of all factors that act upon a species and thus include interactions between species and between stressors. Regression analyses and multivariate techniques with large-scale field data offer a useful means to identify and predict effects of chemical stressors apparent in benthic populations' distribution patterns (Clarke, 1999; Anderson et al., 2006; Crane et al., 2007; Thrush et al., 2008).

2.1. Field based species sensitivity studies.

In the studies presented here, field collected monitoring data are used to derive protection levels for marine sediments that integrate chemical and biological criteria. In **Paper I** we introduced a novel method, namely field-based species sensitivity distribution (f-SSD), to derive ecologically relevant and site-specific SQGs using concurrently collected field data on both benthic species populations and concentrations of common contaminants in sediments. The method alleviates several of the problems of current laboratory-driven methodologies for deriving SQGs that extrapolate laboratory-based observations to field situations, from a few species to many species, and from a single chemical to a mixture. **Paper I** introduces an alternative thinking on the way which environmental standards are derived and interpreted. The traditional mean response between chemical concentration (dose) and abundance (response) may however not be applicable to field conditions where important variables are not

controlled (Kaiser et al., 1994; Terrell et al., 1996; Thomson et al., 1996; Scharf et al., 1998; Cade et al., 1999).

The above studies and three review articles (Huston, 2002; Cade and Noon, 2003; Austin, 2007) have drawn attention to the impact of assuming Liebig's Law of the Minimum is operating when modeling biological responses to environmental predictors and species spatial distributions. Limiting factors are variables that determine the potential limit of a biological response (e.g. reproduction, growth, abundance) and whether an organism lives in an area or not. In relation to plant growth and nutrients Liebig's law states that "if one crop nutrient is missing or in short supply, plant growth will be poor, even if all other elements are abundant" (Figure 8, left panel). At other sampling locations (Figure 8, upper right panel) other nutrients may be the limiting factors (i.e. missing or scarce) and thus when plotting plant growth (response) against the first nutrient (predictor) one may get a scatter diagram with large unequal variation (Figure 8, lower right panel).

In paper II and III quantile regression methods (Cade et al., 1999; Koenker, 2005) were incorporated in the f-SSDs method to model the limiting function of species response along the contamination gradients. The rationale for focusing on the limiting function instead of the mean is that changes near the limiting function would be expected when chemicals included in the model actively limited species abundance. The Law of the Minimum is easily translated into any biological processes that are regulated by more than one factor (Huston, 2002). Schröder et al.

(2005) provide an example of the application of quantile regression to species abundance data (fen plants) in relation to environmental gradients (flooding and

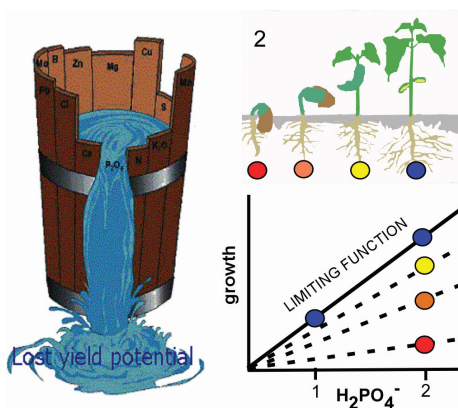


Figure 8. Liebig's Law of the minimum illustrated by loss of potential yield due a limiting factor (left panel). Sampling across a gradient where other resources are less than optimal will constrain growth to less than maximal possible response (right panels). The dotted regression lines illustrate: (1) how it is possible to estimate different part of the response distribution, and (or) (2) consequence if the predictor variable is not the limiting factors (note also that if only the red samples were taken from site 2 it would appear that growth decline with phosphate concentration).

phosphate). Luoma, (1996) is apparently the first to relate the declining limiting function in field data as a measure being equivalent of the dose response. He makes no connection to Liebig's Law or quantile regression but it is of interest to follow his arguments using an example from benthic monitoring data (Figure 8).

The data are from the Ekofisk 1987 survey; a well-known and probably the best studied contaminant gradient on the NCS (Hobbs, 1987; Gray et al., 1990; Clarke, 1999; Clarke and Gorley, 2006). The study of this gradient by Gray et al. (1990) played an important role in the formation of legislations imposed by the Norwegian Control Authority in the 1990s and final banning discharges of oil based mud (OBM) cuttings in January 1993 (Gray et al., 1999).

Luoma (1996) states: "a high unequal variance in response is typically in low pollutant concentrations" (Figure 9 a, b). He further suggests that, at low concentrations, many levels of response, driven by factors other than pollutants exposure are possible when pollutant is not a controlling factor in the process. Following the logic of limiting factors; there must be some other factors than barium (Ba) that constrain the abundance of the brittle star *Amphiura filiformis* to locally lower levels in the sediment samples (e.g. the five blue samples in Figure 8a); in this case possibly lead (Pb; Figure 9b). In samples where it is Ba that affects abundance, this effect may be manifested in what

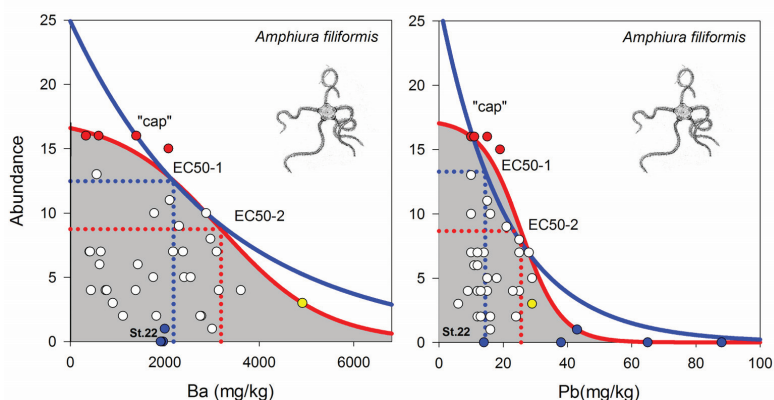


Figure 9. Species distributions of *A. filiformis* along a particular "dose-response" gradient (Ekofisk 1987) quantified by Ba (left panel) and Pb (right panel). Two models are illustrated; a logistic model (red) and an exponential decay model (blue). *A. filiformis* is a characteristic species in many habitats on the NCS (Petersen, 1913). As an adult it is a suspension feeder but will change to deposit feeding in stagnant water or areas of very low water flow (cf. Skoeld et al., 1994). Its bioturbating capabilities have been well documented (Solan and Kennedy, 2002) as well as its functional importance in total benthic respiration in sediments (Solan et al., 2004).

Luoma called a “cap”, i.e. the limiting function. He suggested that the “cap” may describe the dose-response relationship, which is what we try to model using quantile regression. In an ideal situation with many sediment samples with low or no correlation between contaminants the limiting function (the “cap”) will approximate the effects of a single contaminant. Also ecotoxicological studies may profit from using quantile regression to describe the dose-response relationship since in the real world, also bioassay results may show heterogeneous response functions (especially if tests are done in different laboratories).

The Ekofisk-field was around 17 years old in 1987 and inner stations were heavily contaminated by OBM in the three previous years (ca. 900 tons). This yielded a sharp and relative abrupt THC-gradient and high oil concentrations at four of the monitoring stations closest to the installations. Thus, THC is possibly most likely to be the limiting factor in the five samples and to a large extent determines Pb limiting function. More samples would therefore be necessary to ensure that the limiting functions actually reflect Pb as the limiting factor (i.e. samples with higher Pb concentrations and lower THC levels). Increasing the sampling area means however that one must move into another geographical area and habitats with different biogeochemical conditions (and thus introducing new confounding factors). On the other hand it will increase the number of sensitive species possible to estimate an effect concentration from and thus increase the representativeness of test species in the f-SSDs. Increasing the sampling area (number of gradients) made it necessary to use simple statistical models to describe the limiting functions (we used an exponential decay model or a linear). None of these have the ability to depict the observed asymptotes (Figure 9). The latter was adjusted for by applying a correction formula: $EC50 = B_1 - b1^{-\ln(0.5)}$, where B_1 is the background concentration on the NCS and $b1$ is quantile regression slope estimate (EC50-2s in Figure 8 would be 2295 mg/kg and 24 mg/kg, for Ba and Pb, respectively). In total, our obtained effective concentration (f-EC50s) estimates for *A. filiformis* for Ba and Pb on the whole NCS was 4688 mg/kg and 24 mg/kg, respectively.

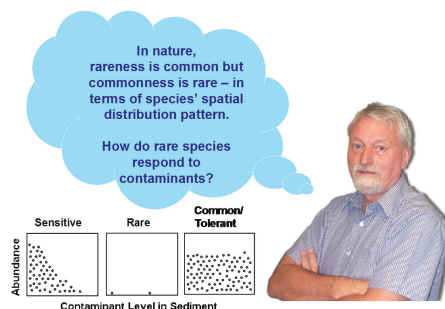
As noted by Austin (2007), quantile regression is not simply about failure to include important variables in the regression models but also that unmeasured or unknown variables almost invariably reduce the abundance of the dependent variable and thus may obscure the nature of the relationship (e.g. synergetic effects, variable

bioavailability, food a competition, predations and facilitation). In many cases it is not possible to attribute a high response (i.e. the perceived disconnection between the response and predictor variable) to any of the measured factors due to unknown or logistic constrain, e.g. sediment sample St. 22 (Figure 9), could not be attributed to any of the contaminants measured and the high response (i.e. low abundance) may be due to chance or some unmeasured factors or possible multiplicative biological or abiotic interactions.

The f-SSD approach is a specific measurement of the biological community response to a specific chemical in the presence of other chemicals in the sediment that takes into account varying physical and biotic factors. Quantile regression reduces the intervariable collinearity between substances by giving data points with negative residuals much lower weight, and thereby magnifies the effect of the contaminant in question in the models (II). The magnitude of the bias from other contaminates (or unmeasured factors) is not known, but as Cade et al. (2005) showed we can be confident that estimates of the limiting functions are less biased than the mean. The degree of correlation may be high in the most contaminated areas, and this will further complicate the task of quantifying a single contamination contribution to the observed response (Cade et al., 2005). This has important implication in interpretation, that is, single chemical criteria versus mixture chemical criteria. Contaminates occur in sediments as a complex mixture of covarying substances and their derivatives. Thus, the ecological effects result from the cumulative toxicological actions of multiple contaminants. This will make field based chemical criteria more protective than chemical criteria based upon only single-chemical approaches (Long et al., 1995). If a chemical criteria is derived from determination of toxicological effects caused by a single pollutant it will greatly underestimate ecological effects in the field since the actual observed effect is caused by contamination in a mixture. On the other hand, since our chemical criteria are estimated based on correlation of ecological effect and concentration of a single substance in sediments, it will greatly overestimate the effects caused by the single substance. Swartz (1999) called this the “mixture paradox”. Since the question we want to answer is; “what is the risk of contamination for populations and communities in the field”, it seems reasonable that potential ecological impact of multiple stressors should be taken into account when deriving chemical criteria. If one compares each single chemical criteria of predicted or measured effect concentrations in

the field, one may get a wrong picture of potential hazard and risk for the species living there.

Rare species are the dominant species group in all multispecies communities and is one of ecology's true universal laws (McGill et al., 2007). Rare species are at greater risk to extinction, and once they go locally extinct, they take longer to re-immigrate than common species (Gaston, 1994; Hubbell, 2001; Volkov et al., 2003).



Two important questions are therefore: (1) how do rare species respond to contaminants, and (2) is it possible to protect 95% of species in the natural biological assemblages including rare species by setting and enforcing sediment quality criteria at Hazardous Concentration of 5% (i.e. HC5). The sensitivities of rare species are, nonetheless, much more difficult to identify and predict because of their low spatial occupancy and low abundance in natural benthic environments. Given the robustness of the f-SSD approach and the abundant availability of field data from the Norwegian Continental Shelf, this study investigated these important questions through understanding how the inclusion of rare benthic species into the original f-SSDs (i.e. from **II**) constructed with common and sensitive species would affect the HC5 estimate. Specific research objectives include: (1) examination of the distribution of rare benthic species at different contaminant concentrations in the sediment along pollution gradients and (2) investigation on how the inclusion of rare species can affect the f-SSD using both computer simulation and actual field data. Based on our results, rare species generally evenly spread across the contamination gradient, suggesting that rare benthic species on the NCS are relative tolerant towards contamination. Inclusion of rare species only had little effect on both the shape of the original f-SSD and the HC5 estimate for all six examined chemicals except barium.

In our study of rarity and commonness in marine and terrestrial species assemblages, we showed that it was possible to divide the relative abundance of species assemblages into log-normally distributed groups of rare and common species based on their occurrences (Gray et al., 2005; Gray et al., 2006b). The species abundance

distributions (SADs) are affected by sampling and binning method (Gray et al., 2006a). Although preliminary analysis suggested the species constituting the different groups were not consistent as sample size increased. A shift in moderate rare species may also be a problem in the rare species analysis (V). Due to low occupancy it was not possible to quantify the sensitivity to single rare species and a more detailed analysis is therefore necessary to investigate how (and if) this shifting of species within bins stabilizes at a given sampling area at different scales.

2.2. Multivariate analysis and the biotic index (BIOSTRESS)

Multivariate analysis is regarded as the most sensitive technique for distinguishing site groupings of disturbed assemblages related to oil activity. Subtle effects induced by pollutants may be reflected in changes in the community composition that may be identified using the biological criteria discussed above (e.g. similarity matrixes, species response models) together with multivariate techniques (e.g. ordination and classification). Our study showed that the effect threshold levels electing effects on the fauna varies between habitats and illustrated that it is difficult to determine generic and fixed sediment quality guidelines for the whole NCS (V). Due to the large number of gradient analyzed, the derived threshold levels are believed to provide a good reference for when to expect an effect on benthic communities in different habitats on the NCS (V).

In the f-SSDs studies we use a 50% decrease in abundance of 5% of the taxa as a measure of effect (HC5), while in these two studies (V, VI) we used SIMPROF as provided in the CLUSTER routine in PRIMER to determine different fauna assemblages (Clarke and Gorley, 2006). The lack of a quantification of the size of the disturbance prevented us to estimate the concentration level in the slightly disturbed fauna assemblages in terms of hazardous concentrations; i.e. HC5, HC10, or possible HC25? ANOSIM provided a standardized measure of group differences (the R-statistic) based on the average ranked within-group and between group similarities (see also Willis et al., 2004), but it is not scaled and was difficult to implement for different gradients as the fauna group sizes was different.

Predicting effects of pollutants in marine sediments is a difficult task and it is often a conflict between repeatability reliability and relevance (cf. Calow, 1992). The simplest method to assess sediment quality is by comparing pollutant concentration

with concentrations levels in control samples. Although neither scientifically sound nor novel, our hypothesis of that setting a SQG of 4-times background concentrations will give sufficient protection for the fauna from metal contamination worked reasonable well. The biotic index BIOSTRESS is also a simple measure, biological criteria, which give a score on pollution and showed promising result compared with NMDS. Further tests on a larger geographical scale is needed to explore what the values means in terms of the severity of disturbance.

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