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N E W S L E T T E R F R O M

RF - Akvamiljø

RF-Akvamiljø is a Centre for environmental research in aquatic environment. The three affiliated companies: RF-Rogaland Research, Akvamiljø a/s and Akvamiljø Caspian AS with 45 scientists and engineers provide advanced research and services within ecotoxicology, environmental risk assessment, monitoring, field- and laboratory experiments and analyses. Akvamiljø Caspian performs environmental consultancy/research in the Caspian region and operates a laboratory - in Baku, Azerbaijan - according to GLP standards.

The Comet Assay – a promising tool for detection of DNA damage

In most living organisms, genetic information is stored in the DNA molecule. DNA carries the information needed to direct protein synthesis and replication. DNA is composed of two antiparallel strands which wind about a common axis to form a double helix. However, numerous pollutants released into the marine environment have the potential to interact with DNA. Therefore, when evaluating environmental risk, the use of a sensitive assay to detect possible genetic impact is essential.

The comet assay is one of the methods established at RF-Akvamiljø to reveal DNA damage. The comet assay, also called the alkaline single-cell gel electrophoresis (SCGE) technique, measures DNA damage in individual cells from experimental animals exposed to potentially genotoxic compounds. The comet assay can be used on all types of nucleated cells.
The comet assay is capable of detecting various forms of DNA damage, e.g. single strand breaks, oxidative DNA base damage and single strand breaks associated with incomplete excision repair sites. Relative to other DNA damage tests, the comet assay has been demonstrated to be a sensitive and rapid technique and it is widely used in areas as human and environmental biomonitoring and genetic toxicology (e.g. to identify mutagens and carcinogens).
When analysing with the comet assay, a small number of cells are embedded in a thin gel on a microscope slide and the cells are treated to liberate DNA. The double helical DNA structure is subsequently unwound and subjected to an electric field where damaged DNA (negatively charged DNA fragments) migrates from the nucleus towards the anode, producing a shape of a "comet" with a head (cell nucleus with intact DNA) and a tail (DNA fragments). Examination of DNA damage is done under a fluorescence microscope after staining with a fluorescent DNA binding dye. The DNA damage is scored using a computerized image analysis system.
RF-Akvamiljø has established the comet assay for fish, mussels and sea urchins, mainly studying the potential of styrene, plasticizers, flame retardant and dispersed crude oil to cause genetic damage.

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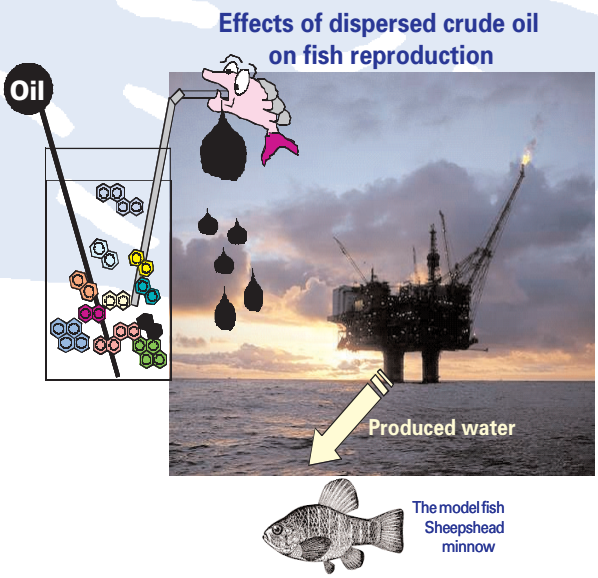

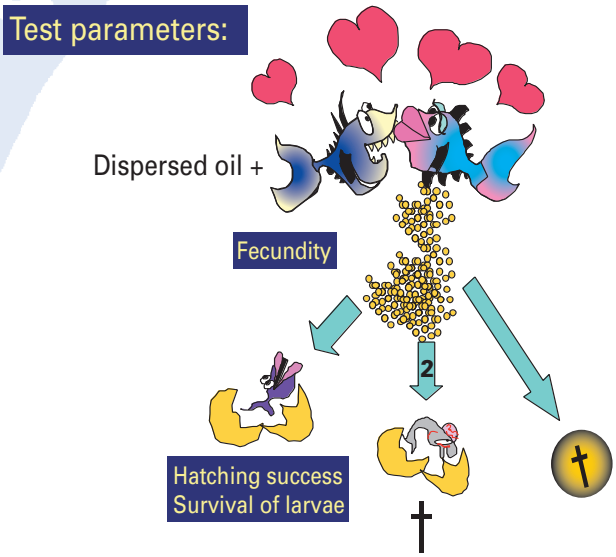

Produced water is the largest wastewater stream in the oil exploration and production process. The chemical composition of the discharged water is complex, including dispersed oil, dissolved hydrocarbons, organic acids, phenols, metals, and traces of chemicals added in the separation and production line.

RF-Akvamiljø has performed a series of fish reproduction experiments in order to test the potential effects of important components of produced water. These experiments were done as part of the **DREAM** (**D**ose related **R**isk and **E**ffect **A**ssessment **M**odel for chronic discharges) program. The objective of the DREAM model is to estimate the environmental risk of produced water discharges. The main question was, how does an increase in exposure concentration and exposure time affect egg production and hatching success. The compounds tested in the DREAM program may not be the most harmful components of produced water, hence the objective of the "DREAM validation project" (financed by Total, and performed in 2002) was to validate the effects observed in the DREAM experiments. In this validation project fish were exposed to dispersed oil, because that is thought to be a sound approximation to produced water. The sheepshead minnow *(Cyprinodon variegatus)* was used as test species. This tiny fish has a short life cycle which gives the opportunity to study effects on ecologically relevant parameters in a rapid and cost efficient way .Like in the DREAM experiments effects on egg production and hatching success of the larvae were studied. Eggs produced by the fish exposed to oil were transferred to clean water to study whether contaminants transferred from the mother (or the health of the parents) may affect the percentage of eggs that hatch. Survival of larvae two weeks after hatching was also studied.

It is important to acquire new information about possible links between biomarker responses and effects at higher levels of biological organisation. Analyses of biomarker responses can be used in biomonitoring, while studies of effects of contaminants on egg production and hatching success have to be done in the laboratory. Contaminants may have specific effects on the reproductive ability of the organisms (e.g. endocrine disruption), or they may cause genetic damage with the possibility of effects in the next generation, or development of tumours. Analysis of PAH metabolites in the bile has proved to be a sensitive biomarker of exposure to oil. PAH metabolites in the bile have been analysed both by the fast and simple semi quantitative fixed wavelength fluorescence method and a quantitative method (GC-MS) to find whether the fast method give a good estimation of the relative concentrations of metabolites in the bile. Analysis of DNA adducts has been included to detect whether the oil can cause genetic damage (co-operation with W. Reichert, NOAA, USA).

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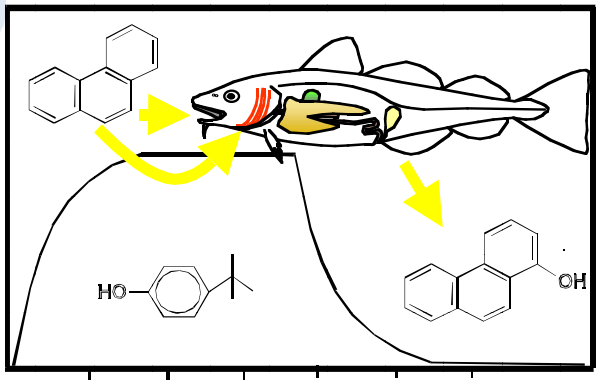
Uptake and excretion of toxic compounds

Several factors may affect the capacity of a contaminant to cause harmful effects. One of them is a contaminant's ability to enter organisms (bioavailability). Tissue distribution and the organism's ability to excrete the compound are other important aspects that will affect potential effects. Availability of such information is important when assessing environmental risk.

Laboratory facilities at RF-Akvamiljø are equipped for exposures and analysis involving isotopes. The techniques used provide high sensitivity and therefore extremely low contaminant concentrations can be tested. Several groups of chemical compounds including *PAHs* and *Alkylated Phenols* are tested on a regular basis and versatile exposure facilities allow us to test a vide variety of organisms including fish and crustaceans.

Recently an extensive study funded by OLF (Norwegian Oil Industry Association) has been conducted. The scope of the study was to measure uptake, tissue distribution and elimination of alkylated phenols in cod. Data will be included in future assessment studies addressing possible environmental impacts of alkylated phenols from produced water.



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NFR-project "Validation of Environmental Risk Assessment"

The oil and gas industry is required by regulations to perform environmental risk assessment prior to oil field developments. The operators must be able to predict potential impacts of regular and possible irregular discharges. Therefore the industry has developed risk assessment tools and obtained toxicological data based on laboratory tests. However, these data must be validated as for their ability to express effects of environmental concern. The overall regulatory requirement is that discharges shall not imply harmful effects.
Most existing literature data is related to **acute toxicity.** However, current environmental concern has shifted towards **chronic effects** and the fitness of organisms has come into focus. Presently emphasis is put on the effects of long term / low dose exposures of parent organisms and the transfer of dispositions to the offspring and effects during their most vulnerable life stage. Field validation of effects found in laboratory requires adequate field measurement methods. This is also crucial for the capacity to predict and measure the *absence* of harmful effects in the field.

Therefore the project aims to develop links between the different tools for risk assessment and monitoring. The laboratory effect studies are based on chronic exposures, and the field monitoring methods are based on biological response methods that can build a **"bridge"** between **laboratory** and **field** measurements. In bridging this gap the methods commonly known as **"biomarkers"** are emphasised. The project will contribute to interpret biomarkers in relation to oil industry discharges, and attempt to put them in a dose: response context where they can be related to the fitness parameters that current environmental risk assessment is based on.
This three-year project is financed by the Norwegian Research Council program PROOF (7 million NOK). In order to obtain optimal use of these resources, the experimental work will be connected to different industry financed projects. *Project title: Validation of methods and data for Environmental Risk Assessment offshore*
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Need for oxygenation in fish cage farms !
A joint project between OxSeaVision – EWOS – RF:

The project started in 2001 by monitoring the environment at the EWOS Innovation cage farms in Oltesvik and Grotnes in Rogaland. A special focus on dissolved oxygen concentrations inside and outside salmon cages, was performed at these and other farms, both in Norway and Canada. Associated parameters, such as temperature, salinity and current velocity and direction were also studied. Strong daily and seasonal fluctuations of dissolved oxygen (DO) were observed: In spring - autumn, oxygen could vary between 40 – 50% of DO saturation within a few hours due to algal activity (or up-welling of deeper water deficient in oxygen (see Figure). Even at low temperature in late winter, abrupt DO reduction from supersaturation 130-140% to 60-70% has been observed. Fluctuating oxygen concentrations, especially at high temperature, affect the appetite and growth of the fish stock. At elevated temperature the stressing conditions caused a considerable mortality (See Figure)

On average, the oxygen saturation seems to be reduced by 10 – 20% in the salmon cages in late summer (> 14 °C, 40 kg fish/cu.m, 2 – 5 cm/s current velocity).

The effects of reduced DO on post-smolt Atlantic salmon were also studied in tanks at EWOS Innovation Dirdal. At 8 – 10°C the trial clearly demonstrated lack of appetite, reduced growth and feed utilisation when oxygen concentrations were kept below 80 – 100% saturation. Between 55 % - 80 % saturation the growth rate decreased about 40% and the feed conversion ratio increased nearly 20%

The focus onwards will be to study the effects of adding oxygen using OSV generated equipment, in periods of oxygen deficit in salmon cages. Monitoring at other EWOS cage farms abroad, e.g. in British Columbia and in Southern Chile, has also clearly indicated the need of adding oxygen to optimise the environmental conditions.

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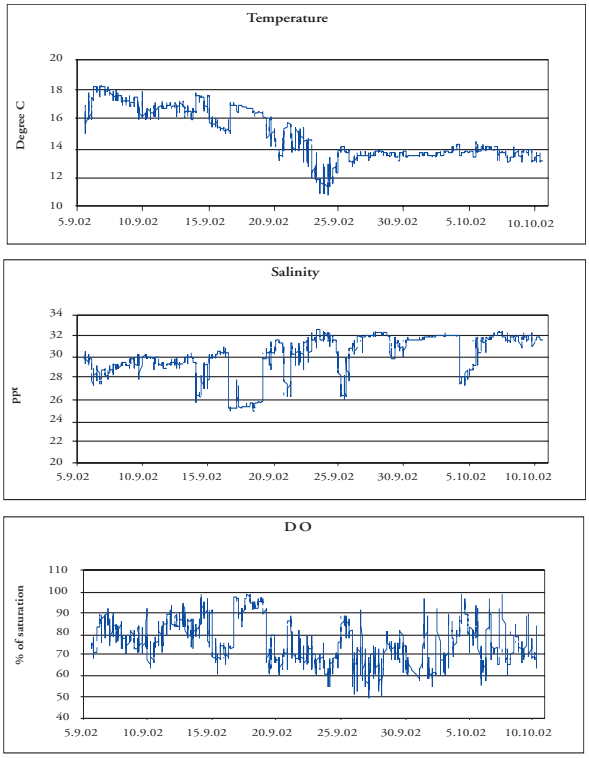
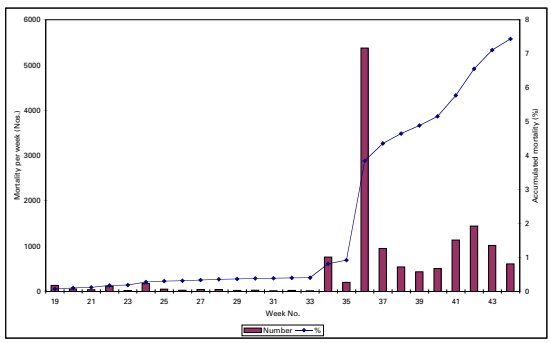


Hydrographic monitoring at 5 m depth outside fish cages EWOS Innovation Oltesvik; 5 September – 10 October 2002. (Week No. 36 – 41). Monitoring frequency: 30 min



Mortality of salmon smolt , EWOS Innovation Oltesvik, during the period Week No. 19 - 43 (May – October)

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